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Antimicrobial and Antioxidant Activities of Algerian *Lavandula Stoechas* Essential Oil

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Abstract: *Lavandula stoechas* is one of the most traditionally used plants in Algeria, for the treatment of painful diseases such as inflammatory diseases, cystitis, nephritis and rheumatic arthritis. Our present study focuses on the evaluation of antimicrobial and antioxidant activity of the essential oil extracted by hydrodistillation from the aerial parts of *Lavandula stoechas* samples harvested in May 2022 at Bouhinoune, a village in Tizi ouzou located at 300 m altitude.The chemical composition of the essential oil was evaluated by GC/MS and the antioxidant activity by the DPPH method. The antimicrobial activity was tested against three gram positive and two gram negative bacterial by the use of the disc diffusion method. GC-MS analysis showed that our essential oil is rich in fenchone, camphor and eucalyptol. The essential oil of *Lavandula stoechas* showed good antioxidant activity compared to ascorbic acid and an interesting inhibitory effect against gram-positive bacteria with inhibition diameters greater than or equal to that recorded with the reference antibiotic. This valuable antibacterial activity is undoubtedly linked to the strong presence of fenchone, eucalyptol, camphor, linalool and linalyl acetate in the essential oil.

Keywords: Lavandula stoechas, Essential oil, Antioxidant activity, Antimicrobial activity, Disc diffusion

Introduction

Bioactive compounds derived from medicinal plants have garnered increasing interest within the scientific community, leading to numerous studies and research efforts. Thus, the investigation of bioactive compounds from medicinal plants has become a focal point (Apretna, 2005). This approach not only contributes to scientific and technological innovation but also to the preservation and sustainable use of biodiversity. Among these plants, lavender stands out not only for its well-known relaxing effect but also for its varied medicinal properties. Indeed, lavender has beneficial effects against lung infections, inflammatory diseases, diabetes, chills, and depression (Andrew, 2001).

Additionally, it presents a promising cytotoxic potential, thereby opening interesting perspectives for the development of anticancer treatments (Boukhatem, 2020). The study of natural products aims to harness the riches offered by nature to discover new molecules with medical, industrial, and environmental applications. This field of research also enables a better understanding of the fundamental biological processes at work in nature. In this context, medicinal plants play a crucial role, particularly the Lamiaceae family, which is extremely diverse and important. They are widely cultivated for their essential oils and therapeutic properties.

Method

Plant Material and Essential Oil Extraction

Leaves and flowers were collected on May 2022 in the region of Bouhinoune Tizi Ouzou province, Algeria. The essential oil extraction was carried out by steam distillation. 100 g of dry material were distilled for 2 hours in 1L of water. The steam, laden with the essence of the distilled material, condensed in the coil of the still before being collected in a decanter flask. Once the distillation was complete, the essential oil was dried with Na_2SO_4 and then stored at 4°C in amber glass bottles to prevent any potential oxidation or contamination.

CG-MS Characterization

The essential oil (EO) diluted with hexane at a 2:20 (v/v) ratio was analyzed using gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis was conducted on a Bruker Scion 436-GC SQ MS system (Bremen, Germany) equipped with a fused silica Bruker BR-5ms capillary column (30 m × 0.25 mm, 0.25 µm film thickness). The mass spectrometer operated with an ionization voltage of 70 eV and scanned a spectral range of 50–300 m/z in full scan mode. The oven temperature was initially maintained at 60 °C for 1 minute, then ramped up to 170 °C at a rate of 2 °C/min, and finally increased to 220 °C at a rate of 15 °C/min, holding this temperature for 1 minute. Helium was used as the carrier gas at a flow rate of 1.0 mL/min. The detector and injector temperatures were set to 300 °C and 250 °C, respectively. The injector operated in split/splitless mode, with an injection volume of 1 µL and a split ratio of 1:20.

Antioxidant Activity

The antioxidant activity was evaluated in vitro using DPPH method. To determine the antioxidant power of *Lavandula stoechas* essential oil (LSEO), 50 µl of each LSEO sample and ascorbic acid at different concentrations (10-100 mg/ml and 10-100 µg/ml respectively) were added to 1 ml of 0.1 mM DPPH. The absorbance was measured at 517 nm after 30 minutes of incubation (Blois.M.S, 1958). For the control, 50 µl of methanol solution without the test substance were added to 1 ml of DPPH, and the percentage of inhibition was calculated according to the following formula: $\mathbf{\% \ Inhibition = [(A_c - A_e)/A_c] * 100}$
With A_c: Absorbance of the control, A_e: absorbance of the sample.

