Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2016, 8(3):249-253
(http://derpharmachemica.com/archive.html)

Reveal antifungal activities of essential oils from *Lavandula dentata* L. a way of valuing the arganeraie

Ali Asdadi[1*], Aicha Hamdouch[1], Said Gharby[2], Radouane Moutaj[3], Bouchra Chebli[4] and Lalla Mina Idrissi hassani[1]

[1]*Laboratoire de Biotechnologies Végétales, Equipe Planta Sud, Faculté des Sciences d'Agadir, Université Ibn Zohr, B.P 28/S, Agadir, Maroc*
[2]*Laboratoire de Chimie des Plantes et de Synthèse Organique et Bioorganique, Faculté des Sciences, Université Mohammed V-Agdal, BP 1014- Rabat, Morocco*
[3]*Laboratoire de Parasitologie et de Mycologie Hôpital Militaire Avicenne, CHU Med VI. Marrakech, 40 000. Maroc*
[4]*Laboratoire de Mécanique de Procédé de l'Energie et de l'Environnement, Ecole Nationale des Sciences Appliquées, Université Ibn Zohr, BP 1136, Agadir, Morocco*

ABSTRACT

The Argane tree localized mostly in the southwest of Morocco, under its shade, original micro-ecosystem of medicinal and aromatic plants and characterized by an extraordinary endemism whose forms part Lavandula dentata L. To preserve the Argane forest, and develop its surrounding ecosystem, our study aims to valorising the essential oils of the Lavandula dentata L, studying the antifungal activities of these essential oils on Candida species clinically isolated from patients with candidiasis nosocomial, comparing it to that of two conventional antifungal drugs: Amphotericin B and Fluconasole. Indeed Emerging nosocomial candidiasis is a major issue for health care professionals by their poor prognosis and resistance to conventional antifungal agents used in the current practice of hospitals. The minimum inhibitory concentration was determined by the method of macro dilution broth in agreement with the guidelines of NCCLS M38P for yeasts. The results are very convincing indeed the antifungal activity of Essential oil is important with an minimum inhibitory concentration of 0.26 mg/ml against all Candida non-albicans found resistant to fluconazole, and with an minimum inhibitory concentration of 0,53mg/ml against Candida albicans studied.

Key words: arganeraie, *Lavandula dentata* L., essential oils, antifungal activity, *Candida species.*

INTRODUCTION

The "arganeraie", offers the coexistence of a large number of plants some of which offer significant potential that can be exploited in the immediate future as aromatic plants and /or medicinal who is our plant *lavandula dentata* L., found in particular in the "Arganeraie" of mountain [1]. This plant should benefit from an operating plan that will greatly expand its potentiality and the valuing in view of a sustainable development in the south of Morocco. *Lavandula dentata* L. is very used in traditional medicine of Maghreb. Its inflorescence, much rich in aromatic compounds that the rest of the plant [2]. The plant contains an essential oil of variable composition depending on the place of harvesting. The plant also contains cumarinas (herniarine, ombelliferone); acids ursolique and betulique [2]. In this perspective, we have tried to promote this plant by studying the antifungal activity of its essential oils *in vitro* against the species of *Candida* isolated in patients with nosocomial candidiasis: *Candida albicans, Candida glabrata, Candida krusei* and *Candida tropicalis* and also the effect of two conventional antifungals: Fluconazole and amphotericin B against these same species of *Candida*, which are responsible of nosocomial infections and fungal emerging which are a major challenge for health professionals by their bad prognosis [3,4], and also by their

resistance to conventional antifungal agents used in the current practice of hospitals [5,6]. Despite the therapeutic progress marked by the emergence of new antifungal molecules wih a high cost to use it normally [7], the mortality rate by these infections is always around 50% [8-11]. Therefore these disadvantages well justifies the search for new therapeutic approaches such as the use of essential oils as antifungal agents, these essential oils should benefit from extensive studies in order to test and prove their antifungal activities *in vitro* and *in vivo*, and enhance the area of the Moroccan "Arganeraie".

MATERIALS AND METHODS

1. Collection of plant materials and essential oil extraction

The study was carried out on essential oils (EOs) sample obtained from aerial parts of *Lavandula dentata* L.(Ld) growing wild in south-western of Morocco during May 2014, and dried inshadow followed by grinding and submitted to hydrodistillation for 3h using Clevenger-type apparatus, according to the European Pharmacopoeia [12,13]. The obtained EOs were weighed, filtrated on anhydrous sodium sulfate, and kept in an amber vial at 4°C until used. Taxonomic identification of the species was confirmed and deposited in the Biotechnology Laboratory and Natural Resource Valuation of University Ibn Zohr, Planta Sud unity at Faculty of Sciences in Ibn Zohr University, Agadir, Morocco.

