

## Antimicrobial activity and chemical constitution of essential oil from Moroccan thyme (*Thymus satureioides* C.) on five microbial contaminants

Kamal OULED TAARABT<sup>1</sup>, Rachida OUAABOU<sup>2</sup>,  
Rachid LAHLALI<sup>3</sup>, Said ENNAHLI<sup>3\*</sup>

<sup>1</sup>Chouaib Doukkali University, Faculty of Sciences of ElJadida, Department of Biology, Morocco; [Taarabtkamal@gmail.com](mailto:Taarabtkamal@gmail.com)

<sup>2</sup>Cadi Ayyad University, Faculty of Science Semlalia, Department of Chemistry, Marrakesh, Morocco; [rachaouaabou@gmail.com](mailto:rachaouaabou@gmail.com)

<sup>3</sup>National School of Agriculture, Meknes, Morocco; [lahlali.r@gmail.com](mailto:lahlali.r@gmail.com); [ennahlisaid@gmail.com](mailto:ennahlisaid@gmail.com) (\*corresponding author)

### Abstract

Antimicrobial preservatives are widely added to health products to prevent microbial contamination. However, because of the risks attributed to several synthetic agents, manufacturers are searching for new natural agents. Therefore, this work investigated the different physicochemical characteristics of one of those natural ingredients, the essential oil (EO) of Moroccan thyme (*Thymus satureioides* C.) and its antimicrobial potential against five microbial strains. The physicochemical parameters (density, refractive index, optical rotation, miscibility, acid value, ester value, and flash point) were measured and the chemical composition was determined by GC/MS. The antimicrobial activity was assessed using disc diffusion method and a macrodilution broth method. The EO yield was 1.01% compared to the dry matter. Fifty-two compounds were identified. The major compounds were thymol (28.66%), borneol (21.16%) and  $\alpha$ -terpineol (12.33%). The disc diffusion method revealed that all the strains tested showed sensitivity to the EO at concentration of 1636  $\mu\text{g}$  per disc. For the minimum inhibitory concentration (MIC) and the minimum microbicidal concentrations (MMC), similar results were obtained for *Staphylococcus aureus* and *Escherichia coli* (640  $\mu\text{g}/\text{mL}$ ), for *Pseudomonas aeruginosa* (960  $\mu\text{g}/\text{mL}$ ) and for *Candida albicans* (800  $\mu\text{g}/\text{mL}$ ) baring *Aspergillus brasiliensis*, which had 480  $\mu\text{g}/\text{mL}$  and 640  $\mu\text{g}/\text{mL}$  for the MIC and MMC, respectively.

**Keywords:** antibacterial; chemical composition; essential oil; MIC; MMC; thyme (*Thymus satureioides* C.)

### Introduction

Antimicrobial preservatives are widely used in various health products and are cheap and effective ingredients against a wide range of spoilage organisms. When a health product does not itself have adequate antimicrobial activity, antimicrobial preservatives may be added, to protect it from microbiological growth or from microorganisms that are introduced inadvertently during or subsequently in the manufacturing process (Meyer *et al.*, 2007; Khorshidian *et al.*, 2018).

Received: 11 May 2021. Received in revised form: 21 Jul 2021. Accepted: 11 Aug 2021. Published online: 18 Aug 2021.

From Volume 13, Issue 1, 2021, Notulae Scientia Biologicae journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

All antimicrobial preservatives used must be proven to be nontoxic for human. The safety and efficiency of preservatives are the fundamental factors to be considered for long-term preservation of health products. However, some synthetic preservatives are not inconsequential, primarily due to their excessive use not only in health products, but also in food and household products. Due to increased public concerns the demand for natural or label-friendly alternatives has drastically increased (Falleh *et al.*, 2020).

Chemical components of plant extracts have the potential to provide a green alternative to conventional chemical antimicrobial preservatives. Recently, there is more focus on replacing chemical preservatives, which might have adverse effects on human health and environment with those of plant-based natural ones (Munda *et al.*, 2019; Lal *et al.*, 2019, 2020). Increasing demand for natural health product preservatives has resulted in essential oils (EOs) becoming more popular. The EOs are gaining much recognition as a potential source of natural bioactives. As part of this study, an attempt was made to develop effective and safe preservatives from natural products by selecting thyme from the *Lamiaceae* family as a representative of Moroccan medicinal plants and aromatics. Generally, thyme species were considered to be medicinal plants because of their pharmacological and biological properties (Al-Maqtari *et al.*, 2011).

Among these endemic species, *Thymus satureioides* C. (*T. satureioides* C.) was known locally in Morocco as “Azukni” or “Zaitra”, and worldwide as “Moroccan thyme”. This species is widespread in the Mediterranean basin of North Africa (Sakkas and Papadopoulou, 2017), typical of arid habitats (Ismaili *et al.*, 2004). In Morocco, *T. satureioides* C. grows spontaneously in the low and middle mountains of the High Atlas and Anti-Atlas (Laila *et al.*, 2014). *T. satureioides* Coss. is a perennial shrub, about half a meter in height with spatulate leaves and purple corolla. It is locally used as a food spice, and essential pain-killer in folk medicine for healing gastrointestinal disorders, antiseptic (Bellakhdar *et al.*, 1991; Fatima-Zahra *et al.*, 2017), diabetes (Tahraoui *et al.*, 2007), respiratory disorders (Rebbas and Bounar, 2014), and to warm the body (Elkhoudri *et al.*, 2016).