Antibacterial Study

Qualitative Study

The agar diffusion method is used to test bacterial sensitivity to antimicrobial substances (Rota.M.C, 2008). The process begins by incubating bacterial strains at 37°C for 18 to 24 hours to obtain isolated colonies. These colonies are then homogenized in a 0.9% saline solution and adjusted to a concentration of 10^8 CFU/ml using spectrophotometry. Under aseptic conditions, a sterile swab is dipped into the bacterial suspension, drained, and used to spread the bacteria on an agar plate by rotating the plate to ensure even distribution.

Discs containing the test substances are applied to the agar with a sterile clamp. The plates are refrigerated for 2 hours to allow diffusion of the substances before being incubated at 37°C for 24 hours. After incubation, zones of inhibition around the discs are measured to determine the effectiveness of the substances against the bacteria. This method ensures reliable and reproducible results by maintaining aseptic conditions, accurate bacterial concentrations, and proper diffusion time.

Quantitative Study

The Minimum Inhibitory Concentration (MIC) is defined as the lowest concentration of a substance that inhibits visible bacterial growth (Nikolić, 2014). To determine the MIC, bacterial suspensions and essential oil dilutions are prepared in a controlled and sterile environment. First, a bacterial suspension is prepared from isolated colonies homogenized in a 0.9% saline solution, adjusted to a concentration of 10^8 CFU/ml using spectrophotometry at 620 nm. Next, 10 ml of Mueller-Hinton (MH) broth are prepared in sterile tubes to dilute the bacterial suspension. Simultaneously, a stock solution of essential oil is prepared by mixing 500 µl of essential oil with 500 µl of MH broth containing 1% Tween 80, a surfactant that helps dissolve the essential oil in the aqueous broth.

The bacterial suspension is diluted in the MH broth to achieve the desired concentrations, and serial dilutions of the essential oil stock solution are performed. The bacteria and essential oil solutions are combined in sterile tubes or microplates, each well or tube containing a different concentration of essential oil, then incubated at 37°C for 24 hours. After incubation, the tubes or wells are examined for bacterial growth. The MIC is determined as the lowest concentration of essential oil that completely inhibits visible bacterial growth, allowing for an accurate assessment of the essential oil's antimicrobial efficacy.

Results and Discussion

GC-MS Results

The result of GC-MS analysis (Tables 1 and 2) show that lavender essential oil (leaves and flowers) is rich in monoterpenes (M) and oxygenated monoterpènes (OM). According to previous studies, most of the essential oils isolated from *Lavandula stoechas* plant collected all over the world, especially the Mediterranean countries such as Spain (Carrasco, 2015), Greece (Skoula, 1996), Turkey (Giray, 2008), Italy (M. Zuzarte, 2013), Morocco (S. Zrira, 2003), Tunisia (C. Messaoud, 2012) and Portugal (F. Matos, 2009). The GC-MS analysis reveals a predominance of monoterpenes, characteristic of the fenchone chemotype (Amara, 2017; Hassiotis, 2018 & Sebai, 2013). *Lavandula stoechas* gathered in other countries outside the Mediterranean such as Pakistan and Iran, presented the predominance of Camphor and Linalool respectively (Khan, 2023; Khavarpour, 2019; Asghari, 2016).

Table 1. Chemical composition of the essential oils extracted from flowers of *Lavandula stoechas*

Compounds	**Formula**	**%**	**Class**
1R-.alpha.-Pinene	$C_{10}H_{16}$	3.11	M
(+)-Camphene	$C_{10}H_{16}$	2.22	M
D-Limonene	$C_{10}H_{16}$	2.27	M
Eucalyptol	$C_{10}H_{18}O$	2.09	OM
Fenchone	$C_{10}H_{16}O$	28,13	OM
Camphor	$C_{10}H_{16}O$	23.46	OM
Borneol, acetate,	$C_{12}H_{20}O_2$	3.51	OM
Myrtenyl acetate	$C_{12}H_{18}O_4$	4.99	OM

Table 2. Chemical composition of the essential oils extracted from leaves of *Lavandula stoechas*

Compounds	**%**	**Formula**	**Class**
1R-.alpha.-Pinene	2.93	$C_{10}H_{16}$	M
Camphene	2.38	$C_{10}H_{16}$	M
m-Cymene	3.00	$C_{10}H_{16}$	M
Eucalyptol	4.75	$C_{10}H_{18}O$	OM
Fenchon	25.54	$C_{10}H_{16}O$	OM
camphor	37.53	$C_{10}H_{16}O$	OM
p-Cymen-8-ol	2.09	$C_{10}H_{14}O$	OM
(-)-Bornyl acetate	2.88	$C_{10}H_{20}O$	OM

