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Antibacterial and Antiviral Activities of Essential Oils of Northern Moroccan Plants

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Authors' contributions

This work was carried out in collaboration between all authors Author NEM has performed the overall practical study, interpreted and wrote the first draft of the manuscript. Author GS has designed the protocol of the antiviral activity and read the respected outcomes. Author AIBNM has read the result of the chemical composition of essential oils. Authors RA and JA have designed the protocol of antibacterial activity. Author EOK provided technical advices. All authors read and approved the final manuscript.

Research Article

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ABSTRACT

Aims: This study was designed to evaluate the antimicrobial activity of five essential oils (EOs) extracted from the aerial parts (leaves and flowering tops) of three species growing in the north of Morocco: *Origanum elongatum*, *Thymus capitatus* and *Mentha suaveolens*.
Study Design: Chemical analysis of EOs, antibacterial and antiviral activities of EOs.
Place and Duration of Study: Department of Biology (Faculty of Sciences), Institute of Agrochemistry and Food Technology (IATA), between September 2009 and December 2009.
Methodology: The EO constituents were extracted by hydrodistillation and analysed by

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GC-MS. The antibacterial activity of EOs was tested against three reference strains, *Salmonella enterica* subsp. *enterica* CECT 915^T, *Listeria monocytogenes* CECT 4031^T, and *Escherichia coli* O157:H7 CECT 4267, and two food isolated strains *Salmonella* sp. S64 and *Listeria monocytogenes* L23, using the diffusion method and the microtitration assays. The antiviral effect of EOs was evaluated for the inactivation of murine norovirus (MNV-1), a human norovirus surrogate.

Results: GC and GC/MS analyses revealed that thyme EO predominantly contains carvacrol (58.77-68.63%), *p*-cymene (4.84-5.63%), γ-terpinene (2.78-3.75%) and β-caryophyllene (2.62-2.91%). Oregano EO was mainly constituted by carvacrol (19.21-40.12%), thymol (3.57-14.24%), *p*-cymene (16.08-16.19%) and γ-terpinene (7.27-13.48%). While, mint EO was characterized by piperitenone oxide (41.84%), (-)-isopulegol (11.95%) and limonene (7.35%). All extracts exhibited an antibacterial activity at different levels against strains reported as the causal agents of foodborne diseases, but a low antiviral activity (0.87-0.50 $\log_{10}$ $TCID_{50}$/ml reduction) was observed.

Conclusion: Results suggest the potential use of tested EOs as bio-preservatives in the food industry. However, their antiviral activity needs to be further investigated.

Keywords: Origanum elongatum; Thymus capitatus; Mentha suaveolens; essential oil; antibacterial and antiviral activity.

1. INTRODUCTION

Since society is experiencing a trend toward 'green consumerism', with a desire of fewer synthetic food additives and products with a smaller impact on the environment, the use of naturally derived antimicrobials has substantially increased in the last decade.

In this sense, biopreservatives are a wide range of natural products that can be used to reduce or eliminate pathogen populations while increasing food quality. Among this type of antimicrobials, essential oils (EOs) have long been applied as flavouring agents in food, and due to their content in antimicrobial compounds, they have potential as natural agents for food preservation [1].

Essential oils or their main active compounds have been reported to possess a wide spectrum of antibacterial [2,3], antiviral [4,5], antifungal [6], antioxidant [7], antiparasitic [8], insecticidal [9] and cytotoxic properties [10].

Essential oils are the volatile oily liquids of the secondary metabolism of scented plants, which are obtained from different plant parts, such as flowers, leaves, seeds, bark, fruits and roots. They are also widely used as food flavours and preservatives to prevent growth of food-borne bacteria and moulds, and so to extend the shelf life of processed foods [1]. Recently, the antiviral effects of different EOs have been evaluated on human norovirus surrogates [11,12].

We report here the antimicrobial properties and the chemical composition of essential oils of three plants (leaves and flowering tops). The antibacterial activity of EOs was evaluated on three reference strains, *Salmonella enterica* subsp. *enterica* CECT 915^T, *Listeria monocytogenes* CECT 4031^T, and *Escherichia coli* O157:H7 CECT 4267, and two food isolated strains, *Salmonella* sp. S64 and *Listeria monocytogenes* L23. We determined the zone of growth inhibition, minimum inhibitory and bactericidal concentrations (MIC and

MBC). The antiviral activity was tested on the murine norovirus (MNV-1) a human norovirus surrogate by determining the 50% tissue culture infectious dose ($TCID_{50}$) in RAW 264.7 cells.

