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EVALUATION OF ANTIMICROBIAL AND ANTI-INFLAMMATORY PROPERTIES OF *LAVANDULA STOECHAS* L. ESSENTIAL OIL

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Abstract

Description of the subject: Wild lavender (*Lavandula stoechas*) is a medicinal and aromatic plant. It is interesting to know its therapeutic virtues, in order to enhance its use in the production of phyto-pharmaceutic molecules.
Objective: In the present work, the antimicrobial and anti-inflammatory activities of *Lavandula stoechas* essential oils were evaluated, in order to find new bioactive natural products.
Methods: *Lavandula stoechas* essential oil was obtained by hydrodistillation from dry flowers. The antimicrobial activity of this oil was evaluated against ten microorganisms using the paper disk agar diffusion method. The minimum inhibitory and bactericidal concentration (MIC and MBC) were also tested. The anti-inflammatory activity was investigated using the model of plantar edema induced in mice by carrageenan.
Results: The results of the antimicrobial activity showed that Gram-positive bacteria were strongly inhibited by *L. stoechas* essential oil. Thus, the MIC values range from 10 µg/ml to 0.3125 µg/ml. the oral administration of *L. stoechas* EO in the doses of 600 and 800 mg/kg reduced significantly plantar edema ($P < 0.05$) comparable to the diclofenac group.
Conclusion: The essential oil of *L. stoechas* presents a real interest and potential for its antimicrobial effect and an anti-inflammatory. It is used as a natural antimicrobial agent against *Candida albicans*.
Keywords: *Lavandula stoechas*; essential oil; Antimicrobial activity; Anti-inflammatory activity; Minimal Inhibitory Concentration; Minimal Bactericidal Concentration.

ÉVALUATION DE L'ACTIVITÉ ANTIMICROBIÈNNE ET ANTI-INFLAMMATOIRE DE L'HUILE ESSENTIELLE DE *LAVANDULA STOECHAS* L.

Résumé

Description du sujet : La lavande sauvage (*Lavandula stoechas*) est une plante médicinale et aromatique. Il est intéressant de connaître ses effets thérapeutiques, afin d'améliorer son utilisation dans la production de molécules phytopharmaceutiques.
Objectif : Dans le présent travail, les activités antimicrobiennes et anti-inflammatoires de l'huile essentielle de *Lavandula stoechas* ont été évaluées afin de valoriser de nouveaux produits naturels bioactifs.
Méthodes : L'huile essentielle de *Lavandula stoechas* a été obtenue par hydrodistillation à partir de fleurs sèches. L'activité antimicrobienne de cette huile a été évaluée contre dix microorganismes en utilisant la méthode de diffusion en disque. La concentration minimale inhibitrice et bactéricide (CMI et CMB) a également été testée. L'activité anti-inflammatoire a été étudiée en utilisant le modèle d'œdème plantaire induit chez la souris par le carraghénine.
Résultats : Les résultats de l'activité antimicrobienne ont montré que les bactéries à Gram-positif étaient fortement inhibées par l'huile essentielle de *Lavandula stoechas*. Ainsi, les valeurs de CMI vont de 10 µg/ml à 0,3125 µg/ml. L'administration orale de l'huile essentielle de *Lavandula stoechas* aux doses de 600 et 800 mg / kg a réduit significativement l'œdème plantaire ($P < 0.05$) comparable au groupe du diclofénac.
Conclusion : L'huile essentielle de *Lavandula stoechas* présente un intérêt réel et potentiel par ses effets antimicrobien et anti-inflammatoire. Elle est utilisée comme agent antimicrobien naturel contre *Candida albicans.*
Mots clés: *Lavandula stoechas*, huile essentielle, Activité antimicrobienne, activité anti-inflammatoire, Concentration Minimale Inhibitrice, Concentration Minimale Bactéricide.

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INTRODUCTION

For a very long time, natural remedies, and especially medicinal plants, were the main and the only recourse available to human [1]. Today, many molecules, isolated from plants, have become drugs [2].

Lavender belongs to the Lamiaceae family. The genus Lavandula L. is composed of about 39 species. The most well-known and valued economically are *Lavandula augustifolia*, *Lavandula x intermedia* and *Lavandula stoeches* [3]. Lavender is as well widespread in the tropical zones as in the temperate zones of the world [4; 5].

