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Phytochemical evaluation and in-vitro antibacterial activity of ethanolic extracts of Moroccan *Lavandula* x *intermedia* leaves and flowers

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Abstract. *Fliou J, Spinola F, Riffi O, Zriouel A, Amechrouq A, Nalbone L, Giuffrida A, Giarratana F. 2023. Phytochemical evaluation and in-vitro antibacterial activity of ethanolic extracts of Moroccan* Lavandula *x* intermedia *leaves and flowers*. *Biodiversitas 24: 5788-5795.* This study performed a preliminary evaluation of the phytochemical composition and *in vitro* antibacterial activity of ethanolic extracts of *Lavandula* x *intermedia* leaves and flowers collected in the Fez-Meknes region of Morocco. Phytochemical analyses comprised qualitative colorimetric determinations of alkaloids, anthraquinones, and terpenes and quantitative analysis of total polyphenols, flavonoids, and condensed tannins by UV spectrophotometer. Antibacterial activity was evaluated by determining minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values against different ATCC bacterial strains. The phytochemical analysis showed a high amount of total polyphenols, flavonoids, and tannins in the leaf extract and a higher amount of terpenes based on colorimetric reaction than the flower extract. A positive colorimetric reaction for alkaloids and anthraquinones was detected for both extracts. The antibacterial activity of leaves and flower extract was not different against Gram-positive and Gram-negative strains ($p<0.05$). The results of the present study suggest the possible use of ethanolic extracts of *L.* x *intermedia* collected in the Fez-Meknes region of Morocco as a natural agent against bacterial pathogens.

Keywords: Antimicrobial activity, *Lavandula* spp., lavender, lavandin, UV spectrophotometric analysis, MIC, MBC

Abbreviations: PL: powders of leaves; PF: powders of flowers; LiLE: *Lavandula* x *intermedia* extract of leaves; LiFE: *Lavandula* x *intermedia* extract of flowers; FC: Folin-Ciocalteu; GAE: gallic acid equivalent; DP: dry plant; QE: quercetin equivalent; CE: catechol equivalent; BHIb: Brain Heart Infusion broth; ATCC: American Type Culture Collection; CCUG: Culture Collection of the University of Gothenburg; ECT: Colección Española De Cultivo Tipo; MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; TTC: 2,3,5-diphenyltetrazolium chloride

INTRODUCTION

One of the main concerns of modern infectious disease is the growing incidence of antibiotic resistance in bacteria (WHO 2017; Larsson and Flach 2022). The excessive and inappropriate use of antibiotics in humans and animals has led to the development of different defense strategies in many bacterial species, resulting in higher resistance to standard therapies being less effective and even ineffective (Hansan et al. 2020). Furthermore, in recent decades, the inappropriate use of antibiotics to control disease in livestock has increased bacterial resistance (Zhao et al. 2021). The World Health Organization has warned about the spread of multidrug-resistant bacteria and requires urgent multisectoral actions to overcome this global health threat (WHO 2021).

Although bacterial resistance has become a major concern for the scientific community and the public, overcoming bacterial resistance has still not been achieved. One of the modern research approaches currently proposes using different natural compounds as alternatives to conventional antibiotics (Pancu et al. 2021). Not by chance, ethnobotany and ethnopharmacology, which aim to study and develop natural drugs and validate the traditional use of medicinal plants, are gaining interest nowadays (Ed-Dra et al. 2021; Anand et al. 2019). In this regard, there are numerous studies that have reported a significant antimicrobial activity for different plant-derived products, such as essential oils, spices and extracts, against a wide range of bacterial species, Gram-positive and Gram-negative (Ed-Dra et al. 2021; Vaou et al. 2021; Nefzi et al. 2023). The use of natural products for food preservatives is in increasing demand as natural alternatives to common chemical preservatives to extend the shelf-life of products or against food-borne pathogens (Nalbone et al. 2022).

The antimicrobial compounds in plants are usually used through the preparation of their extracts (Álvarez-Martínez et al. 2021). Plant extracts are obtained by processing the whole plant or single parts with different solvents that extract and concentrate biologically active compounds. Ethanol is one of the most widely used solvents for preparing plant extracts since it effectively extracts

hydrophilic and lipophilic compounds and is easy to obtain and safe (Wendakoon et al. 2021).

In traditional medicine, lavender (*Lavandula* spp., Linneo 1753) has been used for centuries due to its beneficial effects, such as anticonvulsant, antispasmodic, anxiolytic, analgesic, anti-inflammatory, antioxidant, antifungal and antimicrobial (Koriem 2021; Batiha et al. 2023). *Lavandula* spp. has a high diversity of species and subspecies, but the most widely used are *L. angustifolia*, *L. latifolia*, and *L.* x *intermedia* (Blažeković et al. 2018; Batiha et al. 2023). Although the chemical composition of *Lavandula* spp. varies considerably based on the geographical origin, age of the plant, harvesting season, and collecting procedure; however, the main components responsible for its biological properties are derivates of the shikimate pathway, including phenolic compounds (flavonoids and tannins) and alkaloids and compounds generated by the mevalonate pathway such as anthraquinones and terpenes (linalool, linalyl acetate, camphor, 1,8-cineol, and isoborneol) (Garzoli et al. 2019). The antimicrobial activity of ethanolic extracts of *Lavandula* spp. has been carried out against a wide range of bacteria both Gram-negative and Gram-positive (Batiha et al. 2023). However, only a few studies have evaluated the antibacterial properties of ethanolic extracts of *L.* x *intermedia* (commonly known as lavandin), and little is known about which aerial part of the plant has the most remarkable antimicrobial efficacy (Pokajewicz et al. 2023). To the best of the authors' knowledge, there are no studies on the antimicrobial properties of ethanolic extracts of *L.* x *intermedia*, which grows wild and widespread in Morocco. Therefore, this study aimed to compare the phytochemical profile and antimicrobial activity of ethanolic extracts from leaves and flowers of *L.* x *intermedia* from Morocco.