Recent studies have shown that *Thymus* species have high antioxidant, anti-haemolytic (Ramchoun *et al.*, 2015), hypolipidaemic (Ramchoun *et al.*, 2012), anti-inflammatory (Khouya *et al.*, 2019), anticancer and bio-insecticidal potential (Alaoui-Jamali *et al.*, 2018). Furthermore, several reports highlighted the antimicrobial activity of EO of Moroccan thyme (El Bouzidi *et al.*, 2013; Boubaker *et al.*, 2016; Chraibi *et al.*, 2016). In order to emphasize the benefits of Moroccan thyme, this study aimed at determining the physicochemical parameters and chemical composition of EO extracted from *T. satureioides* C., as well as the *in vitro* study of its antimicrobial activity against the microbes involved in the deterioration of health products.

## Materials and Methods

### *Plant material*

The leafy stems of *T. satureioides* C amounting to 5 kg were collected in 2018 from Al Haouz province, in the Tidili Mesfioua district (31°26'37.3" N, 07°36'26.5" W, altitude 1119 m), Morocco, during flowering period. Samples were dried at 105 °C until constant weight and cut into small pieces. The dried thyme was kept in a stainless-steel container for later analysis.

### *Essential oil isolation*

Thyme essential oil was isolated by hydro distillation of the plant material. Briefly, about 500 g of fresh botanical material were transferred to Clevenger apparatus and 3 L of distilled water was added. The Clevenger apparatus was set at boiling temperature of water for 3 h and thyme essential oil was distilled. The EO was spontaneously separated from the water solution and collected in a 50 mL flacon tube containing 0.5 g anhydrous sodium sulfate. The extract was vortexed for 30 s and centrifuged at 2000 g for 10 min at 5 °C. The EO sample was conditioned in a sealed tinted vial and stored in a refrigerator at 4 °C.

#### *Physicochemical parameters*

The average yield was calculated from the weight of the EO obtained at the end of isolation using the equation below:

$$\text{Percentage yield} = [\text{weight of the extracted oil (g)} / \text{dry weight of the sample (g)}] \times 100 \quad (1)$$

The appearance, colour, smell, consistency and solubility of the EO were recorded. The standards used to determine physicochemical parameters were NF ISO 280 for the refractive index, NF ISO 592 for the optical rotation, NF EN ISO 660 for the acid value, NF ISO 709 for the ester value, NF ISO 1241 for the ester value after acetylation, the AFNOR standard for ethanol miscibility, the “Closed Cup” method for flash point measurement and the electronic density meter for relative density.

#### *Gas chromatography analyses (GC/MS)*

Gas Chromatography-Mass Spectrometry analysis was carried out using an HP 6890 apparatus and a 20 m × 0.18 mm DB-5ms (cross-linked 5% phenyl/95% dimethyl arylenopolysiloxane) column with 0.18 µm film thickness (Agilent). Carrier gas was helium, the flow rate was 3 mL/min. Column temperature was initially kept at 50 °C for 3min, then gradually increased to 320 °C at a rate of 10 °C/min. Oil samples (2 µL, neat) were injected using the split mode (1:50 ratio). Mass spectra were taken over the m/z 30-500 range with an ionizing voltage of 70 eV. Kovat's retention index was calculated using cochromatographed standard hydrocarbons. The individual compounds were identified by MS and their identity was confirmed by comparison of their RIs, relative to C8-C32 n-alkanes, and mass spectra with those of authentic samples or with data already available in the NIST 2007 Mass Spectral Library and the literature (Adams, 2005).

Samples were analysed in triplicate.

#### *Antimicrobial activity*

##### Microbial strains

Reference strains, representing likely contaminants to health products, were obtained from the American Type Culture Collection (ATCC) according to the recommendations of the challenge test of the *European Pharmacopoeia*. Three bacterial strains: *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8739), and two fungal strains: *Candida albicans* (ATCC 10231), and *Aspergillus brasiliensis* (ATCC 16404) were used in this study.

##### *Disc diffusion method*

The antimicrobial activity of the EO was determined by disc diffusion method EUCAST (EUCAST, 2019) with some modifications. In a nutshell, a suspension containing  $1 \times 10^8$  to  $2 \times 10^8$  colony-forming units (CFU)/mL of bacteria cells and 1 to  $2.5 \times 10^5$  spores/mL of fungi were inoculated on Petri dishes containing 4 mm thick Mueller-Hinton agar (MHA) and Potato dextrose agar (PDA) medium, respectively. The 6 mm filter paper discs were impregnated with 15 µl of stock solutions (1636 µg) of EO determined by weighing. The discs were aseptically placed on the surface of the growth-medium with sterile forceps and gently pressed to ensure contact with agar, which had previously been inoculated with the selected test microorganism. Positive control consisted of 0.2% chlorhexidine digluconate solution (20%), a synthetic preservative. Discs without EO served as a negative control. The 15-15-15-minute rule was respected. Inoculum suspension was prepared within 15 minutes, discs were applied within 15 minutes of inoculation and plates were incubated within 15 minutes of discs application. The plates were incubated at 35 °C for 16-24h for bacteria, 24h for *C. albicans* at 35 °C and 24-48 h for *A. brasiliensis* at 25 °C. EO activity was assessed by measuring the inhibition zone diameters, including the diameter of discs.

