CHEMICAL PROFILING AND ANTIBACTERIAL EFFICACY OF *LAVANDULA PINNATA* L. ESSENTIAL OIL WITH CONVENTIONAL ANTIBIOTICS: SYNERGISTIC INTERACTIONS

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Abstract. The study evaluates the phytochemical composition of *Lavandula pinnata* essential oil (LPEO) and its interactions with three common antibiotics against various bacterial strains. The strains include

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Gram-negative species (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae*) and Gram-positive species (*Staphylococcus aureus, Micrococcus luteus, Bacillus subtilis*). The study aims to find innovative solutions in the field of antimicrobial agents, by combining LPEO with antibiotics to optimize their efficacy. LPEO was extracted by hydrodistillation, followed by qualitative and semi-quantitative GC-MS analysis. The modified medium dilution method was used to determine the minimum inhibitory concentrations (MICs) of LPEO and the antibiotics, with interaction analysis using the fractional inhibitory concentration index (FICI). The results show that LPEO, composed of 24 main compounds including beta-linalol, eucalyptol, camphor and borneol, has MICs of 100 to 1000 µg/mL, with variable sensitivity depending on the strain. Synergistic effects were observed between LPEO and ampicillin on three strains, and partially synergistic effects with amoxicillin and erythromycin. No antagonistic effects were observed. Combinations of LPEO and antibiotics could be effective antimicrobial agents against certain bacterial strains. This promising approach merits further investigation to improve antibacterial therapies and explore potential therapeutic applications.
Keywords: *phytochemicals, Gram-negative bacteria, Gram-positive bacteria, therapy combining, synergistic interaction*

Introduction

Antibiotic resistance constitutes a significant public health concern, posing a substantial threat to the efficacy of bacterial infection treatments (Xia et al., 2020). Antibiotics, the most employed pharmaceuticals for addressing bacterial infections, are progressively losing their effectiveness against resistant bacterial strains (Álvarez-Martínez et al., 2020). This bacterial resistance often arises from inappropriate or excessive antibiotic use, as well as natural selection mechanisms, such as genetic mutation (Lopatkin et al., 2021). Various classes of antibiotics exist, including beta-lactams (e.g., penicillin and cephalosporins), macrolides (such as erythromycin and azithromycin), quinolones (e.g., ciprofloxacin), and others (Teratani et al., 2019). As a result, the decreasing efficiency of antibiotics is becoming a concerning reality. In response to this challenge, scientific research is exploring new avenues. An innovative approach involves synergizing traditional antibiotics with essential oils extracted from plants (Bhattacharya et al., 2021). This combination may help to combat antibiotic resistance by harnessing the natural antimicrobial properties of plant-derived essential oils. Furthermore, the study of aromatic and medicinal plants (AMPs) and their bioactive compounds has revealed significant potential for the discovery of new antimicrobial substances (Mothana et al., 2011; Elbouzidi et al., 2024). AMPs offer an alternative and sustainable source of active molecules, promising prospects in the fight against microbial infections while mitigating the selective pressure that promotes antibiotic resistance.

Lavandula pinnata L. f. (syn *L. pinnata* Lundmark.), often known as Fern Leaf Lavender, is a rare endemic species to the Canary Islands. It is highly valued for its attractive lace-like leaf. This thick and compact evergreen shrub has finely cut, pinnate silver-grey leaves with wide lobes (Haddou et al., 2024). The whole plant is coated with short white hairs, giving it a silvery, felt-like look. It blooms consistently from late spring through summer, producing single or triple-headed flower spikes on stalks measuring 8-14 inches (20-35 cm) in length. It is a hardy plant that tolerates drought and hot weather, belonging to the *Lavandula* genus, traditionally used to treat various conditions, including skin and respiratory infections (Argentieri et al., 2016).

The aim of this study is to assess the phytochemical composition of *Lavandula pinnata* essential oil (LPEO) and its interactions with three commonly used antibiotics in the treatment of bacterial infections (amoxicillin, ampicillin, and erythromycin) against a variety of bacterial strains. The bacterial strains included in this research involve a wide range of Gram-negative pathogens, most notably *Escherichia coli, Pseudomonas*

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aeruginosa, and *Klebsiella pneumoniae*, all of which are clinically relevant due to their frequent association with nosocomial infections and other diseases. Additionally, three Gram-positive counterparts were examined: *Staphylococcus aureus*, *Micrococcus luteus*, and *Bacillus subtilis*, all of which are also significant in the context of bacterial infections. Considering the escalating significance of antibiotic resistance, this study aspires to actively contribute to the exploration of innovative solutions in the field of antimicrobial agents, while providing insights into how natural products such as LPEO could be synergistically integrated with existing antibiotics to optimize their efficacy.

Materials and Methods

Plant Material

The plant samples used in this study were collected from twenty individuals to ensure a representative sample of *Lavandula pinnata*, within the same location in Zegzel (N 34° 50’ 34.202’’, W 2° 21’ 3.504’’), a rural commune located in Berkane prefecture, in the Oriental region of Morocco, in November 2023. These specimens were selected based on the presence of flowers to minimize variability. Following collection, these samples were transported to the Faculty of Sciences at University Mohammed Premier Oujda (Morocco) for precise taxonomic identification. A voucher specimen number CLP-2 was deposited in the same faculty. The leaves and flowers of the plants were subjected to a deliberate drying process in shaded conditions.

