HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb

Original article http://dx.doi.org/10.1016/j.apjtb.2016.08.002

Antimycobacterial natural products from Moroccan medicinal plants: Chemical composition, bacteriostatic and bactericidal profile of *Thymus satureioides* and *Mentha pulegium* essential oils

Marwa Chraibi[1], Abdellah Farah[2], Sara Lebrazi[1], Oumaima El Amine[1], Mohammed Iraqui Houssaini[1], Kawtar Fikri-Benbrahim[1*]

[1]*Laboratory of Microbial Biotechnology, Faculty of Science and Technology Saïss, Sidi Mohamed Ben Abdellah University, P.O. Box 2202, Fez, Morocco*

[2]*Laboratory of Aromatic Plants, Medicinal and Natural Substances, National Institute of Medicinal and Aromatic Plants, Phytochemistry Department, P.O. Box 159, Taounate – Principal, Morocco*

ARTICLE INFO

Article history:
Received 12 Nov 2015
Received in revised form 30 Nov 2015
Accepted 27 Dec 2015
Available online 25 Aug 2016

Keywords:
Thymus satureioides
Mentha pulegium
Essential oil
Chemical composition
Antimycobacterial activity

ABSTRACT

Objective: To evaluate the susceptibility of *Mycobacterium aurum* and *Mycobacterium smegmatis in vitro* to the essential oils obtained from two medicinal plants: *Thymus satureioides* (*T. satureioides*) and *Mentha pulegium* (*M. pulegium*), and to study their chemical composition.

Methods: The aerial parts of *T. satureioides* and *M. pulegium* (leaves and stems) were hydro-distillated using a Clevenger-type apparatus and essential oils were analyzed and identified by gas chromatography-mass spectrometry. Antimycobacterial screening of essential oils was performed on the basis of the inhibition zone diameter by disc diffusion method against two mycobacterial strains whereas the minimal inhibitory concentration and minimal bactericidal concentration were determined by using the micro-dilution method.

Results: Chemical analysis of their aerial part's essential oils gave as major compounds, borneol (34.26%), carvacrol (31.21%) and thymol (3.71%) for *T. satureioides* and R(+)-pulegone (75.48%), carvone (6.66%) and dihydrocarvone (4.64%) for *M. pulegium*. Thereafter their antimycobacterial effect evaluation, using the micro-dilution method, indicated that minimal inhibitory concentration values of *T. satureioides* essential oil ranged from 0.062% to 0.015% (v/v) and from 0.125% to 0.031% (v/v) for *M. pulegium* respectively against *Mycobacterium aurum* and *Mycobacterium smegmatis*.

Conclusions: It is clearly evident from the results obtained that the Moroccan medicinal plants have great potential to be used as anti-tuberculosis agents. These findings may help scientists to undertake several research projects to discover useful natural product as new anti-tuberculosis drug.

1. Introduction

The infectious killer disease, tuberculosis (TB), is the leading death cause worldwide from a single human pathogen. It still remains a great public health problem and comes at the second rank of death causes by infectious diseases worldwide.

Mycobacterium tuberculosis (*M. tuberculosis*), discovered by Robert Koch in 1882, is the usually responsible organism which typically affects the lungs (pulmonary TB), but can also affect other sites (extrapulmonary TB) [1]. In addition, the AIDS epidemic and the emergence of bacilli multi-resistant to antibiotics aggravates the impact of this disease. Indeed, HIV and Koch's bacillus make a dangerous combination, each of these two infectious agents helping the growth of the other [2].

According to the latest World Health Organization estimates, there were 9.0 million new TB cases in 2013 and 1.5 million TB deaths (1.1 million among HIV-negative people and 0.4

*Corresponding author: Kawtar Fikri-Benbrahim, Laboratory of Microbial Biotechnology, Faculty of Science and Technology Saïss, Sidi Mohamed Ben Abdellah University, P.O. Box 2202, Fez, Morocco.
Tel: +212 661 216 598
E-mail: kawtar.fikribenbrahim@usmba.ac.ma

Foundation Project: Supported by the Cooperation for Innovation in the Agro-Food Domain Project within the 7th Research and Development Framework Programme FP7-INCO-2013-9 (Grant agreement number 609495).