MATERIALS AND METHODS

Plant collection and study area

The plants of *Lavandula x intermedia* used for this study were harvested three times during the spring of 2019 in Tahla (34° 03′ north, 4° 25′ west, 606 m), Fez-Meknes region, Taza province (Figure 1). Plant identification was carried out by the botanist Hassan Khamar, Professor at the Rabat Institute of Science (Morocco). The sample was deposited in the herbarium of the Faculty of Science of Meknes under the voucher number "113709".

Moisture content

100 g of fresh plant was dried in an oven at 105°C until a constant weight. The moisture content (expressed as a percentage) of the collected plants was calculated using the following formula:

$$\text{Moisture content} = [(M_i - M_f / M_i) \times 100]$$

Where "*Mi*" is the initial weight of the sample while "*Mf*" is the final weight after drying. The analyses were carried out on the plants collected in the three sampling sessions. Three replications were performed for each sample. The mean value was obtained from three replicates expressed as a percentage.

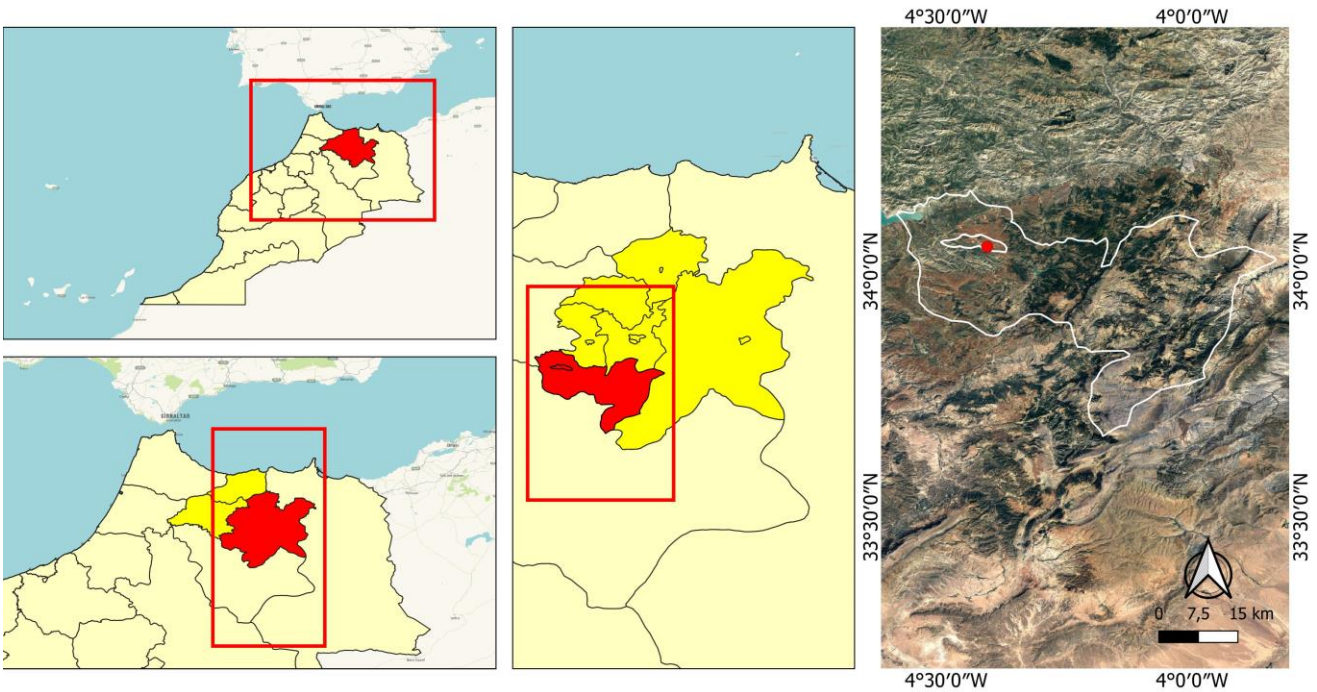

Figure 1. Collection site of *Lavandula x intermedia*, Tahla (34° 03′ north, 4° 25′ west, 606 m), Fez-Meknes region, province of Taza Morocco

Extraction by maceration and yield determination

Plant samples from three sampling sessions were mixed and then washed and air dried at room temperature (25±2°C) in an aired and dark place to avoid the loss of the active compounds. Samples of leaves and flowers were ground separately. The powders were stored in closed containers and kept away from light.