Essential Oil Extraction

LPEO was extracted by hydrodistillation, according to the method described by Haddou et al. (2023). A modified Clevenger device was used, consisting of a 2 L flask, a water-cooled condenser, and a graduated separator. 100 g of dried *L. pinnata* aerial parts (80% leaves and 20% flowers by weight), previously grounded with a mortar, were weighed. Then, 1000 mL of distilled water was added to the flask, followed by the introduction of the plant material. The device was heated on a magnetic stirrer at a temperature of 100 °C for 3 hours. The steam entrained the essential oil, which condensed in the condenser and separated from the water in the separator. EO was collected in a flask. The extraction yield was calculated as a percentage relative to the initial mass of the plant.

Qualitative and Semi-Quantitative Analysis of LPEO

The examination of LPEO was performed using a gas chromatograph equipped with a mass spectrometer detector, as reported in the earlier study by Taibi et al. (2024). A gas chromatograph coupled to a mass spectrometer was used to identify and separate the compounds of the LPEO (GC Shimadzu system with a MS QP2010). The capillary column used was a BPX25, with a coating of 95% dimethyl diphenylpolysiloxane. The column had a length of 30 meters, an internal diameter of 0.25 millimeters, and a film thickness of 0.25 micrometers. The carrier gas utilized was pure helium with a purity of 99.99%. It was maintained at a constant flow rate of 3 milliliters per minute. The experimental parameters were as follows: the injection temperature, ion source temperature, and interface temperature were set at 250 °C. The column oven was initially programmed to maintain a temperature of 50 °C for a duration of 1 minute. The given components underwent ionization by electron impact (EI) at an energy level of 70 electron

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volts (eV). The ions' mass was analyzed within the range of 40 to 300 m/z. In order to create the essential oil samples, we added each oil to the chamber at a volume of 1 liter, while also diluting it with an appropriate solvent. Subsequently, we introduced 1 µL of the prepared essential oil into the system using the split mode, employing a split ratio of 90:1. Three assessments were conducted for each sample to guarantee the precision and consistency of the results. Subsequently, the chemicals in the EO were identified by comparing their retention durations and mass spectra with standards and references included in the NIST database. The data was collected and analyzed using the Laboratory Solutions program (v2.5).

Determination of the MIC

The minimum inhibitory concentration (MIC) of LPEO and three antibiotics (amoxicillin, ampicillin, and erythromycin) was determined using a modified broth dilution technique, as described in Taibi et al. (2024). LPEO and antibiotics were solubilized in dimethyl sulfoxide (DMSO) and diluted in Muller Hinton broth (MHB) at a 2-fold dilution factor, resulting in concentration ranges of 3000 to 25 µg/ml for LPEO and 5 to 0.0025 µg/ml for antibiotics. Six bacterial strains, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Micrococcus luteus*, and *Bacillus subtilis*, were selected for the study. These strains were pre-cultured on nutrient agar media and adjusted to McFarland standard 0.5. Each diluted solution of LPEO or antibiotic, totaling 100µL, was added to sterile tubes containing 90 µL of MHB and 10 µL of bacterial inoculum. The tubes were then incubated at 37 °C for 24 hours. Positive controls (bacterial inoculum + MHB) and negative controls (MHB alone) were also included. After incubation, 40 µL of a 0.2% solution of p-iodonitrotetrazolium violet (INT) were added to each tube and incubated for an additional 20 minutes. The MIC was defined as the lowest concentration of LPEO or antibiotic inhibiting bacterial growth, as indicated by the absence of a color change in the culture medium. The experiments were conducted in triplicate, and the mean values were reported.

Synergistic Studies of LPEO with Synthetic Antibiotics

The interaction between LPEO and three synthetic antibiotics, namely amoxicillin, ampicillin, and erythromycin, was examined using the broth dilution technique. Preparations were made by combining 50 µL of each antibiotic dilution, 90 µL of Muller-Hinton broth, and 10 µL of microbial suspension from various bacterial strains. The mixtures were then incubated at a temperature of 37 °C for a duration of 24 hours. Subsequently, a volume of 40 µL of p-iodonitrotetrazolium violet solution at a concentration of 0.4 mg/mL was introduced to facilitate the identification of bacterial proliferation by the observed change in color. The fractional inhibitory concentration index (FICI) was calculated to evaluate the interaction between LPEO and the antibiotics, according to the following formula:

$$\mathrm{FICI} = \frac{\mathrm{MIC_I}}{\mathrm{MIC_I}^{\mathrm{alone}}} + \frac{\mathrm{MIC_{II}}}{\mathrm{MIC_{II}}^{\mathrm{alone}}} \qquad \text{(Eq.1)}$$

where MIC_I and MIC_{II} are the minimum inhibitory concentrations of LPEO and the antibiotics respectively, when they are used in combination, and MIC_I^{alone} and MIC_{II}^{alone} are the minimum inhibitory concentrations of LPEO and the antibiotics respectively, when they are used alone.

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A synergy was considered when the FICI was less than or equal to 0.5, a partial synergy when the FICI was between 0.5 and 0.75, no effect when the FICI was between 0.75 and 2, and an antagonism when the FICI was greater than 2 (Hall et al., 1983). All experiments were performed in triplicate.