##### *Determination of the minimum inhibitory concentration (MIC)*

The MIC was defined as the lowest concentration of an antimicrobial agent that prevents visible growth of a micro-organism. A macro-dilution broth was used to measure quantitatively the *in vitro* activity of the EO

against the studied micro-organism, as reported in CLSI standards (formerly the NCCLS) with some modifications. The methods M07-A9 (CLSI, 2012), M27-A2 (NCCLS, 2002a), M38-A (NCCLS, 2002b) were used for bacteria, yeasts and filamentous fungi, respectively. Briefly, a series of 10 tubes was prepared with a broth (Mueller-Hinton for bacteria and RPMI-1640 for fungi) with various concentrations of the EO ranging from 160 to 1600  $\mu\text{g}/\text{mL}$  (Table 1). The dimethyl sulfoxide (DMSO) was used to solubilize the EO. The final concentration of DMSO was reduced to 1%.

The tubes were inoculated with a standardised suspension of the test organisms. Tested strains were prepared by adjusting the turbidity of each microbial culture to reach an optical density of 0.5 McFarland standards. These resulted in a suspension containing approximately  $1$  to  $2 \times 10^8$  CFU/mL for bacteria,  $1$  to  $5 \times 10^6$  CFU/mL for yeast *C. albicans* and  $10^6$  spores/mL for *A. brasiliensis*. After incubation for 16-20 h at 35 °C for bacteria and 46-50 h for fungal strains at 25 °C, the tests were examined and the MIC was determined. The controls of growth, solvent and sterility were prepared. The determinations of MIC values were done in triplicate.

**Table 1.** Diagram of preparation of the dilution range of the EO used in macro-dilution

Tube N°	VEO ( $\mu\text{L}$ )	VDMSO ( $\mu\text{L}$ )	[EO] <sub>Initial</sub> (%, v/v)	[EO] <sub>Final</sub>	
				(%, v/v)	( $\mu\text{g}/\text{mL}$ )
1	450	2550	15	0.15	1600
2	405	2595	13.5	0.135	1440
3	360	2640	12	0.12	1280
4	315	2685	10.5	0.105	1120
5	270	2730	9	0.09	960
6	225	2775	7.5	0.075	800
7	180	2820	6	0.06	640
8	135	2865	4.5	0.045	480
9	90	2910	3	0.03	320
10	45	2955	1.5	0.015	160

VEO: Volume of EO contained in the stock solution.

VDMSO: Volume of the solvent contained in the stock solution.

[EO]<sub>Initial</sub>: EO concentration when initially mixing with the solvent for the preparation of the stock solution.

[EO]<sub>Final</sub>: EO concentration in the incubation tube expressed in (% v/v) and converted to ( $\mu\text{g}/\text{mL}$ ) because the density of the EO is known ( $d = 0.9379$ ).

#### *Determination of the minimum microbicidal concentration (MMC)*

The MMC is the lowest concentration of EO capable of reducing microorganisms to zero. The MMC is measured by subculturing the broths used to determine MIC onto fresh agar plates. An aliquot was taken with a calibrated loop on the tubes used in MIC assay from the last tube provided with microbial growth and spread on MHA for 24 h at 35 °C for bacteria, and on Sabouraud agar for 24-72 h at 25 °C for fungi. The MMC of EO was determined from the first microbial-free box. The EO of thyme was considered as microbicide if the MMC/MIC ratio=1, or as bacteriostatic or fungistatic if the MMC/MIC ratio  $\geq 2$  (Konaté *et al.*, 2012). The determinations of MMC values were performed in triplicate.

#### *Statistical analysis*

The EO samples were analysed in triplicate and the data are reported as mean  $\pm$  SD. Statistical analysis was carried out by a Fisher's test using the SPSS statistical software v 22. Values of  $P < 0.05$  were considered statistically significant.

## Results and Discussion

### *Physicochemical parameters*

Hydro distillation of the dried leaf stems of *T. satureioides* C. yielded yellow liquid oil, mobile, and immiscible with water. The oil had an aromatic herbaceous smell characteristic of the plant. The essential oil yield was  $1.01\% \pm 0.14$  on dry weight basis. The physicochemical parameters are shown in Table 2.

Organoleptic characteristics were identical to a study reported on the *T. satureioides* Moroccan thyme (Kasrati *et al.*, 2014). According to previous studies carried out on the same plant in Morocco, the yield ranged from 0.2% to 3.3% (Taoufik *et al.*, 2017). Ramzi *et al.* (2017) attributed these differences in yield to the plant phenological stages. In regards to physicochemical parameters of the EO of thyme, to the best of our knowledge, no research has been conducted. The majority of studies were focused exclusively on the chemical composition of Moroccan thyme.

**Table 2.** Physicochemical parameters of the EO of *T. satureioides* C. leafy stems

Parameter	Value
Relative density at 20 °C	0.9379
Refractive index at 20 °C	1.4928
Optical rotation at 20 °C	+3.38
Miscibility with ethanol 80% V/V at 20 °C	> 10
Acid value in mg KOH/gr	8.9
Acid value in % of oleic acid	4.5
Ester value	18
Ester value after acetylation	234
Flash point (closed cup method) in °C at 101.3 kPa	14

Tests were performed in triplicate and modal values are presented.

