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Essential oil composition and antioxidant activity of five Lamiaceae species

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Abstract
The chemical composition of five Lamiaceae species essential oils, isolated by hydrodistillation, was analyzed by gas chromatography–mass spectrometry (GC-MS). Significant variation of essential oil composition was observed between species. *T. capitatus* and *L. multifida* were distinguished by high amounts of carvacrol (75.6 and 87.5%). Camphor (16.7%), α-pinene (13.6%) and 1.8-cineole (13.9%) were the main constituents of *T. algeriensis* essential oils, while the dominant compounds in *L. stoechas* were fenchone (59.1%) and camphor (23.7%). The highest amount of 1.8 cineole (51.6% and 32.5% for var.*typicus* and var. *troglodytorum*, respectively) was detected in *Rosmarinus officinalis* essential oil. Essential oils were assessed for their antioxidant capacity (DPPH, carotene bleaching test, Ferrous Ion-Chelating Ability Assay). The level of antioxidant activity among species was linked to that of their essential oil composition. The best activity was observed for essential oils characterized by the highest carvacrol content.

Keywords: Lamiaceae, essential oil, GC-MS, antioxidant activity

Introduction
The most important major essential oil bearing plants are dominated within certain plant families such as Lamiaceae, Apiaceae and Lauraceae [1]. Lamiaceae (Labiatae) is an important plant family that consists of 250 genera and more than 7000 species. Distribution is throughout the world but is particularly well represented in tropical and temperate areas [2]. Most of the species are aromatic and valuable in food, cosmetics, and pharmaceutical industries [3]. Some of the major genera belonging to Lamiaceae family are *Thymus*, *Rosmarinus* and *Lavandula*. Secondary metabolites of these plants, such as terpenoids and phenolics, are well known to have multiple biological activities [1].
Essential oils of *Thymus* species were more and more assessed because for their biological properties. They exhibit, antioxidant, antimicrobial, insecticidal, antiseptic and antispasmodic, properties [4, 5, 6].
Pottier-Alapetite [7] recognized for Tunisia one *Rosmarinus officinalis* L. species including four varieties: var. *typicus* Batt., var. *laxiflorus* De Noé, var. *troglodytorum* Maire and var. *lavandulaceum* Batt. [synonym R. *tournefortii* De Noé], *Rosmarinus* species have been used in folk medicine for their antimicrobial, antiulcerogenic, digestive and anti-inflammatory properties [8].
In Tunisia, the genus *Lavandula* includes four wild species: L. *stoechas* subsp. *stoechas* L., *Lavandula multifida* L., *Lavandula coronopifolia* Poir. (=L. *stricta* Del.) and *Lavandula dentata* L [7]. Lavender's essential oils are employed for their antioxidant, antimicrobial, antifungal, anticholinesterase, and anti-inflammatory and antileishmanial effects [9, 10, 11].
The aim of this study was (1) to compare the chemical composition of five Tunisian species from Lamiacea family, and (2) to evaluate their antioxidant, in order to select the most attractive chemotypes.

Materials and methods
Plant material
Lavandula stoechas, Thymus algeriensis and *Rosmarinus officinalis* var. *typicus* were harvested from Korbous

(Longitude 10°35', latitude 36°50'). *Rosmarinus officinalis var. troglodytorum* was collected from Matmata (longitude 9°58′, 33°32′). *Thymus capitatus* and *Lavandula multifida* were collected from Sousse (longitude 10°50', latitude35°30').
The collected plants were identified by Pr. M. Boussaid from the INSAT (Department of Biology), and voucher specimens were deposited in the Herbarium of the National Institute of Applied Science and Technology of Tunis. For each species, ten plants at the flowering phase were sampled at random. The samples were air dried at room temperature for two weeks, then ground to powder before analysis.

Extraction of essential oil and gas chromatography analyses
One hundred grams of dried leaves from each individual were submitted to hydro-distillation for 3 h using a Clevenger apparatus. Oils were recovered directly, using a micro-pipette from above the distillate, and stored in dark vials at 4 °C. GC–MS analyses were performed with a gas chromatograph (Agilent 7890A) equipped with a HP-5MS capillary column (30 m × 0.25 mm) and associated with a mass selective detector (Agilent 5975C inter MSD). The flow of the carrier gas (helium) was 0.8 mL/min.
The oven temperature was programmed from 60 to 240°C at 4°C/ min. The injector temperature was maintained at 250° C. Temperatures of the quadrupole and the source were 150 and 230°C, respectively. The mass scan ranged from 50 to 550 m/z at 70 eV. Essential oil components were identified by comparison of their retention times with those of authentic standards available in our laboratory, and also by comparison of their retention indices according to the literature. The retention indices were calculated according to a series of n-alkanes (C9-C24). The identification was also completed by comparison of their masss spectra with those stored in NIST08 and W8N08 libraries.