Results

Phytochemical Composition of the LPEO

Table 1 and *Figure 1* depicts the chemical composition of LPEO. The essential oil comprises 24 compounds, identified through gas chromatography coupled with mass spectrometry (GC-MS). Retention time (R.T) represents the duration required for a compound to traverse the chromatographic column. The percentage of surface area (Area %) signifies the relative proportion of each compound in the essential oil. Analysis of the table reveals that LPEO is predominantly composed of monoterpenes and sesquiterpenes. The most abundant compounds include beta-Linalool (28.10%), Eucalyptol (16.83%), Camphor (16.21%), and Borneol (9.09%).

Table 1. *Chemical composition of the studied L. pinnata essential oil*

No.	Compounds	RT (min)	% Area
1	α-pinene	5.213	0.27
2	Camphene	5.475	0.36
3	β-pinene	5.942	0.37
4	β-myrcene	6.115	0.65
5	*n*-Hexyl acetate	6.462	0.21
6	2-Carene	6.590	0.16
7	β-cymene	6.740	0.53
8	Eucalyptol	6.850	16.83
9	α-methyl-α-[4-methyl-3-pentenyl]oxiranemethanol	7.545	2.21
10	Linalool Oxide	7.803	2.06
11	α-Linalool	8.006	28.10
12	Camphor	8.802	16.21
13	Borneol	9.200	9.09
14	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)	9.335	4.87
15	*p*-Menth-1-en-8-ol, (S)-(-)-	9.566	7.92
16	Isopropenyl-5-methyl-4-hexenyl acetate	10.886	1.31
17	Thymol	11.170	3.10
18	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)	11.974	0.88
19	2E)-3,7-Dimethyl-2,6-octadienyl methyl carbonate	12.242	1.52
20	Caryophyllene	12.983	0.24
21	,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, (E)	14.753	0.48
22	Caryophyllene oxide	15.228	1.05
23	γ-Cadinene	15.918	0.92
24	α-Bisabolol	16.357	0.66

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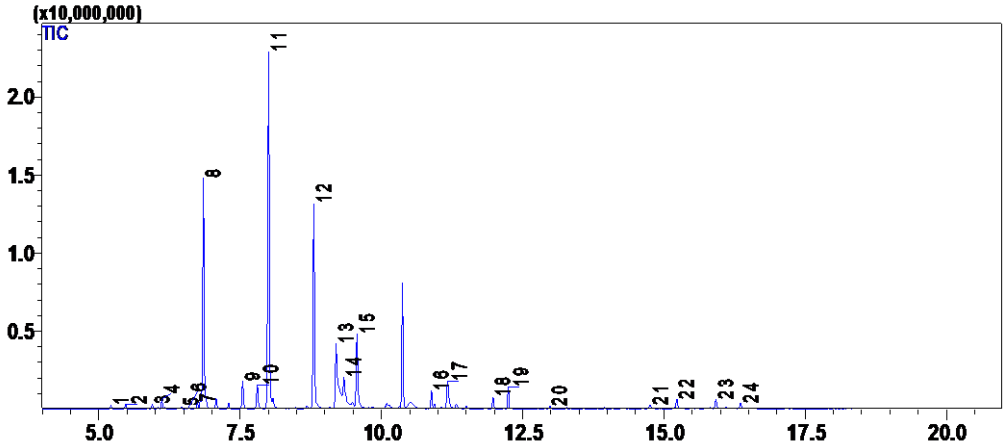

Figure 1. *GC-MS chromatogram of the chemical composition of LPEO*

Antibacterial Activity of LPEO and Conventional Antibiotics

Table 2 presents the minimum inhibitory concentrations (MIC) of LPEO and three conventional antibiotics (ampicillin, amoxicillin, and erythromycin) against different bacterial strains. LPEO exhibits MICs ranging from 100 to 1000 µg/mL. The bacterial strain *B. subtilis* displays the highest sensitivity, with an MIC of 100 µg/mL, whereas *P. aeruginosa* shows relatively higher resistance with an MIC of 1000 µg/mL.

Table 2. *Minimum inhibitory concentration of L. pinnata essential oil (LPEO), and three conventional antibiotics (ampicillin, amoxicillin, and erythromycin) against different bacterial strains*

EO/Antibiotics	MIC (µg/mL)					
	S. aureus	***E. coli***	***B. subtilis***	***P. aeruginosa***	***K. pneumoniae***	***M. luteus***
LPEO	150	150	100	1000	500	250
Ampicillin	0.50	0.75	0.50	1.25	0.30	1.00
Amoxicillin	2.25	0.75	1.50	1.3	1.50	0.75
Erythromycin	1.50	1.25	0.50	0.50	1.75	0.75

Ampicillin demonstrates notable efficacy, with MICs ranging from 0.30 to 1.25 µg/mL. *K. pneumoniae* is particularly sensitive with an MIC of 0.30 µg/mL, while *P. aeruginosa* exhibits increased resistance with an MIC of 1.25 µg/mL. Amoxicillin shows MICs ranging from 0.75 to 2.25 µg/mL. *E. coli* and *M. luteus* exhibit relative sensitivity with MIC of 0.75 µg/mL, whereas *S. aureus* presents a higher MIC of 2.25 µg/mL. Erythromycin demonstrates MICs ranging from 0.50 to 1.75 µg/mL. *B. subtilis* is particularly sensitive with an MIC of 0.50 µg/mL, while *K. pneumoniae* shows relative resistance with an MIC of 1.75 µg/mL.

