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Chemical Composition, Cytotoxic and Antioxidant Activity of the Essential Oil of *Lavandula dentata*

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Abstract: The compositions of the essential oils from the aerial part and flowers of *lavandula dentate,* collected in eastern Morocco (Taforalt, Talazart), were analysed by GC/FID and GC–MS, 42 constituents (84,76% of the total oil) and 47 constituents (85.48% of the total oil) were identified in aerial part and flowers, respectively. Of which β-pinene (27.08 and 30.06 %), Pincarveol (14.77 and 8.59 %), myrtenal (8.18 and 6.81%), α-pinene (7.78 and 8.38%) and 1,8-cineole (5.53 and 5.47%) were the main compounds in entire pant and flowers, respectively. The oils of aerial part and flowers were subjected to screening for their possible antioxidant activities by using 2.2-diphenyl-1-picrylhydrazyl (DPPH). In the former case, the essential oils from aerial part and flowers showed free radical scavenging activity with IC50 value of 32,12±0,574 and 41,29±1,208 µl/ml respectively. The in vitro cytotoxicity of both oils on five (P388D1, PC3, V79, U-373 MG, MCF7) human cancer cell lines were also examined. The cytotoxicity of the Flower oil on two cancer cell lines (P388D1, U-373 MG) was significantly stronger than that of the oil from the aerial part of *Lavandula dentata.*

Key words: Essential oils · *Lavandula dentata* · Cytotoxicity · Antioxidant activities

INTRODUCTION

The genus *Lavandula* L. belongs to Lamiaceae and is native to the Mediterranean region, Canary Islands and India, although it is now cultivated in different regions of the world.

A vast literature exists on the essential oils in *Lavandula*, reviewed by [1]. 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 [2].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 [3].These inherent problems of variation mean that other classes of chemical constituents such as flavonoids are often of greater use and signifcance to the systematist.

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 [4].In food manufacturing, lavender essential oil is employed in flavoring beverages, ice-cream, candy, baked goods and chewing gum [5].Essential oils of plants are of growing interest both in the industry and scientific research because of their antibacterial, antifungal and antioxidant properties and make them useful as natural additives in foods [6].

Recently, aromatherapy is becoming increasingly popular and lavender is used in aromatherapy as a relaxant [7,8].Several therapeutic effects of lavender, such as sedative, spasmolytic, antiviral and antibacterial activities have been reported [9,10].

Several plants of this genus have been studied from the chemical, biological and pharmacological point of view [7,8,11-20].

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Lavender essential oils are advocated for their use as an antibacterial agent in both early and modern aromatherapy texts [21, 22].

Therefore, the aim of the present work was carried out to study in vitro antioxidant, cytotoxicity and to evaluate the component of essential oils by GC-FID and GC–MS.

MATERIALS AND METHODS

Essential Oil Isolation: The dried aerial parts were submitted to Hydrodistillation for 3h using Clevenger type apparatus, according to the European Pharmacopoeia (1996) [23]. 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 sulphate. The oil was stored at 4°C until analysis by GC/FID and GC/MS (Table I).

Gas Chromatography: Essential oil samples (0.1 µl) were injected neat into an HP 6890 gas chromatography equipped with a flame ionisation detector (FID) and a 30 m x 0.25 mm HP-5 (cross-linked Phynel-Methyl Siloxane) column with 0.25 µm film thickness (Agilent) was used for the study. Helium was used as carrier gas, the flow through the column was 1,4 ml min^{-1} and the splitless mode was used. The column was maintained at 40°C for 5 min, increased to 230°C at rate of 10°C min^{-1} and finally raised from 230 to 280 at rate of 30°C min^{-1}.

Mass Spectrometry Analysis: The oil was analysed by gas chromatography-mass spectrometry (GC-MS) using a Hewlett Packard 6890 mass selective detector coupled with a Hewlett Packard 6890 gas chromatograph. The MS operating parameters were as follows: ionisation potential, 70 eV; ionisation current, 2 A; ion source temperature, 200°C, resolution, 1000. Mass unit were monitored from 30 to 450 m/z. Identification of components in the oil was based on retention indices relatives to n-alkanes and computer matching with the WILLEY 275.L library, as well as by comparison of the fragmentation patterns of mass spectra with those reported in the literature [24]. The chromatographic conditions were identical to those used for GC analysis.

