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Antibacterial and α-amylase inhibitory activities of selected *Lamiaceae* species essential oils

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Abstract
The *in vitro* antibacterial and α-amylase inhibitiory activities of the essential oils isolated from five Lamiaceae species (*Thymus capitatus, Thymus algeriensis, Lavandula. stoechas, Lavandula multifida, Rosmrinus officinalis var. typicus, and var. troglodytorum*), were evaluated.
Antibacterial effects of essential oils have been studied against seven strains (*Staphylococcus aureus*, *Streptococcus feacalis*, *Bacillus cereus*, *Staphylococcus epidermis* (gram-positive), and *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumonia* (gram-negative).
The highest antibacterial activity was recorded for *T. capitattus* oil. The Results indicate a high variation of MIC and MBCs among tested species. *T. capitatus* sample showed the best bacteriostatic and bactericidal activities (MIC=0.05-0.1 µl/ml, MBC=0.125-0.5 µl/ml). The uppermost α-amylase inhibitiory capacity was also observed by *T. capitatus* essential oil, rich in carvacrol.

Keywords: Essential oils, Lamiaceae, antibacterial, α-amylase inhibitiory

Introduction
Nowadays, medicinal plants are intensely screened and applied in the fields of pharmacology, medical and clinical microbiology, due to their potential as a source of natural biologically active compounds [1]. Plant secondary metabolites are an enormously variable group of phytochemicals in terms of their number, structural heterogeneity and distribution [2]. Biological activities of secondary metabolites were found to be regularly matched with their chemical composition which is strongly affected by several factors such as plant phenological stages, plant parts and ecological and genetic factors [3, 4].
Lamiaceae family has several species of aromatic plants that are applied in traditional medicine and in the pharmaceutical and food industries because of their biological properties [5, 6, 7]. The plant family Lamiaceae, formerly called Labiatae, for its flowers are characterized by a bilabiate corolla. Lamiaceae species are widely distributed around the globe, with various heights and habitats and greater abundance in the Mediterranean region [8]. In terms of chemical composition, several species of this family have been the subject of many studies centered on: essential oils, flavonoids, iridoids, sterols, diterpenoids, for the pharmaceutical, food and cosmetics industries. The secondary metabolites from Lamiaceae species have revealed important activities such as antispasmodic, antiseptic [9], antidiabetic, antihypertensive [10] anti-inflammatory, antimicrobial, antioxidant, hepatoprotective, insecticide, etc [11].
The aim of this study was to evaluate the antibacterial and α-amylase inhibitory activities of five members of Lamiaceae family belonging to the genera *Thymus* (*T. capitatus, T. algeriensis*), *Lavandula* (*L. stoechas, L. multifida*), *Rosmarinus* (*R. officinalis var.typicus, and var.troglodytorum*).

Material and methods
Plant material
Five *Lamiacea* species *(Thymus capitatus, Thymus algeriensis, Lavandula multifida, Lavandula stoechas, Rosmarinus*

officinalis var. *typicus* and *Rosmarinus officinalis var. troglodytorum)* were harvested from different Tunisian regions [12].

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. 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 [12].

Antibacterial activity assays

Bacterial strains

The antimicrobial activity was tested against 7 standards bacteria, namely *Staphylococcus aureus*, *Streptococcus feacalis*, *Bacillus cereus*, *Staphylococcus epidermis* (gram-positive), and *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella pneumonia* (gram-negative).

The antibacterial activity was determined through the agar disc diffusion and the broth dilution methods. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined. All tests were performed in duplicate.

Antibacterial tests were carried out by disc-diffusion method [13]. Using 100 µl of suspension of the tested microorganisms containing $5*10^5$CFU/ml of bacterial strains spread on the nutrient agar. The filter paper discs (6 mm in diameter) were impregnated with 10 µl of each essential oils and then placed onto the agar plates. Before incubation, all Petri dishes were kept in the refrigerator (4 °C) for 2 h and incubated after at 37 °C for 24 h for bacteria growth. After incubation, the diameters (mm) of the inhibition zones were measured including the diameter of discs. The diameters of inhibition zones were used as a measure of antibacterial activity and each assay was repeated two times. Gentamicin (30 µg/disc) was used as positive control.