2. MATERIALS AND METHODS

2.1 Plant Materials and Hydrodistillation

Three plants were collected during the April-August period of 2009 in the areas around Tetouan, and Al-Housaima (northern Morocco). They were identified by Prof. M. Kadiri (Department of Biology, Faculty of sciences, Tetouan, Morocco) and Prof. A. Ennabili (National Institute of Medicinal and Aromatic Plants- Taounate, University Sidi Mohamed Ben Abdellah, Fes, Morocco). Voucher specimens were deposed in the herbarium of this Institute of Taounate: *Thymus capitatus* (L.) Hoffmanns. & Link, (leaves, flowering tops) (INP 1235), *Origanum elongatum* (Bonnet) Emb. & Maire (leaves, flowering tops) (INP 1234) and *Mentha suaveolens* Ehrh. (leaves) (INP 1237).

Aerial parts of collected plants were air-dried at room temperature and stored in a dry place prior to use, then grossly pulverized and subjected to hydrodistillation for 3 h using a Clevenger apparatus.

2.2 Essential Oils Chemical Analysis

The chemical composition of essential oils were analysed using a gas chromatograph (*TRACE GC Ultra*) fitted to a mass spectrometer (*Polaris Q-Ion Trap MS*). Operating in electron-impact EI (70 eV) mode. VB-5 (Methylpolysiloxane 5% phenyl) and a column (30 m × 0.25 mm × 0.25 µm thickness) were used (*Centre National pour la Recherche Scientifique et Technique- CNRST*, Rabat, Morocco). The chromatographic conditions were as follows: Injector and detector temperatures at 220 and 300℃, respectively; carrier gas, helium at flow rate of 1.4 ml/min; temperature program ramp from 40 to 300℃ with gradient of 4℃/min (holding the initial and final temperature for 4 min). The relative amount of individual components of the total oil was expressed as a percentage peak area relative to total peak area. Library search was carried out using the combination of NIST MS Search and literature.

2.3 Bacterial Strains

Experiments were performed on three reference strains supplied by the Spanish Type Culture Collection (CECT): *Salmonella enterica* subsp. *enterica* CECT 915^T, *Listeria monocytogenes* CECT 4031^T, and *Escherichia coli* O157:H7 CECT 4267, and two food isolates supplied by the Public Health Service of Valencia (Spain), one *Salmonella* sp isolated from mayonnaise S64 and one *Listeria monocytogenes* L23 from seafood salad.

Stock bacterial inoculum suspensions were obtained from 18 h culture in Lysogeny broth (LB) at 37ºC. Afterwards, the strains were re-inoculated and incubated approximately for three hours for *E. coli* and *Salmonella*, and five hours for *Listeria*. Those final suspensions served for the inocula preparation. The cell density of each suspension was determined spectrophotometrically, and then adjusted to a concentration of 10^6 CFU/ml.

2.4 Virus and Cell line

The cytopathogenic murine norovirus (MNV-1) (kindly provided by Prof. H. W. Virgin, Washington University School of Medicine, USA) was propagated and assayed in RAW 264.7 cells. Semi-purified stocks were subsequently produced from the same cells by centrifugation of infected cell lysates at 660 × g for 30 min. Infectious viruses were enumerated by determining the 50% tissue culture infectious dose ($TCID_{50}$) with eight wells per dilution and 20 µl of inoculum per well.

RAW 264.7 cells were maintained in Dulbecco's Minimum Essential Medium (DMEM, HyClone), supplemented with 10% of fetal bovine serum (FBS, HyClone), 100 U/ml penicillin, 100 µg/ml streptomycin (HyClone), 10 mM hydroxyethyl piperazine ethane sulphonic acid buffer (HEPES, HyClone) and 2 mM L-glutamine (HyClone). RAW 264.7 cells were grown in humidified atmosphere of 5% carbon dioxide at 37ºC.