Cell Lines and Culture: The human tumour cell lines PC3 (prostate), U-373 MG (human, Caucasian, V79 (fibrobasts) and MCF-7(Human breast cancer cell lines), P388D1 (murine macrophage) were grown and maintained in a humidified incubator at 37°C and in 5% CO2 atmosphere.

Table 1: Essential oil components of aerial part and flowers of *L.dentata*

	Aerial part			Flower		
Components	RT	KI	%	RT	KI	%
tricyclene	8,16	915,218	0.3	8,16	915,21	0.34
alpha.-pipene	8,49	929,56	7.78	8,48	929,13	8.38
camphene	8,81	943,48	1.13	8,81	943,48	0.79
beta.-pinene	9,55	975,65	27.08	9,53	974,78	30.06
beta myrcene	-	-	-	9,83	987,82	0.31
1,3,8-para-menthatriene	10,07	998,26	Tr	-	-	-
1,5,8-p-menthatriene	-	-	-	10,07	998,26	tr
alpha.-terpinene	10,32	1011,23	0.35	-	-	-
2-(bromomethyl)-1,3,						
3-trimethylcyclohexene	-	-	-	10,32	1009,13	tr
1,8 cineole	10,62	1027,27	5.53	10,61	1026,74	5.47
gamma.-terpinene	11,14	1055,08	Tr	11,14	1055,08	tr
o-cresol	11,32	1064,71	Tr	-	-	-
cis-linalool oxide	11,43	1070,59	1.81	11,41	1069,52	0.98
trans-linalool oxide	11,71	1085,56	2.13	-	-	-
linalool	11,98	1100	4.7	11,95	1098,39	4.46
alpha.-campholene						
aldehyde	-	-	-	12,35	1122,15	0.54
veratrole	-	-	-	12,45	1128,14	0.24
Pincarveol	12,69	1142,51	14.77	12,64	1139,52	8.59
verbenol	12,73	1144,91	0.31	-	-	-
camphor	-	-	-	12,7	1143,11	2.32
cryptone	12,92	1156,29	0.3	-	-	-
pinocarvone	13,00	1161,08	2.36	12,98	1159,88	2.44
borneol	13,07	1165,27	2.56	13,04	1163,47	1.66
4-terpineol	13,22	1174,25	0.58	13,21	1173,65	0.41
myrtenal	13,56	1194,61	8.18	13,54	1193,41	6.81
alpha.-terpineol	-	-	-	13,44	1187,42	0.5
verbenone	13,76	1207,75	0.73	13,74	1206,34	0.5
cis-carveol	13,90	1217,60	0.57	13,87	1215,49	0.39
cuminic aldehyde	14,18	1237,32	0.27	-	-	-
d-carvone	14,24	1241,55	0.26	14,23	1240,84	0.24
perilla alcohol	-	-	-	15,15	1305,84	tr
perilla aldehyde	14,69	1273,24	0.38	14,68	1272,53	0.47
l-bornyl acetate	14,83	1283,1	tr	-	-	-
hexyl tiglate	-	-	-	15,42	1325,54	0.43
perillol	15,11	1302,92	0.66	-	-	-
Hexyl hexanoate	-	-	-	16,16	1379,56	tr
piperitenone	15,28	1315,33	tr	-	-	-
alpha.-cubebene				16,36	1394,16	tr
hexyl tiglate	15,42	1325,55	0.37	-	-	-
Hexyl hexanoate	16,16	1379,56	tr	-	-	-
alpha.-cedrene	16,47	1402,38	tr	-	-	-
trans-caryophyllene	-	-	-	16,71	1421,43	tr
beta.-sesquiphellandrene	-	-	-	16,95	1440,48	0.23
Cis alpha.-bergamotene	16,60	1412,7	tr	16,6	1412,7	tr
gamma.-elemene	16,79	1427,78	tr			
beta.-cubebene	-	-	-	17,04	1447,62	tr

