INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 12, No. 1, p. 187-192, 2018

RESEARCH PAPER OPEN ACCESS

Chemical composition, antibacterial and antioxidant activities of *Lavandula pubescens* Decne essential oil from Algeria

Djamila Hamada[1], Ladjel Segni[1], Mohammed Bilal Goudjil[1], Salah Eddine Benchiekh[1], Noureddine Gherraf*[2]

[1]*Labaratory of Process Engineering, Faculty Applied Sciences, Ouargla University, Ouargla, Algeria*

[2]*Laboratoire des Ressources Naturelles et Aménagement des Milieux Sensibles, Larbi ben M'hidi University, Oum El bouaghi, Algeria*

Key words: *Lavandula pubescens,* Hydro-distillation, Essential oils, Antimicrobial, Antioxidant

http://dx.doi.org/10.12692/ijb/12.1.187-192 Article published on January 22, 2018

Abstract

Lavandula pubescens Decne is one of five *Lavandula* species growing wild in Algeria. The plant is widely used in traditional medicine. In this work, the essential oils of *L. pubescens* collected from El-mermothia locality in Tebessa (Algeria) were obtained by hydro-distillation, and subjected to antimicrobial and antioxidant assays. The antimicrobial activity was tested using the agar disc diffusion method, by determining the inhibition zone. The most important activity was recorded against *Enterococcus faecalis (ATCC 29212)* and *Escherichia coli (ATCC 25922)*. The antioxidant activity was assessed using two methods namely DPPH and Reducing power and the results revealed significant potency with IC_{50}values of 17.24µg/mL and 33.38 µg/mL respectively; but still lower than that found for the standard ascorbic acid.(8.86 µg/mL and 20.06 µg/mL)

* **Corresponding Author:** Noureddine Gherraf ✉ ngherraf@yahoo.com

Introduction

The use of medicinal plants has been increasing steadily with notable use in the pharmaceutical, cosmetic, and food industries (Christaki *et al*, 2012). Their essential oils and extracts as natural sources of antimicrobial and antioxidant compounds were reported worldwide (Sagdic *et al,* 2002). Essential oils are volatile, natural, complex compounds characterized by a strong odor and are produced by aromatic plants as secondary metabolites (Bakkali *et al*, 2008). They are usually obtained by hydro-distillation, steam distillation or dry distillation of a whole plant or just some parts (Usfda, 2006).

Lavandula pubescens known as "Zaetereljbal" has been traditionally used in folk medicine as a carminative, insect repellent, and antiseptic (Dubai and Alkhulaidi, 1997). The genus consists of about 39 species some of which being used for centuries, either dried or as essential oils (Wood, 1997).

In Algerian flora, there are five species from which three are endemic (Quezel and santa, 1963). Large part of the aroma and flavor of *Lavandula* genus is due to the presence of essential oils, some constituents of which have also shown to have biological activity and could be responsible for the plants use in folk medicine (Ghazanfar, 1994).

The aim of this work was to evaluate the in vitro antibacterial and antioxidant properties of the essential oils, obtained by hydro-distillation. The in vitro antioxidant activities were determined by using two complementary assays; namely inhibition of DPPH radical and reducing power.

Materials and methods

Vegetal material

Leaves of *L. pubescens* aerial parts were collected from El-mermothia locality in Tebessa (Algeria) in November 2016. The plant was taxonomically identified by Pr. Omar Idoude Biology Department, Ouargla University. A voucher specimen was deposited in department herberieum under the number DH 7.

Preparation of essential oils

100 g of air dried *L. pubescens* were well crushed prior to be submitted to hydro distillation using a Clevenger-type apparatus for three hours. The obtained oils were dried over anhydrous sodium sulfate and after filtration stored at 4°C until being tested and analyzed.

Antibacterial activity

Bacterial strains

The tests were applied to the following strains: *Escherichia coli* (ATCC 25922), *pseudomonas aeriginosa* (ATCC 9721). *Staphylococcus aureus* and *Enterococcus faecalis (ATCC 29212).*

Disc diffusion method

The evaluation of the antibacterial activity of essential oil was performed using the disc diffusion method according to the National Committee for Clinical Laboratory Standards recommendations (NCCLS., 2000).

The bacterial strains were spread on the Mueller Hinton Agar (MHA). Discs (6 mm Ø; Whatman No. 3) impregnated with the essential oil were placed on the surface of such media and incubated at 37°C for 24 h. The tests were performed in triplicate.