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

million among HIV-positive people) [1]. These alarming statistics indicate the devastating nature of TB.

The majority of *Mycobacterium* species are resistant to the most widely used therapeutic agents in the treatment of tuberculosis which is hydrazide of isonicotinic acid (isoniazid) [3]. Thus, there is an urgent need to search for and develop new, effective and affordable anti-TB drugs. Hence, recently growing interest has focused on naturally occurring molecules; in particular plant oils and crud extracts which have been used for a wide variety of purposes for many years.

The objective of this work is to explore opportunities, for drug discovery by researchers, which are offered by Morocco represented by its rich culture, traditions and natural biodiversity. So, this investigation was aimed to study the chemical composition of *Thymus satureioides* (*T. satureioides*) and *Mentha pulegium* (*M. pulegium*) essential oils, followed by a screening of the antimycobacterial activity. Furthermore, the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined.

2. Materials and methods

2.1. Plant material

The plants used in this work were *T. satureioides* and *M. pulegium*, freshly harvested and collected respectively from Ljoukak (30°59′50.365″ N, 8°9′45.13″ W, altitude 1 210 m) and Oued Laou (35°26′24″ N, 5°4′48″ W, altitude 69 m). The botanical authentication was confirmed at the National Institute of Medicinal and Aromatic Plants, Morocco.

2.2. Essential oils extraction

The aerial parts of *T. satureioides* and *M. pulegium* (leaves and stems) were hydro-distillated using a Clevenger-type apparatus to recover the essential oils for 3 h. The distilled essential oils were kept in dark at 4 °C until further use.

2.3. Gas chromatography-mass spectrometry (GC–MS) analysis conditions

The essential oil was analyzed using GC–MS (Polaris Q ion trap MS). Hence, analyses were performed on a Hewlett-Packard (HP 6890) gas chromatograph (flame ionization detector), equipped with a 5% phenyl methyl silicone HP-5 capillary column (30 m × 0.25 mm × film thickness 0.25 μm). The temperature was programmed from 50 °C after 5 min initial hold to 200 °C at 4 °C/min. Chromatography carrier gas was N_2 (1.8 mL/min); split mode was used with a flow of 72.1 mL/min and a ratio of 1/50; temperature of injector and detector was 250 °C, and final hold time was 48 min. The machine was led by a computer system type "HP Chem Station", managing its functioning and allowing to follow the evolution of chromatographic analyses. Diluted samples (1/20 in methanol) of 1 μL were injected manually.

2.4. Microbial strains

The essential oils of *T. satureioides* and *M. pulegium* were tested for their antimycobacterial activity against two reference microbial strains.

Mycobacterium aurum A+ (*M. aurum*) is a non-pathogenic *Mycobacterium* species with a generation time of approximately 6 h. This strain was used as a model to evaluate the effect of active substances on the growth of *M. tuberculosis*.

Mycobacterium smegmatis mc2-155 (*M. smegmatis*) is a non-pathogenic atypical mycobacterial strain with a generation time of approximately 3 h.

These strains were maintained in 20% glycerol at −20 °C and sub-cultured before use. The mycobacteria were cultivated at 37 °C on Sauton's medium for 48–72 h [4,5]. The turbidity was adjusted at 10^6 UFC/mL (estimated by absorbance at 600 nm).

2.5. Disc diffusion method

A primary antimycobacterial screening was performed using the disc diffusion method according to National Committee for Clinical Laboratory Standards [6]. Briefly, Petri dishes containing Sauton agar culture medium were inoculated with a previously prepared mycobacterial inoculum. The discs (filter paper, 6 mm of diameter) placed in the center of each plate were impregnated with 5 μL of each essential oil. Petri dishes were placed at 4 °C for 2 h to allow a better diffusion of molecules and then incubated at 37 °C for 48–72 h.

The antimycobacterial activity was evaluated by measuring the diameter of inhibition zone in millimeters. All experiments were conducted in triplicates.