The powders of leaves (PL) and flowers (PF) were macerated, according to Romani et al. (2006), with slight modifications. 20 g of PL or PF were macerated at room temperature (25±2°C) for 2.5 h with 100 mL of an aqueous solution of 70% ethanol (*v*/*v*). After filtration, the filtrate was centrifugated for 20 min at 4000 rpm at room temperature. The obtained extract was filtered again on filter paper and stored at 4°C for further use in a closed container.

The *L.* x *intermedia* extract of leaves (LiLE) and flowers (LiFE) were subsequently evaporated to dryness and weighed to obtain the yield. The yield percentage is a percentage of the initial weight of leaves and flowers.

Phytochemical screening

Phytochemical analyses were carried out to reveal the presence (qualitative analyses) and amount (quantitative analyses) of secondary metabolites in the LiLE and LiFE.

Qualitative analyses: Alkaloids, anthraquinones and terpenes

Colorimetric methods were performed for alkaloids, anthraquinones, and terpenes. It would be possible to predict the amount based on the intensity of the color. The results were classified as follows: very positive (the solution changed color clearly and intensely), positive (the solution changed color clearly), moderately positive (the solution changed color only slightly), and negative (no color variation).

Determination of alkaloids

The presence of alkaloids was determined: 500 µL of extract was added to 1.5 mL of distilled water and adjusted to pH 2 using 5% sulfuric acid (Sigma Aldrich, St. Louis, MO, USA). Then, the solutions were extracted by liquid-liquid extraction using dichloromethane (Sigma Aldrich, St. Louis, MO, USA). The dichloromethane extract was concentrated using rotavapor and resolubilized in dichloromethane. The extract was added with Dragendroff reagent (Sigma Aldrich, St. Louis, MO, USA) (Zhang et al. 2021). The appearance of yellow-orange color confirmed the presence of alkaloids.

Determination of anthraquinones

The presence of anthraquinones was carried out according to the protocol described by El-Mawla et al. (2003). 500 µL of extract was mixed with 1500 µL methanol (Sigma Aldrich, St. Louis, MO, USA), 1500 µL of hydrochloric acid 6M (Sigma Aldrich, St. Louis, MO, USA) and 100 µL ammonium hydroxide (Sigma Aldrich, St. Louis, MO, USA). The appearance of a red color confirmed the presence of anthraquinones.

Determination of terpenes

Determination of terpenes was conducted according to the Salkowski method as used by Ahmad et al. (2018). 500 µL of the extract was mixed with 200 µL of chloroform (Sigma Aldrich, St. Louis, MO, USA) and 300 µL of sulphuric acid (Sigma Aldrich, St. Louis, MO, USA). The appearance of a reddish-brown color confirmed the presence of terpenes.

Quantitative analyses: Total polyphenol, total flavonoids, and condensed tannins

The amount of total polyphenols, total flavonoids, and condensed tannins was determined based on absorbance analysis by UV spectroscopy. Different calibration curves were constructed using standard compounds to determine the amount of each compound.

Determination of total polyphenols

Total polyphenols were determined according to the Folin-Ciocalteu (FC) method described by Kamal (2011).

100 µL of extract was mixed with 500 µL of FC reagent (Sigma Aldrich, St. Louis, MO, USA) and 400 µL of 7.5% sodium carbonate (Sigma Aldrich, St. Louis, MO, USA) solution (*w*/*v*). The mixtures were stirred and incubated in the dark at room temperature for 10 min. The absorbance of a blue-colored chromophore was measured at 765 nm by a UV spectrophotometer (Biobase, Shandong, China). A calibration curve was constructed using gallic acid in methanol/water (50:50 v/v) solution at 0 (blank), 50, 100, 150, 200, and 250 mg/L. The total polyphenol was calculated by interpolation to the calibration curve. Results were expressed as mg of gallic acid equivalent (GAE)/g of dry matter.

Determination of total flavonoids

The total flavonoids determination was performed by mixing 500 µL of the extract with 1500 µL of 95% methanol (*v*/*v*), 100 µL of 10% aluminum chloride (Sigma Aldrich, St. Louis, MO, USA) (*w*/*v*), 100 µl of 1 M sodium acetate (Sigma Aldrich, St. Louis, MO, USA) and 2.8 mL of distilled water (Dehpour et al. 2003). The solution was stirred and incubated in the dark at room temperature for 30 min. The absorbance of the solution was measured at 415 nm using the UV spectrophotometer. A calibration curve was constructed using quercetin (Sigma Aldrich, St. Louis, MO, USA) methanol/water (50:50 *v*/*v*) solution at concentrations of 0 (blank), 50, 100, 150, 200, and 250 mg/L. The total flavonoids were calculated by interpolation to the calibration curve. The results were expressed in mg quercetin equivalent (QE)/g dry matter.

Determination of condensed tannins

Condensed tannins were determined using the vanillin reagent method, as described by Palacios et al. (2021). Vanillin reagent was made by mixing an equal volume of 8% hydrochloric acid (*v*/*v*), 37% methanol (*v*/*v*), and 4% vanillin (Sigma Aldrich, St. Louis, MO, USA) in methanol (*w*/*v*) and then kept at 30°C before the analysis. 200 µL of extract was mixed with 1000 µL of vanillin reagent and incubated in the dark at 30°C for 20 min. The tannin

content was determined by measuring the absorbance at 500 nm by the UV spectrophotometer. A calibration curve was constructed using methanol/water (50:50 *v*/*v*) solutions of catechol (Sigma Aldrich, St. Louis, MO, USA) at 0 (blank), 50, 100, 150, 200, and 250 mg/L. The condensed tannins were calculated by interpolation to the calibration curve. Results were expressed in mg of catechol equivalent (CE)/g dry matter.