Antioxidant activity

Antiradical activity of the oils was carried out using DPPH assay according to Barry (AL. Barry and Thornsberry, 1991). The test was based on the scavenging activity of the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical. Ascorbic acid was used as a positive control. 1 ml of essential oils diluted in ethanol at different concentrations is added to 1 ml of the DPPH solution prepared at 0.4 mM in ethanol. After 30 min of incubation in the dark, the absorbance was read at 517 nm.

The reduced level of these molecules by DPPH is expressed in percentage according to the following formula:

$$DPPH^{\cdot} \text{ scavenging effect } (\%) = ((A_{Control} - A_{Sample})/A_{Control}) \times 100$$

where, $A_{Control}$ is the absorbance of the control reaction and A_{Sample} is the absorbance of the tested essential oils or Ascorbic acid. The oil concentration providing 50% inhibition (IC_{50}) was calculated from the curves of linear regression. Tests were carried out in triplicate.

Reducing power

The ability of the essential oils to reduce iron (III) was assessed by the method of**.** A Sample of 1.0 mL of various dilutions of essential oils was mixed with 1 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 1 mL (1%) of potassium ferricyanide (K3Fe(CN)6). The mixture was incubated at 50°C for 20 min, then acidified with 1 mL of TCA (10%). Finally, 0.25 mL of $FeCl_3$ (0.1%) were added to this solution. Distilled water was used as blank and for control. Absorption of this mixture was measured at 700 nm using a UV spectrophotometer (Oyaizu, 1986). Ascorbic acid was used as a positive control. The EC_{50} was obtained by interpolation from linear regression analysis (Piaru *et al.*, 2012). Increased absorbance of the reaction mixture indicated an increased reducing power (Singh *et al,* 2011).

Results and discussions

Antibacterial activity

The diameters of the zones of inhibition of essential oils for the tested microorganisms are shown in Table 1. The essential oils showed a significant inhibitory effect against gram-positive and gram-negative bacteria with inhibition zones ranged from 8 to 24.56 mm.

Table 1. Antibacterial activity of the essential oils of the *Lavandula pubescens*.

Micro-organisms	Inhibition zones (mm)
Escherichia coli (ATCC 25922)	20.2
Enterococcus faecalis (ATCC 29212)	24.5
Pseudomonas aeruginosa (ATCC 27853)	7.1
Staphylococcus aureus (ATCC 25923)	15.3

The most important activity was recorded against *Enterococcus faecalis (ATCC 29212)* and *Escherichia coli (ATCC 25922)*. *Pseudomonas aeruginosa* behaves more resistant due its greater ability to develop resistance vis-à-vis many antimicrobial agents, hence their frequent involvement in hospital infections. In fact, the resistance of this strain is not surprising; as these bacteria have an intrinsic opposition to biocides, which is related to the nature of their outer membranes formed by impermeable barrier to hydrophobic compounds (Mann *et al,* 2000). In the presence of permeabilizing agents of the outer membrane, inactive substances against these bacteria become active (Goudjil *et al.*, 2015).

Table 2. Antioxidant activities of essential oils of *Lavandula pubescens*.

	Scavenging activity	
	DPPH IC_{50} (µg / mL)	FRAP EC_{50} (µg /mL)
Essential oil	17.24	33.38
Ascorbic acid	8.86	20.06

The potent antimicrobial activity of the oils may be attributed to the higher percentage of oxygenated terpenes (87.1 %) (Knobloch *et al.*, 1988). Carvacrol as the main compound was reported to exert specific effects on *S. aureus* that destroys the viability of biofilm and the cell morphology in typical biofilm Architecture (Nostro *et al.*, 2009).

The presence of a phenolic hydroxyl group in carvacrol particularly, is credited with its activity against pathogens (Ultee *et al.*, 2002).

Antioxidant activity

Antioxidant activity of *L. pubescens* essential oil was determined by two different methods namely free radical-scavenging assay (DPPH) and ferric reducing power. The results are reported in Table 2.

