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Physico-Chemical Compositions and Antimicrobial Activity of Essential Oil of Eastern Moroccan *Lavandula dentata*

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ABSTRACT

The present study describes the phytochemical profile and antimicrobial activity of *Lavandula dentata* essential oil, collected in eastern Morocco (Taforalt, Talazart). The sample of essential oil was obtained from the aerial parts of the plant by hydrodistillation and analyzed by GC–MS. From the 29 compounds representing 99.87% of the oils: 1, 8 cineol (41.28%), sabinene (13.69%), bicycle [3.1.0] hexan-3-Ol, 4-methylene-1-(1-methylethyl) (6.76%), myrtenal (5.11%) and α-pinene (4.05%) appear as the main components. The oil also contained smaller percentages of borneol, linalool oxide cis, linalool, myrtenol, bicyclo [3.1.1] heptan-2-one, 6, 6-dimethyl-, (1r) and pinocarvone. Furthermore, antimicrobial activity of the oil was evaluated using agar diffusion and broth microdilution methods. The antimicrobial test results showed that the oil had antimicrobial activity against all 22 bacteria strains included in the study, except *Pseudomonas aeruginosa*. Results, suggest potential antimicrobial activity of the essential oil of *L. dentata,* which may find its application in future research for the food and pharmaceutical industry.

Key Words: *Lavandula dentata*; Essential oil; Antimicrobial activity; GC-MS

INTRODUCTION

The genus *Lavandula* (lavender, Lamiaceae) is distributed from the Canary and Cape Verde Islands and Madeira, across the Mediterranean Basin, North Africa, South, West Asia, the Arabian Peninsula and tropical NE Africa with a disjunction to India. Although the name of the genus is familiar to many people, only a few species are well known e.g., *L. angustifolia* Miller or English lavender, *L. latifolia* or spike lavender and *L. stoechas* or French lavender. The English lavender is widely cultivated in gardens and is also an important essential oil crop from which many cultivars have been selected.

A vast literature exists on the essential oils in *Lavandula*, reviewed by Boelens (1995). For taxonomists, use of essential oils as characters for the classifcation is limited by inherent problems of natural variability, although at lower taxonomic levels this can be used to help recognize cultivars (Grayer *et al.,* 1996). The chemical composition and ratios of the individual components making up the oils are also known to change in response to environmental conditions, such as water and nutrient stress or time of year (Ross & Sombrero, 1991). These inherent problems of variation mean that other classes of chemical constituents such as flavonoids are often of greater use and significance to the systematist.

With regard to the genus *Lavandula*, no flavonoid survey of the genus has yet been undertaken, although some species have been investigated as part of wider taxonomic surveys of the family. The following flavonoids were reported from *L. dentata*: genkwanin (apigenin 7-methyl ether), luteolin, apigenin, luteolin 7-glucoside, apigenin 7-glucoside, luteolin 7-rutinoside, vitexin and vicenin-2.

Lavender is one of the most useful medicinal plants. Commercially, the lavender is an important source of essential oil that is widely used in fragrance industry including soaps, colognes, perfumes, skin lotions and other cosmetics (Paul *et al*., 2004). In food manufacturing, lavender essential oil is employed in flavoring beverages, ice-cream, candy, baked goods and chewing gum (Kim & Lee, 2002). Recently, aromatherapy is becoming increasingly popular and lavender is used in aromatherapy as a relaxant (Lis-Balchin & Hart, 1999; Ghelardini *et al*., 1999). Several therapeutic effects of lavender, such as sedative, spasmolytic, antiviral and antibacterial activities have been reported (Gamez *et al*., 1990; Buchbauer *et al*., 1991).

Several plants of this genus have been studied from the chemical, biological and pharmacological point of view (Pascual *et al*., 1983; Gamez *et al*., 1987; Kokkalou, 1988;

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Guillemain *et al*., 1989; Valentini *et al*., 1993; Lis-Balchin & Hart, 1999; Ghelardini *et al*., 1999; Gilani *et al*., 2000; An *et al*., 2001; Cavanagh & Wilkinson, 2002; Nogueira & Romano, 2002).

Lavender essential oils are advocated for their use as an antibacterial agent in both early and modern aromatherapy texts (Lawless, 1992; Gattefosse, 1995). Gattefosse, for example in his 1937 aromatherapy text describes the use of essences of lavender essential oils as an antiseptic mouthwash and in embalming (Gattefosse, 1995). Similarly Lis-Balchin and Deans (1997) and Lis-Balchin *et al*. (1998) showed that lavandin, French lavender, spike lavender, Bulgarian lavender and generic 'lavender' (type unspecified) essential oils all have activity against a large number of bacteria and fungi. For example, Bulgarian lavender essential oil inhibited 23 of 25 different bacteria, while lavandin inhibited 17 of 25 bacteria.