Synergistic Potential of LPEO with Conventional Antibiotics

The effect of various combinations of Lavandula pinnata essential oil (LPEO) with ampicillin, amoxicillin, and erythromycin on the growth of different bacterial strains is summarized in *Tables 3, 4,* and *5*.

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Table 3. *Effect of combinations between L. pinnata essential oil (LPEO), and ampicillin*

Bacterial strain	Combinations	MICa [a] (µg/mL)	MICc [b] (µg/mL)	FIC [c]	FICI [d]	Interaction
S. aureus ATCC 25923	*L. pinnata* Ampicillin	150 0.50	100 0.1	0.67 0.20	0.87	No effect
E. coli ATCC	*L. pinnata* Ampicillin	150 0.75	50 0.1	0.33 0.13	0.46	Synergy
B. subtilis ATCC	*L. pinnata* Ampicillin	100 0.50	25 0.1	0.25 0.20	0.45	Synergy
P. aeruginosa ATCC 27853	*L. pinnata* Ampicillin	1000 1.25	100 0.3	0.10 0.24	0.34	Synergy
K. pneumoniae	*L. pinnata* Ampicillin	500 0.30	400 0.2	0.80 0.67	1.47	No effect
M. luteus	*L. pinnata* Ampicillin	250 1.00	150 0.1	0.60 0.10	0.70	Partial synergy

[a] MICa: MIC of single component tested alone; [b] MICc: MIC of each component in the association at the most effective inhibition growth; [c] FIC: Fractional inhibitory concentration is determined by the ratio MICc/MICo; [d] FICI (fractional inhibitory concentration index): FIC of Ampicillin + FIC of LPEO

Table 4. *Effect of combinations between L. pinnata essential oil (LPEO), and amoxicillin*

Bacterial strain	Combinations	MICa [a] (µg/mL)	MICc [b] (µg/mL)	FIC [c]	FICI [d]	Interaction
S. aureus ATCC 25923	*L. pinnata* Amoxicillin	150 2.25	50 0.30	0.33 0.13	0.47	Synergy
E. coli	*L. pinnata* Amoxicillin	150 0.75	75 0.10	0.50 0.13	0.63	Partial synergy
B. subtilis	*L. pinnata* Amoxicillin	100 1.50	150 0.20	1.50 0.13	1.63	No effect
P. aeruginosa ATCC 27853	*L. pinnata* Amoxicillin	1000 1.00	350 0.20	0.35 0.20	0.55	Partial synergy
K. pneumoniae	*L. pinnata* Amoxicillin	500 1.50	500 0.70	1.00 0.47	1.47	No effect
M. luteus	*L. pinnata* Amoxicillin	250 0.75	100 0.20	0.40 0.27	0.67	Partial synergy

[a] MICa: MIC of single component tested alone; [b] MICc: MIC of each component in the association at the most effective inhibition growth; [c] FIC: Fractional inhibitory concentration is determined by the ratio MICc/MICo; [d] FICI (fractional inhibitory concentration index): FIC of amoxicillin + FIC of LPEO

LPEO and Ampicillin

- Synergistic effect on *E. coli, B. subtilis,* and *P. aeruginosa*.
- Partially synergistic effect on *M. luteus*.
- No effect on *S. aureus* and *K. pneumoniae*.
- No antagonistic effects observed.

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LPEO and Amoxicillin

- Synergistic effect on *S. aureus*.
- Partially synergistic effect on *E. coli*, *P. aeruginosa*, and *M. luteus*.
- No effect on *B. subtilis* and *K. pneumoniae*.
- No antagonistic effects observed.

LPEO and Erythromycin

- Synergistic effect on *E. coli*, *P. aeruginosa*, and *M. luteus*.
- Partially synergistic effect on *S. aureus*.
- No effect on *B. subtilis* and *K. pneumoniae*.
- No antagonistic effects observed.

Table 5. *Effect of combinations between L. pinnata essential oil (LPEO), and erythromycin*

Bacterial strain	Combinations	MICa [a] (µg/mL)	MICc [b] (µg/mL)	FIC [c]	FICI [d]	Interaction
S. aureus ATCC 25923	*L. pinnata* Erythromycin	150 0.50	50 0.1	0.33 0.20	0.53	Partial synergy
E. coli	*L. pinnata* Erythromycin	150 0.75	25 0.2	0.17 0.27	0.43	Synergy
B. subtilis	*L. pinnata* Erythromycin	100 0.50	75 0.4	0.75 0.80	1.55	No effect
P. aeruginosa ATCC 27853	*L. pinnata* Erythromycin	1000 1.25	100 0.5	0.10 0.40	0.50	Synergy
K. pneumoniae	*L. pinnata* Erythromycin	500 0.30	250 0.1	0.50 0.33	0.83	No effect
M. luteus	*L. pinnata* Erythromycin	250 1.00	50 0.2	0.20 0.20	0.40	Synergy

