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Composés bioactifs

Phytochemical components and antioxidant and antimicrobial activities of essential oils from native Tunisian *Thymus capitatus* and *Rosmarinus officinalis*

Composition phytochimique et activités antioxydante et antimicrobienne des huiles essentielles de *Thymus capitatus* et de *Rosmarinus officinalis* de Tunisie

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Abstract ***Introduction***. Essential oils and their components are currently of great interest as a potential source of highly bioactive natural molecules. They are being studied for their possible use as safe alternative for food protection against oxidation and microbial spoilage. **Objective.** This study aimed on the phytochemical prospection of *Thymus capitatus* and *Rosmainus officinalis* essential oils and their oral toxicity evaluation. ***Material and methods***. Chemical analysis of tested essential oils was carried out using gas chromatography combined to mass spectroscopic (GC-MS). Their safety limit was evaluated by acute toxicity. The antioxidant activity was estimated using *in vitro* methods. The antimicrobial activity was evaluated against twelve pathogenic germs. ***Results***. Results showed that carvacrol and 1,8-cineol were the major compounds of *Thymus capitatus* and *Rosmarinus officinalis* essential oils. Acute toxicity results exhibited that both tested essential oils were inoffensive at 2000 mg/kg. Additionally, *Thymus capitatus* essential oil presented higher antioxidant activity than *Rosmarinus officinalis:* 2,2-diphényl-1-picrylhydrazyl (DPPH) assay results showed lower IC_{50} for *Thymus capitatus* essential oil than *Rosmarinus officinalis*. Concerning the antimicrobial results, *Thymus capitatus* essential oil presented greater efficacy than *R. officinalis.* Indeed, the minimal growth inhibition diameter generated by thyme essential oil exceeded 38 mm (except for *Salmonella typhirium*) and reached 60 mm (against *C. tropicalis* and *C. albicans*). However, the maximal growth inhibition diameter generated by R. officinalis essential oil was limited to 36 mm (against *Shigella sonnei*). ***Conclusion.*** Overall, *Thymus capitatus* and *Rosmarinus officinalis* essential oils have strong potential applicability

for pharmaceutical industries.

Key words: *Acute toxicity, Antioxidant Activity, Antimicrobial Activity, Essential Oils, GC-MS, Rosmarinus*

Résumé ***Introduction.*** Les huiles essentielles et leurs composants ont actuellement beaucoup d'intérêts en tant que source potentielle de molécules naturelles hautement bioactives. Elles font l'objet d'étude pour leur éventuelle utilisation comme alternative pour la protection des aliments contre l'oxydation et le pourrissement. ***Objectif.*** L'objectif de cette étude est de déterminer la composition chimique, les potentialités antioxydantes et antimicrobiennes ainsi que la toxicité des huiles essentielles de *Thymus capitatus* et de *Rosmarinus officinalis* de Tunisie. ***Matériel et méthodes.*** La composition chimique a été étudiée par chromatographie gazeuse couplée à la spectrographie de masse (GC-MS). La toxicité aigüe a été évaluée *in vivo* sur la souris. Les activités antioxydantes des deux huiles essentielles ont été mesurées *in vitro*. De même, leur activité antimicrobienne a été déterminée contre 12 germes pathogènes. ***Résultats*** Le carvacrol et le 1,8 cinéol sont les composés majeurs des huiles essentielles de *Thymus capitatus* et de *Rosmainus officinalis.* Chez la souris, ces deux huiles sont innofensives à la dose de 2000 mg/kg. En outre, l'huile essentielle de *Thymus capitatus* montre une meilleure activité antioxydante évaluée par le test DPPH (2,2-diphényl-1-picrylhydrazyl) et une plus forte activité antimicrobienne que l'huile essentielle de *Rosmarinus officinalis*. En effet, les zones d'inhibition de la croissance microbienne générées par l'huile essentielle du Thym ont dépassé 35 mm (contre *E. coli*), alors que la plus large zone d'inhibition générée par l'huile essentielle du Romarin n'a pas dépassé le seuil de 36 mm. ***Conclusion.*** Les huiles essentielles de *Thymus capitatus* et de *Rosmainus officinalis* montrent des potentialités biologiques intéressantes pour l'industrie pharmaceutique.