In this study, antibacterial activity of *L. dentata* oil was examined using different bacterial species. In addition, composition of volatile compounds, were also determined.

MATERIALS AND METHODS

Plant material. Samples of *Lavandula dentata* were collected in eastern Morocco (Taforalt, Talazart) in July 2007. Identification of the species was confirmed and a voucher specimen was preserved in scientific institute in Rabat.

Experimental Apparatus

Essential oil isolation. The dried aerial parts were submitted to Hydro distillation for 3 h using Clevenger type apparatus, according to the European Pharmacopoeia (1996). Briefly, the plant was immersed in water and heated to boiling, after which the essential oil was evaporated together with water vapour and finally collected in a condenser. The distillate was isolated and dried over anhydrous sodium sulfate. The oil was stored at 4°C until analysis by GC–MS (Table I).

Gas chromatography-mass spectrometry (GC-MS). The essential oil of *L. dentata* was subjected to GC-MS analysis using Trace GC ULTRA /Polaris Q (GC-MS, ThermoElectron). The column was a VB-5 (5% phenyl/95% dimethylpolysiloxane) with film thickness of 0.25 µm, a length of 30 m and an internal diameter of 0.25 µm was used with helium as carrier gas. The GC oven temperature was kept at 50°C for 5 min and programmed to 250°C for 3 min at rate of 4°C/min and programmed to 300°C at rate of 25°C/min. The injector temperature was set at 250°C. Split flow was adjusted at 50 mL/min. MS were taken at 70eV. Mass range was from uma 20 to 350. A library search was carried out using the "Wiley GC/MS Library", Nist and Pmw. The sample was dissolved in Hexane. The components from the gas chromatography-mass spectral analysis are reported in Table I.

Antimicrobial activity. The essential oils were individually tested against Gram+ and Gram-bacteria. Some bacterial strains used in this study were obtained from American type culture collection (ATCC), National Institute of Healthy (NIH), USA. All bacteria were stored in trypticase soy (Sanofi Diagnostic Pasteur, France) broth containing 25% (v/v) glycerol (Sigma-Aldrich) at -20°C. Prior to use, the cultures were propagated twice in the appropriate media as mentioned above to make them physiologically active.

Two techniques were used to test the antimicrobial activity of *L. dentata* oil. A 16 h culture was diluted with sterile physiological saline solution with reference to the MC Farland 0.5 standard to achieve an inoculum of approximately 10^6 CFU/mL a suspension was swabbed in three directions on 4 mm thick Mueller Hinton agar (MHA) (Oxoid, England) with a cotton swap. Sterile, 6 mm diameter stainless steel cylinders were placed on plats of MHA. To each of the duplicated cylindress, 100 µL of filter sterilised test E.O (dilution with tween 80 at 2%) were added. The plates were then incubated for 24 h at 37°C. The results were recorded by measuring the zones of growth inhibition surrounding the cylinders.

In addition to the solid medium diffusion procedure, the micro plate bioassay (micro dilution) was used, as recommended by NCCLS, for determination of minimum inhibitory concentration (MIC) (NCCLS; 1999). The MIC was defined as lowest concentration of *L. dentata* oil inhibiting visible bacterial growth after incubation for 20h at 37°C. Into each well 100 µL of Brain Heart Infusion broth (BHI, Difco Laboratories, Detroit, MI, USA) inoculated with the bacteria inoculum prior to the essay. An aliquot (100 µL) of the essential oil was added in first well. Geometric dilutions ranging from 0,041 mg/mL to 21 mg/mL of the essential oils were prepared in a 96 well micro titre plate, including one growth control (BHI +Tween 80)and one sterility control (BHI+Tween 80+test oil). The contents of the wells were mixed and micro plates were incubated at 37°C for 24 h. The MIC was determined by quantitative tetrazolium based colorimetric method. Ten microliters of 4 mg/mL solution of 3-(4, 5-dimethylthiazo-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT; Biochemika, Fluca) in distilled water were added to each well. Plates were incubated at 37°C. After a few minutes at room temperature; the plates were read. A colour change from blue to purple was indicative of bacterial growth.