L. stoechas is a tender plant, preferring sunny places and siliceous soils [6]. Flowering is extended from March to May [7; 8]. *L. stoechas* is native to countries bordering the western Mediterranean, the Atlantic islands, Turkey, Pakistan, India and Europe. It grows spontaneously in Algeria [9; 10]; [11]

L. stoechas is used in the treatment of several diseases, such as respiratory (expectorant), gastric (antispasmodic), urinary and wound healing problems [12; 13].

Previous studies have shown that essential oil of *L. stoechas* has an antimicrobial [12; 14; 15; [16] and anti-inflammatory properties [17; 18].

The objective of this study is to evaluate the antimicrobial and anti-inflammatory effect of *L. stoechas* essential oil harvested at Chrea mountains in Blida- Algeria.

MATERIALS AND METHODS

The flowers of *L. stoechas* were harvested from Chrea mountain at Beni Ali station (36°27’08.7’’N 2°51’47.4’’E 800 meters) Blida-Algeria. A specimen of this plant was identified at the Agronomy National higher School, Algiers- Algeria. Inflorescences were dried and kept in canvas bags.

1. Extraction of essential oil

The hydrodistillation of the dry flowers of *L. stoechas* is carried out using a Clevenger. The dry plant material (100 g) was boiled for 4 h. The yield of *L. stoechas* EO’s was calculated.

2. Evaluation of antimicrobial activity

The microorganisms studied are: five Gram-negative bacteria (*Escherichia coli* ATCC 4157, *Pseudomonas aeruginosa* ATCC 4352, *Salmonella typhi* ATCC 6539, *Salmonella enteritidis* ATCC 13076 and *Enterococcus feacalis* ATCC 29212), four Gram-positive bacteria (*Bacillus sutilis* ATCC 9372; *Bacillus cereus* ATCC 10876, *Staphylococcus aureus* ATCC 6538 and MRSA ATCC 43300) and yeast (*Candida albicans* ATCC 24433). They are all reference strains. It’s were provided to us by the central laboratory of Frantz-Fanon hospital in Blida-Algeria. The antimicrobial activity was determined by the method of direct contact in a solid medium. The protocol followed is that of Bauer *et al.* [19]:

2.1. Disc diffusion assay (Qualitative study)

The culture mediums Mueller Hinton (MH) for the bacteria and Sabouraud for the yeast are poured into Petri dishes. The antimicrobial activity consists in depositing a sterile disc impregnated with 12 µl of *L. stoechas* EO’s on the surface of an agar. The latter are previously inoculated with the microorganisms to be tested. After incubation, the results are read by measuring the diameter "mm" of the inhibition zone. According to Leclerc [20]**.**, strains with a diameter of the inhibition zone greater than or equal to 15 mm are said to be sensitive, and these one are the subject of a quantitative study. Each test was performed in three replicates [20].

2.2. Determination of the minimum inhibitory and bactericidal concentration (MIC and MCB)

They were determined according to the CLSI test with some modification [21]. The technique consists to prepare dilutions of EO (varying from 20 µg/ ml to 0.3125 µg/ ml) in agar medium. A disk impregnated with the microbial suspension (12 µl) was deposited on the appropriate agar and previously cast. Then, the Petri dishes were incubated. The results are reflected in the absence of microbial growth visible to the naked eye.

On the basis of the results of the MIC assay, the MBC was assayed by transferring the discs showing complete absence of growth to MH or Sabouraud solid agar plates. The MBC was defined as the lowest essential oil concentration at which no growth could be observed after incubation at previously-mentioned conditions.

3. Evaluation of anti-inflammatory activity

The anti-inflammatory properties was carried out on Albino mice (males and females of 20-23g) using the plantar edema model [22]. This technique consists in administering *L. stoechas* EO's by oral route 30 minutes before the injection of carrageenan at 1%.