[a] MICa: MIC of single component tested alone; [b] MICc: MIC of each component in the association at the most effective inhibition growth; [c] FIC: Fractional inhibitory concentration is determined by the ratio MICc/MICo; [d] FICI (fractional inhibitory concentration index): FIC of erythromycin + FIC of LPEO

Discussion

Phytochemical Composition of the LPEO

The phytochemical analysis of LPEO reveals a distinctive composition, with a high concentration of beta-Linalool (28.10%), Eucalyptol (16.83%), Camphor (16.21%), and Borneol (9.09%). These compounds significantly contribute to the essential oil's characteristic scent and are associated with its broad spectrum of biological activities, including antibacterial, antioxidant, anti-inflammatory, and analgesic effects (Carrasco et al., 2016; Cardia et al., 2018). The observed chemical profile of LPEO notably diverges from prior research findings. For instance, Argentieri et al. (2016) identified carvacrol as the principal compound in LPEO. Whereas Figueiredo et al. (1995) reported p-phellandrene as the predominant component in essential oils of *L. pinnata* from Portugal. These discrepancies in chemical composition can be attributed to various factors, including genotypic and phenotypic diversity within the plant species, environmental

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influences, and differing cultivation conditions (Tak et al., 2016; Vaičiulytė et al., 2017). Environmental factors, such as soil composition, climate, altitude, and seasonal changes, have a significant impact on the chemical composition and yield of essential oils (Aboukhalid et al., 2017; Khalil et al., 2020). Moreover, genetic variations within the species can lead to different chemotypes, which are distinguished by distinct dominant compounds (Rabotyagov et al., 2018). These factors affect the production of key compounds and biological activities, emphasizing the need to optimize growing conditions to achieve desired oil qualities. Understanding these influences is crucial for maximizing the therapeutic and commercial potential of essential oils.

Antibacterial Activity of LPEO and Conventional Antibiotics

The diversity in the chemical composition of LPEO suggests a complex synergy among its various constituents, which may explain the observed variations in antimicrobial activity against different bacterial strains. Compounds such as linalool, borneol, thymol, and caryophyllene, known for their antimicrobial properties, likely contribute to this efficacy (Kotan et al., 2007; Dahham et al., 2015; Zhang et al., 2021). The genus *Lavandula* is renowned for its notable antibacterial properties. For instance, *Lavandula angustifolia* essential oil is highly effective against *Staphylococcus aureus*, enhancing macrophage responses and reducing inflammation (Giovannini et al., 2016). Similarly, *Lavandula stoechas* essential oil, has shown strong antibacterial effects against pathogens like *Escherichia coli* and *Pseudomonas aeruginosa*, proving its utility in both healthcare and food safety applications (Bouyahya et al., 2017). The MIC values indicate that LPEO has a broad spectrum of antibacterial activity, particularly against *B. subtilis*, with an MIC of 100 µg/mL. This suggests that LPEO could be considered for therapeutic applications where traditional antibiotics might face resistance issues. For instance, *P. aeruginosa*, known for its resistance to many antibiotics, shows the highest MIC against LPEO, highlighting the challenge in treating infections caused by this bacterium. Ampicillin's MIC range of 0.30 to 1.25 µg/mL confirms its effectiveness, especially against *K. pneumoniae*. In contrast, *P. aeruginosa*'s higher MIC of 1.25 µg/mL aligns with its known resistance profile, suggesting the need for alternative or adjunct therapies. Amoxicillin's MIC range from 0.75 to 2.25 µg/mL and erythromycin's from 0.50 to 1.75 µg/mL provide insights into their relative efficacy against various strains. The sensitivity of *E. coli* and *M. luteus* to amoxicillin and *B. subtilis* to erythromycin emphasizes their potential as effective treatments for infections caused by these bacteria. Overall, these findings underscore the significance of considering the specificity of antimicrobial agents against different bacterial strains. The potential synergistic interactions between LPEO and conventional antibiotics could offer novel strategies in antimicrobial therapy, particularly in combating resistant bacterial strains.

Synergistic Potential of LPEO with Conventional Antibiotics

Confronted by the escalating challenge of antibiotic resistance, a strategic therapeutic paradigm emerges as a promising resolution. This approach entails the synergistic integration of artificially synthesized antibiotics with naturally occurring compounds, exemplified by the incorporation of essential oils (Silva et al., 2019). These combinations of antimicrobial agents can elicit various effects, ranging from synergy to antagonism, as well as addition or indifference. Synergistic effects are particularly noteworthy as they enhance the efficacy of the combinatorial approach, surpassing that of its individual components. This enables a more effective and less toxic means of combating bacterial