The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined according to Okeke et al. [14]. Serial dilutions of 1/2, 1/4, 1/8 and 1/10 were made with dimethylsulphoxide (DMSO) and 10 µl of each dilution were puted on sterile paper discs (6 mm diameter) placed on the surface of inoculated Petri dishes. The MIC was defined as the lowest concentration of the total essential oil at which the microorganism does not demonstrate visible growth [14]. Referring to results of the MIC assay, the minimum bactericidal concentration (MBC) was determined.

50 µL from each dilution of essential oil, showing growth inhibition zone in disc diffusion method, were added to 5 ml of TSA broth tubes then incubated at 37 °C for 24 h in an incubator shaker. The MBC was announced when there is no growth on disks.

α-amylase inhibition Assay

The α-amylase inhibitory assay of the essential oils was carried out as described previously by Kim *et al*. [15]. Essential oil (100µl, at different concentrations in 5% methanol in 0.02 M sodium phosphate buffer) was mixed with 25 µl α-amylase solution (1U) and 200 µl of sodium phosphate buffer (0.02 M, pH 6.9). After pre-incubation during 10 min at 37°C, 180 µl of starch solution in sodium buffer (0.02 M) was added. The resulting mixture was incubated at 37°C for 20 min and then stopped with 180 µl 3, 5-dinitrosalicylic acid color reagent. After incubation in heating bath (90 °C) for 15 minutes and cooled to room temperature, the reaction mixture was diluted with 600 µl of deionized water, and absorbance was measured at 540 nm. The percentage of inhibition was estimated by IC_{50}.

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.

Results and discussion

Chemical composition of the isolated essential oils

GC/MS chemical analyses of essential oils revealed that *T. capitatus* and *L. multifida* were distinguished by high amounts of carvacrol (75.6 and 87.5%). *T. algeriensis* essential oil was characterized by high levels of camphor (16.7%), α-pinene (13.6%) and 1.8-cineole (13.9%), while the dominant compounds in *L. stoechas* were fenchone (59.1%) and camphor (23.7%). *Rosmarinus officinalis* essential oils exhibited the highest amount of 1.8 cineole (51.6% and 32.5% for var.*typicus* and var. *troglodytorum*, respectively) [12].

Antibacterial activity

The antibacterial activity of essential oils estimated by the diameter of inhibition varied according to species and bacteria strains (Table 1). The highest antibacterial activity was recorded for *T. capitattus* oil. The inhibition zones varied between 24 and 31 mm observed against *S. faecalis* and *B. cereus*, respectively. *T. algeriensis* exhibited a slight to moderate activity with a growth zone ranging from 7.5 to 13.5 mm. The inhibition zones varied between 7.5 and 17 mm for *R. officinalis* var *typicus* and 7.5 and 14.5 mm for var. *troglodyturum*. All tested bacteria were more susceptible to Gentamycin (18-25 mm) than to the essential oils tested. *S.epidermidis* and *S. feacalis* being the most resistant to both Gentamycin and oils.

Table 1: Antibacterial activity estimated by the inhibition diameters (mm) of the essential oils of the selected species

		Rosmarinus officinalis		***Thymus***		***Lavandula***		
Bacteria	Source no.	***R.troglodyturum***	***R. typicus***	***T. capitatus***	***T. algeriensis***	***L. stoechas***	***L. multifida***	***Gentamycin (30µg/disc)***
Gram-positive								
S. aureus	ATCC6538	14.5±1.5	17±1	26.5±1.5	13.5±0.5	15±1	ND	22
S.epidermidis	ATCC12228	7.5±0.5	8.5±0.5	25.5±1.5	7.5±0.5	8.5±0.5	ND	19
S.feacalis	ATCC10541	8±0	7.5±0.5	24±0	8±1	7.5±0.5	ND	18
Gram-negative								
B. cereus	ATCC11778	12±0	10±0	31±1	10±0	10±0	ND	25
E. coli	ATCC10536	10±0	9±0	27±1	11.5±0.5	8.5±0.5	ND	20
K. pneumoniae	ATCC10031	13±0	11.5±0.5	28.5±1.5	11±1	13.5±0.5	ND	23
P. aeroginosa	ATCC9027	12±0	12±0	26.5±0.5	9.5±0.5	12±0	ND	23

ND: not determined
Means followed by different letters within the same column are significantly different ($p<0.05$).
Values represent mean ± standard deviation of experiments in duplicate.

The bacteriostatic and bactericidal effectiveness of oils estimated by MIC and MBC are shown in Table 2. The Results indicate a high variation of MIC and MBCs among tested species.