Antibacterial activity

Preparation of the strains

The antibacterial activity of LiLE and LiFE extract was performed in the Laboratory of Food Microbiology of the Department of Veterinary Science of the University of Messina against 19 different bacteria (4 Gram-positive and 15 Gram-negative) (Table 1).

All the working cultures used in this study were prepared by inoculating bacterial isolate from a frozen stock (−80°C) into Brain Heart Infusion broth (BHIb; Biolife, Milan, Italy) and incubated at 37°C for 24 h, except the *Aeromonas* strains that were incubated at 30°C for 24 h.

Determination of MIC and MBC

The determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) was assessed by microdilution assay following Ed-Dra et al. (2021). Wells in 96-microwell plates (Biosigma, Cona, Italy) were filled with 100 µL of BHIb at decreasing concentrations of LiLE and LiFE (final concentrations ranged from 2.5 to 0.0001 mg/mL). A positive control (BHIb without plant extracts) and a blank (BHIb plus plant extract without bacteria) were performed in each isolate. A fresh inoculum of each bacterial broth culture was inoculated in the microwell plates at ~10^4 CFU/mL in each well. Then, microwell plates were incubated at 37 °C for 24 h, except the *Aeromonas hydrophila* strains that were incubated at 30°C for 24 h. The MIC was established by evaluating the media turbidity that indicated bacterial growth. The lowest concentration in which there was no visible growth (turbidity of the broth medium) was the MIC.

After MIC evaluation, the MBC was carried out by adding 40 µL of 2,3,5-diphenyltetrazolium chloride (TTC) (Sigma-Aldrich, Buchs, Switzerland) with a concentration of 0.2 g/mL followed by incubation for 30 min at 37°C (except the *A. hydrophila* strains that were incubated at 30°C for 24 h). The appearance of red color indicated the presence of live bacteria. All assays were performed in triplicate.

Data analysis

The Shapiro-Wilk test tested the normal distribution of the MIC and MBC values. Different nonparametric tests were carried out to compare the antimicrobial activity between LiLE and LiFE against Gram-positive and Gram-negative bacteria. The Wilcoxon test was used to compare the MIC and MBC between LiLE and LiFE for all bacterial strains. The same test was used to evaluate differences between the MIC and MBC values of LiLE and LiFE within each group of Gram-positive and Gram-negative bacteria. The Mann-Whitney test was used to compare MIC and MBC values of LiLE and LiFE between Gram-positive and Gram-negative bacteria. The significant level (p) was set at 5% (0.05), and all tests were performed in two-tailed. All the statistical analyses were done using Graph Pad Prism 9 software (San Diego, CA, USA).

Table 1. Bacterial strains tested for antimicrobial activity of the *Lavandula x intermedia* extract of leaves (LiLE) and flowers (LiFE)

Gram	Strains
Positive	*Listeria monocytogenes* ATCC 13932
	Listeria monocytogenes ATCC 7644
	Staphylococcus aureus ATCC 6538
	Staphylococcus aureus ATCC 25923
Negative	*Salmonella enterica* subsp. *Enterica* serovar Enteritidis ATCC 13076
	Salmonella enterica subsp. *Enterica* serovar Typhimurium ATCC 14028
	Escherichia coli ATCC 8739
	Escherichia coli ATCC 35218
	Pseudomonas aeruginosa ATCC 27853
	Vibrio parahaemolyticus ATCC 17802
	Vibrio parahaemolyticus CCUG 43363
	Yersinia enterocolitica subsp. *enterocolitica* ATCC 23715
	Yersinia enterocolitica subsp. *enterocolitica* ATCC 9610
	Aeromonas hydrophila ATCC 7966
	Aeromonas hydrophila ATCC 35654

Note: ATCC: American Type Culture Collection; CCUG: Culture Collection of the University of Gothenburg; CECT: Colección Española De Cultivo Tipo

RESULTS AND DISCUSSION

Moisture content and extraction yield

The moisture content of the fresh plant of *L.* x *intermedia* was 74.10%. The yield of ethanolic extract of leaves and flowers was 6.6% and 4.2%, respectively.

Phytochemical screening

Qualitative analyses: Alkaloids, anthraquinones and terpenes

The results of phytochemical screening showed that LiLE and LiFE contained alkaloids, anthraquinones, and terpenes (Table 2). Although the reactions were positive, the color changed only moderately during the determination of alkaloids and anthraquinones in both LiLE and LiFE. Instead, the color obtained for terpenes determination in the LiLE was more intense than in LiFE.

Quantitative analyses: Total polyphenol, total flavonoids, and condensed tannins

The total polyphenol in LiLE (96.21 mg GAE/ g dry matter was higher than that in the LiFE (41.03 mg GAE/g dry matter) (Table 3.). The flavonoid content in LiLE was higher than that in LiFE, with a concentration of 10.86 mg QE/g dry matter and 7.32 mg QE/g dry matter, respectively. A higher concentration of condensed tannins was also detected in the LiLE than in the LiFE, with values of 3.92 mg CE/g DP and 1.39 mg CE/g DP, respectively.