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infections. These findings align with previously documented results concerning various essential oils extracted from medicinal plants (Boonyanugomol et al., 2017; El Atki et al., 2019; Jugreet and Mahomoodally, 2020). The synergies observed between LPEO and antibiotics of the penicillin class (ampicillin and amoxicillin) or macrolides (erythromycin) exhibit variable effects, ranging from synergistic to partial or null concerning the tested bacterial strains. The cooperative interactions indicate a possible alignment between the ways antibiotics work, and the bioactive components found in the essential oil. Essential oils include secondary metabolites that use a wide range of ways to combat germs. Phenols and terpenes hinder the development of bacterial cells by interfering with essential metabolic processes (Magi et al., 2015). Certain substances disrupt the process of bacterial DNA and RNA production, while others impede the activity of crucial enzymes (Ait Said et al., 2015; Soulaimani et al., 2021). Essential oils often blend these compounds, so increasing their effectiveness by specifically targeting different elements of bacterial metabolism. Nevertheless, the precise methods by which essential oils exert their effects may differ based on the chemical makeup of the oil and the susceptibility of the bacteria being targeted (Sharma et al., 2023). Simultaneously, antibiotics also use a variety of ways to combat germs. Ampicillin and amoxicillin primarily work by blocking the formation of the bacterial cell wall, which weakens the germs and makes them susceptible (Araz et al., 2018). In contrast, erythromycin functions by impeding the production of bacterial proteins by its interaction with ribosomes, which hinders the creation of essential proteins necessary for bacterial development and viability (Magi et al., 2015).

The observed synergistic effects suggest that LPEO can potentiate the efficacy of conventional antibiotics, offering a less toxic and more effective means of treating bacterial infections. This is particularly significant for strains like *E. coli* and *P. aeruginosa,* which are known for their resistance to multiple antibiotics (Strateva and Yordanov, 2009; Kresken et al., 2023). the combination of LPEO with conventional antibiotics holds potential as a natural antimicrobial strategy or as an adjuvant therapy to enhance antibiotic efficacy. This approach is particularly relevant in the context of growing antibiotic resistance, highlighting the need for innovative solutions in antimicrobial therapy.

Conclusion

The results from the study on various combinations of *Lavandula pinnata* essential oil with conventional antibiotics reveal diverse effects on the growth of different bacterial strains. Notably, synergistic interactions were observed against *E. coli, B. subtilis, P. aeruginosa, S. aureus,* and *M. luteus*. These synergies resulted in a significant reduction in the minimum inhibitory concentrations of each component when used in combination, highlighting the enhanced efficacy of LPEO-ampicillin, LPEO-amoxicillin, and LPEO-erythromycin mixtures compared to their individual use. These findings are consistent with prior research on essential oils from medicinal plants, reinforcing the potential of these natural compounds as alternatives or adjuncts to conventional antibiotics. The observed synergistic effects suggest that combining LPEO with antibiotics could be a viable strategy for developing potent antimicrobial agents targeting specific bacterial strains. This approach is particularly promising in the context of antibiotic resistance, which is a growing global health challenge. The study underscores the importance of exploring the synergy between natural compounds and antibiotics to

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enhance antibacterial treatment efficacy. These results advocate for further research into the specific mechanisms of action and potential clinical applications of LPEO-antibiotic combinations. While this study utilized bacterial strains that are not classified as multi-resistant, the observed synergistic effects between LPEO and conventional antibiotics suggest promising potential. Future research should include multi-resistant bacterial strains to evaluate the full therapeutic potential of LPEO-antibiotic combinations in combating antibiotic-resistant infections. This approach will provide a more comprehensive understanding of the clinical relevance and efficacy of these synergistic treatments. The promising results from this study highlight the need for continued research into LPEO and its synergistic interactions with antibiotics. This line of investigation could contribute significantly to the development of more effective and less toxic antimicrobial therapies, addressing the urgent need for novel strategies to combat antibiotic-resistant bacterial infections.

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REFERENCES

[1] Aboukhalid, K., Al Faiz, C., Douaik, A., Bakha, M., Kursa, K., Agacka-Mołdoch, M., Machon, N., Tomi, F., Lamiri, A. (2017): Influence of environmental factors on essential oil variability in *Origanum compactum* Benth. growing wild in Morocco. – Chemistry & Biodiversity 14(9): e1700158.

[2] Ait Said, L., Zahlane, K., Ghalbane, I., El Messoussi, S., Romane, A., Cavaleiro, C., Salgueiro, L. (2015): Chemical composition and antibacterial activity of *Lavandula coronopifolia* essential oil against antibiotic-resistant bacteria. – Natural Product Research 29(6): 582-585.

[3] Álvarez-Martínez, F. J., Barrajón-Catalán, E., Micol, V. (2020): Tackling antibiotic resistance with compounds of natural origin: A comprehensive review. – Biomedicines 8(10): 405.

[4] Araz, K., Patel, S., Khajehdehi, N., Wade, C. B., Thomas, J., Krajnyk, I. (2018): Interactions between lemongrass and lavender essential oils in combination with ampicillin influencing antibacterial activity on *Sporosarcina ureae* and *Serratia liquefaciens*. – Western Undergraduate Research Journal: Health & Natural Sciences 9(1).

[5] Argentieri, M. P., De Lucia, B., Cristiano, G., Avato, P. (2016): Compositional analysis of Lavandula pinnata essential oils. – Natural Product Communications 11(3): 1934578X1601100305.

[6] Bhattacharya, R., Rolta, R., Dev, K., Sourirajan, A. (2021): Synergistic potential of essential oils with antibiotics to combat fungal pathogens: Present status and future perspectives. – Phytotherapy Research 35(11): 6089-6100.

[7] Boonyanugomol, W., Kraisriwattana, K., Rukseree, K., Boonsam, K., Narachai, P. (2017): In vitro synergistic antibacterial activity of the essential oil from Zingiber cassumunar Roxb against extensively drug-resistant Acinetobacter baumannii strains. – Journal of Infection and Public Health 10(5): 586-592.