The mice were divided into five groups of five mice each. The control group «1» received the physiological water, the groups «2», «3» and «4» were treated with 400, 600 and 800 mg/kg body weight (BW) respectively of *L. stoechas* EO's, the last group «5» was treated with diclofenac at 2mg/kg of BW, a non-steroidal anti-inflammatory reference. The percentage of the increase edema is calculated according to the following formula:

$$\% = \frac{\textbf{Mass of the left paw} - \textbf{mass of the right paw}}{\textbf{mass of the right paw}} \times 100$$

After 4 hours of carrageenan injection, the percentage of reduction of edema was calculated as follows: % of control batch% of batch treated /% of control batch × 100

4. Statistical analysis

The data was carried out using the systat7. The degree of variability of the result is expressed as means ± standard deviation. The difference between the samples was determined using the Tukey HSD test. The difference was considered significant when $p < 0.05$.

RESULTS

1. Yield of essential oil

The dry flowers of *L. stoechas* has a good yield of HE, R = 1.13R ± 0.11% w / w. The EO obtained from *L. stoechas* presents organoleptic characteristics specific to the Lavandula genus (Table 1).

Table I: Organoleptic characteristics of the essential oil of *L. stoechas*

Aspect	Color	Odour	Flavor
Mobile Liquid	Yellow	Pleasantly strong	Fresh

2. Antimicrobial activity

The antimicrobial activity of *L. stoechas* EO's against the employed microorganisms was qualitatively assessed by the presence or absence of inhibition zones. *In vitro* testing of *L. stoechas* EO showed a significant inhibitory effect on the majority of microorganisms used (Table 2). Among the four Gram positive bacteria, the species *B. cereus, S. aureus* and MRSA are very sensitive to *L. stoechas* EO; *B. subtilis* is also considered to be sensitive. Gram negative bacteria exhibited slight sensitivity to our EO, except for *E. feacalis*, which was strongly inhibited by our EO. The following bacteria: *S. enteritidis* and *P. aeruginosa* were resistant to *L. stoechas* EO. The yeast *C. albicans* is strongly inhibited by the tested EO.

Comparison of means (Tukey's HSD test) between *L. stoechas* EO and all of the antibiotics tested revealed a highly significant difference ($p<0.05$) for all bacteria studied; except for *S. enteritidis*, which exhibited a non-significant difference between the EO and chloramphenicol. *L. stoechas* EO showed greater inhibitory activity than the antibiotics for all of the bacteria, with the exception of *S. enteritidis* and *S. typhi*.

Table 2: results of the diameters of inhibition zone of *L. stoechas* EO's and the positive controls (Antibiotics).

Strains	*L. stoechas* EO	Antibiotics						
		AMC	P	IMP	OX	VA	OFX	C
B. cereus	57.7±0.6	6±0*	-	-	-	17.3±0.6*	-	-
S. aureus	50.7±0.6	-	-	-	24.7±0.6*	17.3±0.6*	-	-
SARM	37±1	-	-	-	19±1*	21±0*	-	-
B. subtilis	25.3±0.6	6±0*		-	-	17.3±0.6*	-	-
E. faecalis	29.3±0.6	-	-	34,3±1.2*	-	18.7±0.6*	-	-
E. coli	13.3±0.6	6±0*	6±0*	-	-	-	-	-
S. typhi	12,7±1.2	-	-	-	-	-	37.3±1.5*	32,7±0.6*
S. enteritidis	9±0	-	-	-	-	-	31±0*	10,7±1.2
P. aeruginosa	6±0	6±0	6±0	-	-	-	-	-
C. albicans	29,5±0.7	-	-	-	-	-	-	-

AMC: Amoxicillin + clavulanic acid, P: Penicillin, IMP: Imipenem, OX: Oxacillin, VA: Vancomycin, OFX: Ofloxacin, C: Chloramphenicol. *Significant difference between *L. stoechas* EO and the antibiotic.

The analysis of variance (Fig. 1) showed a significant difference between bacterial species studied (p<0.05).