Antioxidant activity evaluation
From each sample, different concentrations of essential oils were prepared. The antioxidant activity was carried out using three different methods: metal chelating, free radical-scavenging activity using DPPH (2, 2-diphenyl-1-picrylhydrazyl) and Carotene bleaching test.

Free radical scavenging activity
The free radical scavenging activity of extracts were measured by 1,1-diphenyl-2-picryl-hydrazil (DPPH) using the method of Zaouali *et al*. [12]. $4*10^{-5}$ M solution of DPPH was prepared and 3 ml of this solution was added to 1 ml of oils at different concentrations. The mixture was vortexed and maintained at room temperature for 30 min in the dark. The absorbance was measured at 517 nm against a blank. Trolox was used as positive control.
All experiments were carried out in triplicate and results were expressed as IC_{50} (the efficient concentration corresponding to 50% of DPPH present in the test solution).

Carotene bleaching test
The inhibition of β-carotene bleaching by the essential oils was carried out according to Zaouali *et al*. [12]. The antioxidant activity was determined in terms of the bleaching of the β-carotene using the formula: % Inhibition=100x $[(As_t-C_t)/(C_0-C_t)]$, where As_t and C_t are the absorbance measured after incubation for 120 min for the sample and the control, respectively, and C_0 is the absorbance of the control measured at zero time. Results were expressed as IC50 An extract concentration providing 50% inhibition (IC_{50}) was obtained, plotting inhibition percentage versus extract solution concentrations.

Ferrous Ion-Chelating Ability Assay
Ferrous ion (Fe^{2+}) chelating activity of different concentrations of essential oil samples was measured by inhibiting the formation of Fe^{2+}-ferrozine complex after treatment of test material with Fe^{2+}, following the method of Zhao *et al*. [13]. The percentage of inhibition of ferrozine Fe^{2+} complex was estimated by IC_{50} using the following formula: metal chelating effect (%) = [(AB-AA)/AB] × 100; where AB is the absorbance of the ferrozine-ferrous ion complex and AA is the absorbance of the sample. All analyses were performed in triplicate.

Statistical analysis
The significance of the variation in essential oil composition and their biological activities among species were determined using one-way Analysis of Variance (ANOVA) followed by Duncan's multiple range tests, using SAS version 9. A Canonical Correspondence Analyses (CCA) were carried out by using the MVSP 3.1 program (Kovach Computing Services, Pentraeth, Wales).

Results and discussion
Variation of essential oil composition
The essential oils extracted by hydrodistillation from the dried aerial parts of different species, ranged from 0.04% to 0.8% for *L. multifida* and *T. capitatus*, respectively (Table 1). The essential oil composition varied significantly ($p < 0.05$) among the analysed species. A total of 33 compounds of the essential oils analyzed were identified. Most of them commonly found in essential oils (Table 1). Each particular species had a distinct major chemical composition.
The highest amount of 1.8 cineole was detected in *Rosmarinus officinalis* essential oils with 51.6 and 32.5% for var. *typicus* and *troglodyturum*, respectively. Furthermore, these samples were found to be rich in camphor, α- pinene, borneol and camphene. At the species level, our results on the composition of Tunisian *R. officinalis* oils were in accordance with those previously reported for other Italian and Iranian rosemary samples [14, 15].