Considering Algeria's area, the variation of the chemical composition is able to observed, other chemotypes is able to recorded, the isolated EO of the plant harvested in the west marked the predominance of the eucalyptol chemotype (Boukhatem, 2020), on the eastern side it is the linalyl acetate chemotype (Barkat, 2012) from this comparison we can notice how the climate and the type of soil can have an impact on the chemical composition of essential oils (Hadef, 2007). Furthermore, others factors can qualitatively and quantitatively alter the chemical composition of essential oils such as the state of growth of the plant (Gouyon, 1986), collection period, and the geographic location (Hadef, 2007 & Senatore).

Antioxidant Activity

The antioxidant activity of the essential oil appears related to its chemical composition (M. Lahlou, 2004)]. Our study revealed a result of 43.84 ± 2.09 mg/ml (Table 3). It has been demonstrated that the lower IC50 value indicates greater radical scavenging activity (Baali, 2019). Baali et al (2019) obtained a result of 4.04 ± 0.0017 mg/ml, which is explained by the high presence of fenchone at 50.29%, a major active compound directly associated with improved antioxidant activity (Rubert, 2000).

Table 3. Half maximal inhibitory concentration (IC50) of LSEO. Ascorbic acid was used as positive control.

Compounds	**IC50% (mg/ml)**
Ascorbic acid	0.062 ± 0.003
Essential oil (LS)	44.06 ±2.09

Antibacterial Activity

LSEO presented (Table 4) an interesting effect against Gram+ bacteria such as staphylococcus aureus and Bacillus with inhibition diameters (11.5±0.70) mm and (17.5±2.12) mm respectively. It is noted that the inhibition diameter given by Enterobacteria was (17.5±0.70) mm. On the other hand, the activity recorded against Gram- negative bacteria, LSEO presented more or less interesting effect with inhibition diameters (6.85±0.21) mm finally E,coli (10.5±0.70)mm. These results were confirmed by the values provided by the minimum inhibitory concentration (MIC) presented in Table 5.

Table 4. Result disk diffusion test of *Lavandula stoechas* EO

Strain		**Inhibition diameter(mm)**	**SD**	**ATB (mm)**	**% inhibition**
Pseudomonas aeruginosa	Gram -	6.85	0.21	27	7.61
klebsiella pneumoniae		8	1.41	15	8.88
Eschrichia Coli		10.5	0.70	23	11.66
Enterococcus feacalis		17.5	0.70	13	19.44
Staphylococcus aureus		11.5	0.70	24	12.77
Staphylococcus aureus	Gram +	9.5	0,70	25	10.55
Bacillus cereus		17.5	2.12	28	19.44

Table 5. MIC result of *Lavandula stoechas* EO

Strain		**MIC (mg/ml)**
Pseudomonas aeruginosa	Gram -	49.37±1.10
klebsiella pneumoniae		1.23±0.53
Eschrichia Coli		3.7±3.20
Staphylococcus aureus	Gram +	2.46±1.06
Bacillus cereus		1.23±0.53

However, it has been shown that the antibacterial activity depends on the chemical composition of the LSEO. According to a comparison of the antibacterial activity between an essential oil of Bulgarian origin (52.1% linalool; 9.5% linalyl acetate) and another EO of French origin (43.2% linalool and 29.1% linalyl acetate), it has been demonstrated that the EO of Bulgarian origin was better than the HE of French origin against 25 bacteria (Lis-Balchin, 1998). Several studies have demonstrated that Linalool and linalyl acetate have a powerful effect against microorganisms (Jianu, 2013). Other compounds have also been recognized for their antibacterial activity such as caryophylline (Jianu, 2013; Hendry, 2009). The antibacterial effect against E.Coli and Staph auereus can be justified by the presence of the Eucalyptol molecule [29] . In addition, α-terpenoel has been found to possess significant micro-biostatic activity against S aureus, E.Colie, Pseudo (Bouzouita, 2005).

Conclusion

Our study aimed to determine the effect of LSEO essential oil against Gram-positive and Gram-negative bacteria, as well as to evaluate its antioxidant activity against the DPPH radical. The results showed that LSEO essential oil has a promising effect against Gram-positive strains, with particularly notable antibacterial activity. Among the five strains tested, Bacillus was identified as the most sensitive to LSEO. These findings suggest that LSEO essential oil could be an interesting candidate for the development of new antibacterial strategies, especially against infections caused by Gram-positive bacteria.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Acknowledgements or Notes

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