2.5 Antibacterial Activity Tests

2.5.1 Agar diffusion method

The disk-diffusion assay was used to test antibacterial activity of the essential oils against five bacteria according to Bauer et al. [13]. Sterile filter paper disks (6 mm in diameter) impregnated with 10 µl of EO were placed on nutrient agar medium uniformly seeded with a broth culture (10^6 CFU/ml) of the test microorganisms. These plates were then kept at low temperature (4ºC) for 2 h to allow maximum diffusion, and incubated at 37ºC for 24 h. The diameter in millimetre of the inhibition zones around the disks was recorded. All the tests were performed in triplicate. ANOVA test was used with STATISTICA 6.0 software. Differences were considered significant if $p \leq 0.05$.

2.5.2 Microtitration method

Essential oils were diluted in Lysogeny broth (LB) supplemented with bacteriological agar 0.15% (w/v). Serial twofold dilutions, ranging from 2 % to 0.0039% (v/v) of essential oil, were prepared in a 96-well microtitre plate, volume being 50 µl. Wells were then inoculated with 50 µl of microbial suspension at final concentration of 10^6 CFU/ml. The covered microplates were incubated overnight at 37ºC. To assess microbial growth, 10 µl of resazurin were added to the wells. After incubation at 37ºC for 2 h, the minimum inhibitory concentration (MIC) was then determined as the lowest essential oil concentration prevented change of colouring of resazurin [14]. The minimum bactericidal concentration (MBC) corresponded to the lowest concentration of the essential oils yielding negative subcultures after incubation at appropriate temperature for 24 h. It was determined in broth dilution tests by subculturing 10 µl from negative wells on PCA medium [14].

2.6 Antiviral Activity Assays

EOs at 2% were added to MNV suspensions in DMEM with 2% FCS and further incubated at 37ºC for 1 h. After that, RAW 264.7 cells were inoculated with serial dilutions of MNV-1 and EOs mixture from 10^0 to 10^{-8}. Positive controls were MNV suspensions without EO. Infectious viruses were enumerated by cell-culture assays as described above. Virucidal activity of EOs was estimated by comparing the number of infectious viruses on suspensions

without EOs and on the EO-treated virus suspensions. The log reduction was calculated as follows: Reduction = $\log_{10}$ (*treated cells / untreated cells*).

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Essential Oils

Essential oils were isolated by hydrodistillation from the aerial parts of *Thymus capitatus* (leaves, flowering tops), *Origanum elongatum* (leaves, flowering tops) and *Mentha suaveolens* (leaves). The mains components are summarized in Table 1.

Thymus capitatus EO was mainly constituted by carvacrol (58.77-68.63%), along with other components to relatively low levels of *p*-cymene (4.84-5.63%), γ-terpinene (2.78-3.75%) and β-caryophyllene (2.62-2.91%). It was substantially similar to that of EOs originating in Tunisia collected during different phases of plant development, and from different locations [15]. Many authors have found that carvacrol is the main component of thyme EOs: El Ajjouri et al. [16] (70.92%); Faleiro et al. [17] (79%); Hedhili et al. [18] (53.71%); Bouzouita et al. [2] (62-83%); Benjilali et al. [19] (78%). Similarly, Turkish thyme EO was dominated by only 35.6% of carvacrol followed by 18.6% of thymol [20]. However, the main constituents of thyme EO from Sardinia are thymol (29.3%) and *p*-cymene (26.4%), while, carvacrol represents only 10.8% of the species [21].

Besides, the major components of oregano EO were carvacrol (19.21-40.12%), thymol (3.57-14.24%), *p*-cymene (16.08-16.19%) and γ-terpinene (7.27-13.48%). Several authors have characterized an oregano EO rich of carvacrol at different levels (36.6-76.6% [22], 62.8-79.2% [23] and 24.5-51.6% [24]). In addition, the two components *p*-cymene (16.08%) and γ-terpinene (7.27%) of oregano EO obtained from flowering tops was near to those analysed by Figueredo et al. [23]. Also, the EO obtained from leaves differed appreciably with non-negligible levels of thymol (14.24%) like it found by Figueredo et al. [23] with two samples (13.6% and 17.2%). These analyses indicate that thyme [21] and oregano EOs [23] are not always dominated by carvacrol, as we found for oregano EOs.