RESULTS

Chemical composition of the essential oil. The results obtained by GC–MS analyses of the essential oil of *L. dentata* are presented in Table I. Twenty nine compounds were identified in the essential oil. As a result of GC–MS analyse, *L. dentate* contained, 1, 8-cineole (41.28%), sabinene (13.89%), bicyclo [3.1.0] hexan-3-Ol, 4-methylene-1-(1-Methylethyl) (6.76%), myrtenal (5.1%) and α-pinene (4.05%) as the major compounds. Other significant constituents were verbenol (2.1%), linalool oxide (2.49%), linalool oxide Cis (2.66%), bicyclo [3.1.1] heptan-2-one, 6,

6-dimethyl (2.36%) myrtenol (2.75%) and borneol L (2.84%).

Antimicrobial activity. Table II presents the inhibition zone of essential oil determined for 24 of Gram positive or gram negative bacteria using the diffusion technique on solid media. The results showed that the essential oil had a substantial inhibitory effect on all assayed bacteria strains noted by large growth inhibition halos. The data indicated that Gram-positive *L. monocytogenes* was the most sensitive strain tested to the oil of *L. dentata* with the strongest inhibition zone (70 mm). The *Streptococcus sp* was, in general, found to be more sensitive among Gram-positive bacteria with inhibition zone of 50 mm. The oil also exhibited high antimicrobial activity against *S. pneumoniae*. Modest activities were observed against *S. aureus*, with inhibition zones of 16–22 mm. Among these, Gram-negative strains also displayed variable degree of susceptibility against investigated oil. Maximum activity was observed against *N. meningitides (1), H. influezae (2)* with inhibition zone of 50 mm, followed by *K. pneumoniae, Salmonella sp (1), P. mirabilis, Pantoea sp* and *E. cloacae.* Modest activities were observed against important food pathogens such as *E. coli*, with inhibition zones of 11-20 mm.

The results of the MIC are presented in Table III. The data indicate that the oil exhibited varying levels of antimicrobial activity against the investigated food pathogens. MIC values showed by the essential oil were in the range of 0.041 to 10 mg/mL. In liquid medium, the essential oil was active against all the test strains. The Gram negative *Ps. aeruginosa* seemed to be resistant to the investigated oil with a MIC of 10 mg/mL. Maximum activity was observed against the *S. aureus, S. pneumoniae, S. sp* with a MIC of 0.041 mg/mL. *Streptococcus* sp *(2)* was the least sensitive bacteria with MIC of 0.338. *S. epidermidis*, *H. influenza* and *P. mirabilis* showed similar susceptibility to the investigated oil, ranging from 0.167 mg/mL.

The oil exhibited the highest inhibitory effect against Gram negative bacteria *Escherichia coli (2), N. meningitidis* and *Pantoea sp* in a range between 0.083 and 0.041 mg/mL.

Table I. Phytochemical composition of *Lavandula dentata essential* oil (%)

Phytochemicals	*RT*	*%*
α-Pinene	8.81	4.05
Camphene	9.36	0.78
Verbenene	9.59	0.19
Sabinene	10.48	13.89
P-Cymene	12.43	0.46
1,8-Cineole	12.65	41.28
Linalool Oxide	14.33	2.49
Linalool Oxide Cis	14.94	2.66
Linalool	15.45	2.76
α-Campholene Aldehyde	16.33	0.92
Bicyclo[3.1.1] Heptan-2-One, 6,6-Dimethyl-, (1r)-	16.67	2.36
Bicyclo [3.1.0] Hexan-3-Ol, 4-Methylene-1-(1-Methylethyl)-, (1à,3à,5à)-	16.81	6.76
Camphor	16.96	1.48
Verbenol	17.09	1.19
Pinocarvone	17.67	1.76
Borneol L	17.84	2.84
Terpinol-4-Ol	18.28	0.88
Pentalene, Octahydro-1-(2-Octyldecyl)- (Cas)	18.39	0.17
(1r)-(-)-Myrtenal	18.90	5.11
Myrtenol	19.01	2.75
Verbenone	19.38	1.02
P-Menth-4(8)-En-9-Ol (Cas)	20.10	0.37
-(P-Chlorophenyl)-2,6-Diphenylpyridine	23.87	0.96
α-Selinene	28.68	0.46
1,1, 1, 3, 5, 7, 9, 9,9-Nonamethylpentasiloxane	29.32	0.57
Mesityl Acetyl Propynylphosphine	31.58	0.45
α-Eudesmol	33.45	0.45
3-Methyl-5-Propyl-4-Butylidene-Cyclohex-2-Ene-1-One	34.46	0.48