Antibacterial activity

The results of the antimicrobial activity of the LiLE and LiFE against Gram-positive and Gram-negative bacteria are presented in Table 4. There was no significant difference in the MIC value (p=0.2764) and the MBC value (p=0.1680) between LiLE and LiFE for Gram-positive and Gram-negative bacteria. The average MIC and MBC values for LiLE against all bacteria were 1.08±0.91 mg/mL and 1.31±1.09 mg/mL, respectively. The average MIC and MBC values for LiFE against all bacteria were 1.10±0.28 mg/mL and 1.50±0.57 mg/mL, respectively. The MIC value between Gram-positive and Gram-negative bacteria was not significantly different (p=0.0761) in LiLE and LiFE. The average MIC value was 1.50±0.53 mg/mL for Gram-positive bacteria and 0.98±0.66 for Gram-negative bacteria. The average MBC value for Gram-negative bacteria was 1.10±0.85 mg/mL, significantly lower (p=0.0491) than for Gram-positive bacteria (1.80±0.58 mg/mL).

The average MIC value of LiLE was significantly lower (p=0.0447, U=6) against Gram-negative bacteria (0.71±0.81 mg/mL) than Gram-positive bacteria (2.00±0.00 mg/mL). There was no significant difference (p=0.0901, U=9) between the average MBC values against Gram-positive and Gram-negative bacteria, i.e., 2.25±0.29 mg/mL and 0.97±1.07 mg/mL, respectively. The MIC value of LiLE against Gram-positive *Staphylococcus aureus* and *Listeria monocytogenes* was 2 mg/mL. Instead, the MBC obtained for *S. aureus* 25923 and *L. monocytogenes* 7644 was 2.5 mg/mL, while against *S. aureus* 6538 and *L. monocytogenes* 13932 was 2 mg/mL. The MIC values of LiLE against Gram-negative varied between 0.0005 mg/mL and 2 mg/mL, while the MBC ranged between 0.001 mg/mL and 2.5 mg/mL.

There was no significant difference in the average MIC value of LiFE (p=0.7905, U=18) between the mean MIC values of LiFE against Gram-positive (1.00 ± 0.00 mg/mL) and Gram-negative (1.15±0.34 mg/mL) bacteria. There was also no significant difference (p=0.0769, U=8) in the mean MBC values between Gram-positive (2.00±0.00 mg/mL) and Gram-negative bacteria (1.32±0.56 mg/mL). The MIC and MBC of LiFE against Gram-positive bacteria were 1 mg/mL and 2 mg/mL, respectively, for *L. monocytogenes* and *S. aureus.* The MIC values for LiFE against Gram-negative bacteria varied between 1 mg/mL and 2 mg/mL, while the MBC ranged between 1 mg/mL and 2.5 mg/mL.

Table 2. Phytochemical screening of ethanolic extracts of leaves and flowers of *Lavandula x intermedia* from Fez-Meknes region of Morocco

Secondary metabolite	Leaves ethanolic extract	Flowers ethanolic extract
Alkaloid	+	+
Anthraquinones	+	+
Terpenes	++	+

Note: ++: Positive reaction; +: Moderately positive reaction

Table 3. The content of total polyphenol, total flavonoids, and condensed tannins in ethanolic extracts of leaves and flowers of *Lavandula x intermedia* from the Fez-Meknes region of Morocco

Ethanolic extract	Total polyphenols (mg GAE/g DP)	Flavonoids (mg QE/g DP)	Condensed tannins (mg CE/g DP)
Leaves	96.21±4.93	10.86±1.72	3.92±1.03
Flowers	41.03±3.55	7.32±1.65	1.39±0.46

Note: Results are expressed as mean ± standard deviations of three replicates

Table 4. Antibacterial activity of ethanolic extracts of leaves and flowers of *Lavandula x intermedia* from the Fez-Meknes region of Morocco against Gram-positive and Gram-negative bacteria. Data was obtained from three replicates

	Bacteria strains	Ethanolic extract of leaves		Ethanolic extract of flower	
		MIC	MBC	MIC	MBC
Gram-positive	*Staphylococcus aureus* 25923	2	2.5	1	2
	Staphylococcus aureus 6538	2	2	1	2
	Listeria monocytogenes 13932	2	2	1	2
	Listeria monocytogenes 7644	2	2.5	1	2
Gram-negative	*Salmonella* Enteritidis 13076	2	2.5	1	2
	Salmonella Typhimurium 14028	2	2.5	1.5	2
	Escherichia coli 8739	1	1	1	1
	Escherichia coli 35218	2	2.5	2	2.5
	Pseudomonas aeruginosa 27853	1	1	1	1
	Vibrio parahaemolyticus 17802	0.01	0.01	1	1
	Vibrio parahaemolyticus 43363	0.0005	0.001	1	1
	Yersinia enterocolitica 23715	1	1	1	1
	Yersinia enterocolitica 9610	0.1	0.1	1	1
	Aeromonas hydrophila 7966	0.0005	0.01	1	1
	Aeromonas hydrophila 35654	0.0005	0.01	1	1