Table 1: Continued

1,4-dihydro-3, 5-dimethoxy- 2-methylnaphthalene	-	-	-	17,21	1461,11	tr
Trans alpha.-bergamotene	16,86	1433,33	tr	16,87	1434,13	0.38
Beta funebrene	16,95	1440,48	tr	-	-	-
epi-bicyclosesquip -hellandrene	17,04	1447,62	tr	-	-	-
trans-.beta.-farnesene	17,51	1484,92	tr	17,51	1484,92	0.27
beta.-selinene	17,57	1489,68	0.59	17,58	1490,47	0.9
germacrene d	-	-	-	17,9	1516,66	0.4
beta.-bisabolene	17,78	1506,67	tr	17,78	1506,66	tr
cis-.alpha.-bisabolene	-	-	-	18,19	1540,83	0.3
gamma.-Cadinene	17,89	1515,83	tr	-	-	-
calamenene	18,00	1525	0.38	18,01	1525,83	0.85
4,7-dimethyl-1-tetralon				18,28	1548,33	0.86
caryophyllene oxide	18,77	1589,17	0. 42	18,77	1589,17	1.04
beta.-eudesmol	19,56	1658,41	0.68	19,57	1659,29	1.36
bicyclo[4.4.0]dec-1-en, 2-isopropyl-5-methyl -9-methylene-	-	-	-	19,41	1645,13	0.48
1,4-dimethyl-7- (1-methylethyl)azulene	19,81	1680,53	tr	-	-	-
4-dimethyl-7 -(1-methylethyl)azulene	-	-	-	19,81	1680,53	0.21
alpha.-bisabolol	-	-	-	19,87	1685,84	0.77
14-norcadin-5-en -4-one isomer	-	-	-	20	1697,34	1.1
Total	84,76			85,48		

Minimum Essential Medium (MEM) (500 ml) supplemented with 25 ml foetal bovine serum (FBS), 10 ml L-glutamine and with antibiotics penicillin – streptomycin (10ml) and Gentamycine (1ml). Cells were grown in 25 cm^2 flasks and detached with trypsin. Split ratio was once per week and the medium was changed 1–2 times per week. P388D1 (murine macrophage) cell lines was maintained in suspension cultures in complete MEM medium and sub-cultured every 2–3 days.

MTT Assay: This assay detects the reduction of MTT [3-(4, 5-dimethylthiazolyl) - 2,5-diphenyltetrazolium bromide] by mitochondrial dehydrogenase, to blue formazan product, which reflects the normal functioning of mitochondrial and cell viability [25]. Briefly, after being harvested from culture flasks the cells were counted. For P388D1($6x10^5$)and PC3 ($4x10^5$) cells were incubated in 96-well plates, containing 100µl of the growth medium per well. While for V79 ($2.4 x10^5$), U-373 MG (human, Caucasian)and MCF7 ($5x10^5$) cells were seeded per well. Cells were permitted to adhere for 24 h and then treated with the essential oil dissolved in medium for 48 h; 200 µl of 0, 5 mg/ml MTT in phosphate buffered saline (PBS) was added to each well and the plate was incubated at 37°C for 4 h. The medium was removed and 100µl DMSO was then added to each well. After incubation at 37°C for 10 min, absorbance in the control (treated with 0.1% DMSO) and in the essential oil-treated cells was measured at 540 nm, using a microplate reader (Labsstem iMEMS reader/dispenser MF). The effect of essential oil on the proliferation of cancer and normal cells was expressed as relatively cell viability, using the following formula: Percent viability = OD of drug treated sample/OD of none treated sample) * 100 [26].

Free Radical-Scavenging Activity: DPPH assay: The free radical scavenging activity of *L.dentata* essential oil was measured using the DPPH (2.2-diphenyl-1-picrylhydrazyl) method of Brand-Williams *et al.* (1995) [27] with some modifications.

Different aliquots of pure essential oil (2–40µl) were mixed with 50 µl of methanol and were placed in 96-well microplates. 100 µl of 0.008% methanolic solution of DPPH (Sigma–Aldrich) was added and allowed to react in the dark at room temperature and then added to 100µl of 0.008% DPPH (Sigma–Aldrich) in Methanol, to give final concentrations of 8, 20, 32, 40 and 60µl /ml. The decrease in absorbance of DPPH at 517 nm.

The radical-scavenging activities of samples, expressed as percentage inhibition of DPPH, were calculated according to the formula: Inhibition percentage (Ip) = [(AB - AA)/AB] * 100 [28].Where AB and AA are the absorbance values of the blank sample and of the tested samples checked after 60 min, respectively. The IC50 value represented the concentration of the essential oil that caused 50% inhibition. All samples were analysed in triplicate. The authentic Alpha –tocopherol (2.3µM) and acide ascorbique (5.67µM) were used as a positive control.

RESULTS AND DISCUSSIONS

Chemical Composition of the Essential Oils: Aerial and flowers part of *L.dentata* were subjected to hydro distillation and the yellowish essential oils were obtained with the yields of 1.41% and 1.207%, respectively.