T. capitatus sample showed the best bacteriostatic and bactericidal activities (MIC=0.05-0.1 µl/ml, MBC=0.125-0.5 µl/ml). This oil exhibited high amounts of carvacrol. Oils, rich in this compound were known to possess a high antimicrobial activity [16]. Some studies suggest that carvacrol is capable of disintegrating the outer membrane of Gram-negative bacteria and increasing the permeability of the cytoplasmic membrane to adenosine triphosphate [17]. MICs and MBCs values of other tested species ranged from 1.66 to 5 µl/ml and from 2.5 to 10 µl/ml, respectively. Essential oils from var. *typicus* showed a low bactericidal effect (MBCs = 10 µl/ml). However, oils from var. *troglodytorum*, showed a moderate bactericid activity (MBC = 5 µl/ml). Our results were similar to those previously reported by Zaouali *et al*. [18] and those of Gachkar *et al*. [19] for *R. officinalis* from Iran.

Table 2: Antibacterial activity expressed as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) (µl/ml)

	Rosmarinus officinalis				***Thymus***				***Lavandula***	
	R.troglodyturum		R. typicus		T. capitatus		T. algeriensis		L. stoechas	
	CMI	CMB	CMI	CMB	CMI	CMB	CMI	CMB	CMI	CMB
Gram-positive										
S. aureus	5	5	5	10	0.05	0.5	2.5	2.5	2.5	2.5
S.epidermidis	5	5	5	10	0.1	1	5	10	-	-
S.feacalis	5	5	5	10	0.1	0.5	5	5	-	-
Gram-negative										
B. cereus	2.5	5	2.5	10	0.1	0.25	1.66	2.5	2.5	5
E. coli	2.5	5	5	10	0.05	0.25	2.5	10	2.5	5
K. pneumoniae	5	5	5	5	0.05	0.5	2.5	2.5	2.5	2.5
P. aeroginosa	5	5	5	10	0.1	0.125	2.5	2.5	2.5	2.5

α-amylase inhibitory activity

α-amylase inhibitory activity of the essential oils varied between 0.05 and 0.11 mg/ml (Figure 1). The uppermost activity was observed by *T. capitatus* essential oil. However, this activity was lower than that of standard acarbose (50.13 µg/ml) widely used and marketed anti-diabetic drug.

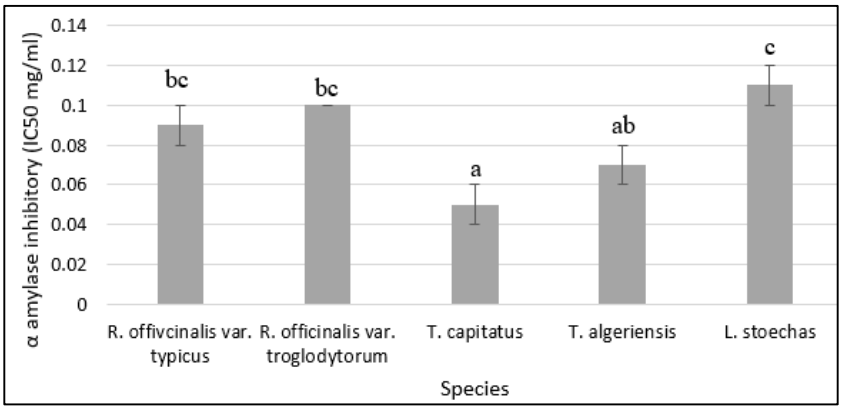

Fig 1: α-amylase inhibitory activity of the selected species essential oils (IC_{50} mg/ml)

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

Lower paiproperty inhibitory property was reported for *Thymus* species essential oils growing wild in Portugal with 0.8 and 1.1mg/ml for *T. capitata* and *T. caespititius* oils, respectively. This activity can be attributed to carvacrol [20].

Natural α-amylase inhibitors from plant sources offer an attractive strategy for the treatment of type 2 diabetes and obesity diseases [21].

Conclusion

This study was carried out in order to describe the antibacterial and α-amylase inhibitory activities of five Lamiacea species essential oils. The level of biological activities among species was linked to that of their essential oil composition. The best activity was observed for T. capitatus essential oil characterized by the highest carvacrol content. These results may highlight the use of this specie in in a large field of application including agroalimentary, pharmaceutical and biological defense instead of toxic synthetic compounds.

Conflicts of interest

The authors declare no conflict of interest.

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