[8] Bouyahya, A., Chamkhi, I., Balahbib, A., Rebezov, M., Shariati, M. A., Wilairatana, P., Mubarak, M. S., Benali, T., El Omari, N. (2017): Lavandula stoechas essential oil from

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_51695182

Morocco as novel source of antileishmanial, antibacterial and antioxidant activities. – Biocatalysis and Agricultural Biotechnology 12: 179-184.

[9] Cardia, G. F. E., Silva-Filho, S. E., Silva, E. L., Uchida, N. S., Cavalcante, H. A. O., Cassarotti, L. L., Salvadego, V. E. C., Spironello, R. A., Bersani-Amado, C. A., Cuman, R. K. N. (2018): Effect of lavender (*Lavandula angustifolia*) essential oil on acute inflammatory response. – Evidence-Based Complementary and Alternative Medicine 2018(1): 1413940.

[10] Carrasco, A., Martinez-Gutierrez, R., Tomas, V., Tudela, J. (2016): Lavandin (Lavandula× intermedia Emeric ex Loiseleur) essential oil from Spain: determination of aromatic profile by gas chromatography–mass spectrometry, antioxidant and lipoxygenase inhibitory bioactivities. – Natural Product Research 30(10): 1123-1130.

[11] Dahham, S. S., Tabana, Y. M., Iqbal, M. A., Ahamed, M. B. K., Ezzat, M. O., Majid, A. S. A., Majid, A. M. S. A. (2015): The anticancer, antioxidant and antimicrobial properties of the sesquiterpene β-caryophyllene from the essential oil of *Aquilaria crassna*. – Molecules 20(7): 11808-11829.

[12] El Atki, Y., Aouam, I., El Kamari, F., Taroq, A., Nayme, K., Timinouni, M., Lyoussi, B., Abdellaoui, A. (2019): Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics. – Journal of Advanced Pharmaceutical Technology & Research 10(2): 63.

[13] Elbouzidi, A., Taibi, M., Baraich, A., Haddou, M., Loukili, E. H., Asehraou, A., Mesnard, F., Addi, M. (2024): Enhancing Secondary Metabolite Production in Pelargonium graveolens Hort. Cell Cultures: Eliciting Effects of Chitosan and Jasmonic Acid on Bioactive Compound Production. – Horticulturae 10(5): 521.

[14] Figueiredo, A. C., Barroso, J. G., Pedro, L. G., Sevinate-Pinto, I., Antunes, T., Fontinha, S. S., Looman, A., Scheffer, J. J. C. (1995): Composition of the essential oil of Lavandula pinnata L. fil. var. pinnata grown on Madeira. – Flavour and Fragrance Journal 10(2): 93-96.

[15] Giovannini, D., Gismondi, A., Basso, A., Canuti, L., Braglia, R., Canini, A., Mariani, F., Cappelli, G. (2016): *Lavandula angustifolia* Mill. essential oil exerts antibacterial and anti-inflammatory effect in macrophage mediated immune response to Staphylococcus aureus. – Immunological Investigations 45(1): 11-28.

[16] Haddou, M., Taibi, M., Elbouzidi, A., Loukili, E. H., Yahyaoui, M. I., Ou-Yahia, D., Mehane, L., Addi, M., Asehraou, A., Chaabane, K. (2023): Investigating the Impact of Irrigation Water Quality on Secondary Metabolites and Chemical Profile of Mentha piperita Essential Oil: Analytical Profiling, Characterization, and Potential Pharmacological Applications. – International Journal of Plant Biology 14(3): 638-657.

[17] Haddou, M., Elbouzidi, A., Taibi, M., Baraich, A., El Hassania, L., Bellaouchi, R., Saalaoui, E., Asehraou, A., Salamatullah, A. M., Bourhia, M., Nafidi, H. A., Addi, M., El Guerrouj, B., Chaabane, K. (2024): Exploring the Multifaceted Bioactivities of *Lavandula pinnata* L. Essential Oil: Promising Pharmacological Activities. – Frontiers in Chemistry 12: 1383731.

[18] Hall, M. J., Middleton, R. F., Westmacott, D. (1983): The fractional inhibitory concentration (FIC) index as a measure of synergy. – Journal of Antimicrobial Chemotherapy 11(5): 427-433.

[19] Jugreet, B. S., Mahomoodally, M. F. (2020): Essential oils from 9 exotic and endemic medicinal plants from Mauritius shows in vitro antibacterial and antibiotic potentiating activities. – South African Journal of Botany 132: 355-362.

[20] Khalil, N., El-Jalel, L., Yousif, M., Gonaid, M. (2020): Altitude impact on the chemical profile and biological activities of *Satureja thymbra* L. essential oil. – BMC complementary Medicine and Therapies 20: 1-11.