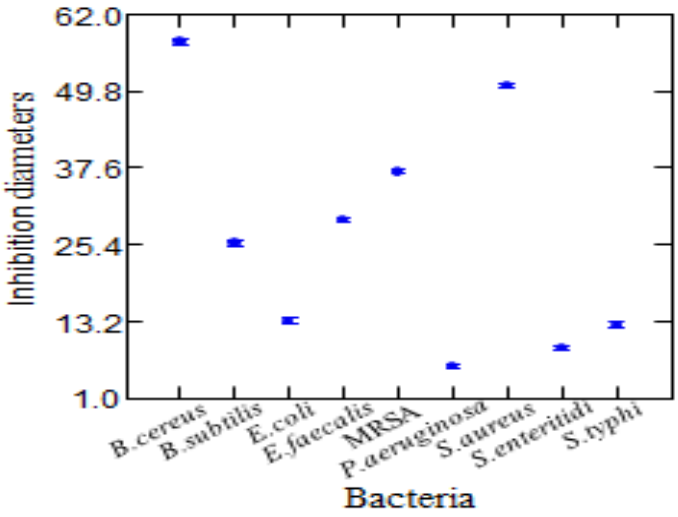

Figure 1: Results of the effect of *L. stoechas* EO's on bacterial strains.

The evaluation of antimicrobial activity is also based on MIC and MBC values (Table 3). They represent the inhibitory and bactericidal strength of *L. stoechas* EO.

Table 3: Results of the MIC and MBC of *L. stoechas* EO's.

Strains	MIC (µg/ml)	MBC (µg/ml)
E. coli	2.5	5 µg/ml
P. aeruginosa	-	-
B. sutilis	< 0.3125	1.25
B. cereus	0.625	10
S. aureus	1.25	2.5
MRSA	0.625	10
S. typhi	2.5	2.5
S. enteritidis	2.5	2.5
C. albicans	1.25	2.5

3. Anti- inflammatory activity

The anti-inflammatory action was carried out *in vivo* by carrageenan induced mouse paw edema. The results obtained from three doses of *L. stoechas* EO (800, 600 and 400 mg/kg BW) were compared with the results from diclofenac (2 mg/kg) and a control (Water).

Table 4 shows that oral administration of diclofenac, 800 mg/kg of essential oil and 600 mg/kg BW of EO resulted in a 55%, 58% and 48% reduction in edema, respectively (Fig. 2).

Table IV: Results of reduction of edema of *L. stoechas* EO's.

Lots (n=5)	Percent reduction in edema
Placebo (Water)	80.84 %
Diclofénac 2mg/kg	55.22%*
EO at 800 mg/kg	58.30%*
EO at 600 mg/kg	48.52%*
EO at 400 mg/kg	30.85%

* Significant difference between lots and diclofenac (Tukey test)

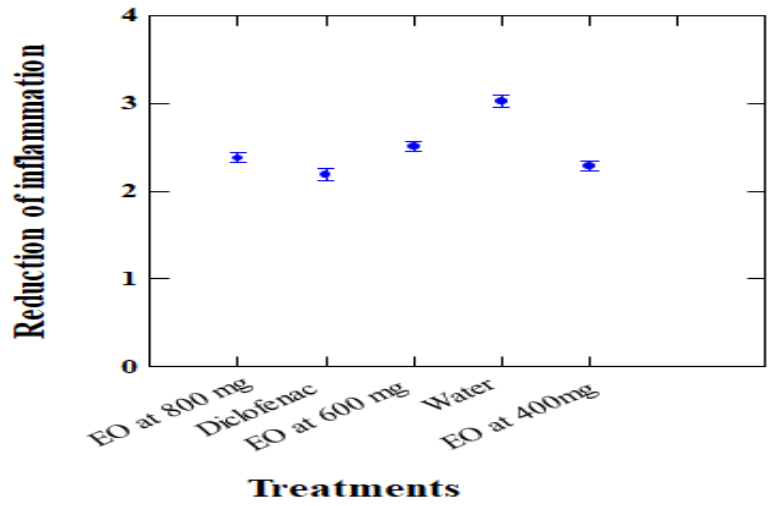

Figure 2: Results of reduction of inflammation of *L. stoechas* EO's

DISCUSSION

The hydrodistillation of dry flowers of *L. stoechas* provides an essential oil yield lower than that obtained by [23; 6]. In 2013, the work carried out in Portugal by Zuzarte *et al.* [16] shows a low yield of the aerial part of the same plant (0.7%). *L. stoechas* of Greece and Algeria showed a low yield of EO [24; 15].