2. Gas chromatography/mass spectrometry (GC/MS) analysis of essential oils

The chemical compositions of essentials oils were analyzed using a gas chromatograph (TRACE GC Ultra) fitted to a mass spectrometer (Polaris Q-Ion Trap MS). Operating in electronimpact EI (70 eV) mode. VB-5 (Methylpolysiloxane 5% phenyl) and a column (30m x 0.25mm x 0.25µm thickness) were used (National Center for Scientific research and Technology (CNRST), Rabat, Morocco). The chromatographic conditions were as follows: Injector and detector temperatures at 220 and 300°C, respectively; carrier gas, helium at flow rate of 1.4 ml/min; temperature program ramp from 40 to 300°C with gradient of 4°C/min (holding the initial and final temperature for 4 min). The relative amount of individual components of the total essential oil was expressed as a percentage of peak area relative to the total peak area. Library search was carried out using the combination of data already available in the NIST 2005 and Mass Spectral Library in the literature [14].

3. Test organisms and inoculums preparation

In this study, twenty clinical isolates of *Candida* (C) species, including *C. albicans* (12), *C. glabrata* (3), *C. krusei* (2) and *C. Tropicalis* (3) were obtained from patients with nosocomial candidiasis. These strains were isolated in the laboratory of parasitology/mycology and bacteriology at Avicenna military hospital, Marrakech, Morocco, on Sabouraud dextrose agar (SDA) supplemented with chloramphenicol (Bio-RAD), and then incubated at 30°C for 48 hours to ensure that yeast cells were actively divided, then adjusted between: 2.16×10^5cells/ml to 5.22×10^5cells/ml for fungal strains with counting with haemocytometer for each repetition. In order to identify the yeasts, germ tube test, API 20C AUX (Bio-Merieux, Marcy-l'Étoile, France) according to the Manufacturer's recommendations and chromogenic medium CandiSelect 4 (Bio-RAD, Marnes-la Coquette, France) were carried out.

4. Broth macrodilution method

This method was used to determine the minimum inhibitory concentration (MIC) according to Clinical and Laboratory Standards Institute-CLSI reference document (formerly NCCLS- M27-A3 guidelines) [15] with modifications. Stock solutions of EOs and antifungal drugs were prepared in DMSO (10%) to enhance essential oil solubility where the final concentration never exceeded 2% and in sterile Sabouraud dextrose broth. Serial dilutions of stock solutions of EOs were prepared for a final oil concentration ranging from 8.46 to 0.07 mg/ml, of Fluconazole (FLC) ranging from 0.75 to 0.012 mg/ml and Amphotericin B (AMB) ranging from 0.33 to 0.01 mg, then after 200 ml of each strain of yeast suspension previously adjusted was added to each tube and all tubes were incubated at 37°C for 48 h in an orbital shaking incubator (at 100 rpm). The lowest EOs concentrations inhibiting fungal growth were identified as MIC. To determine MFC, a loopful of broth was removed from each individual tube and spot inoculated on individual Sabouraud dextrose agar plates. The plates were incubated at 37°C for 48 hours, and MFC was determined as the lowest concentration of EOs and antifungal drugs completely inhibiting the growth of *Candida* species.

For each strain studied, the growth conditions and the sterility of the medium were checked in two control tubes. The safety of DMSO was also checked at the highest examined concentration. These experiments were performed in triplicate as well.

RESULTS AND DISCUSSION

1. Chemical composition of essential oil of *Lavandula dentata* L.

Table 1 shows the the qualitative and quantitative compositions of the oil analyzed namely percentage compositions as well as the identities of compounds present in the EOs of Ld. The yield of the EOs was 1.44% (v/w) calculated on a dry weight basis. Chemical composition of EOs of Ld was determined by the GC/MS analyzes.