Note: Concentrations refer to mg/mL

Discussion

The biological activities of *L.* x *intermedia* have long been investigated, and the antimicrobial activity has varied (Pokajewicz et al. 2023). However, the antibacterial activity has been studied mainly by testing its free or encapsulated essential oils, while fewer data are available for aqueous and ethanolic extracts (Dobros et al. 2022). This study is the first study reporting the antibacterial activity of ethanolic extracts of flowers and leaves of *L.* x *intermedia* from the Fez-Meknes region of Morocco. Overall, the antibacterial activity reported in the literature for lavandin ethanolic extracts is highly variable, with sometimes conflicting results between studies. The recent review by Pokajewicz et al. (2023) showed that lavandin ethanolic extracts were active against Gram-positive and Gram-negative bacteria. However, several studies reported that lavandin ethanolic extracts were active against only active against Gram-negative bacteria. A study by Moon et al. (2006) showed that ethanolic leaf extracts of lavandin were only active against Gram-negative bacteria, such as *Proteus vulgaris* and *Pseudomonas aeruginosa*, and there is no antibacterial activity against the Gram-positive *S. aureus* and *Streptococcus pyogenes*. The results of Moon et al. (2006) partially agree with the results of the present study, i.e., no significant difference in MIC values between Gram-positive and Gram-negative bacteria. Still, based on the MBC value, the ethanolic extract was more active against Gram-negative bacteria.

The antimicrobial activity of *L.* x *intermedia* ethanolic extracts varies due to the bacteria's characteristics and the compounds' biological activities (Vitalini et al. 2022). The cell surface is the first interaction site between external agents and bacteria. The cell wall of Gram-positives is uniform and relatively thick and consists of numerous layers of peptidoglycan, which are intersected by teichoic and teichuronic acids (Vadillo-Rodríguez et al. 2021). Instead, the peptidoglycan in Gram-negative is surrounded by an outer membrane consisting of an inner phospholipid layer and an outer layer of lipopolysaccharide anchored to each other through protein structures that extend into the extracellular space (Madigan et al. 2017). Bacterial surfaces possess hydrophilic or hydrophobic, acidic or basic functional groups that confer hydrophobicity and electric negative charge (Hamadi et al. 2008; Goulter et al. 2009). These structural and functional surface properties regulate the interaction between bacteria and external agents. The selective activity of the ethanolic extract of lavandin against Gram-negative or Gram-positive bacteria was due to the mechanism of antibacterial activity.

In the present study, the antibacterial activity of the extracts was carried out against Gram-negative and Gram-positive bacteria. The results suggest that the mechanism of action of the antibacterial compound in the extract is more related to the antibacterial properties of the compound. The chemical composition of the ethanolic extracts plays an important role in the antibacterial activity and its variability. Therefore, the same extraction method of lavandin ethanolic extracts might have different antimicrobial activity since the compounds and the amounts extracted vary according to numerous factors influencing plant growth (Gyawali and Ibrahim 2014). Soil nutritional conditions, air composition, temperature, and, in general, all environmental factors that interact with plants are variables that influence the production of secondary metabolites such as polyphenolic compounds, flavonoids, tannins, alkaloids, anthraquinones, and terpenes (Pant et al. 2021). The results of the antibacterial activity in this study are different from ethanolic extracts of flowers and leaves of lavandin from Croatia and tested against the same bacterial strains (Blazekovic et al. 2011). In detail, the MIC and MBC detected by Blazekovic et al. (2011) for *L. monocytogenes* 7644, *S. enteritidis* 13076, *P. aeruginosa* 27853, and *S. aureus* 25923 were higher than the values obtained in the present study. Furthermore, Blazekovic et al. (2011) observed that the antimicrobial activity of the flower extract was higher than that of the leaf extract in contrast to

the results of the present study, where there was no significant difference in the antibacterial activity of the two extracts.

Phytochemical analysis revealed the presence of polyphenolic compounds, flavonoids, tannins, alkaloids, anthraquinones, and terpenes in both extracts. Several studies reported the antimicrobial activity of these secondary metabolites; therefore, these compounds might be responsible for the antibacterial activities (Compean and Ynalvez 2014). This study showed a high content of total polyphenols, flavonoids, and tannins in the leaf extract and a high content of terpenes based on the colorimetric method. Despite the higher content of secondary metabolites in the leaf extract than in the flower extract, there was no significant difference in the antibacterial activity. It might be due to a synergistic mechanism of the various constituent compounds rather than the single compound's activity. Blazekovic et al. (2011) showed that HPTLC analyses revealed the presence of flavonoids and higher amounts of terpenes and polyphenolics in flower ethanolic extracts than in leaf extract of lavandin. Environmental factors influence secondary metabolites produced by plants. Therefore, the results in this study differed from those of flower ethanolic extract obtained from lavandin collected in Poland in the same season. Dobros et al. (2022) reported that the total polyphenols of flower ethanolic extract of lavandin was 19.7 mg GAE/g DP and flavonoids of 13.83 mg QE/g DP. These values differed from those in this study (41.03 mg GAE/g DP and 7.32 mg QE/g DP, respectively).