Table 1: Mean percentage of the essential oil compounds at the species levels

Compound	*Rosmarinus officinalis*		*Thymus*		*Lavandula*	
	Var. *typicus*	Var. *troglodytorum*	*algeriensis*	*capitatus*	*stoechas*	*multifida*
Yield (%)	0.76	0.64	0.41	0.8	0.58	0.04
α-thujene	-^{a}	0.5^{b}±0.1	-^{a}	0.5^{b}±0.2	-^{a}	-^{a}
α-Pinene	9.2^{b}±1.2	10.9^{c}±0.4	13.6^{d}±2.0	-^{a}	-^{a}	0.1^{a}±0.01
Camphene	3.8^{d}±0.8	11.0^{e}±0.4	3.2^{c}±0.7	0.1^{a}±0.0	0.6^{b}±0.1	-^{a}
β-pinene	3.2^{d}±0.3	0.5^{b}±0.0	2.9^{c}±0.4	-^{a}	-^{a}	-^{a}
sabinene	-^{a}	-^{a}	0.8^{c}±0.2	0.2^{b}±0.2	-^{a}	-^{a}
β-myrcene	0.9^{d}±0.1	0.6^{c}±0.0	0.2^{b}±0.1	0.7^{c}±0.0	-^{a}	0.7^{c}±0.0
α-terpinene	0.4^{b}±0.0	-^{a}	0.1^{a}±0.1	1.0^{c}±0.1	-^{a}	-^{a}
1.8-cineole	51.6^{e}±2.9	32.5^{d}±1.3	13.9^{c}±2.0	-^{a}	8.0 ^{b} ±2.6	-^{a}
p-cymene	1.9^{c}±0.1	2.7^{d}±0.1	0.6^{b}±0.1	5.3^{e}±0.9	-^{a}	-^{a}
α-ocimene	-^{a}	-^{a}	0.1^{a}±0.2	-^{a}	-^{a}	5.1^{b}±0.5
Y-terpinene	0.5^{b}±0.0	-^{a}	0.4^{b}±0.0	5.7^{c}±0.3	-^{a}	-^{a}
α-terpinolene	3.9^{d}±0.1	1.3^{c}±0.1	1.3^{c}±0.0	0.3^{b}±0.0	-^{a}	0.3^{b}±0.1
Linalool	0.4^{b}±0.2	0.1^{a}±0.1	2.5^{e}±0.5	1.2^{d}±0.1	0.9^{c}±0.0	0.4^{b}±0.0
Pinocarveol	-^{a}	-^{a}	1.5^{b}±0.1	-^{a}	-^{a}	-^{a}
Camphor	5.5^{b}±0.6	29.5^{e}±0.1	16.7^{c}±0.6	-^{a}	23.7^{d}±2.7	0.2^{a}±0.0
Borneol	11.7^{e}±1.6	6.5^{d}±0.2	5.9^{c}±0.2	0.5^{b}±0.3	0.8^{b}±0.4	^{a}
α-terpineol	3.3^{b}±0.1	-^{a}	2.6^{b}±0.2	-a	-^{a}	0.2^{a}±0.0
Fenchone	1.5^{b}±0.5	-^{a}	-^{a}	-^{a}	59.1^{c}±3.4	0.4^{a}±0.0
Carveol	-^{a}	-^{a}	0.3^{b}±0.2	-^{a}	-a	-^{a}
Pinocarvone	-^{a}	-^{a}	0.6^{b}±0.0	-^{a}	-^{a}	-^{a}
Verbenone	-^{a}	-^{a}	0.9^{b}±0.1	-^{a}	-^{a}	-^{a}
Bornyl acetate	0.6^{b}±0.2	1.1^{c}±0.0	2.1^{d}±0.2	-a	-^{a}	-a
Myrtenal	-^{a}	^{a}	1.2^{b}±0.1	-a	3.1^{c}±0.8	-a
Carvacrol	-^{a}	-^{a}	-^{a}	76.5^{b}±4.0	-^{a}	87.5^{c}±1.5
Carvacryl acetate	-^{a}	-^{a}	-^{a}	1.3b±1.2	-^{a}	-^{a}
Myrtenyl acetate	0.2^{b}±0.1	0.1^{ab}±0.1	-^{a}	-^{a}	1.6^{c}±0.8	-^{a}
β-Caryophyllène	1.2^{c}±0.6	0.6^{b}±0.0	2.1^{d}±1.4	2.8^{e}±0.2	-^{a}	-^{a}
γ-Muurolene	-^{a}	-^{a}	-^{a}	-^{a}	0.4^{b}±0.0	-^{a}
Caryophyllene oxide	0.3^{a}±0.0	-^{a}	6.8^{b}±0.6	0.5^{a}±0.1	-^{a}	-^{a}
γ-selinene	-^{a}	-^{a}	1.3^{b}±0.2	-^{a}	-^{a}	-^{a}
β-panainsene	-^{a}	-^{a}	3.3^{b}±0.9	-^{a}	-^{a}	-^{a}
β-eudesmol	-^{a}	-^{a}	0.6^{b}±0.3	-^{a}	-^{a}	-^{a}
Elemol	-^{a}	-^{a}	8.2^{b}±2.3	-^{a}	-^{a}	-^{a}
All identified components	98.1±9.6	97.9±2.8	93.7±13.8	96.6±7.8	98.2±10.8	94.9
Monoterpens hydrocarbons(%)	18	23.5	21.3	8.2	0.6	5.9
Oxygenated monoterpenes (%)	74.8	69.8	46.1	79.5	97.2	88.7
Sesquiterpens hydrocarbons(%)	3.1	1.9	4.7	3.1	0.4	0.3
Oxygenated Sesquiterpens (%)	0.3	0	15.6	0.5	0	0
Others (%)	1.9	2.7	6	5.3	0	0