### *Chemical composition of the essential oil*

The identified principal components of the *T. satureioides* C. essential oil is listed in Table 3. The GC/MS analyses of the essential oil, lead to the identification of fifty-two compounds representing 96.38% of the total oil composition.

Major compounds of the *T. satureioides* C. were thymol (28.66%) followed by borneol (21.16%),  $\alpha$ -terpineol (12.33%),  $\beta$ -caryophyllene (6.39%) and carvone (5.49%), while other compounds were present under 2%. The EO of *T. satureioides* C. displayed a high content of monoterpenes (85.05%) and low contents of sesquiterpenes (11.33%). The major constituents of oxygenated monoterpenes were alcohols (37.55%) followed by phenols (30.62%) and ketones (5.91%). A small number of esters (0.96%) and ether-oxides (0.656%) were detected.

The total of chemical compounds (52 compounds) reported in this study were close to those reported by Taoufik *et al.* (2017) who reported 56 compounds. However, other authors reported a lower number (20 compounds) (Chraïbi *et al.*, 2016).

The quantitative and qualitative chemical composition showed a significant variability. It is impossible to find a chemically homogeneous and standardised EO for thymus species in its natural habitat (Aïcha *et al.*, 2013), nonetheless, the presence of common compounds such as carvacrol, borneol, and thymol, which are ubiquitous major components in varying amounts, cannot be ruled out (Jaafari *et al.*, 2007; Lahnine *et al.*, 2017; Rahman, 2018). Also, the current study found that, borneol is the only compound with a percentage that consistently exceeds 20%, while it reported at lower percentage (around 10%) in *Thymus capitatus* and *Thymus bleicherianus* (Aïnane *et al.*, 2019). Therefore, it can be concluded that *T. satureioides* C has a borneol chemotype.

**Table 3.** Chemical composition of the essential oil of *T. satureioides* C. leafy stems by GC/MS

Compounds	RT	RT*	Conc. %	Compounds	RT	RT*	Conc. %
Tricyclene	4.96	5.53	0.064	$\alpha$ -Terpineol	9.94	15.21	12.33
$\alpha$ -Thujene	5.06	5.62	0.109	Cis-Dihydro carvone	9.96	15.41	0.24
$\alpha$ -Pinene	5.2	5.85	0.911	Verbenone	10.12	15.97	0.041
Camphene	5.54	6.26	1.825	Nerol	10.34	16.97	0.023
Sabinene	6.01	6.91	0.068	BornyleFormate	10.4	17.36	0.359
$\beta$ -Pinene	6.1	7.04	0.252	Carvone	10.54	17.53	5.498
Octene-3-ol	6.19	7.04	0.018	Linalyl Acetate	10.62	--	0.082
Myrcene	6.36	7.43	0.337	Isothymol	11.15	20.14	0.109
3-Octanol	6.52	7.44	0.09	Bornyl Acetate	11.2	19.62	0.247
$\alpha$ -phellandrene	6.66	7.85	0.038	Thymol	11.32	19.71	28.66
$\delta$ -3-carene	6.7	8.10	0.023	Carvacrol	11.41	20.14	1.854
$\alpha$ -Terpinene	6.87	8.30	0.333	$\alpha$ -Terpinyl Acetate	12.06	--	0.276
<i>p</i> -Cymene	7.02	8.53	2.768	$\alpha$ -Copaene	12.45	23.49	0.071
Limonene	7.1	8.69	0.403	$\beta$ -Bourbonene	12.57	24.05	0.035
$\beta$ -phellandrene	7.12	8.70	0.077	$\beta$ -Caryophyllene	13.05	25.36	6.395
Eucalyptol	7.16	8.76	0.656	Aromadendrene	13.25	26.27	2.234
(E)- $\beta$ -Ocimene	7.42	9.42	0.011	$\alpha$ -Humulene	13.49	26.82	0.317
$\gamma$ -Terpinene	7.63	7.78	1.879	Allo-Aromadendrene	13.54	27.07	0.019
Cis-Sabinene hydrate	7.85	10.2	0.048	Germacrene-D	13.81	28.15	0.012
Terpinolene	8.1	10.98	0.165	$\gamma$ -cadinene	14.2	29.35	0.142
<i>p</i> -Cymenene	8.19	11.09	0.036	$\delta$ -cadinene	14.25	29.72	0.106
Linalol	8.37	11.32	2.467	Spathulenol	14.96	31.96	0.039
Cis-Thujone	8.45	11.56	0.007	Caryophyllene Oxide	15.04	32.16	1.179
Trans-Thujone	8.69	12.00	0.008	Caryophylla-4(12),8(13)-diene5- $\beta$ -ol	15.67	34.40	0.351
Camphre	9.15	--	0.117				
Borneol	9.59	14.29	21.158	Epi- $\beta$ -Cadinol	15.71	34.38	0.429
Terpinene-4-ol	9.68	16.66	1.46	Total (%)			96.376

RT: Retention time

RT\*: Retention time from literature (Adams, 2017, NIST Chemistry Web Book, SRD 69).