Mots clés : *Toxicité aigüe, Activité antioxydante, Activité antimicrobienne, Huiles essentielles, GC-MS, Rosmarinus officinalis*, *Thymus capitatus*

Introduction

The fast-growing microbial resistance has become a great concern for food producers and consumers due to its huge impact on human health. Regrettably, industrial food companies remain unable to deal with such threat using conventional conservators [1]. Therefore, new technologies and alternatives designs, which can provide advanced solutions for this problem, are required [2].

In this context, essential oils have been used for centuries in different applications, including treatment of various ailments/diseases. Due to their high content of bioactive compounds (phenols, flavonoids, terpenes and their derivatives), essential oils have great and diverse biological activities. It is well established that most of essential oils have a wide spectrum of antimicrobial activity against food-borne pathogens and spoilage bacteria [3]. Most essential oils possess, at least, a limited antibacterial activity, with some oils and components exhibiting greater degree of efficiency. This activity varies from one essential oil to another and from one tested microbial strain to another, but it is always dose dependent [4]. Besides, essential oils of various medicinal and aromatic plants are known for their ability to prevent fatty acids from oxidative decay [5]. In this context, numerous studies were focused on EOs antioxidant properties. It is well established that volatile phenolics with conjugated double bonds (commonly present in EOs) usually show substantial antioxidative properties [6]. For instance, carvacrol, which is predominant in *Thymus*, is responsible for the distinguished antioxidant activities in the obtained EO. Accordingly, scientists are considering the idea to incorporate essential oils (EOs) as natural antioxidant/antimicrobial agents for food preservation [7]. As a matter of fact, the remarkable efficiency of most EOs against a wide range of pathogenic microorganisms, responsible of food spoilage and generally involved in food poisoning, was reported repeatedly [8,9]. Despite thyme and rosemary EOs

are generally recognized as safe (GRAS) [10], appropriate dose for food incorporation should be determined by preliminary studies of acute toxicity. Such studies are primordial to prevent any overdose which may undervalue the targeted results. As a matter of fact, it is dangerous to assume, just because thyme and rosemary tea or alcoholic extract could be harmless, that their essential oil is also safe [11]. Redoubtable EOs toxicity may be related to their high concentration of pure molecules either with potential toxic effects, which react, singly or in combination, with biomolecules to produce the intended biological response [12].

This study was focused, firstly, on the phytochemical prospection of *Thymus capitatus* and *Rosmainus officinalis* EOs, and on their oral acute toxicity determination. Moreover, antioxidant and antimicrobial potencies of both tested EOs were examined.

Material and methods

Plant sampling

Plant aerial parts were collected, from one naturally diversified mountain in Nabeul "Sidi Abderrhamane mountain" in the north-east of Tunisia.

Essential oil extraction

The dried material was cut into small pieces and subjected to hydrodistillation, using a Clevenger type apparatus. The obtained EOs were collected and stored at -20°C in amber vials before analysis. EO yields, expressed as the amount of oil (ml) obtained per 100 g of dry plant material, were measured to evaluate each studied plant capacity to produce EO.

GC-MS analysis

EOs were analyzed using a gas chromatograph (HP 5890-SERIE II) coupled to a mass spectrometer (HP-MSD 5972 A) equipped with an HP INNOWAX polar column (30 m × 0.25 mm, film thickness, 0.25 µm). Helium (1.2 ml/min) injection was set in the split mode (1/10). Injector and detector temperatures were 250 and 280°C, respectively. Ionization was by electron impact at 70eV, and the ion source temperature was 175°C. Mass spectral data were acquired in the scan mode in the m/z range of 50 to 550. The components were identified by comparing their relative retention times and mass spectra with the data from the Baser library of essential oil constituents, Wiley, Mass Finder and Adams GC/MS libraries. Peak areas were quantified as a percentage of the total ion count. Peaks contributing to the total area by more than 0.01% were identified. The linear retention indices (I.r.i.) were calculated according to the formula: I.r.i. = $100n + 100\ (t_x - t_n) / (t_{n+1} - t_n)$

t_n and t_{n+1} : retention times of the reference n-alkane hydrocarbons eluting immediately before and after the target chemical compound "X"; t_x : retention time of compound "X".