2.6. Determination of MIC

The MIC values, which represent the lowest essential oil concentration that completely inhibits the growth of mycobacteria, were performed in 96 well-microplate using the microdilution assay according to the protocol previously described by Bouhdid *et al.* [7] with slight modifications.

Due to the immiscibility of essential oils with water and thus the culture medium, each essential oil was serially diluted in Sauton broth supplemented with agar 0.15% (w/v), used as an emulsifier, in which the final concentration of the essential oil was between 8.000% and 0.007% (v/v) for *T. satureioides* and *M. pulegium*. The 12th well was considered as growth control (it contained only the culture medium and strain). Then, 50 μL of bacterial inoculum was added to each well at a final concentration of 10^6 CFU/mL. After incubation at 37 °C for 48–72 h, 10 μL of rezasurin were added to each well as mycobacterial growth indicator. After further incubation at 37 °C for 2 h, the bacterial growth was revealed by the change of coloration from purple to pink [7]. Experiments were carried out in triplicates to minimize experimental error.

2.7. Determination of MBC

The MBC was determined by inoculating 3 μL from each negative well, which were spotted on Sauton plates and incubated at 37 °C for 48–72 h. The MBC corresponded to the lowest concentration of the essential oil at which the incubated microorganism was completely killed [8]. Tests were performed in triplicates.

3. Results

3.1. Composition of essential oils

The results of chromatographic analysis of *M. pulegium* essential oils are presented in Table 1, which showed that 18 volatile compounds accounting for 98.77% of this essential oil were detected. The main components were R(+)-pulegone (75.48%), carvone (6.66%) and dihydrocarvone (4.64%). Beside other compounds with relatively low levels, including *p*-mentha-3,8-diene (2.44%), limonene (1.88%), pinocarvone (1.27%), α-peperitone (1.13%), octanol-3 (1.86%) were found.

Chemical analysis of *T. satureioides* essential oil revealed 20 different compounds accounting for 96.17% of its composition (Table 2). This essential oil contained a complex mixture consisting mainly by oxygenated monoterpenes, such as borneol (34.26%), carvacrol (31.21%), thymol (3.71%) and α-terpineol (1.16%), with borneol, carvacrol and E-caryophyllene (6.32%) as the major constituents.

Table 1
Chemical composition of *M. pulegium* essential oil.

RI	Compounds*	Percentage (%)**
939	α-Pinene	0.52
952	Cyclohexanone-3-methyl	0.26
980	β-Pinene	0.39
993	Myrcene	0.16
994	Octanol-3	1.86
1001	δ-2-Carene	0.07
1031	Limonene	1.88
1072	*p*-Mentha-3,8-diene	2.44
1154	Menthone	0.19
1168	Pinocarvone	1.27
1173	Menthol	0.72
1194	Dihydrocarvone	4.64
1238	R(+)-pulegone	75.48
1242	Carvone	6.66
1252	α-Peperitone	1.13
1419	Caryophyllene	0.33
1630	γ-Eudesmol	0.28
1649	α-Eudesmol	0.49
	Total	98.77

RI: Retention index; *: Constituents identified by GC–MS; **: Percentages of compounds provided by GC.

Table 2
Chemical composition of *T. satureioides* essential oil.

RI	Compounds*	Percentage (%)**
931	α-Thujene	0.04
939	α-Pinene	2.14
967	Verbenene	2.34
976	Sabinene	1.24
980	β-Pinene	1.44
1011	δ-3-Carene	0.34
1026	*p*-Cymene	2.29
1031	Limonene	0.38
1033	1,8-Cineole	1.34
1062	γ-Terpinene	2.12
1087	Fenchone	2.54
1139	Trans-pinocarveol	0.87
1143	Camphre	0.43
1165	Borneol	34.26
1189	α-Terpineol	1.16
1290	Thymol	3.71
1295	Bornylacetate	1.97
1298	Carvacrol	31.21
1418	E-caryophyllene	6.32
1440	β-humulene	0.03
	Total	96.17

RI: Retention index; *: Constituents identified by GC–MS; **: Percentages of compounds provided by GC.