Qualitative and quantitative analytical results by GC/FID and GC–MS were shown in Table 1. 42 constituents (84,76% of the total oil) and 47 constituents (85.48% of the total oil) were identified

in aerial part and flowers, respectively. Of which beta.-pinene (27.08 and 30.06 %), Pincarveol (14.77 and 8.59 %), myrtenal (8.18 and 6.81%), alpha- pipene (7.78 and 8.38%) and 1,8-cineole (5.53 and 5.47%) were the main compounds in entire pant and flowers, respectively.

The other chemical components were linalool, linalool oxide cis, trans linalool oxide, pinocarvone, borneol, Camphor and camphene caryophyllene oxide.

The components of essential oils were separated into five classes, which were monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes and others (Table 1). As it can be seen in Table 1, except for some quantitative differences between the essential oils studied, both oils have highly similar chemical compositions. Additionally, camphor, trans-linalool oxide, alpha.-campholene aldehyde, alpha.-terpinene, 14-norcadin-5-en-4-one isomer and alpha.-terpineol could not be found in both oils.

Another study from Algeria has investigated the main constituents in the oil of *Lavandula 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 [29].

It was noteworthy that the compositions of the lavandula oil in Morocco were in partial agreement with the previous report [29].There were, however, significant differences between main components. On the contrary, beta-pinene witch was the major constituant in our reported was not found in the previous report [29].

These changes in the essential oil compositions might arise from several environmental (climatical, seasonal, geographical) and genetic differences [30].

Several investigations [11, 31-38] 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.

Antioxydant Assays: DPPH free radical scavenging activity: Free radical scavenging capacities of the essential oils, measured by DPPH assay are shown in Table 2. In the DPPH assay, the ability of the examined essential oils to act as donor of hydrogen atoms or electrons in transformation of DPPH* into its reduced form DPPH-H was investigated.

The examined *L.dentata* essential oils were able to reduce the stable, purple-colored radical DPPH into yellow-colored DPPH-H.

Table 2: IC50 values (µl/ml) of antioxidant activity of oils from L.dentata.

Sample	IC50 (µl/ml)[a]
aerial part oil (n=5)	32,12±0,574
Flower oil (n=5)	41,29±1,208

Table 3: Cytotoxicity of the essential oils from the rhizome and the aerial part of *L.dentata* on five cancer cell lines

	IC50 (µg/ml)[a]	
Cell lines	Leaf oil	Flower oil
MCF-7	101±4.402	98.5±1.025
PC3	59±8.083	91.33±1.116
U-373	72±1.571	46±1.571
V79	73±1.528	158±13.2
P388D1	>600	52±1.155

L.dentata essential oils obtained from aerial part showed greater radical-scavenging activity than those collected from Flower, exhibiting IC50 values for 32,12±0,574 and 41,29±1,208 µl/ml, respectively ($P<0.005$).

whereas those of the synthetic antioxidant of Ascorbate and Alpha tocopherol activity were 23.18±0.113 and 23±0.132µg/ml, respectively. *L. dentata* essential oils contained monoterpenes and oxygenated terpenes such as beta -pinene, alpha pinene and Pincarveol myrtenal,myrtenol, 1.8-cineole, linalol and linalool oxide. Moreover, trying to correlate the observed activity with the chemical composition of the oils, it is noteworthy to cite the work of Ruberto and Baratta (2000) [39], who studied the antioxidant activity of 98 pure essential oils chemical components and showed that monoterpene hydrocarbons had a significant protective effect, with several variants due to the different functional groups. Furthermore, some researchers show that some essential oils rich in nonphenolic compounds also have antioxidant potentials [40].Table 1 shows that essential oils of *L.dentata* are markedly rich in nonphenolic components. Because of this, activities of L.dentata essential oils can be attributed to the nonphenolic constituents.

Data are given as means ± SEM (n = 5). Ascorbic acid (IC50 23.18 ± 0.113µl/ml) and Alpha tocopherol (23 ± 0.132µg/ml) were used as positive controls for antioxidant Assay.

In vitro cytotoxicity of the aerial part and Flower oil: The MTT in vitro cytotoxicity assay was used to compare the aerial part and Flower oil of lavandula dentata. The results of cytotoxicities of the both oil on five human cell lines (P388D1, PC3, V79, U-373 MG, MCF7) are shown in Table 3.