Oral acute toxicity

The safety limit of Thyme and Rosemary EOs was determined by recording their lethal dose $(LD)_{50}$ values on mice [13]. Female mice (*Mus musculus* L.) with an average weight, and age (35±6g; 3±0.2 months) were selected as test candidates. For each tested EO, 6 groups of animals, obtained from the animal experimental center of Pasteur (Tunis), were housed 8 per plastic cage. The photoperiod was set as one light cycle (from 6:00 to 18:00h) and air changes and room temperature were controlled too (22±1°C). All animals had free access to tap water and at *ad-libitum* feeding; except for short fasting period (12 hours) before the treatment with the single dose of tested EOs, in order to prevent mice from being disturbed by digestion processes. Requisite amount of *T. capitatus* or *R. officinalis* EOs were mixed properly with an appropriate amount of corn oil (as neutral and safe carrier) to obtain 6 different solutions containing desired concentration of tested EO (50; 100; 200; 500; 1000; 2000 mg/kg of mice body weight). From each EO solution, 0.5 mL was orally administered through a syringe with catheter to each group. In control group, pure corn oil was given to mice. After a single dose administration, the general behavior of mice was observed continuously for 1h after treatment, intermittently for 4h and over period of 24h. The mice were observed for 14 days following treatment, and all signs of toxicity and deaths, and their latencies were recorded. All experiments were conducted in accordance with the Official Journal of the European Committee in 1991 [8].

Antioxidant activities

DPPH free radical-scavenging assay. The evaluation of EO free-radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was made as follow [14]: 250 µl of *T. capitatus* and *R. officinalis* EOs dilutions were added, on a 96 wells plate, to 50µl of a DPPH solution (0.2 mM in methanol). The mixture was left standing in the dark for 30 min. The absorbance was then measured at 517 nm employing a Varioskan Flash spectral scanning multimode reader. The ability to scavenge DPPH was expressed as inhibition percentage that was calculated using the following equation:

DPPH scavenging effect (%) = $[(A_0-A_1)/A_0] *100$

A_0: absorbance of the control; A_1: absorbance of the sample.

Results were expressed as Inhibition Capacity $(IC)_{50}$ value (µg/mL).

Iron reducing power. The reducing power of *T. capitatus* and *R. officinalis* Eos were determined through the transformation of Fe^{3+} to Fe^{2+} [15]. Sample solutions at different concentrations were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of potassium ferricyanide (1%, w/v). The mixture was incubated at 50°C for 20min. Afterwards, 2.5 ml of TCA (10%) were added and the mixture was centrifuged for 10min at 1000×g. Supernatant (2.5 ml) was mixed with distilled water (2.5 mL) and 0.5 ml of ferric chloride (0.1%, w/v), and the absorbance was read at 700 nm against ascorbic acid, as authentic standard. Higher absorbance of the reaction mixture indicates greater reducing power. Results were expressed as IC_{50} value (µg/mL).

β-Carotene Bleaching Inhibition Capacity. The capacity of *T. capitatus* and *R. officinalis* EOs to inhibit the β-carotene bleaching was determined [16]. Two mg of *β*-carotene were dissolved in 20 mL of chloroform, and 2 mL of this solution were added to Tween 40 (200 mg) and linoleic acid (20 mg). After evapora-ting chloroform, 50 mL of oxygenated water were added. Afterwards, appropriate dilutions of the tested samples (10µl) were distributed in 96-wells plate and 150 µl of the formed emulsion were added. Five replicates were prepared for each sample concentration. The microplate was incubated at 50°C for 120 min, and the absorbance was measured at 470nm, using Varioskan Flash spectral scanning multi-mode reader (Thermo Electron, Vantaa, Finland). Readings were performed both immediately and after 120 min of incubation. Samples antioxidant activity was evaluated as β-carotene bleaching inhibition using the following formula:

$(\%) = [(S-A_{120})/(A_0- A_{120})] *100$

A_0 and A_{120} : absorbances of the control at 0 and 120 min;

S *: sample absorbance at 120 min*.