With the DPPH essay, we obtained a stable radical, purple in solution and has a maximum absorption characteristic at 517 nm. The routine protocol applied is based on the disappearance of the radical when the DPPH is reduced by a compound with a free-radical property, causing the transformation of the color from purple to yellow. The IC_{50} is defined as the concentration of the sample required to achieve a 50% decrease in the absorbance of the initial solution of DPPH. The IC_{50} values are inversely proportional to the scavenger effect whose low values reflect a significant anti-radical effect. DPPH inhibitory activity of *L. pubescens* essential oils was evaluated and compared with ascorbic acid as a positive control. From Table 2, the IC_{50} values obtained show that the ascorbic acid has a low rate of IC_{50}, with a value of (IC_{50} = 8.86 ± 0.36 µg/ml) in comparison with *L. pubescens* essential oils (IC_{50}= 17.24 µg/ml), reflecting a significant anti-radical potential. This antiradical activity is likely due to the high content of the phenolic components. Our results were in agreement with the reported antioxidant activity of essential oils of *Lavandula* species. The *L. angustifolia, L. stoechas* and *L. pedunculate* oils were reported as highly antioxidant species by DPPH radical scavenging, inhibition of lipid peroxidation and DNA protection assays (Rafael *et al.*, 2015).

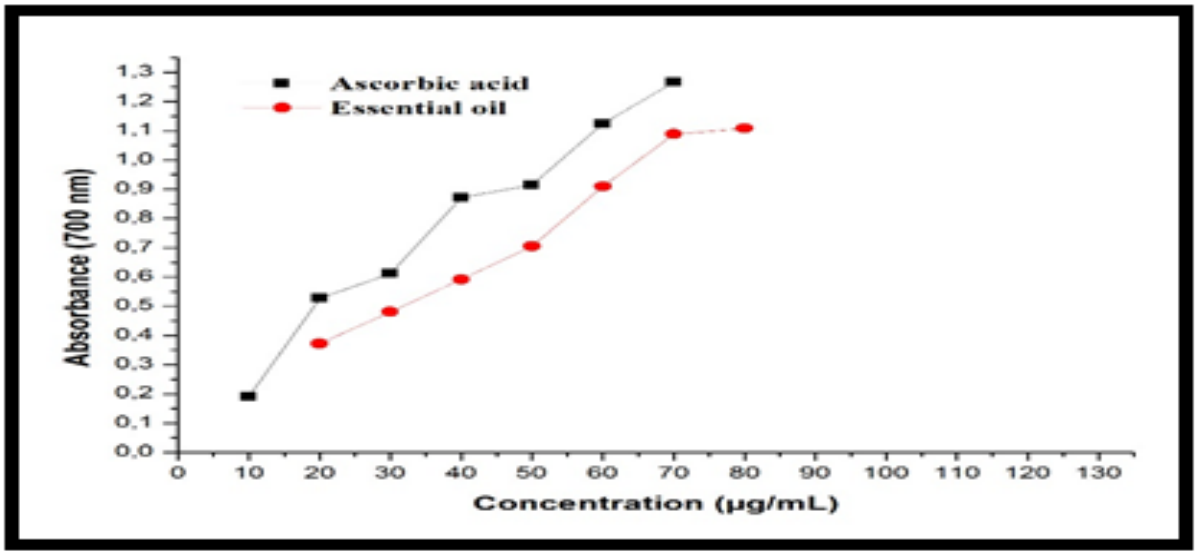

Fig. 1. Reducing power of *L. pubescens* essential oil.

For reducing power, the reducing properties are associated with the presence of compounds which exert their action by breaking the free radical chain by donating a hydrogen atom, or reacting with certain precursors of peroxide to prevent peroxide formation (Gulcin *et al,* 2010).

In this assay, the ability of essential oil to reduce iron (III) to iron (II) was determined and compared to that of ascorbic acid, which is known to be a strong reducing agent. Our essential oil showed antioxidant activity with an EC_{50} value of 33.38 µg/mL, while the ascorbic acid as a positive control gave a value of 20.09 µg/mL. Figure 1 indicates that, the reducing power increased with the concentration of sample and clearly demonstrated that the essential oils have significant reducing activity.

Conclusion

These results confirm the use of this plant by the ancient people as a medicinal plant with antiseptic effects since it has an interesting effect on a variety of microbial species such as *Escherichia coli* and *Enterococcus faecalis*. Considerable antioxidant

activities were found in the essential oil; evaluated in this study. Our results clearly demonstrate that the essential oils of *Lavandula pubescens* can present an interesting natural alternative, for food preservation and pharmaceutical treatment.

References

Bakkali F, Averbeck S, Averbeck D, Idaomar M. 2008. Biological effects of essential oils. A review. Food and Chemical Toxicology **46,** 446–475. https://doi.org/10.1016/j.fct.2007.09.106

Barry AL, Thornsberry C. 1991. Susceptibility test: diffusion test procedures. Manual of clinical microbiology, American society for microbiology, 5th Ed. Washington DC: American Society for Microbiology 1117–1125.