*Antimicrobial activity of the EO of Thymus satureioides C.*

Disc diffusion is one of the most largely used antimicrobial susceptibility tests. It is suitable for the majority of micro-organisms in the range of antimicrobial agents that can be tested and requires no special equipment.

The antimicrobial activity of *T. satureioides* C. EO by disc diffusion method against five microbial strains, are shown in Table 4. At dose of 1639  $\mu$ g/disc the EO of thyme shows antimicrobial activity against the five tested strains.

The data indicated that maximum activity was observed against *A. brasiliensis* (32.00 mm), followed by *C. albicans* and *E. coli* (13.00 mm). Moderate activity was observed against *S. aureus*, with inhibition zone of (8.50 mm). *P. aeruginosa* was considered as resilient since a small inhibition zone (7.50 mm) was observed.

The MIC and MMC results are summarized in Table 5. *P. aeruginosa* remained the most resilient strain to the EO of thyme compared to other strains. The growth of *P. aeruginosa* was inhibited at 960  $\mu$ g/mL, while *A. brasiliensis* was inhibited at 480  $\mu$ g/mL. The EO had a bacteriostatic action at a concentration of 640  $\mu$ g/mL for *S. aureus* and *E. coli* and a fungistatic action at a concentration of 800  $\mu$ g/mL for *C. albicans*. MMC showed that all micro biostatic concentrations were microbiocidal, except for *A. brasiliensis*. The fungicidal action

requires a higher concentration (640 µg/mL), which is normal since the fungus developed a form of resistance to adverse conditions.

The intraspecific chemical variability of the EO of the *T. satureioides* C. was no longer surprising and can be attributed to a set of factors, including genetic factors, environmental conditions (soil, precipitation, temperature, light, stress, etc.), geographical origin, vegetative cycle, biotic effects (the harvest period and the methods used for conservation and isolation) (Jaafari *et al.*, 2007; Aicha *et al.*, 2013; Kasrati *et al.*, 2014).

**Table 4.** Antimicrobial activity of *T. satureioides* C. EO by disc diffusion method against five microbial strains

	<i>S. aureus</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>	<i>A. brasiliensis</i>
Ø <sub>m</sub>	8.5±0.1 <sup>c</sup>	13±0.0 <sup>b</sup>	7.5±0.1 <sup>cd</sup>	13±0.2 <sup>b</sup>	32±0.4 <sup>a</sup>

Ø<sub>m</sub>: mean diameter of the halo of inhibition of the EO of the *Thymus satureioides* C. measured in mm.

Means followed by the same letter in the same row are not significantly different (P < 0.05).

**Table 5.** Minimum inhibitory and minimum microbicidal concentration of *T. satureioides* EO against five microbial strains

[EO] <sub>Final</sub> (µg/mL)	<i>S.aureus</i>		<i>E. coli</i>		<i>P. aeruginosa</i>		<i>C. albicans</i>		<i>A. brasiliensis</i>	
	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC	MIC	MMC
1600	–	–	–	–	–	–	–	–	–	–
1440	–	–	–	–	–	–	–	–	–	–
1280	–	–	–	–	–	–	–	–	–	–
1120	–	–	–	–	–	–	–	–	–	–
960	–	–	–	–	–	–	–	–	–	–
800	–	–	–	–	+	+	–	–	–	–
640	–	–	–	–	+	+	+	+	–	–
480	+	+	+	+	+	+	+	+	–	+
320	+	+	+	+	+	+	+	+	+	+
160	+	+	+	+	+	+	+	+	+	+

+: Presence of microbial growth (sedimentation of pellet for de *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans* and a supernatant ring for *A. brasiliensis*).

–: No microbial growth.

Antimicrobial susceptibility of EO of *T. satureioides* C. was evaluated by two methods (disc diffusion method and macrodilution). The results showed that all the microbial strains studied were sensitive to EO, except *P. aeruginosa* which exhibited a resilience. Strains of *Pseudomonas aeruginosa* are known to have multidrug resistance for antibiotics (Mayaud *et al.*, 2008; Kasrati *et al.*, 2014). It seems that its external membrane and efflux mechanisms protect it against the actions of the EO (Mayaud *et al.*, 2008). These results align with several studies on antimicrobial activity of EO of *T. satureioides* C. Oussalah *et al.* (2007) showed that the EO of thymus, was able to inhibit the growth of *S. aureus* and *E. coli*. El Bouzidi *et al.* (2013) and Boubaker *et al.* (2016) reported an inhibitory effect of EO of Moroccan thymus on *C. albicans* and fungi (especially citrus pathogens), respectively. The MIC of *C. albicans* is higher relative to that of *S. aureus* and *E. coli*. This result is in line with previous work which concluded that the EO of *T. satureioides* C had moderate anti-fungal activity (Ait-Sidi-Brahim *et al.*, 2018). The comparison of MIC values is complex because of the variability of the methods used (concentrations used, volume of inoculum, culture medium, pH, temperature, incubation time, etc.) (Lawal and Ogunwande, 2013) and the variability of the microbial strains tested. However, it seems that the difference in antimicrobial activity may have been related to the concentration, composition, functional groups, and structural configuration of the compounds and their possible synergistic interactions (Oussalah *et al.*, 2007).