Results were expressed as IC_{50} value (µg/ml).

Antimicrobial activities

Bacterial strain. The antimicrobial potencies of *T. capitatus* and *R. officinalis* EOs were assessed against 8 food borne bacteria: *Pseudomonas aeruginosa* (ATCC 8166), *Escherichia coli* (ATCC 35218), *Staphylococcus aureus* (ATCC 6583), *Bacillus subtilis* (ATCC), *Enterococus faecalis* (ATCC 29212), *Salmonella typhirium* (ATCC 13311), *Shigella sonnei* (ATCC 29930), *Micrococcus luteus* (NCIMB 8166) and *Klebsiella sp* (ATCC 700843), and 4 pathogenic yeasts: *Candida krusei* (ATCC 6258), *Candida albicans* (ATCC 2091), *Candida glabrata* (ATCC 35218), and *Candida tropicalis* (06-085).

Disc Diffusion Method. A loopful of the bacterial working stocks were enriched on a tube containing 9 ml of Mueller-Hinton broth, then incubated at 37°C for 18–24 h. The overnight cultures were used for the antibacterial activity of the essential oils tested in this study and the optical density was adjusted at 0.5 McFarland turbidity. The inoculum of the respective bacteria was streaked onto Mueller-Hinton agar plates using a sterile swab. Sterile filter discs (diameter 6 mm, Whatman paper N°5) were impregnated with 10 µl of *T. capitatus* or *R. officinalis* EOs, and then placed on the inoculated agar. The dishes were incubated at 37°C for 18–24 h. The diameter of the inhibition zones around each of the discs was taken as measure of the antimicrobial activity [17]. Each experiment was carried out 6 times and the mean diameter of the inhibition zone was recorded.

Determination of minimum inhibitory (MIC) and bactericidal (MBC) concentrations. The antimicrobial activity of *T. capitatus* and *R. officinalis* EOs was also evaluated by determining MICs and MBCs. MIC was defined as the lowest concentration of the test agent that gives restricted growth and MBC was defined as the lowest concentration that allowed no visible growth on agar (99.9% inhibition). MBC concentrations were usually higher than the MIC ones. Each tested EO was serially diluted in sterile Mueller Hinton broth in 96 well plates. Each well was inoculated with 5µl of standardized cell suspension (10^5 CFU/ml) and incubated at 37°C overnight. The highest dilution where no growth occurred was recorded as the MIC. For MBC testing, aliquots (200 µl) of broth from wells containing no growth were plated onto MH agar and again incubated overnight at 37°C. The highest dilution where there were no survivors was recorded as the MBC. In both of the above methods, controls for each organism were performed using sterile water in place of the tested antimicrobials and the purity of cultures was confirmed by plating growth from wells. Each experiment was carried out three times and the results were recorded.

Statistical analysis

For all tested parameters, at least three replicates were used. Means were compared using one way-ANOVA analysis (Duncan test), significant differences were found at 5% of confidence level using the

Statistical package SAS 9.1 (2002, 525). Graphics were obtained using Microsoft Office Excel 2003 (Microsoft Corp. Washington, USA).

Results

Characterization and yield measurement of essential oils

The hydrodistillation of both *T. capitatus* and *R.*

Table 1. Chemical composition of *T. capitatus* and *R. officinalis* Eos expressed as % of constituents by GC-MS