Leaves and flower ethanolic extracts of lavandin from Morocco possess antibacterial properties. Different qualitative and quantitative phytochemical analyses showed the presence of several secondary metabolites, which are probably responsible for the antibacterial activity. Leaves and flower extracts of *L.* x *intermedia* could be proposed as natural preservatives against different bacterial pathogens, especially *Vibrio* spp. and *Aeromonas* spp. but they are also against *Salmonella* spp. and *Listeria monocytogenes*. Further studies are needed to determine the biological properties and the chemical compound responsible for the biological activity of the ethanolic extract of lavandin.

REFERENCES

Ahmad R, Parveen G, Gauri NA, Wal N. 2018. Phytochemical screening, sugar content, total protein and antimicrobial activity of three important medicinal plants. Intl J Fauna Biol 5 (6): 125-139.

Álvarez-Martínez FJ, Barrajón-Catalán E, Herranz-López M, Micol V. 2021. Antibacterial plant compounds, extracts and essential oils: An updated review on their effects and putative mechanisms of action. Phytomedicine 90: 153626. DOI: 10.1016/j.phymed.2021.153626.

Anand U, Jacobo-Herrera N, Altemimi A, Lakhssassi N. 2019. A comprehensive review on medicinal plants as antimicrobial therapeutics: Potential avenues of biocompatible drug discovery. Metabolites 9 (11): 258. DOI: 10.3390/metabo9110258.

Batiha GES, Teibo JO, Wasef L, Shaheen HM, Akomolafe AP, Teibo TKA, Al-kuraishy HM, Al-Garbeeb AI, Alexiou A, Papadakis, M. 2023. A review of the bioactive components and pharmacological properties of *Lavandula* species. Naunyn-Schmiedeb Arch Pharmacol 396 (5): 877-900. DOI: 10.1007/s00210-023-02392-x.

Blazekovic B, Stanic G, Pepeljnjak S, Vladimir-Knezevic S. 2011. *in vitro* antibacterial and antifungal activity of *Lavandula* x *intermedia* Emeric ex Loisel. 'Budrovka'. Molecules 16 (5): 4241-4253. DOI: 10.3390/molecules16054241.

Blaženković B, WeiFeng Y, Yi W, Chun L, Kindl M, Pepeljnjak S, Vladimir-Knežević S. 2018. Chemical composition, antimicrobial and antioxidant activities of essential oils of *Lavandula* x *intermedia* 'Budrovka' and *L. angustifolia* cultivated in Croatia. Ind Crops Prod 123: 173-182. DOI: 10.1016/j.indcrop.2018.06.041.

Compean KL, Ynalvez RA. 2014. Antimicrobial activity of plant secondary metabolites: A review. Res J Med Plant 8 (5): 204. DOI: 10.3923/rjmp.2014.204.213.

Dehpour AA, Ebrahimzadeh MA, Fazel NS, Mohammad NS. 2009. Antioxidant activity of the methanol extract of *Ferula assafoetida* and its essential oil composition. Grasas y aceites 60 (4): 405-412. DOI: 10.3989/gya.010109

Dobros N, Zawada K, Paradowska K. 2022. Phytochemical profile and antioxidant activity of *Lavandula angustifolia* and *Lavandula* x *intermedia* cultivars extracted with different methods. Antioxidants 11 (4): 711. DOI: 10.3390/antiox11040711.

Ed-Dra A, Filali FR, Presti VL, Zekkori B, Nalbone L, Elsharkawy ER, Bentayeb A, Giarratana, F. 2021. Effectiveness of essential oil from the *Artemisia herba-alba* aerial parts against multidrug-resistant bacteria isolated from food and hospitalized patients. Biodiversitas 22 (7): 2995-3005. DOI: 10.13057/biodiv/d220753.

El-Mawla A, Backheet EY, El-Emary NA. 2003. Anthraquinones production in aerial parts-derived callus of *Rubia tinctorum* L. Bull Pharm Sci 26 (1): 49-54.

Garzoli S, Turchetti G, Giacomello P, Tiezzi A, Laghezza Masci V, Ovidi E. 2019. Liquid and vapour phase of lavandin (*Lavandula* × *intermedia*) essential oil: Chemical composition and antimicrobial activity. Molecules 24 (15): 2701. DOI: 10.3390/molecules24152701.

Goulter RM, Gentle IR, Dykes GA. 2009. Issues in determining factors influencing bacterial attachment: A review using the attachment of *Escherichia coli* to abiotic surfaces as an example. Lett Appl Microbiol 49 (1): 1-7. DOI: 10.1111/j.1472-765X.2009.02591.x.

Gyawali R, Ibrahim SA. 2014. Natural products as antimicrobial agents. Food Control 46: 412-429. DOI: 10.1016/j.foodcont.2014.05.04.

Hamadi F, Latrache H, Zahir H, Elghmari A, Timinouni M, Ellouali M. 2008. The relation between *Escherichia coli* surface functional groups' composition and their physicochemical properties. Braz J Microbiol 39: 10-15. DOI: 10.1590/S1517-83822008000100003.

Hansan T, Al-Harmoosh R. 2020. Mechanisms of antibiotics resistance in bacteria. Sys Rev Pharm 11: 817-823. DOI: 10.31838/srp.2020.6.118.

Kamal J. 2011. Quantification of alkaloids, phenols and flavonoids in sunflower (*Helianthus annuus* L.) Afr J Biotechnol 10 (16): 3149-3151. DOI: 10.5897/AJB09.1270.