Classically, the antibacterial action of EO relies primarily on lipophilicity, which allows EO to interact with cell membranes and position itself between lipid chains (Sokolik *et al.*, 2018), thus causing a series of

cellular disturbances, leading to the inhibition of growth, or even bacterial destruction. Some studies suggest that the high thymol content confers this antimicrobial potential to the EO being studied (El Bouzidi *et al.*, 2013), while others have shown that thymol has an antibacterial and antifungal activity (Marchese *et al.*, 2016). Furthermore, other studies highlighted the mechanism of action of Thymol *in vitro* against *Actinobacillus pleuropneumoniae*. They reported that thymol disrupts the integrity of the bacterial membrane, leading to the leakage of cell components ultimately causing cell death (Wang *et al.*, 2017). Moreover, other molecules may be implicated in this antimicrobial activity, such as carvacrol and carvone (Zhou *et al.*, 2014; Hakemi-Vala *et al.*, 2017).

## Conclusions

Moroccan thyme can yield 1.01% of EO. The latter has a borneol chemotype characterised by its richness in phenols and monoterpene alcohols. The extracted EO have significant antimicrobial activity at acceptable levels, particularly against microbial strains responsible for the deterioration of health products. The EO of *T. satureioides* C. represent a natural source of antimicrobial substances that exhibited potential for use in pharmaceutical and cosmetic industries, generally, in the field of antimicrobial preservation. The main active components of EO of *T. satureioides* C. could be used as natural alternatives for protection of health products. However, the use of the EO of *T. satureioides* C in its raw form, can pose technical problems due to its insolubility in water, high volatility, aroma, colour and, interference, etc. Hence, encapsulation is one valuable option to solve these issues. Also, molecular screening the EO to identify and extract specific active components that exclusively have the antimicrobial activity would be invaluable.

## Authors' Contributions

KOT: hypothesized and drafted the manuscript. RO: guided drafting of the manuscript. RL: helped in the drafting. SE: Writing - review and editing. All authors read and approved the final manuscript.

## Acknowledgements

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

## References

- Adams RP (2005). Identification of essential oil components by gas review: Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publishing Corporation, Carol Stream IL.
- Aicha N, Chendid Rachida T, Meskaoui Abdelmalek E, Ben Abdellah Fez Morocco M (2013). Micropropagation of *Thymus satureioides* Coss. an endangered medicinal plant of Morocco. Journal of Agriculture and Technology 9:487-501.



- Ainane A, Talbi M, Khammour F, Hamad M, Kouali EL, Abba EH, Cherroud S, Ainane T (2019). Composition chimique et activité anti insecticide des huiles essentielles de *Thymus* du Maroc: *Thymus capitatus*, *Thymus bleicherianus* et *Thymus satureioides*. Proceedings BIOSUNE 1:96-100.
- Ait-Sidi-Brahim M, Markouk M, Larhsini M (2018). Moroccan medicinal plants as anti-infective and antioxidant agents, in: new look to phytomedicine: advancements in herbal products as novel drug leads. Elsevier, pp 91-142. <https://doi.org/10.1016/B978-0-12-814619-4.00005-7>
- Al-Maqtari MAA, Alghalibi SM, Alhamzy EH (2011). Chemical composition and antimicrobial activity of essential oil of *thymus vulgaris* from Yemen. Turkish Journal of Biochemistry 36:342-349.
- Alaoui-Jamali C, Kasrati A, Leach D, Abbad A (2018). Étude comparative de l'activité insecticide des huiles essentielles des espèces de thymus originaires du Sud-Ouest marocain. Phytothérapie 16:268-274. <https://doi.org/10.3166/s10298-016-1051-6>
- Bellakhdar J, Claisse R, Fleurentin J, Younos C (1991). Repertory of standard herbal drugs in the Moroccan pharmacopoea. Journal of Ethnopharmacology 35:123-143. [https://doi.org/10.1016/0378-8741\(91\)90064-K](https://doi.org/10.1016/0378-8741(91)90064-K)
- Boubaker H, Karim H, El Hamdaoui A, Msanda F, Leach D, Bombarda I, ... Ait Ben Aoumar A (2016). Chemical characterization and antifungal activities of four *Thymus* species essential oils against postharvest fungal pathogens of citrus. Industrial Crops Production 86:95-101. <https://doi.org/10.1016/j.indcrop.2016.03.036>
- Chraïbi M, Farah A, Lebrazi S, El Amine O, Iraqui Houssaini M, Fikri-Benbrahim K (2016). Antimycobacterial natural products from Moroccan medicinal plants: Chemical composition, bacteriostatic and bactericidal profile of *Thymus satureioides* and *Mentha pulegium* essential oils. Asian Pacific Journal of Tropical Biomedicine 6:836-840. <https://doi.org/10.1016/j.apjtb.2016.08.002>
- CLSI (2012). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; Approved Standard-Ninth Edition. CLSI Doc. M07-A9. Wayne, PA Clin. Lab. Stand. Inst.
- El Bouzidi L, Jamali CA, Bekkouche K, Hassani L, Wohlmuth H, Leach D, Abbad A (2013). Chemical composition, antioxidant and antimicrobial activities of essential oils obtained from wild and cultivated Moroccan thymus species. Industrial Crops Production 43:450-456. <https://doi.org/10.1016/j.indcrop.2012.07.063>
- Elkhoufri N, Baali A, Amor H (2016). Maternal morbidity and the use of medicinal herbs in the city of Marrakech, Morocco. Indian Journal Traditional Knowledge 15:79-85.
- EUCAST EC on AST (2019). Determination of antibiotic sensitivity. CASFM / EUCAST French Society of Microbiology.
- Falleh H, Ben Jemaa M, Saada M, Ksouri R (2020). Essential oils: A promising eco-friendly food preservative. Food Chemistry 330:127268. <https://doi.org/10.1016/j.foodchem.2020.127268>
- Fatima-Zahra E, Fouzia RF, Abdelilah R (2017). Ethnobotanical study of medicinal plants used in traditional medicine in the province of Sidi Kacem, Morocco. Asian Journal of Pharmacy and Clinical Research 10:121-130. <https://doi.org/10.22159/ajpcr.2017.v10i1.14326>
- Hakemi-Vala M, Rafati H, Aliahmadi A, Ardalan A (2017). Nanoemulsions: a novel antimicrobial delivery system. In: Nano- and Microscale Drug Delivery Systems. Elsevier, pp 245-266. <https://doi.org/10.1016/B978-0-323-52727-9.00013-3>
- Ismaili H, Milella L, Fkih-Tetouani S, Ildirissi A, Camporese A, Sosa S, Altinier G, Della Loggia R, Aquino R (2004). *In vivo* topical anti-inflammatory and *in vitro* antioxidant activities of two extracts of *Thymus satureioides* leaves. Journal of Ethnopharmacology 91:31-36. <https://doi.org/10.1016/j.jep.2003.11.013>
- Jaafari A, Mouse HA, Rakib EM, M'Barek LA, Tilaoui M, Benbakhta C, Boulli A, Abbad A, Ziad A (2007). Chemical composition and antitumor activity of different wild varieties of Moroccan thyme. Brazilian Journal of Pharmacognosy 17:477-491. <https://doi.org/10.1590/S0102-695X2007000400002>
- Kasrati A, Alaoui Jamali C, Fadli M, Bekkouche K, Hassani L, Wohlmuth H, Leach D, Abbad A (2014). Antioxidative activity and synergistic effect of *Thymus satureioides* Coss. essential oils with cefixime against selected food-borne bacteria. Industrial Crops Production 61:338-344. <https://doi.org/10.1016/j.indcrop.2014.07.024>
- Khorshidian N, Yousefi M, Khanniri E, Mortazavian AM (2018). Potential application of essential oils as antimicrobial preservatives in cheese. Innovative Food Science and Emerging Technologies 45:62-72. <https://doi.org/10.1016/j.ifset.2017.09.020>
- Khouya T, Ramchoun M, Hmidani A, El moualij B, Amrani S, Harnafi H, ... Alem C (2019). Acute toxicity and antiproliferative and procoagulant activities of fractions derived from *Thymus satureioides* of the Moroccan High Atlas. South African Journal of Botany 121:568-576. <https://doi.org/10.1016/j.sajb.2019.01.005>