Constituents	*I.r.i.*	% of constituents	
		T. capitaus	*R. officinalis*
Tricyclene	928	Nd	0.2± 0.07
α-thujene	933	0.6± 0.01	0.3± 0.01
α-pinene	941	0.4± 0.02	8.4± 0.00
Camphene	955	0.2± 0.07	5.7± 0.00
β-pinene	982	0.3± 0.01	7.2± 0.05
Myrcene	993	1.1± 0.01	1.2± 0.01
3-octanol	995	0.1± 0.04	Nd
α-phellandrene	1006	0.2± 0.02	0.2± 0.1
α-terpinene	1020	1.3± 0.00	0.8± 0.01
p-cymene	1027	3.9± 0.14	0.7± 0.06
1.8-cineole	1034	Nd	33.8± 0.03
(E)-β-ocimene	1052	Nd	0.1± 0.11
γ-terpinene	1063	6.7± 0.02	1.5± 0.09
cis-sabinene hydrate	1070	0.1± 0.01	0.3± 0.01
Terpinolene	1090	0.1± 0.04	0.7± 0.03
Linalool	1101	1.8± 0.1	0.8± 0.06
Camphor	1145	Nd	10.7± 0.01
Borneol	1168	1.2± 0.02	12.3± 0.1
4-terpineol	1178	1.3± 0.00	1.3± 0.00
α-terpineol	1190	0.2± 0.05	3.7± 0.03
bornyl acetate	1287	Nd	5.1± 0.7
Thymol	1292	0.3± 0.12	Nd
Carvacrol	1301	76.1± 0.01	Nd
β-caryophyllene	1419	2.7± 0.05	2.8± 0.01
α-humulene	1455	Nd	0.4± 0.02
Bicyclogermacrene	1496	0.2± 0.00	Nd
caryophyllene oxide	1582	0.3± 0.01	0.8± 0.01
Monoterpene hydrocarbons		15.1	27.6
Oxygenated monoterpenes		81.0	68.0
Sesquiterpene hydrocarbons		2.9	3.2
Oxygenated sesquiterpenes		0.3	0.8
Diterpenes		0.0	0.0
Phenylpropanoids		0.0	0.0
Other derivatives		0.1	0.0
Total identified		99.4	99,6

I.r.i.: linear retention indices.

officinalis aerial parts produced each apale-yellow liquid. *T. capitatus* plant produced higher amount of EO (yield: 2.3% v/w), as compared to *R. officinalis* (yield: 1.6% v/w). GC-SM data are represented in **Table 1**. Accordingly, oxygenated monoterpenes constituted the major fraction of both tested EOs (81 and 68%, respectively), distantly followed by monoterpene hydrocarbons (15.1 and 27.6%, respectively). Also, both *T. capitatus* and *R. officinalis* EOs presented low concentration of sesquiterpene hydrocarbons (2.9 and 3.2% respectively). **Table 1** showed that from the 29 components of *T. capitatus* EO, carvacrol was clearly distinguished as the major molecule representing more than 75% of the total components.

Focusing on rosemary, identification suggested that its EO was composed also of 29 components with 1,8-cineole as the major compound representing more than 33% of the total essential oil components, followed by borneol (12.3%), and camphor (10.7 %).

Determination of *T. capitatus* and *R. officinalis* essential oils safetv limits

The oral acute toxicity results exhibited that both *T. capitatus* and *R. officinalis* EOs were inoffensive at all tested concentrations (from 50 – 2000 mg/kg of mice BW). All mice exposed to each EO presented no alteration in general behavior when compared to the controls. Indeed, no toxic signs, such as death occurrence, piloerection, abdominal contortions, locomotion, convulsions or muscle tone were observed in members of any groups at all tested periods. Thus, *T. capitatus* and *R. officinalis* EOs did not present any extensive toxic effect in rodents, since their LD_{50} in mice was much higher than 2000 mg/kg of mice BW.

Antioxidant activities of *T. capitatus* and *R. officinalis* Essential Oils

DPPH free radical-scavenging assay. DPPH assay results exhibited that *T. capitatus* and *R. officinalis* Eos presented interesting capacities in free radical-scavenging with significant differences (p<0.05) bet-

Table 2. **Antiradical, *β*-Carotene bleaching inhibition and reducing capacities expressed as IC_{50} and EC_{50}values (µg.ml^{-1}) of *T. capitatus* and *R. officinalis* EOs**

	DPPH radical-scavenging	*β*-Carotene bleaching inhibition capacities	Iron reducing power
T. capitatus	300^{a}	200^{b}	900^{a}
R. officinalis	460^{b}	150^{a}	1500^{b}

Values followed by the same letter, within a column, are not significantly different at 5% of confidence level.