IC50 values calculated at 72 h exposure period. It induced a concentration-dependent inhibitory effect on all cell lines tested in the dilutions ranging from 0.01 to 100µg/ml. According to the data, U-373 was most susceptible to the cytotoxic effects of the oils of aerial part and flowers of *L.dentata,* whereas MCF-7 was most insensitive.

The overall general rating for sensitivity of the cell lines tested to the aerial part and flower oil were PC3>U373>V79>MCF7>P388D1 andU373>P388D1>PC3> MCF7>P388D1 respectively.

The mean IC50 range of aerial part and flower oil in the cell lines tested were 59±8.083->600 µg/ml and 46±1.571-158±13.2 µg/ml respectively.

Data are presented as mean values ± SEM. Different uppercase letters (A–D) within columns indicate statistically significant ($p < 0.05$) difference between values.

The cytotoxic activity of the both oil of L.dentata may be attributed to specific components of the oil. A few of the compounds found in L.dentata essential oil have been tested for cytotoxic properties. It has been reported that a-humulene (5.87%) shows activity against MCF-7, PC3, A-549, DLD-1, M4BEU and CT-26 cell lines [41].There is still controversy over whether caryophyllene oxide (0.47%) is cytotoxic or not (Jie *et al*,2006). Kubo *et al.* (1996) and Sibanda *et al.* (2004) [42, 43] reported that it exhibited a modest cytotoxic activity, while some reports also showed that it was inactive against tumor cell lines [44,41,45]. ã-Elemene (0.39%) and ä-elemene (0.06%) have also been reported to be cytotoxic [46].Altogether, the compounds with cytotoxic activity of the oil are mainly sesquiterpenes. In fact, it has been reported that sesquiterpenes are responsible for the cytotoxic activity of Myrica gale L. essential oil [47]. However, the low concentration of caryophyllene oxide, alpha.-bergamotene, gamma.-elemene, Beta funebrene, beta.-selinene, beta.-bisabolene, gamma.-Cadinene, calamenene cannot fully explain the cytotoxic activity of L. dentata essential oil, which means some other compounds, probably sesquiterpenes, are active in the essential oil. In addition, minor components could also contribute to cytotoxic activity of the oil. It is also possible that the minor components may be involved in some type of synergism with the other active compounds [48, 49]. Perillyl alcohol, a component of the EO of lavender as well as the most important metabolite of d-limonene, is currently under investigation as a chemo-preventative and chemo-therapeutic agent. A pharmacokinetic study has been performed using stable-isotope labelled internal standards. Two new major metabolites besides intact perillyl alcohol, perillic acid and *cis*- and *trans* dihydro perillic acid have been found in human plasma [50]. Another study, dealing with the same monoterpene alcohol, was designed to test its chemo preventative potential especially in a lung tumour-bioassay. Perillyl alcohol is an inhibitor of farnesyl transferase. In the early development stages of mouse lung carcinogenesis the *ras*-protein undergoes a series of modifications and farnesylation at the cysteine is one of these, which leads to the anchoring of *ras*-p 21-gen to the plasma membrane in its biologically active state. Perillyl alcohol administered to test mice showed a 22 per cent reduction in tumour incidence and a 58 per cent reduction in tumour multiplicity [51]. Perillyl alcohol also inhibited significantly the incidence (percentage of animals with tumours) and multiplicity (tumour/animals) of invasive adenocarcinomas of the colon and exhibited increased apoptosis of the tumour cells. Therefore consumption of diets containing fruits and vegetables rich in monoterpenes, such as d-limonene, as well as using also the EO of lavender, for example, as a flavour compound in the provençal kitchen [52],reduces the risk of developing cancer of the colon, mammary gland, liver and lung [53],Not only perillyl alcohol, but also other terpene alcohols, such as nerolidol, beta-citronellol, linalool and menthol, showed inhibitory activities on induced neoplasia of the large bowel and duodenum [54].

CONCLUSION

Antioxidants and cytotoxicity properties of the essential oils and various extracts from many plants have recently been of great interest in both research and the food industry, because their possible use as natural additives emerged from a growing tendency to replace synthetic antioxidants with natural ones. Most components of *L.dentata* essential oil were identified and their cytotoxic and antioxidant activity investigated.

Both entire and flower oils obtained from *L.dentata exerted* antioxidant radical-scavenging effects when tested by DPPH assay. According to the data, U-373 was most susceptible to the cytotoxic effects of the oils of aerial part and flowers of *L.dentata,* whereas MCF-7 was most insensitive. Further studies are needed to obtain more information regarding the practical effectiveness of these oils in animal models.

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