- Konaté K, Mavoungou JF, Lepengué AN (2012). Antibacterial activity against  $\beta$ - lactamase producing methicillin and ampicillin-resistants *Staphylococcus aureus*: Fractional Inhibitory Concentration Index (FICI) determination. *Annals of Clinical Microbiology and Antimicrobials* 11:18.
- Lahnine L, Mghazli S, Mahrouz M, Hidar N, Ouhammou M, Mouhib M, Zantar S, ... Misdaq MA (2017). Decontamination by gamma irradiation at low doses of *Thymus satureioides* and its impact on physico-chemical quality. *Food Bioproduction Process* 104:48-56. <https://doi.org/10.1016/j.fbp.2017.04.006>
- Laila Z, Fatima G, Rachid M, Abdelaziz B, Chaouki AF (2014). Essential oil composition and biomass productivity of Moroccan endemic *Thymus satureioides* Coss. Ball. growing in the Agoundis Valley. *Journal of Medicinal Plants Research* 8:504-512. <https://doi.org/10.5897/jmpr2014.5373>
- Lal M, Baruah J, Begum T, Pandey SK (2020). Identification of a novel myrcene and methyl iso-eugenol rich essential oil variant (Jor Lab L-11) of lemongrass (*Cymbopogon flexuosus* L.). *Journal of Essential Oil-Bearing Plants* 23:660-668. <https://doi.org/10.1080/0972060X.2020.1823893>
- Lal M, Borah A, Pandey SK (2019). Identification of a new high geraniol rich variety 'Jor Lab L-15' of lemongrass [*Cymbopogon khasianus* (Hack) Stapf (ex Bor)]. *Journal of Essential Oil-Bearing Plants* 22:972-978. <https://doi.org/10.1080/0972060X.2019.1661797>
- Lawal OA, Ogunwande IA (2013). Essential oils from the medicinal plants of Africa. *Medicinal Plant Research in Africa: Pharmacology and Chemistry* 203-224. <https://doi.org/10.1016/B978-0-12-405927-6.00005-9>
- Marchese A, Orhan IE, Daglia M, Barbieri R, Di Lorenzo A, Nabavi SF, ... Nabavi SM (2016). Antibacterial and antifungal activities of thymol: A brief review of the literature. *Food Chemistry* <https://doi.org/10.1016/j.foodchem.2016.04.111>
- Mayaud L, Carricajo A, Zhiri A, Aubert G (2008). Comparison of bacteriostatic and bactericidal activity of 13 essential oils against strains with varying sensitivity to antibiotics. *Letters in Applied Microbiology* 47:167-173. <https://doi.org/10.1111/j.1472-765X.2008.02406.x>
- Meyer BK, Ni A, Hu B, Shi L (2007). Antimicrobial preservative use in parenteral products: Past and present. *Journal of Pharmaceutical Science* 96:3155-3167. <https://doi.org/10.1002/jps.20976>
- Munda S, Dutta S, Pandey SK, Sarma N, Lal M (2019). Antimicrobial activity of essential oils of medicinal and aromatic plants of the North East India: A biodiversity hot spot. *Journal of Essential Oil-Bearing Plants* 22:105-119. <https://doi.org/10.1080/0972060X.2019.1601032>
- NCCLS (2002a). Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts. *Approv. Stand. Ed. Doc. M27-A2* [ISBN 1-56238-469-4]. NCCLS, 940 West Val. Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
- NCCLS (2002b). NCCLS. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi. *Approv. Stand. Doc. M38-A* [ISBN 1-56238-470-8]. NCCLS, 940 West Val. Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA.
- Oussalah M, Caillet S, Saucier L, Lacroix M (2007). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E. coli* O157:H7, *Salmonella typhimurium*, *Staphylococcus aureus* and *Listeria monocytogenes*. *Food Control* 18:414-420. <https://doi.org/10.1016/j.foodcont.2005.11.009>
- Rahman M (2018). Application of computational methods in isolation of plant secondary metabolites. *Computational Phytochemistry* 107-139. <https://doi.org/10.1016/b978-0-12-812364-5.00004-3>
- Ramchoun M, Harnafi H, Alem C, Büchele B, Simmet T, Rouis M, Atmani F, Amrani S (2012). Hypolipidemic and antioxidant effect of polyphenol-rich extracts from Moroccan thyme varieties. *ESPEN Journal* 7. <https://doi.org/10.1016/j.clnme.2012.02.005>
- Ramchoun M, Sellam K, Harnafi H, Alem C, Benlyas M, Khallouki F, Amrani S (2015). Investigation of antioxidant and antihemolytic properties of *Thymus satureioides* collected from Tafilalet Region, south-east of Morocco. *Asian Pacific Journal of Tropical Biomedicine* 5:93-100. [https://doi.org/10.1016/S2221-1691\(15\)30151-9](https://doi.org/10.1016/S2221-1691(15)30151-9)
- Ramzi H, Ismaili MR, Aberchane M, Zaanoun S (2017). Chemical characterization and acaricidal activity of *Thymus satureioides* C. & B. and *Origanum elongatum* E. & M. (Lamiaceae) essential oils against Varroa destructor Anderson & Trueman (Acari: Varroidae). *Industrial Crops Production* 108:201-207. <https://doi.org/10.1016/j.indcrop.2017.06.031>
- Rebbas K, Bounar R (2014). Floristic and ethnobotanical study of medicinal plants of M'Sila (Algeria). *Phytotherapie* 12:284-291. <https://doi.org/10.1007/s10298-014-0872-4>
- Sakkas H, Papadopoulou C (2017). Antimicrobial activity of basil, oregano, and thyme essential oils. *Journal of Microbiology and Biotechnology* 27(3):429-438. <https://doi.org/10.4014/jmb.1608.08024>