Table 3. Inhibition zone diameter (expressed in mm) of *T. capitatus* and *R. officinalis* EOs

Microbial Strains		Inhibition Zone (mm)	
		Thymus capitatus	***Rosmarinus officinalis***
Gram-positive Bacteria	*Staphylococcus aureus*	42^{n}	15^{c}
	Enterococus faecalis	50^{p}	35^{i}
	Micrococcus luteus	40^{m}	14^{b}
Gram-negative Bacteria	*Pseudomonas aeruginosa*	39^{l}	16.33^{d}
	Salmonella typhirium	14.33^{b,c}	12^{a}
	Shigella sonnei	50^{p}	36^{j}
	Klebsiella sp	38^{k}	14.67^{c}
	Esherchia coli	35.67^{j}	21^{f}
Yeast	*Candida tropicalis*	60^{r}	23.33^{g}
	Candida glabrata	44^{o}	27^{h}
	Candida albicans	60^{r}	27.33^{h}
	Candida krusei	57.33^{q}	19.33^{e}

Values followed by the same letter are not significantly different at 5% of confidence level.

ween tested samples (**Table 2**). *T. capitatus* EO displayed better capacity to neutralize $DPPH^{\cdot}$ radical (IC_{50}=300µg/ml), as compared to *R. officinalis* EO (IC_{50} = 460 µg/ml).

β-Carotene Bleaching Inhibition Capacity. Thyme and rosemary EOs capacities to inhibit *β*-carotene bleaching are summarized in **Table 2**. The both tested EOs presented important efficacy with differrent extend. *R. officinalis* essential oil was more efficient in inhibiting *β*-carotene bleaching, as it presented statistically a lower IC_{50} than *T. capitatus* (150 and 200 µg/ml, respectively).

Iron reducing power. Data shown in **Table 2** showed that *T. capitatus* and *R. officinalis* EOs had different and significant potential antioxidant efficiency even at different extend. *Thymus capitatus* essential oil presented lower EC_{50} value (900 µg/ml), as compared to *R. officinalis* EO (EC_{50} = 1500 µg/ml).

Antimicrobial activities

***Disc diffusion method*.** Results suggested that *T. capitatus* EO was more efficient in combating microbial growth as compared to *R. officinalis* one. Indeed, the antimicrobial activity was greater in thecase of thyme (**Table 3**), with minimal growth inhibition diameter exceeding 38 mm (except for *Salmonella typhirium*) and reaching 60 mm (against *C. tropicalis* and *C. albicans*). However, the maximal growth inhibition diameter generated by *R. officinalis* EO was limited to 36 mm (against *Shigella sonnei*).
The focus on bacterial behavior demonstrated that gram-positive bacteria were more sensitive to *T. capitatus* EO than gram-negative ones. As a matter of fact, data statistical analysis (Duncan test) showed significant differences between *T. capitatus* EO efficacy against these two bacterial categories. However, no statistical difference was found in the case of *R. officinalis* EO. *Salmonella typhirium* was the most resistant bacteria toward the tested antimicrobials (14.33 and 12 mm, respectively). On the contrary, *Shigella sonnei* and *Enterococus faecalis* were the most sensitive pathogens to both *T. capitatus* and *R. officinalis* EOs (50 and 35 mm, respectively).
Considering the anti-yeast efficiency (**Table 3)**, *T. capitatus* and *R. officinalis* EOs were interestingly active in inhibiting the growth of the four tested *Candida*. In fact, the least growth inhibition was recorded against *Candida krusei* (19.33 mm using *R. officinalis* EO). While, maximal inhibition was recorded when testing *T. capitatus* EO (60 mm against *Candida tropicalis* and *Candida albicans*). As for the antibacterial activity, *T. capitatus* EO was significantly (p<0.05) more efficient in inhibiting yeast growth with inhibition zones exceeding 44 mm (against *Candida glabatra*), as compared to *R. officinalis* EO which inhibition zones were limited to 27.33 mm (against *Candida albicans*).