Means followed by different letters within the same column are significantly different ($p<0.05$).

T. algeriensis essential oil was found to be rich in camphor (16.7%) followed by α-pinene and 1.8-cineole (13.6 and 13.9%, respectively). This oil was characterized by very high percentage of monoterpenes which constitute the predominant class as was found for the majority of *T. algeriensis* essential oils [16, 17]. The carvacrol presented the major compound of *T. capitatus* essential oils (76.5%). Our results were in agreement with those from previous studies performed on Tunisian *T. capitatus* [18, 19]. Other chemotypes were identified in *Thymus* species, collected from other areas, such as thymol, γ-terpinene, p-cymene and linalool [20, 21, 6].

The comparison of essential oil composition among the analysed *Lavandula* species showed that carvacrol was restricted to *L. multifida* (87.5%). Our results were in accordance with those previously reported for Tunisian oils [22, 23]. *L. multifida* from Portugal was found to be rich in fenchone and 1,8-cineole [9]. Ecological factors, i.e. temperature, altitude, relative humidity and rainfall, associated to genetic ones and to phonological plant stages should be at the origin of these variations [24].

L. stoechas showed the most important contents of fenchone (59.1%) and camphor (23.7%). Oil composition of this species has been widely investigated. Most reports indicate that fenchone is the main specific constituent of *L. stoechas* oils [23]. These results were comparable to that observed for *L. pedunculata* [25] and *L. tenuisecta* [26] essential oils.

The established CCA plot, according to axes 1-2 (84.19% of the total variation) based on the amounts of the major constituents, showed a clear separation among species (Figure 1).

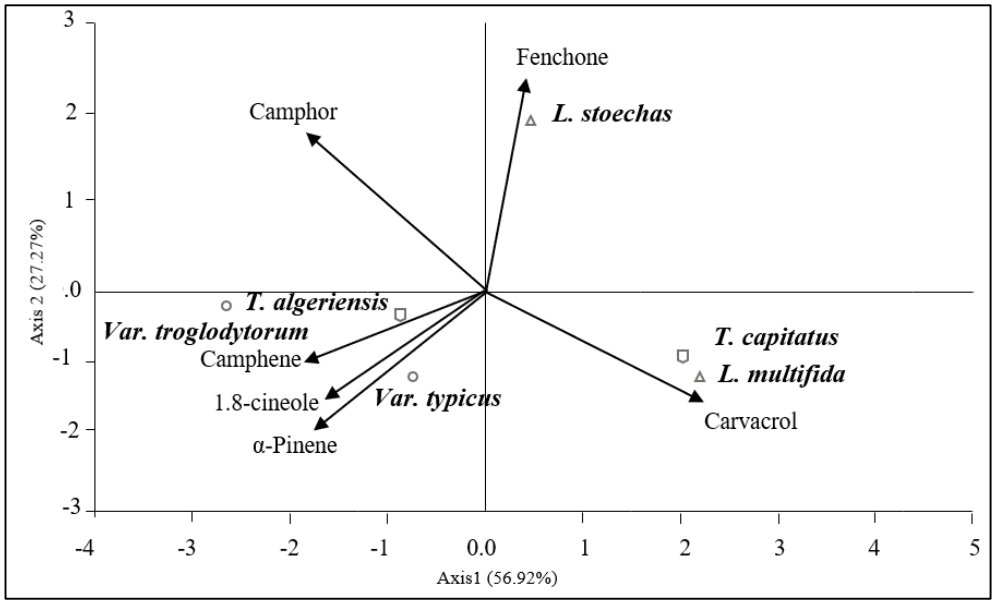

Fig 1: Plot of the Canonical Correspondence Analysis, performed on all species using the major essential oil compounds