- Sokolik CG, Ben-Shabat-Binyamini R, Gedanken A, Lellouche JP (2018). Proteinaceous microspheres as a delivery system for carvacrol and thymol in antibacterial applications. *Ultrasonics Sonochemistry* 41:288-296. <https://doi.org/10.1016/j.ultsonch.2017.09.032>
- Tahraoui A, El-Hilaly J, Israili ZH, Lyoussi B (2007). Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia province). *Journal of Ethnopharmacology* 110:105-117. <https://doi.org/10.1016/j.jep.2006.09.011>
- Taoufik F, Anejjar A, Asdadi A, Salghi R, Chebli B, El Hadek M, Idrissi Hassani LM (2017). Synergic effect between *Argania spinosa* cosmetic oil and *Thymus satureioides* essential oil for the protection of the carbon steel against the corrosion in sulfuric acid medium. *Journal of Materials and Environmental Science* 8:582-593.
- Wang L, Zhao X, Zhu C, Xia X, Qin W, Li M, ... Hu J (2017). Thymol kills bacteria, reduces biofilm formation, and protects mice against a fatal infection of *Actinobacillus pleuropneumoniae* strain L20. *Veterinary Microbiology* 203:202-210. <https://doi.org/10.1016/j.vetmic.2017.02.021>
- Zhou H, Tao N, Jia L (2014). Antifungal activity of citral, octanal and  $\alpha$ -terpineol against *Geotrichum citri-aurantii*. *Food Control* 37:277-283. <https://doi.org/10.1016/j.foodcont.2013.09.057>



The journal offers free, immediate, and unrestricted access to peer-reviewed research and scholarly work. Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.



**License** - Articles published in *Notulae Scientia Biologicae* are Open-Access, distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) License.

© Articles by the authors; SHST, Cluj-Napoca, Romania. The journal allows the author(s) to hold the copyright/to retain publishing rights without restriction.