Table II. Antibacterial activity of essential oil as determined by diffusion technique on solid media

Microorganisms	*Essential oil zone inhibition(mm)*
Salmonella sp (1)	20
Salmonella sp (2)	15
Neisseria meningitidis (1)	50
Neisseria meningitidis (2)	21
Enterobacter cloacae	15
Klebsiella pneumoniae (1)	20
Klebsiella pneumoniae (2)	38
Heamophilus influezae(1)	28
Heamophilus influezae (2)	50
Pseudomonas aeruginosa ATCC12228	0
Pantoea sp	15
Escherichia coli	20
Escherichia coli	12
Escherichia coli ATCC125922	15
Escherichia coli	11
Escherichia coli	11
Proteus mirabilis-	18
Staphylococcus aureus ATCC 25923	22
Staphylococcus aureus	16
Streptococcus sp	50
Streptococcus pneumoniae	43
Listeria monocytogenes	70

DISCUSSION

Another study from Algeria has investigated the main constituents in the oil of *L. dentata*; 1, 8-cineole (38.4%), cis-verbenol (4.3%), p-cymen-8-ol (3.8%) and fenchone (2.3%). Myrtenal (2.0%), pinocarvone (1.9%), α -terpineol (1.8%) and α-terpinen- 7-al (1.8%) were other notable constituents of the oil (Dob *et al*., 2005).

It was noteworthy that the compositions of the *Lavandula* oil in Morocco were in partial agreement with the previous report (Dob *et al*., 2005). There were, however, significant differences between main components. For example, the major constituent of the *Lavandula* oil in our research 1, 8-cineole, was also reported to be the major constituent in previous report (Dob *et al*., 2005). On the contrary, p-cymen-8-ol, pinocarvone and α-terpineol and α -terpinen- 7-al and fenchone, which were not detected in our sample, were found in the previous report (Dob *et al*., 2005). Sabinene, which was present at low concentration (1.4%) in the previous report (Dob *et al*., 2005), represented

at the major constituent in our sample (13.89%). These changes in the essential oil compositions might arise from several environmental (climatical, seasonal, geographical) and genetic differences (Perry *et al*., 1999).

Several investigations(Pascual *et al*., 1983; Kokkalou, 1988; Pascual *et al*., 1989; Valentini *et al*., 1993; Figueiredo *et al*., 1995; Oszagyán *et al*., 1996; Venskutonis *et al*., 1997; Barazandeh, 2002; Nogueira & Romano, 2002) on the essential oils of various *Lavandula* species showed that 1, 8-cineole, linalool, linalyl acetate, fenchone and ß-phellandrene characterise most of these plants.

As far as antimicrobial activity is concerned, *Ps. aeruginosa* was the only bacterium that was not susceptible to the oil, since it is known to have high level of intrinsic resistance to virtually all known antimicrobials and antibiotics due to a combination of a very restrictive outer membrane barrier, highly resistant even to synthetic drugs (Skočibušić *et al*., 2006). *Ps. aeruginosa* was considered resistant to the Tarchonanthus camphorates essential oil and even to the reference antibiotic chloramphenicol, since no inhibition zone was observed (Matasyoh *et al*., 2007). This bacterium has shown resistance to antimicrobial agents and diterpenes present in Salvia species (Darias *et al*., 1990).

It has frequently been reported that Gram (+) bacteria are more susceptible to essential oil than Gram (−) bacteria (Mann *et al*., 2000). The tolerance of Gram (−) bacteria to essential oil has been ascribed to the presence of a hydrophilic outer membrane that blocks the penetration of hydrophobic essential oils into target cell membrane. However, our results (Table I & II) did not show any selectivity towards Gram (+) bacteria. There was no preferential activity against Gram positive versus and Gram negative organisms.

The traditional and anecdotal use of lavender essential oils for their antibacterial activity has been substantiated by this study. We have shown that, with the exception of *Ps. aeruginosa*, all bacteria tested were susceptible to *L. dentata* essential oil. These results open the opportunity for the use of lavender essential oils as an antibacterial ingredient in toiletries, cosmetics and cleaning products.

This high antibacterial activity of *L. dentata* essential oil supports the results found by other researchers. Dadalioğlu and Everendiliek (2004) showed that *L. stoechas* (Spanish lavender) had a strong antibacterial action against Escherichia coli O157:H7, *L. monocytogenes, S. typhimurium* and *S. aureus*. Similarly Lis-Balchin and Deans (1997) and Lis-Balchin *et al*. (1998) showed that lavandin, French lavender, spike lavender, Bulgarian lavender and generic 'lavender' (type unspecified) essential oils all have activity against a large number of bacteria and fungi. For example, Bulgarian lavender essential oil inhibited 23 of 25 different bacteria, while lavandin inhibited 17 of 25 bacteria.