The first group, includes *T. capitatus* and *L. multifida* species characterized by their richness in carvacrol. The second one, situated at the negative side of axis 1, is represented by *T. algeriensis*, *R. officinalis* var *typicus* and var. *trogloditurum* revealing high percentages of 1.8 cineole, α-pinene and camphene. The third group is formed by *L. stoechas*, characterized by a fenchone rich-oil.

Antioxidant activity

In our study, the antioxidant activity of different oils, except that of *L. multifida* that revealed a very low yield for testing, varied significantly among species (Table 2).

The antioxidant variation observed among samples reflects their essential oil composition differences. The IC_{50} was ranged from 0.58 to 20 mg/ml. *T. capitatus* essential oil showed the most efficient IC_{50} values for the free radical scavenging activity (DPPH). The level of the antioxidant activity of *Thymus* oil compared to other species may be primarily attributed to the concentration of their phenolic components such as carvacrol [27].

Notably, *T. capitatus* essential oil was also the most efficient regarding the potential to protect β-carotene from bleaching (IC_{50}=0.50 mg/ml). For other species, *R. officinalis* var *typicus* exhibited the strongest activity (IC_{50}=14 mg/ml), followed by *T. algeriensis* (IC_{50}=16 mg/ml). *L. stoechas* showed the lowest inhibition of carotene bleaching activity with 30.04 mg/ml.

Table 2: Antioxidant activities determined by DPPH. β-carotene and chelating effect for the essential oil of the selected species.

Assays	*Rosmarinus officinalis*		*Thymus*		*Lavandula*	
	typicus	*troglodyturum*	*T. capitatus*	*T. algeriensis*	*Lstoechas*	*multifida*
Antioxidant activity						
DPPH (IC_{50} mg.ml-1)	$20.0^{d} \pm 1.15$	$10.0^{c} \pm 0.6$	$0.58^{a} \pm 0.03$	$10.9^{c} \pm 1.24$	$5.90^{b} \pm 0.51$	ND
β-Carotene (IC_{50} mg.ml-1)	$14.0^{b} \pm 0.8$	$20.0^{b} \pm 1.3$	$0.50^{a} \pm 0.02$	$16.0^{b} \pm 0.8$	$30.0^{c} \pm 2.3$	ND
Chelating effect (IC_{50} mg.ml^{-1})	$8.33^{c} \pm 1.52$	$2.2^{b} \pm 0.45$	$-^{a}$	$6.9^{c} \pm 0.1$	$7.5^{c} \pm 0.2$	ND

ND: not determined

Means followed by different letters within the same column are significantly different ($p<0.05$).

IC_{50} values for the standard Trolox: 75.36 ± 0.12 µg/mL (DPPH scavenging activity).

IC_{50} values for the standard BHT: 29.40 ± 0.11 µg/mL (β-carotene bleaching inhibition).

IC_{50} values for the standard EDTA: 18.91 ± 0.04 µg/mL (chelating ability).

With the exception of *T. capitatus* species, all essential oils showed a significant ability to chelate ferrous ion with IC_{50} value ranging from 2.2 to 8.33 mg/ml. These results were in agreement with those reported by Bounatirou *et al.* [19] which mentioned the absence of this activity in the essential oil of *T. capitatus*. It could be due to the incapacity of the carvacrol the main constituent of this essential oil, a mono-hydroxylated compound, to form a complex with Fe^{2+} [28]. *Rosmarinus officinalis* var. *troglodytorum* essential oil exhibited the most important ferrous ion chelating activity. However, it's difficult to attribute the antioxidant effect of a total essential oil to one or few active compounds. Both minor and major compounds should make a significant contribution to the oil's activity [29].

4. Conclusions

This study was carried out in order to describe the chemical composition and the antioxidant activity of essential oils of five Lamiacea species. Quantitative differences of the essential oil composition were observed. They exhibited a considerable antioxidant capacity. Essential oil of *T. capitatus* revealed the best activity. These results may highlight the use of this specie in diverse industrial fields.

Conflicts of interest

The authors declare no conflict of interest.

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