Koriem KMM. 2021. *Lavandula aetheroleum* oil: A Review on phytochemical screening, medicinal applications, and pharmacological effects. Bioint Res Appl Chem 11 (3): 9836-9847. DOI: 10.33263/BRIAC113.98369847.

Larsson DJ, Flach CF. 2022. Antibiotic resistance in the environment. Nat Rev Microbiol 20 (5): 257-269. DOI: 10.1038/s41579-021-00649-x.

Madigan M, Bender K, Buckley D, Sattley W, Stahl D. 2017. Brock Biology of Microorganisms, 15th edition. Pearson, London.

Moon T, Cavanagh HM, Wilkinson JM. 2007. Antifungal activity of Australian grown *Lavandula* spp. essential oils against *Aspergillus nidulans*, *Trichophyton mentagrophytes*, *Leptosphaeria maculans* and *Sclerotinia sclerotiorum*. J Essent Oil Res 19 (2): 171-175. DOI: 10.1080/10412905.2007.9699253.

Nalbone L, Panebianco F, Cammilleri G, Ferrantelli V, Giarratana F. 2022. Anisakicidal effects of R (+) limonene: An alternative to freezing treatment in the industrial anchovy marinating process. Foods 11 (8): 1121. DOI: 10.3390/foods11081121.

Nefzi N, Pagliari S, Campone L, Megdiche-Ksouri W, Giarratana F, Cicero N, Ziino G, Nalbone L. 2023. Chemical composition and comprehensive antimicrobial activity of an ethanolic extract of propolis from Tunisia. Antibiotics 12 (5): 802. DOI: 10.3390/antibiotics12050802.

Palacios CE, Nagai A, Torres P, Avelino Rodrigues J, Salatino A. 2021. Contents of tannins of cultivars of sorghum cultivated in Brazil, as determined by four quantification methods. Food Chem 337: 127970. DOI: 10.1016/J.foodchem.2020.127970.

Pancu DF, Scurtu A, Macasoi IG, Marti D, Mioc M, Soica C, Coricovac D, Horhat D, Poenaru M, Dehelean C. 2021. Antibiotics: Conventional therapy and natural compounds with antibacterial activity - a pharmaco-toxicological screening. Antibiotics 10 (4): 401. DOI: 10.3390/antibiotics10040401.

Pant P, Pandey S, Dall'Acqua S. 2021. The influence of environmental conditions on secondary metabolites in medicinal plants: A literature review. Chem Biodiver 18: e2100345. DOI: 10.1002/cbdv.202100345.

Pokajewicz K, Czarniecka-Wiera M, Krajewska A, Maciejczyk E, Wieczorek PP. 2023. *Lavandula x intermedia* - a bastard lavender or a plant of many values? Part II. Biological activities and applications of lavandin. Molecules 28 (7): 2986. DOI: 10.3390/molecules28072986.

Romani A, Pinelli P, Cantini C, Cimato A, Heimler D. 2006. Characterization of Violetto di Toscana, a typical Italian variety of artichoke. Food Chem 95: 221-225. DOI: 10.1016/j.foodchem.2005.01.013.

Vaou N, Stavropoulou E, Voidarou C, Tsigalou C, Bezirtzoglou, E. 2021. Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives. Microorganisms 9 (10): 2041. DOI: 10.3390/microorganisms9102041.

Vadillo-Rodríguez V, Cavagnola MA, Pérez-Giraldo C, Fernández-Calderón MC. 2021. A physico-chemical study of the interaction of ethanolic extracts of propolis with bacterial cells. Colloids Surf 200: 111571. DOI: 10.1016/j.colsurfb.2021.111571.

Vitalini S, Nalbone L, Bernardi C, Iriti M, Costa R, Cicero N, Giarratana F, Vallone L. 2022. Ginger and parsley essential oils: Chemical composition, antimicrobial activity, and evaluation of their application in cheese preservation. Nat Prod Res 37: 2742-2747. 10.1080/14786419.2022.2125965.

Wendakoon C, Calderon P, Gagnon D. 2012. Evaluation of selected medicinal plants extracted in different ethanol concentrations for antibacterial activity against human pathogens. J Med Act Plants 1 (2): 60-68. DOI: 10.7275/R5GH9FV2.

WHO [World Health Organization]. 2017. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. World Heal. Organ. 2017. www.who.int/medicines/ publications/global-priority-list-antibiotic-resistant-bacteria/en/.

WHO [World Health Organization]. 2021. Antimicrobial resistance. https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.

Zhang N, Wang M, Li Y, Zhou M, Wu T, Cheng Z. 2021. TLC–MS identification of alkaloids in Leonuri Herba and Leonuri Fructus aided by a newly developed universal derivatisation reagent optimised by the response surface method. Phytochem Anal 32 (3): 242-251. DOI: 10.1002/pca.2970.

Zhao Y, Yang QE, Zhou X, Wang FH, Muurinen J, Virta MP, Brandt KK, Zh YG. 2021. Antibiotic resistome in the livestock and aquaculture industries: Status and solutions. Crit Rev Environ Sci Technol 51 (19): 2159-2196. DOI: 10.1080/10643389.2020.1777815.