Another study has investigated the activity of the disc diffusion assays of nine lavender essential oils against 11 bacteria and the yeast *C. albicans*. There was considerable variability in the zone's size of inhibition with the different oils and range of susceptible organisms for any single oil. *Ps. aeruginosa* was the only bacterium that was not susceptible to the oils; the small zone (6.4 ± 0.9 mm) produced with European *L. angustifolia* oil was not significantly different to the other oils. Overall, no essential oil was observed to be the 'best' against all organisms, with *Strep. pyogenes, Pr. vulgaris* and *C. albicans* having consistently larger zones than other organisms (Moon *et al*., 2006).

Table III. Minimal inhibitory concentration (MIC) of essential oil from *Lavandula dentata*

Microorganisms	***Essential oil***
	MIC (mg/ml)
Staphylococcus aureus ATCC25923	0.041
Staphylococcus epidermidis ATCC12228	0.167
Streptococcus pneumoniae	0.041
Streptococcus sp (1)	0.041
Streptococcus sp (2)	0.338
Heamophilus influezae (1)	0.167
Pseudomonas aeruginosa ATCC27853	10
Escherichia coli (1)	0.167
Escherichia coli (2)	0.041
Proteus mirabilis	0.167
Heamophilus influezae	0.167
Neisseria meningitidis	0.04187
Klebsiella pneumoniae	0.04187
Neisseria meningitidis1	0.041
Pantoea sp	0.083

Some researchers have attempted to correlate antibacterial or antifungal activity with essential oil constituents (Lis-Balchin *et al*., 1998; Griffin *et al*., 2000). It has been suggested that antibacterial activity is associated with high concentrations of monoterpenes; however, these authors conclude that the relationship is complex and could not be easily predicted (Lis-Balchin & Deans, 1997; Lis-Balchin *et al*., 1998). Griffin (2000) in his investigation of the antimicrobial activity of terpenoids showed that antibacterial activity was determined by a complex mix of factors including hydrogen bonding parameters, water solubility and molecular size.

The essential oil evaluated in this work has a great variety of phytochemicals that could be considered as responsible for a larger or smaller part of the antimicrobial activity. Although they usually occur as complex mixtures, their activity can generally be accounted for in terms of their major monoterpenoid components. Research into the antimicrobial actions of monoterpenes suggests that they diffuse into and damage cell membrane structures (Sikkema *et al.,* 1995).

The antimicrobial activity of the oil could be due to the synergistic effects of the diversity of major and minor constituents. As a matter of fact, borneol has been reported to have significant antimicrobial activity (Tabanca *et al*., 2001; Vardar *et al*., 2003). α-pinene, which was found to be in appreciable amounts, has been reported to possess antifungal activity of oil from *Pistacia lentiscus*

(Anacardiaceae) (Magiatis *et al.,* 1999). Pinene-type monoterpene hydrocarbons (α-pinene & β-pinene) are well-known chemicals having antimicrobial potentials (Dorman & Deans, 2000). The major components of this oil, 1,8-cineole, has been known to exhibit antimicrobial activity against the bacterial strains (*E. coli, P. aeruginosa, S. typhi, S. aureus, Rhizobium leguminosarum, Bacillus subtilis*) (Sivropoulou *et al*., 1997). Terpinen-4-ol, although a minor constituent in the oil under study, is known to have very efficient antibacterial properties (Carson & Riley, 1995). This monoterpene, isolated from Achillea species, showed antibacterial activity (Magiatis *et al*., 2002). Another minor monoterpene alcohol, linalool, is reported to have a wide range of antibacterial and antifungal activity (Pattnaik *et al*., 1997).

The synergistic effects of these active chemicals with other constituents of the essential oil should be taken into consideration for the antimicrobial activity. The mechanism of action of terpenes is not fully understood but it is thought to involve membrane disruption by the lipophilic compounds (Cowan, 1999). Antimicrobial activities of essential oils are difficult to correlate to a specific compound due to their complexity and variability.

CONCLUSION

Although tests on food are necessary, the present study indicates that *L. dentata* oil extracts can be considered as an alternative to ‘‘traditional food preservatives’’, eliminating or reducing the growth of important food borne pathogens and spoilage bacteria and contributing to enhance food safety and shelf life.

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