On the other hand, mint EO was characterized by piperitenone oxide (41.84%), (-)-isopulegol (11.95%) and limonene (7.35%). In fact, in most cases, mint EOs were mainly composed by menthanic oxygenated monoterpenes [6,25,26,27,28,29,30,31]. Besides, Oumzil et al. [6] eminent three profiles of mint EOs depending on the subspecies. One of these profiles was dominated by piperitenone oxide (56%) as found in our oil. The same was reported for mint EO from Japan (87.3%) [25].

Table 1. Main components of tested essential oils

RT (min)	Compounds	*M. suaveolens*	*O. elongatum*		*T. capitatus*	
		L	L	FT	L	FT
11.42	*p*-Cymene	0.42	16.19	16.08	4.84	5.63
11.59	Limonene	7.35				
12.68	γ-Terpinene	1.06	13.48	7.27	3.75	2.78
16.83	(-)-Isopulegol	11.95				
20.98	Thymol		14.24	3.57	0.04	0.07
21.28	Carvacrol		19.21	40.12	68.63	58.77
23.24	Piperitenone oxide	41.84				
24.93	β-Caryophyllene	1.32	1.38	0.86	2.91	2.62

L: leaves; FT: flowering tops; RT: retention time.

3.2 Antibacterial Activity

Initial screening of the antibacterial activity of the investigated EOs was studied against five tested microorganisms using the agar disk diffusion assay. The antibacterial activity of EOs can be classified into three levels [32,33]: (i) weak activity (inhibition zone≤12 mm), (ii) moderate activity (12 mm<inhibition zone<20 mm) and (iii) strong activity (inhibition zone≥20 mm). Results from the agar disk diffusion tests for antimicrobial activity of the EOs are shown in Table 2.

Significant difference of activities of the investigated EOs against the tested bacterial strains was observed (P = 0.000). The oregano and thyme EOs showed the highest activity (P = 0.06) against all the tested microorganisms, especially against *L. monocytogenes* and *Salmonella* (zones of inhibition ranged from 21.67 ± 0.58 mm to 34.33 ± 4.04 mm). While, a moderate activity was observed against *E. coli*, with zones of inhibition ranging from 14.33 ± 2.52 mm to 19.67 ± 1.15 mm. Rather, the mint EO showed weak activity against *E. coli* O157:H7 and both *L. monocytogenes* strains. However, moderate activity was observed against *Salmonella* strains.

In this study, the Gram-negative bacteria *Salmonella* was found to be more susceptible to the tested EOs than the Gram-positive bacteria *L. monocytogenes* (P = 0.315), while, *E. coli* O157:H7 was less susceptible (P = 0.001).

Furthermore, the microtitration assays were conducted to determine the MICs and MBCs of the tested EOs. The MICs and MBCs of the EOs against the tested strains are presented in Tables 3 and 4. The MICs and MBCs values confirmed the results obtained by the agar disk diffusion method. Oregano and thyme EOs had the lowest MICs (0.0625-0.125 %) and MBCs (0.125-0.25%) against *Salmonella*, while *Listeria* was inhibited at MICs ranging from 0.0625 to 0.5% and MBCs varied between 0.25% and 1%. Moreover, the mint EO was less active against all the tested microorganisms with MICs and MBCs between 0.5% and >2%. Besides, the tested oils inhibited *E. coli* at MICs and MBCs ranging from 0.125 to 0.5%.

However, we found that *E. coli* was less susceptible to the oils tested in agar medium than *L. monocytogenes* L23, while, in microtitration assays, it was less susceptible. This difference in activity can be explained by the oil solubility and volatility as reported by Bouhdid et al. [14] and Hernandez et al. [34]. Furthermore, the food isolate *L. monocytogenes* L23 was more resistant than the type strain *L. monocytogenes* CECT 4031^T. This fact can be explained by the food origin of the isolate (seafood salad).