Determination of minimum inhibitory (MIC) and bactericidal (MBC) concentrations. As for the disc diffusion assay, the MIC and MBC measurements

Table 4. MIC and MBC concentrations of *T. capitatus* and *R. officinalis* EOs

Microbial Strains		MIC (µg. ml $^{-1}$)		MBC (µg. ml $^{-1}$)	
		T.capitatus	***R.officinalis***	***T.capitatus***	***R.officinalis***
Gram-positive Bacteria	*Staphylococcus aureus*	0.048 ^{a}	0.048 ^{a}	<6 ^{f}	<50 ^{i}
	Enterococus faecalis	0.048 ^{a}	0.097 ^{b}	<6 ^{f}	<50 ^{i}
	Micrococcus luteus	0.097 ^{b}	0.097 ^{b}	<1. 562 ^{d}	<25 ^{g}
Gram-negative Bacteria	*Pseudomonas aeruginosa*	0.048^{a}	0.048 ^{a}	<6 ^{f}	>50 ^{k}
	Esherchia coli	0.048 ^{a}	0.097 ^{b}	<6 ^{f}	25 ^{h}
	Salmonella typhirium	0.048 ^{a}	0.097 ^{b}	3.125 ^{e}	<50 ^{i}
	Shigella sonnei	0.048 ^{a}	0.097 ^{b}	<0.781 ^{c}	<50 ^{i}
	Klebsiella sp	0.048 ^{a}	0.097 ^{b}	3.125 ^{e}	>50 ^{k}
Yeast	*Candida krusei*	0.048 ^{a}	0.048 ^{a}	3.125 ^{e}	25 ^{h}
	Candida albicans	0.048 ^{a}	0.048 ^{a}	3.125 ^{e}	25 ^{h}
	Candida glabrata	0.048 ^{a}	0.097 ^{b}	3.125 ^{e}	>50 ^{k}
	Candida tropicalis	0.048 ^{a}	0.048 ^{a}	3.125 ^{e}	50 ^{j}

For each test, values followed by the same letter are not significantly different at 5% of confidence level.

(**Table 4**) showed interesting efficiency for both tested oils, with a notable superiority in *T. capitatus* EO antimicrobial potency. Considering the minimum inhibitory concentration, *R. officinalis* EO presented the highest values, reaching 0.97 µg/ml, against the majority of tested microbial strains. In the case of *T. capitatus* EO, MICs were more efficient as they were inferior to 0.048 $\mu g.ml^{-1}$, except for *Micrococcus luteus* where MIC was equal to 0.097 µg/ml. The obtained MBCs confirmed that *T. capitatus* EO was noticeably more efficient in destroying bacterial cells, as compared to *R. officinalis* EO, since the former one presented considerably lower bactericidal concentration (< 6 and >50 µg/ml, respectively).

Discussion

The aim of this study was to focus, firstly, on the phytochemical prospection of *Thymus capitatus* and *Rosmainus officinalis* EOs, and on their oral acute toxicity determination. Moreover, antioxidant and antimicrobial potencies of both tested EOs were examined.

As *T. capitatus* is an important medicinal, culinary and aromatic plant, various profiles of its EO were previously described based on its major compounds [18]. Actually, thymol, carvacrol or thymol/carvacrol were often reported as main component in thyme EO depending on several factors, mainly geographical origins of the plant. For instance, carvacrol (62 to 83%) were identified as major compounds of *T. capitatus* EO from Tunisia [18,19], while the Sardinian oil was dominated by thymol [20].

Taking into consideration that rosemary is a well-known species, several chemical compositions of its EO were reported with significant differences in their main components [15]. Nakavuma *et al*., [21] founded that α-pinene was the major compound (26.24%) of their *R. officinalis* EO, with lower amount of 1,8 cineole (24.2%), while, Boroski *et al*., [14] reported higher amount of 1,8 cineole than α-pinene (50.49 and 15.82%, respectively).

Accordingly, Flamini *et al*., [17] classified rosemary EO into two chemotypes: the first one was labeled the α-pinene chemotype with the main compound being α-pinene (>20%)? and the second one was labeled 1,8 cineole chemotype with the main compound being 1,8 cineole (>30%).