Table 1: Chemical composition of essential oil of *Lavandula dentata* L. by GC/MS analyzes

Peak Area (Compounds)	KI	Area %
Monoterpene hydrocarbons		**0,34**
α-Thujene	931	0,04
α-Pinene	939	0,08
Sabinene	979	0,07
β-Pinène	980	0,01
p-Cymene	1026	0,04
Limonene	1030	0,1
Oxygenated monoterpenes		**84,78**
Myrcene	991	0,51
1,8-Cineole	1033	0,74
Linalool	1088	48,02
Camphor	1143	2,51
Borneol	1165	0,07
Menthol	1172	0,04
Terpinene-4-ol	1178	0,82
α-Terpineol	1189	0,67
Myrtenol	1194	0,12
δ-Terpineol	1197	1,47
Linalyl acetate	1257	29,65
Lavandulylacetate	1289	0,14
Bornylacetate	1295	0,02
Phenols		**1,1**
Thymol	1290	0,93
Carvacrol	1292	0,17
Sesquiterpene hydrocarbons		**3,84**
β-Caryophellene	1418	0,14
Germacrene-D	1480	0,11
Bicyclogermacrene	1494	3,4
γ-Cadinene	1515	0,19
Oxygenated sesquiterpenes		**0,95**
Caryophylleneoxide	1580	0,61
α-Eudesmol	1652	0,34
Total identified		**91,01**

Lavandula dentata L. (Ld) were found to have 1.44% (v/w), yield of essential oil.The oil was analyzed by GC and GC/MS, and the qualitative and quantitative compositions are presented in Table 1. The content of particular compounds was calculated from the GC peak areas, using the normalization method. According to the analyses, the following compounds were prevalent in the Ld EOs; in all, Twenty seven compounds were identified, accounting for 91,01% of the total essential oil. Oxygenated monoterpenes were shown to be the main group of constituents (84,78%), with Linalool (48,02%) and Linalyl acetate (29,65%) being the main compounds ; followed by Sesquiterpene hydrocarbons (3,84%), with Bicyclogermacrene (3,4%) being the main compound of the Moroccan Ld EOs similar to those obtained by other authors[16]; followed by the Phenols (1,1%) with thymol (0,93%) being the main compound; followed by the Oxygenated sesquiterpenes (0,95%) with Caryophyllene oxide (0,61%) being the main compound; lastly followed by the Monoterpene hydrocarbons (0,34%) with Limonene as the main compound (0,1%).

In the studied EOs of Ld, Linalool, Linalyl acetate, Bicyclogermacrene were among the major components, together constituting about 81,07% of total .In addition, other compounds were present at lower levels, as thymol, Caryophyllene oxide and Limonene whose chemical polymorphism may be due mainly to the geographic and climatic conditions [17].

2. The broth macro dilution method

Table 2. Antifungal susceptibilities of thirty Candida strains isolates by the CLSI macro dilution method after 48 h of incubation

MIC and MFC (mg/mL) at 48 h						
Species (number of isolates)	**Essential oil & Antifungal agent**	**Range**	**MIC**	**MFC**	**MFC/CMI**	**Interpretation**
Candida albicans(12)	EOs Ld	8.46-0,07	0,26	0.53	2,04	Fungicide
	FLC	0,75-0,012	0,01	0,02	2,00	Fungicide
	AMB	0,33-0,01	0,17	0,17	1,00	Fungicide
Candida glabrata(3)	EOs Ld	8.46-0,07	0,13	0.26	2,00	Fungicide
	FLC	0,75-0,012	0,38	0,38	1,00	Fungicide
	AMB	0,33-0,01	0,17	0,33	1,94	Fungicide
Candida krusei(2)	EOs Ld	8.46-0,07	0,13	0,26	2,00	Fungicide
	FLC	0,75-0,012	0,38	0,75	1,97	Fungicide
	AMB	0,33-0,01	0,17	0,33	1,94	Fungicide
Candida tropicalis (3)	EOs Ld	8.46-0,07	0,13	0,26	2,00	Fungicide
	FLC	0,75-0,012	0,012	0,02	1,67	Fungicide
	AMB	0,33-0,01	0,01	0,02	2,00	Fungicide

The antifungal activity of essential oils of Ld was tested against twenty *Candida* strains that cause nosocomial infections (Table 2). It was found that the *Candida* species examined were sensitive to the essential oil studied. Our study is in good agreement with those already postponed [18].

The results presented in the Table 2 show that the EOs of Ld has a strong antifungal activity compared with the conventional fungicide, against all tested *Candida* species isolated from nosocomial infections in hospitals. We note a very important antifungal activity especially against strains non *Candida albicans* species, this activity extends even to species resistant to conventional antifungal used as control. The antifungal activity of EOs of Ld may be attributed to the presence of major compounds. In the case of EOs of Ld attribution of activity to the major compound (Linalol) seems founded and already reported by Bekkali [18]. Moreover, since the essential oils are complex mixtures of several compounds, it is difficult to attribute their biological activity to a particular constituent. Usually, major compounds are the ones responsible for the antifungal activity of the EOs. The antifungal activity of the EOs of Ld is presumably due to the presence of Linalool, Linalyl acetate, Bicyclogermacrene, thymol, Caryophyllene oxide and Limonene who were reported in previous studies having an antifungal activity [18].