Table 2. Inhibition zones of essential oils against tested bacteria

		Inhibition zones diameter [a] (mm) (mean values ± SD)				
		M. suaveolens	***O. elongatum***		***T. capitatus***	
		L	**L**	**FT**	**L**	**FT**
Salmonella	CECT 915[T]	12.67 ± 4.04	34.33 ± 4.04	31.50 ± 2.78	33.67 ± 5.51	21.67 ± 0.58
	S64	13.00 ± 0.00	28.17 ± 1.61	30.67 ± 5.51	29.00 ± 3.00	22.33 ± 2.31
E. coli O157:H7	CECT4267	6.17 ± 0.26	19.67 ± 1.15	18.00 ± 0.00	14.33 ± 2.52	18.50 ± 1.80
L. monocytogenes	CECT4031[T]	8.33 ± 1.61	34.00 ± 0.00	29.00 ± 1.73	28.67 ± 0.58	28.67 ± 4.16
	L23	3.33 ± 0.61	31.00 ± 3.46	33.00 ± 1.00	25.00 ± 0.00	28.00 ± 2.65

[a] *The diameter of the disks (Ø=6 mm) was not included; L: leaves; FT: flowering tops; T: type strain; Salmonella sp. S64 isolated from mayonnaise; L. monocytogenes L23 isolated from seafood salad.*

Table 3. Minimal inhibitory concentration (MIC) of essential oils (v/v %) against tested bacteria

		MIC (v/v %)				
		M. suaveolens	***O. elongatum***		***T. capitatus***	
		L	**L**	**FT**	**L**	**FT**
Salmonella	CECT 915^{T}	0.5	0.0625	0.0625	0.0625	0.125
	S64	0.5	0.0625	0.0625	0.0625	0.125
E. coli O157:H7	CECT 4267	0.5	0.25	0.25	0.25	0.125
L. monocytogenes	CECT 4031^{T}	1	0.125	0.125	0.0625	0.125
	L23	>2	0.5	0.5	0.5	0.5

L: leaves; FT: flowering tops; T: type strain; Salmonella sp. S64 isolated from mayonnaise; L. monocytogenes L23 isolated from seafood salad.

Table 4. Minimal bactericidal concentrations (MBC) of essential oils (v/v %) against tested bacteria

		MBC (v/v %)				
		M. suaveolens	***O. elongatum***		***T. capitatus***	
		L	**L**	**FS**	**L**	**FS**
Salmonella	CECT 915^{T}	1	0.125	0.125	0.125	0.125
	S64	0.5	0.125	0.125	0.125	0.125
E. coli O157:H7	CECT 4267	0.5	0.5	0.5	0.25	0.125
L. monocytogenes	CECT 4031^{T}	>2	0.125	0.25	0.125	0.125
	L23	>2	0.5	0.5	1	0.5

L: leaves; FT: flowering tops; T: type strain; Salmonella sp. S64 isolated from mayonnaise; L. monocytogenes L23 isolated from seafood salad.

In this study, thyme and oregano EOs showed their best activities as well against Gram-negative and Gram-positive bacteria. Similar results were reported previously for thyme and oregano EOs obtained from different species [2,14,17,35,36,37,38]. While, Bounatirou et al. [15] found that thyme EO had a low activity against *Salmonella*. Besides, mint EO (*M. suaveolens*) had a low activity as observed by Sutour et al. [39].

The inhibitory activity of oregano and thyme EOs is probably mainly due to the phenolic constituents (carvacrol 19.21-68.63% and thymol 14.2-43.57%). Actually, several authors have pointed to the antimicrobial activity of carvacrol and thymol against *E. coli* O157: H7 in *in-vitro* experiments [35,40,41]. These two compounds disintegrate the outer membrane of *E. coli* O157: H7 and release outer membrane-associated material from the cells to the external medium [42]. Moreover, Gill and Holley [43] have found that carvacrol inhibited *E. coli* and *L. monocytogenes* motility, by inhibiting ATPase activity and disrupting the membrane.

Although, comparing oregano and thyme EOs from leaves and flowering tops of the same plant, they showed a slight difference in either minimal inhibitory or bactericidal concentrations, while, the percentage of the main components (carvacrol, thymol and γ-terpinene) varied for oregano EOs, as it was observed by Peñalver et al. [44]. In fact, an additive effect between the main components has been suggested [14,41].

On the other hand, mint EO was mainly composed by peperitenon oxide which was considerate to have a low antimicrobial activity due to the presence of an epoxide between

C1 and C2 [6]. However, mint EO showed the same minimum bactericidal concentration as oregano EO against *E. coli*. This result points especially the important role of minor components [7,45].