3.2. Antimycobacterial activity of essential oils

The antimycobacterial activities of *T. satureioides* and *M. pulegium* essential oils against mycobacteria were examined in the present study and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and their diameter, the MIC and the MBC values.

The study of antimycobacterial power of the two essential oils had shown their effect on *M. smegmatis* and *M. aurum*. This effect resulted in the inhibition of bacterial growth around the disc containing the pure essential oil.

The essential oil of *T. satureioides* showed an important activity against mycobacterial studied strains, inhibition zone diameters ranged from 18.5 to 28.0 mm for *M. smegmatis* and *M. aurum* respectively, likewise *M. pulegium* essential oils had a remarkable activity against the two tested mycobacterial strains, but less important than that of *T. satureioides* oil, with inhibition zone diameters of (12.50 ± 0.07) and (19.50 ± 0.07) mm for *M. smegmatis* and *M. aurum* respectively.

Indeed, the inhibition zone diameters obtained for *T. satureioides* were higher than those obtained for *M. pulegium* and inhibition zone diameter corresponding to *M. aurum* (28.00 ± 0.28) mm were more important than those of *M. smegmatis* (18.50 ± 0.21) mm.

The results of the essential oil's MIC and MBC against *M. aurum* and *M. smegmatis* represented in Table 3 showed that all tested essential oils had an important antibacterial effect. Indeed, the MIC values of *T. satureioides* essential oil ranged from 0.062% to 0.015% (v/v) and from 0.125 to 0.031% (v/v) for *M. pulegium* oil against the mycobacterial studied strains. Hence, *T. satureioides* essential oil exhibited a higher antibacterial effect with MIC values 2 fold least compared to *M. pulegium* essential oil against *M. aurum* and *M. smegmatis*. Furthermore, *M. smegmatis* was more resistant than *M. aurum* to *M. pulegium* oils tested with MIC value of 0.125% (v/v).

Regarding the MBC values of both essential oils tested (Table 3) it seems that they could be similar to their MIC values against *M. aurum* and *M. smegmatis*. In fact, the MBC values of

Table 3
Determination of MIC and MBC values of *T. satureioides* and *M. pulegium* essential oils against *M. smegmatis* and *M. aurum*.

Concentration (v/v) %	*T. satureioides*				*M. pulegium*			
	M. smegmatis		*M. aurum*		*M. smegmatis*		*M. aurum*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
8.000	–	–	–	–	–	–	–	–
4.000	–	–	–	–	–	–	–	–
2.000	–	–	–	–	–	–	–	–
1.000	–	–	–	–	–	–	–	–
0.500	–	–	–	–	–	–	–	–
0.250	–	–	–	–	–	–	–	–
0.125	–	–	–	–	–	–	–	–
0.062	–	–	–	–	+	+	–	–
0.031	+	+	–	–	+	+	–	–
0.015	+	+	–	–	+	+	+	+
0.007	+	+	+	+	+	+	+	+

T. satureioides and *M. pulegium* essential oils were 0.062%, 0.015% and 0.125%, 0.031% (v/v) against *M. smegmatis* and *M. aurum* respectively. So, these essential oils can have a bactericidal action against the studied strains.

4. Discussion

Several substances extracted from plants have been widely used in traditional medicine worldwide for the treatment of various diseases. About 60% of the population use medicinal plants to heal themselves [9]. Indeed, the plants represent a rich source of several biologically active compounds that can serve as treatment against several diseases including TB. In fact *M. tuberculosis* and humans have always cohabited because the natural reservoir of this bacterium is the human species. So, the eradication of TB is therefore a potential target in the medium term future then the discovering of effective new drugs is an indispensable priority.

Many works have been consecrated in recent years to the medical properties of herbs and especially the antibacterial properties of their fragrant extracts: essential oil [9–11]. These volatiles were scientifically proven active against several pathogenic strains and having many activities such as antioxidant, anticancer and antifungal activities [12–16].

Therefore, this study aimed to determinate the chemical composition and antimycobacterial activity of two endemic plants commonly used in the traditional medicine in Morocco.