In our study it was found that *C. glabrata, C. krusei and C. tropicalis* are very sensitive to the essential oil of the Ld compared to *C. albicans*, strains with MIC within a range of 0,13mg / ml to 0,26mg / ml as seen in Table 2. In a more general way, this essential oil has a fungicidal activity at the concentration of 0,53 mg/ml against all the strains of *Candida albicans* tested and non-*albicans*. As for the MIC, our study showed that *Candida non albicans* strains are more sensitive to our essential oil that strains of *Candida albicans* if one refers to the average MIC recorded in all strains of *Candida* non-*albicans* tested.

It should be noted that the report of CMF / MIC of Ld EOs tested against all strains of Candida species tested is greater than or equal to 1. This value allows us to affirm that all tested extracts are fungicides according to Berche et al.[19]

CONCLUSION

These results demonstrated that EOs of Ld are a potential source of bioactive compounds with strong activity against different pathogenic *Candida* species.

Our findings tentatively suggest important therapeutic implication for Ld EOs as a suitable alternative for amphotericin B and fluconazole as well as his EOs could be alternative substances for fungi control, in particular *C. krusei* and strains that have acquired resistance to conventional antifungal agents. These results may form the basis of further *in vivo* studies to purify the major components of these plants and to assess their appreciable antifungal actions against *Candida* species. However, if these EOs are to be used for medicinal purposes, issues of safety and toxicity will need to be addressed. Finally, we recommend the use of this essential oil instead of harmful synthetic antifungal agents. This essential oil is a social and economic interest for sustainable development for the regions of the “Arganeraie” in southern Morocco.

Acknowledgments
Authors represent deep thanks for the plant taxonomist Pr. Msanda Fouad for the taxonomic identification of the sample, in the Biotechnology Laboratory and Natural Resources Valuation at Ibn Zohr University, Agadir, Morocco.

RÉFERENCES

[1] Benabid, A. *Évalua-[6]* **2000**,
[2] Bellakhdar, J. *Plantes médicinales au maghreb et soins de base: Précis de phytothérapie moderne*. Eds Le Fennec: **2006**.
[3] Anane, S.; Khalfallah, F. *Pathologie-biologie* **2007**, *55*5,
[4] Pihet, M.; Marot, A.s. *Revue francophone des laboratoires* **2013**, 450,
[5] Develoux, M.; Bretagne, S. *EMC - Maladies Infectieuses* **2005**, *2*3,
[6] Dannaoui, É. *Revue francophone des laboratoires* **2013**, 450,
[7] Toubas, D. *Revue Francophone des Laboratoires* **2013**, *2013*450,
[8] Poulain, D. *Lett Infectiologue* **2000**, *90*15,
[9] GRANIER, F. *La Presse médicale* **2000**, *29*37,
[10] Mishra, B.B.; Singh, D.D.; Kishore, N.; Tiwari, V.K.; Tripathi, V. *Phytochemistry* **2010**, *71*2-3,
[11] Eggimann P, P.D. *Réanimation* **2002**, *11*3,
[12] Anonymous. *European pharmacopeia*. Maissonneuve SA ed.; Sainte-Ruffine, France, **1975**; Vol. 3.
[13] Council of Europe. European pharmacopoeia. 3rd ed, D., Ed. **1997**.
[14] Adams, R., P., . *Identification of essential oil components by gas chromatography/mass spectrometry*. Allured Publishing Corporation: Illinois, **2007**.
[15] NCCLS, N.C.f.C.L.S. *Approved Standard M38-A, Wayne, PA.* **2002**,
[16] Bouchra, C.; Hmamouchi, M.; Mohamed, A.; Mina, I. *Phytopathologia Mediterranea (Italy)* **2003**,
[17] Cheikh-Rouhou, S.; Besbes, S.; Lognay, G.; Blecker, C.; Deroanne, C.; Attia, H. *Journal of Food Composition and Analysis* **2008**, *21*2,
[18] F. Bakkali.; S. Averbeck.; D. Averbeck.; Idaomar., M. *Food Chem. Toxicol* **2008**, *46*
[19] Berche, P.; Gaillard, J.; Simonet, M. *Médecine & Sciences, Paris* **1991**,