In fact, different activities were observed for γ-terpinene, *p*-cymene, and limonene. Indeed, γ-terpinene did not antagonize the growth of *E. coli* O157:H7 [41] and *Salmonella* Typhimurium [46]. Also, *p*-cymene showed no antibacterial activity against *E. coli* O157:H7 in *in-vitro* experiments [41], while, it was inhibited in *in-vitro* [47] and in apple juice [48]. As well, limonene had moderate antimicrobial activity against *E. coli* O157:H7 [6] and low antilisterial properties [49]. Besides, Dorman and Deans [50] observed that limonene was more active than *p*-cymene.

Moreover, Cristani et al. [47] suggested that the antimicrobial effect of carvacrol, thymol, γ-terpinene and *p*-cymene may result, from a gross perturbation of the lipidic fraction of the cell membrane of the microorganism. In addition, the biological precursor of carvacrol, *p*-cymene causes swelling of the cytoplasmic membrane to a greater extent than does carvacrol [51]. A synergism has been observed when *p*-cymene was combined with carvacrol against *Bacillus cereus in vitro* and in rice [52].

3.3 Antiviral Activity

In this study, the results obtained showed that the EOs tested at 2% had no or low antiviral activity against MNV-1 (Table 5). Mint EO represented a reduction in viral titre of 0.87 $\log_{10}$ $TCID_{50}$/ml. This was followed by flowering tops of oregano and thyme EOs with 0.75 and 0.5 $\log_{10}$ $TCID_{50}$/ml reductions, respectively. However, Elizaquível et al. [12] found that oregano EO decreased MNV titres by 1.04-1.62 $\log_{10}$ $TCID_{50}$/ml and clove EO showed reductions of 0.67 $\log_{10}$ $TCID_{50}$/ml at 37ºC while zataria EO showed no notable reductions in MNV titres. Also, Kovač et al. [11] observed no reduction of norovirus surrogates titres by using hyssop and marjoram EOs at different temperatures and times.

Table 5. Antiviral activity of EOs against MNV-1

	M. suaveolens	***O. elongatum***		***T. capitatus***	
	L	**L**	**FT**	**L**	**FT**
Control	$5.90\ 10^{7}$	$2.50\ 10^{6}$	$1.87\ 10^{7}$	$2.50\ 10^{6}$	$1.87\ 10^{7}$
EO-treated virus	$7.90\ 10^{6}$	$5.90\ 10^{6}$	$3.30\ 10^{6}$	$7.90\ 10^{6}$	$5.90\ 10^{6}$
Antiviral activity	-0.87	0.37	-0.75	0.49	-0.50

L: leaves; FT: flowering tops.

On the other hand, Lee et al. [53] found that pretreated cells with red ginseng extract or ginsenosides reduced the titre of MNV between 0.37-1.48 $\log_{10}$ $TCID_{50}$/ml depending on the virus concentration while co-treatment or post-treatment were not effective.

In addition, it has been reported that chitosan [54], grape seed extract [55], pomegranate juice, pomegranate polyphenols [56,57], cranberry juice and cranberry proanthocyanidins [58,59] reduce to some extent the titre of foodborne viral surrogates. For MNV, the highest reduction was observed by cranberry proanthocyanidins with almost 3 log reduction [58].

In fact, non-enveloped viruses are less susceptible to EOs, due to the lack of the lipid envelope, for example: adenovirus was not affected by eucalyptus EO [4], the same for poliovirus 1 and adenovirus 2 treated with tea tree EO [60].

Behravan et al. [5] suggest that antiviral activities of *Thymus transcaspicus* EO against *Bacillus* phage CP51 were due to the presence of carvacrol and thymol. In addition, Lai et al. [61] found that herpes simplex virus type I HSV-1, an enveloped DNA virus, was 90 % inactivated directly within 1 hour by both carvacrol and thymol. Also, the human rotavirus (RV), a non-enveloped virus, was not inhibited by the Mexican oregano EO, while carvacrol alone exhibited high antiviral activity [62].

In this study, MNV-1 was incubated with EOs prior to host cell infection to investigate if the EOs might interfere directly with virus capsid structures as proposed by others authors [56,63,64]. However, antiviral activity of tested EOs needs to be further investigated.

4. CONCLUSION

In conclusion, the antibacterial and antiviral activities of EOs are affected by their chemical compositions. Based on their varied composition, EOs display different activities on bacteria and virus. The antibacterial and the slight antiviral activity of tested oregano, thyme and mint EOs fostered their potential as bio-preservatives to improve food safety.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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