Regarding the chemical composition of *M. pulegium* essential oil, 18 volatile compounds accounting for 98.77% were detected. Similar chemical composition has been found for this essential oil in Iran, India, Portugal and Turkey especially for the content of pulegone which is reported as its major component, but in different proportions [17–19].

Nonetheless, there is a great variability in the chemical composition of *M. pulegium* essential oil among the studies performed so far [20–22]. Such variability may be related with different plant's vegetative phases and also with environmental conditions (seasonal and geographical variations, soil composition) [23]. However, volatiles of *T. satureioides* essential oil revealed 20 different compounds accounting for 96.17% of its composition whose majority compounds are borneol (34.26%), carvacrol (31.21%) and E-caryophyllene (6.32%). This chemical composition of *T. satureioides* essential oil is broadly similar to that of *T. satureioides* from Ourika mainly composed by carvacrol (26.5%), borneol (20.1%), E-caryophyllene (5.7%) and α-pinene (4.6%) [24] and significantly analogue to the results obtained by El Bouzidi *et al.* [25] who found camphen (at a percentage of 8%) which haven't been detected in our tested essential oil.

The essential oil of *T. satureioides* have a more important inhibitory activity than *M. pulegium* against the two mycobacterial studied strains, especially for *M. aurum* which have shown a high sensitivity and a very low MIC value: 0.015% (v/v). Moreover, the concentration of 0.062% (v/v) was sufficient to stop the growth of *M. smegmatis*.

This antibacterial activity is mainly attributed to various chemical compounds isolated by hydrodistillation. Hence, monoterpenes hydrocarbon, oxygenated monoterpenes such as borneol, pulegone and carvacrol were reported to be responsible for the antimicrobial activity of several essential oils [26]. In fact Sarrazin *et al.* [27] have demonstrated that the thymol is able to alter the outer membrane and the carvacrol can destabilize the cytoplasmic membrane, act on proton exchange and induce depletion of the adenosine triphosphate pool, thereby reducing the pH gradient across the membrane and conducting to cell death. In addition, terpineols are known for their higher efficiency and broader spectrum of antimicrobial activity [28–30].

The importance of the hydroxyl group has been confirmed and its relative position on the phenolic ring does not appear to strongly influence the degree of antibacterial activity [31,32]. However the essential oil's activity is due not only to the major compounds but also to the synergistic effect of minor compounds [33,34] and the major constituents are not necessarily responsible for the total antimicrobial activity; hence, the consideration of antimicrobial power of the minority compounds should be taken into consideration [10].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

This work was co-funded by the Cooperation for Innovation in the Agro-Food Domain Project within the 7th Research and Development Framework Programme FP7-INCO-2013-9 (Grant agreement number 609495).

References

[1] World Health Organization. Global tuberculosis report 2014. Geneva: World Health Organization; 2014. [Online] Available from: http://www.who.int/tb/publications/global_report/gtbr14_main_text.pdf [Accessed on 15th December, 2015]

[2] Naidoo K, Grobler AC, Deghaye N, Reddy T, Gengiah S, Gray A, et al. Cost-effectiveness of initiating antiretroviral therapy at different points in TB treatment in HIV-TB coinfected ambulatory patients in South Africa. *J Acquir Immune Defic Syndr* 2015; **69**(5): 576-84.

[3] Van Ingen J. Microbiological diagnosis of nontuberculous mycobacterial pulmonary disease. *Clin Chest Med* 2015; **36**(1): 43-54.

[4] Fonseca L, Moore D, Durier N. Inventory of methods for mycobacterial culture and phenotypic drug susceptibility testing (DST). MTB culture and phenotypic drug susceptibility testing – methods inventory; 2011. [Online] Available from: http://www.stoptb.org/wg/new_diagnostics/assets/documents/Inventory%20of%20culture%20and%20DST%20methods%20-%20July%2031%202011%20-%20final.pdf [Accessed on 15th December, 2015]

[5] Papa F, Rivière M, Fournié JJ, Puzo G, David H. Specificity of a *Mycobacterium kansasii* phenolic glycolipid (mycoside A) immunoserum. *J Clin Microbiol* 1987; **25**(12): 2270-3.