In *T. capitatus* case, no report on thyme toxicity were reported at least until 5000 mg/kg of mice body weight of *Thymus vulgaris* EO. Similarly, the oral toxicity of *R. officinalis* EO was reported above 5000 mg/kg of mice body weight [22].

Both tested EOs presented interesting antioxidant capacities. Indeed, most of EOs are rich sources of phytochemicals with beneficial antioxidative and free radical scavenging characteristics [23]. In this context, thyme and rosemary EOs noticed antioxidant efficacy could be related to their rich composition on oxygenated monoterpenes known for their substantial antioxidative properties. With this respect, the variability in antioxidant activities between tested EOs is related to the chemical com-position of each one of them. *T. capitatus* EO, with higher content in oxygenated monoterpenes (81%), was more efficient in scavenging DPPH free radicals and in reducing ferrous iron, as compared to *R. officinalis* EO (62% oxygenated monoterpenes). Precisely, the remarkable antioxidant activity of *T. capitatus* EO could be attributed to its high content of carvacrol (>75%). This monoterpenoid phenol has redox properties and plays an important role in neutralizing free radicals and in peroxide decomposition [24]. Indeed, carvacrol works against oxidation by donating its hydrogen atom to lipid free radicals to stop the chain reaction from proceeding further [5].

Although *R. officinalis* EO presented lower antioxidant efficiency than *T. capitatus* EO, its activity was worth noting. Rosemary EO antioxidant activity might be due to the synergy between some or all the components present even in small amount [25]. Borneol and camphor molecules, present in lower concentration in *R. officinalis* EO, own interesting antioxidant activities, too.

The search for natural compounds with efficient antimicrobial activity remains a priority for scientists to overcome antibiotics induced resistance or to prevent some diseases mainly caused by contaminated foods. In this context, obtained results exhibitted that *T. capitatus* and *R. officinalis* EOs showed impressive antimicrobial efficacies. In fact, EOs antimicrobial activity was usually related to their content of monoterpenic molecules [26]. Theoretically, the antimicrobial effect of monoterpenes is based on their abilities to disrupt the microbial cytoplasmic membrane, since if the integrity of such membrane is disturbed it loses its properties as a barrier, matrix for enzymes and energy transducer and the cell viability will be compromised [26].

The focus on *R. officinalis* EO efficiency exhibited an interesting potency, which could be related to its major compound 1,8 cineole (>33%), as several authors discussed that this molecule present potential antimicrobial efficacy [27]. Actually, 1,8 cineole (terpenes) targets the microbial cytoplasmic membrane, and its mechanism of action is likely the accumulation in the cellular membrane, causing a loss of

membrane integrity, the inhibition of respiratory enzymes and the dissipation of the proton-motive force [27].

Otherwise, the gathered results highlighted that *Thymus capitatus* EO presented higher antimicrobial activity against all tested microorganisms, as compared to *R. officinalis* one. This variability could be explained by the difference on chemical nature of each EO compounds. As a matter of fact, EOs chemical structure affects their mode of action in inhibiting microbial growth [28]. Here in, EOs rich in phenol have the highest antibacterial activity followed by those rich in terpenic alcohols. In the same context, *T. capitatus* EO antimicrobial strong potency might be related to the known antimicrobial effect of its major compound, carvacrol [29]. This molecule was generally recognized as very effective against bacterial growth. Indeed, carvacrol provokes the disintegration of the bacterial outer membrane, followed by the release of lipopolysaccharides, resulting in an increase in the ATP permeability of the cytoplasmic membrane and consequently cell death [29].

Conclusion

T. capitatus and *R. officinalis* EOs are studied for their chemical compositions, oral acute toxicity, and for their antimicrobial and antioxidant activities. For both studied species, differences in the oil composition and yields are detected. Besides, *T. capitatus* and *R. officinalis* EOs do not present any toxicity in rodents. As well, these EOs present interesting antioxidant and antimicrobial properties, and thyme has a significantly higher efficiency in all monitored assays. As a whole, *T. capitatus* and *R. officinalis* EOs can be safely recommended as a natural and effective food preservative.

Conflict of interests

The authors declare no conflict of interests.

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