Christaki E, Bonos E, Giannenas I, Paneri PF. 2012. Aromatic plants as a source of bioactive compounds. Agriculture **2**, 228–243. https://doi.org/10.3390/agriculture2030228

Dubai A, Alkhulaidi A. 2005.Medicinal and aromatic plants in Yemen, deployment-components of effective-uses. Ebadi Center for studies and Publishing Sana'a- Yemen. 127.

Goudjil MB, Ladjel S, Bencheikh SE, Zighmi S, Hamada D. 2015.Chemical Composition, Antibacterial and Antioxidant Activities of the Essential Oil Extracted from the *Mentha piperita* of Southern Algeria. Research Journal of Photochemistry, **9(2)**, 79-87**.** https://doi.org/10.3923/rjphyto.2015.79.87

Gulcin I, Huyut Z, Elmastas M. 2010**.** Radical scavenging and antioxidant activity of tannic acids. Arabian Journal of Chemistry, **3(1),** 43-53. https://doi.org/10.1016/j.arabjc.2009.12.008

Ghazanfar SA. 1994**.** Handbook of Arabian Medicinal Plants. CRC press, Inc., USA pp 64-65.

Knobloch E, Pauli A, Iberl B, Wies N, Weigand H. 1988. Mode of action of essential oil components on whole cells of bacteria and fungi in plate tests, P. Schreier (Ed.), Bioflavor '87, Walter de Gruyter Verlag, Berlin, New York (1988), pp. 287-299

Mann CM, Markham JL.1998**.** A new method for determining the minimum inhibitory concentration of essential oils. Journal of Applied Microbiology **84,** 538 544. https://doi.org/10.1046/j.1365-2672.1998.00379.x

Nostro A, Marino A, Blanco AR, Cellini L, Giulio D, Pizzimenti M, Sudano F, Roccaro A, Bisignano G. 2009. *In vitro* activity of carvacrol against staphylococcal preformed biofilm by liquid and vapour contact. Journal of Medicinal Microbiology **58,** 791-797. https://doi.org/10.1099/jmm.0.009274-0.

Oyaizu M, 1986. Studies on products of browning reaction: Antioxydative activities of products of browning reaction prepared from glucosamine. Japanese Journal of Nutrition and Dietetics**, 44,** 307-315. https://doi.org/10.5264/eiyogakuzashi.44.307

Piaru SP, Mahmud R, Abdul Majid AMS, Mahmoud Nassar ZD. 2012. Antioxydant and antiangiogenic activities of essential oil of *Myristica fragrance* and *Morinda citrifolia*. Asian Pacific Journal of Tropical Medicine, **5(4),** 294-298. https://doi.org/10.1016/S1995-7645(12)60042-X

Quezel P, Santa S. 1963. Nouvelle flore de l'Algérie et des régions, désertiques et méridionales, Tome II, éditions CNRS, Paris 675

Rafael B, Ana M, Rita J, Rita A, Aida D, Noélia D, Maria M, Generosa T. 2015**.** Antioxidant and Antimitotic Activities of Two Native *Lavandula* Species from Portugal. Journal of Evidence-Based Complementary and Alternative Medicine. http://dx.doi.org/10.1155/2015/570521

Sagdic O, Karahan AG, Ozcan M, Ozkan G. 2003. Effect of some spice extracts on bacterial inhibition. Food Science and Technology International **9,** 353-356. https://doi.org/10.1177/1082013203038976

Singh R, Shushni MAM, Belkheir A. 2011**.** Antibacterial and antioxidant activities of *Mentha peperita* L. Arabian Journal of Chemistry **8(3),** 322-328 https://doi.org/10.1016/j.arabjc.2011.01.019

Ultee A, Bennik M, Moezelaar R. 2002.The phenolic hydroxyl group of carvacrol is essential for action against the food borne pathogen Bacillus cereus. Applied and environmental microbiology **68,** 1561-1568.
https://doi.org/10.1128/AEM.68.4.1561-1568.2002

USFDA, U.S Food and Drug Administration. 2006. Food additive status list.
www.cfsan.fda.gov/%3Ddms/opaappa.html#ftn.HAccessed20.07.09

Wood JRI. 1997. A Handbook of the Yemen Flora, Whit stable Litho Printers Ltd, Royal Botanic Gardens, Kew. UK. 296.