[6] National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests; approved standard – 11 ed. Wayne: National Committee for Clinical Laboratory Standards; 2012, p. 76. [Online] Available from: http://antimicrobianos.com.ar/ATB/wp-content/uploads/2012/11/01-CLSI-M02-A11-2012.pdf [Accessed on 15th December, 2015]

[7] Bouhdid S, Abrini J, Zhiri A, Espuny MJ, Manresa A. Investigation of functional and morphological changes in *Pseudomonas aeruginosa* and *Staphylococcus aureus* cells induced by *Origanum compactum* essential oil. *J Appl Microbiol* 2009; **106**: 1558-68.

[8] Bassole HN, Kabore ZI, Traore AS. [Study of bacteriostatic and bactericid profiles of plant extracts against pathogenic microorganisms linked to contamination of food of animal origin]. *Pharm Med Trad Afr* 2001; **11**: 113-22. French.

[9] Radji M, Kurniat M, Kiranasar A. Comparative antimycobacterial activity of some Indonesian medicinal plants against multi-drug resistant *Mycobacterium tuberculosis*. *J Appl Pharm Sci* 2015; **5**(1): 19-22.
[10] Sadiki M, Elabed A, Elaabedy A, Elabed S, Farah A, Iraqui M, et al. Characterization and antibacterial activity of the essential oil from *Thymus vulgaris* cultivated in Morocco (Taounate) against ten bacteria. *World J Pharm Res* 2015; **5**: 314-25.
[11] Ouedrhiri W, Bouhdid S, Balouiri M, El Ouali Lalami A, Moja S, Chahdi FO, et al. Chemical composition of *Citrus aurantium* L. leaves and zest essential oils, their antioxidant, antibacterial single and combined effects. *J Chem Pharm Res* 2015; **7**(1): 78-84.
[12] Sarac N, Ugur A, Sen B. *In vitro* antimutagenic activity of *Vitex agnus-castus* L. essential oils and ethanolic extracts. *Ind Crop Prod* 2015; **63**: 100-3.
[13] Al Nomaani RS, Hossain MA, Weli AM, Al-Riyami Q, Al-Sabahi JN. Chemical composition of essential oils and *in vitro* antioxidant activity of fresh and dry leaves crude extracts of medicinal plant of *Lactuca sativa* L. native to Sultanate of Oman. *Asian Pac J Trop Biomed* 2013; **3**(5): 353-7.
[14] Taguchi Y, Ishibashi H, Takizawa T, Inoue S, Yamaguchi H, Abe S. Protection of oral or intestinal candidiasis in mice by oral or intragastric administration of herbal food, clove (*Syzygium aromaticum*). *Nihon Ishinkin Gakkai Zasshi* 2005; **46**(1): 27-33.
[15] Rodríguez O, Sánchez R, Verde M, Núñez M, Ríos R, Chávez A. Obtaining the essential oil of *Syzygium aromaticum*, identification of eugenol and its effect on *Streptococcus mutans*. *J Oral Res* 2014; **3**(4): 218-24.
[16] Timsina B, Shukla M, Nadumane KV. A review of few essential oils and their anticancer property. *J Nat Pharm* 2012; **3**(1): 1-8.
[17] Agnihotri VK, Agarwal SG, Dhar PL, Thappa RK, Baleshwara, Kapahi BK, et al. Essential oil composition of *Mentha pulegium* L. growing wild in the North-western Himalayas India. *Flavour Frag J* 2005; **20**: 607-10.
[18] Aghel N, Yamini Y, Hadjiakhoondi A, Pourmortazavi SM. Supercritical carbon dioxide extraction of *Mentha pulegium* L. essential oil. *Talanta* 2004; **62**: 407-11.
[19] Teixeira B, Marques A, Ramos R, Batista I, Serrano C, Matos O, et al. European pennyroyal (*Mentha pulegium*) from Portugal: chemical composition of essential oil and antioxidant and antimicrobial properties of extracts and essential oil. *Ind Crop Prod* 2012; **36**: 81-7.