[21] Kotan, R., Kordali, S., Cakir, A. (2007): Screening of antibacterial activities of twenty-one oxygenated monoterpenes. – Zeitschrift für Naturforschung C 62(7-8): 507-513.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_51695182

[22] Kresken, M., Wohlfarth, E., Wichelhaus, T. A., Gatermann, S. G., Pfennigwerth, N., Eisfeld, J., Seifert, H., German BL/BLI Study Group (2023): Susceptibility of Multidrug-Resistant Escherichia coli, Klebsiella pneumoniae, and Pseudomonas aeruginosa from Germany to Ceftolozane–Tazobactam, Ceftazidime–Avibactam, and Imipenem–Relebactam. – Microbial Drug Resistance 29(4): 138-144.
[23] Lopatkin, A. J. Bening, S. C., Manson, A. L., Stokes, J. M., Kohanski, M. A., Badran, A. H., Earl, A. M., Cheney, N. J., Yang, J. H., Collins, J. J. (2021): Clinically relevant mutations in core metabolic genes confer antibiotic resistance. – Science 371(6531): eaba0862.
[24] Magi, G., Marini, E., Facinelli, B. (2015): Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant Group A Streptococci. – Frontiers in Microbiology 6: 130454.
[25] Mothana, R. A. A., Kriegisch, S., Harms, M., Wende, K., Lindequist, U. (2011): Assessment of selected Yemeni medicinal plants for their in vitro antimicrobial, anticancer, and antioxidant activities. – Pharmaceutical Biology 49(2): 200-210.
[26] Rabotyagov, V. D., Palii, A. E., Khokhlov, Y. S. (2018): Interspecific Hybridization in Lavandin (Lavandula× intermedia Emeric ex Loisel.) Breeding for Essential Oil Quality. – Agricult. Biol. (Sel'skokhozyaĭstvennaya Biologiya) 53(3): 547-556.
[27] Sharma, N., Sheikh, Z. N., Alamri, S., Singh, B., Singh Kesawat, M., Guleria, S. (2023): Chemical Composition, Antibacterial and Combinatorial Effects of the Essential Oils from Cymbopogon spp. and Mentha arvensis with Conventional Antibiotics. – Agronomy 13(4): 1091.
[28] Silva, D. M., Costa, P. A., Ribon, A. O. B., Purgato, G. A., Gaspar, D.-M., Diaz, M. A. N. (2019): Plant extracts display synergism with different classes of antibiotics. – Anais da Academia Brasileira de Ciências 91(2).
[29] Soulaimani, B., El Hidar, N., El Fakir, S. B., Mezrioui, N., Hassani, L., Abbad, A. (2021): Combined antibacterial activity of essential oils extracted from *Lavandula maroccana* (Murb.), *Thymus pallidus* Batt. and *Rosmarinus officinalis* L. against antibiotic-resistant Gram-negative bacteria. – European Journal of Integrative Medicine 43: 101312.
[30] Strateva, T., Yordanov, D. (2009): Pseudomonas aeruginosa–a phenomenon of bacterial resistance. – Journal of Medical Microbiology 58(9): 1133-1148.
[31] Taibi, M., Elbouzidi, A., Haddou, M., Loukili, E. H., Bellaouchi, R., Asehraou, A., Douzi, Y., Addi, M., Salamatullah, A. M., Nafidi, H. A., Bourhia, M. (2024): Chemical Profiling, Antibacterial Efficacy, and Synergistic Actions of Ptychotis verticillata Duby Essential Oil in Combination with Conventional Antibiotics. – Natural Product Communications 19(1): 1934578X231222785.
[32] Taibi, M., Elbouzidi, A., Haddou, M., Belbachir, Y., Baraich, A., Khalil, Z., Bellaouchi, R., Asehraou, A., Addi, M., AL-Farga, A., Al-Maaqar, S. M., El Guerrouj, B., Chaabane, K. (2024): Phytochemical characterization and multifaceted bioactivity assessment of essential oil from Clinopodium nepeta subsp. ascendens: potential for the food and pharmaceutical sectors. – CyTA-Journal of Food 22(1): 2348735.
[33] Tak, J.-H., Jovel, E., Isman, M. B. (2016): Contact, fumigant, and cytotoxic activities of thyme and lemongrass essential oils against larvae and an ovarian cell line of the cabbage looper, Trichoplusiani. – Journal of Pest Science 89: 183-193.
[34] Teratani, Y., Hagiya, H., Koyama, T., Adachi, M., Ohshima, A., Zamami, Y., Tanaka, H. Y., Tatebe, Y., Tasaka, K., Mikami, N. (2019): Pattern of antibiotic prescriptions for outpatients with acute respiratory tract infections in Japan, 2013-15: a retrospective observational study. – Family Practice 36(4): 402-409.
[35] Vaičiulytė, V., Ložienė, K., Taraškevičius, R., Butkienė, R. (2017): Variation of essential oil composition of *Thymus pulegioides* in relation to soil chemistry. – Industrial Crops and Products 95: 422-433.
[36] Xia, Y., Wang, D., Pan, X., Xia, B., Weng, Y., Long, Y., Ren, H., Zhou, J., Jin, Y., Bai, F. (2020): TpiA is a key metabolic enzyme that affects virulence and resistance to

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_51695182

aminoglycoside antibiotics through CrcZ in Pseudomonas aeruginosa. – Mbio 11(1): 10-1128.

[37] Zhang, Y., Liu, Y., Luo, J., Jie, J., Deng, X., Song, L. (2021): The herbal compound thymol targets multiple salmonella typhimurium virulence factors for Lon protease degradation. – Frontiers in Pharmacology 12: 674955.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2206_51695182