According to Merghache *et al.* [25].variations in EO contents may be due to several ecological and genetic factors. Ecological factors include: maturity of flowers, interaction with the environment (type of climate, soil) and timing of harvest.

The results of antibacterial activity showed that: the Gram positive bacteria seemed to be more easily inhibited than the Gram negative bacteria. This could be explained by the structural differences in the bacterial cell wall. The resistance of the Gram negative bacteria can be attributed to the lipopolysaccharides (LPS) present in their external membrane. This membrane component renders the bacteria intrinsically resistant to external agents, such as hydrophilic components and essential oils, antibiotics and detergents [26].

The differences in the sensitivity of bacteria to *L. stoechas* EO could also be due to variations in the level of penetration of the EO components through the microbial membrane structures [27].

Nevertheless, it must be taken into account that the zone of inhibition depends on the ability of the antibacterial compound to diffuse uniformly through the agar [26].

The species *P. aeruginosa* exhibited the greatest resistance to *L. stoechas* EO. This is due to its high capacity for developing resistance to several antimicrobial agents, which explains why it is frequently associated with hospital-acquired infections [28]. The results from a study conducted in Turkey by Goren *et al.* [12] show growth inhibition of the three bacterial species tested (*E. coli, B. sutilis, S. aureus*).

According to the literature, a significant number of micro-organisms (bacteria and yeasts) are sensitive to *L. stoechas* EO. This is due to the presence of certain chemical compounds, including camphor, linalool, 1,8-cineole, carvacrol, eugenol, terpinen-4-ol [15] and myrtenal [29].

The values of MIC an MBC differ from one species to another. The highest MIC value (2.5µg/ml) was observed in *E. coli, S. typhi* and *S. enteritidis* (Table 2). The MBC values varied, such that *L. stoechas* oil is lethal for *B. cereus* and MRSA at a concentration of 10µg/ml. An MBC value of 2.5µg/ml effectively killed the following species: *S. aureus, S. typhi, S. enteritidis* and *C. albicans.*

The results from studies performed in Tunisia [14], Algeria [15] and Morocco [30] are consistent with our results, in that their EO showed very high effectiveness against Gram positive organisms compared to Gram negative organisms. In Portugal, Zuzarte *et al.* [16] observed a MIC value between 1.25 and 2.50 µl/ml for *C. albicans*. These results agree with this study.

Acute inflammation is a defensive reaction process of the body's immune system to protect it from biological, physical or chemical attack that does not lead to cell death. It is characterized by cardinal signs, the most important being redness and heat. These are due to increased blood circulation and capillary dilation. The swelling is caused by fluid accumulation; the pain is due to the release of chemicals that stimulate the nerve endings [31].

Carrageenan is a sulfated mucopolysaccharide found in red algae. It induces biphasic inflammation linked to the activation of cyclo-oxygenase [32]. It has been shown in mice that, in an initial phase, carrageenan induces the synthesis of chemical mediators such as histamine and serotonin that mediate inflammation [32].

In a second phase, carrageenan induces the synthesis of primarily prostaglandins, as well as proteases and lysosomes. This final step is susceptible to antagonists of prostaglandin synthesis and natural or synthetic anti-inflammatories [33; 34; 35].

For these two models (EO at 800 and 600 mg/kg), edema was significantly reduced compared to mice that received diclofenac (p<0.05). The activity of *L. stoechas* EO was observed from at the same time that prostaglandins were released into the site of inflammation. Thus, this EO contains prostaglandin inhibitors.

These results could be due to the fact that the EO is rich in bioactive compounds (oxygenated monoterpenes). These results indicate that the effect of *L. stoechas* EO is dose-dependent. However, *L. stoechas* EO has anti-inflammatory properties that are markedly weaker than those of diclofenac.

CONCLUSION

The essential oil of *L. stoechas* presents a real and potential interest by its antimicrobial and anti-inflammatory effect. It can be a very important source of plant protection constituents used to eradicate infections and inflammations. Similarly, it would be interesting to consider the use of this essential oil in the formulation of pharmaceutical products such as intimate toilet lotions, as it has an increased sensitivity against *C. albicans*.

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