[20] Kamkar A, Javan AJ, Asadi F, Kamalinejad M. The antioxidative effect of Iranian *Mentha pulegium* extracts and essential oil in sunflower oil. *Food Chem Toxicol* 2010; **48**: 1796-800.
[21] Morteza-Semnani K, Saeedi M, Akbarzadeh M. Chemical composition and antimicrobial activity of the essential oil of *Mentha pulegium* L. *J Essen Oil Bear Plants* 2011; **14**(2): 208-13.
[22] Cherrat L, Espina L, Bakkali M, Pagán R, Laglaoui A. Chemical composition, antioxidant and antimicrobial properties of *Mentha pulegium, Lavandula stoechas* and *Satureja calamintha* Scheele essential oils and an evaluation of their bactericidal effect in combined processes. *Innov Food Sci Emerg Technol* 2014; **22**: 221-9.
[23] Scognamiglio M, D'Abrosca B, Esposito A, Fiorentino A. Chemical composition and seasonality of aromatic Mediterranean plant species by NMR-based metabolomics. *J Anal Methods Chem* 2015; **2015**: 258570.
[24] Kasrati A, Jamali CA, Fadli M, Bekkouche B, Hassani L, Wohlmuth H, et al. Antioxidative activity and synergistic effect of *Thymus satureoides* Coss. essential oils with cefixime against selected food-borne bacteria. *Ind Crop Prod* 2014; **61**: 338-44.
[25] El Bouzidi L, Jamali CA, Bekkouche K, Hassani L, Wohlmuth H, Leach D, et al. Chemical composition, antioxidant and antimicrobial activities of essential oils obtained from wild and cultivated Moroccan *Thymus* species. *Ind Crop Prod* 2013; **43**: 450-6.
[26] Reichling J, Schnitzler P, Suschke U, Saller R. Essential oils of aromatic plants with antibacterial, antifungal, antiviral and cytotoxic properties – an overview. *Forsch Komplementmed* 2009; **16**: 79-90.
[27] Sarrazin SLF, Da Silva LA, Oliveira RB, Raposo JDA, Da Silva JKR, Salimena FRG, et al. Antibacterial action against food-borne microorganisms and antioxidant activity of carvacrol-rich oil from *Lippia origanoides* Kunth. *Lipids Health Dis* 2015; **14**(1): 145-52.
[28] Zengin H, Baysal AH. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. *Molecules* 2014; **19**: 17773-98.
[29] Liu G, Wu G, Jin C, Kong Z. Preparation and antimicrobial activity of terpene-based polyurethane coatings with carbamate group-containing quaternary ammonium salts. *Prog Organ Coat* 2015; **80**: 150-5.
[30] Durán-Peña MJ, Ares JMB, Hanson JR, Collado IG, Hernández-Galán R. Biological activity of natural sesquiterpenoids containing a gem-dimethyl cyclopropane unit. *Nat Prod Rep* 2015; **32**: 1236-48.
[31] Leopoldini M, Pitarch IP, Russo N, Toscano M. Structure conformation, and electronic properties of apigenin, luteolin, and taxifolin antioxidants. A first principle theoretical study. *J Phys Chem A* 2004; **108**: 92-6.
[32] Shan B, Cai YZ, Sun M, Corke H. Antioxidant capacity of 26 spice extracts and characterization of their phenolic constituents. *J Agric Food Chem* 2005; **53**(20): 7749-59.
[33] Ouraini D, Agoumi A, Ismaili-Alaoui M, Alaoui K, Cherrah Y, Alaoui MA, et al. Antifungal activity of oleic acid and essential oils of *Thymus satureioides* L. and *Mentha pulegium* L. compared to antifungal in mycotic dermatitis. *Phytotherapy* 2007; **1**: 6-14.
[34] Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Alvarez J. Antibacterial activity of different essential oils obtained from species widely used in Mediterranean diet. *Int J Food Sci Technol* 2008; **43**: 526-31.