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Original Research Article

Chemical Composition of *Thymus vulgaris*, *Origanum compactum* and *Vetiveria zizanoides* Essential oils and their Antibacterial and Antioxidant Activities

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ABSTRACT

Thanks to their various compounds, essential oils (EOs) are promising innovative resources of antimicrobial and antioxidant agents. This work aims to explore *in vitro* the antibacterial and antioxidant potential of the volatile oils extracted from three Moroccan species: *Thymus vulgaris*, *Origanum compactum* and *Vetiveria zizanoides*. The phytochemistry of these oils attained by hydro-distillation was investigated via GC-MS analysis. Antioxidant potential was evaluated by DPPH and FRAP assays. The antibacterial effect was assessed on *Staphylococcus aureus* and *Salmonella enterica* using broth-microdilution method. Phytochemical analysis allowed to identify 10 components for each EO. Caryophellene (19.93%) and carvacrol (15.35%) were the main identified compounds in *O. compactum* EO, while *T. vulgaris* EO was dominated by caryophyllene (17.925%) and thymol (15.127%). As regard *V. zizanoides* EO, longiverbenone (27.31%) and longipinocarvone (26.87%) were the major compounds. The antibacterial screening demonstrated that these oils exhibited significant inhibitory effects against *S. aureus* and *S. enterica* with MIC values varying from 0.25 to 0.5% and 0.5 to 1% (v/v), respectively. These values are close to those of positive control: tetracycline (MIC values = 0.5 and 0.25% (v/v), respectively). The studied EOs presented promising antioxidant effects by IC_{50} values varied between 93.10 ± 1.3 and 141.5 ± 2.41 µg/mL for DPPH assay, and between 131.79 ± 2.64 and 184.8 ± 1.02 µg/mL for FRAP method.
In light of this work, the volatile compounds of the studied plants could constitute a natural source for antibacterial and antioxidant agents. Presented information could shed light into further studies on these plant Eos.

Keywords: Antioxidant effect, Antibacterial effect, Essential oils, *Origanum compactum, Thymus vulgaris, Vetiveria zizanoides*.

Introduction

Since the ancient civilizations to nowadays, humanity has used medicinal plants for food as well as for care to treat various kinds of diseases. Recently, scientific evidence demonstrated that many medicinal plants and their essential oils have various useful therapeutic properties in prevention and relief of diseases. Indeed, several natural plant components have showed important biological activities, including antioxidant, antifungal, antiviral, anti-inflammatory, antiseptic, and antibacterial properties. The antimicrobial properties of the volatile oils and their compounds from various plant varieties have been well documented.[1,2]
Origanum compactum Benth. locally known as "Zaâter", grows from June to August, on dry, calcareous, and rocky soils; up to 700 meters of altitude. It also grows on forest land and sometimes between trees and shrubs. It is considered as a real medicine in Morocco, and used to cure or relieve symptoms of a large number of diseases such as heart disease, diabetes, inflammation, hypertension, stomach diseases, febrifuge and respiratory diseases. It has also been used against bile acid pathologies and constipation as well as to increase appetite.[3-5] Nowadays, *O. compactum* EOs are used in many industrial fields (food, perfumery, pharmacy...) thanks to their wide spectrum of biological activities, such as antibacterial, anticancer, antiseptic, antifungal and antioxidant.[6,7]The chemical composition of oregano' EOs has been extensively studied and has shown significant fluctuation from one region to another.[8,9]
Thymus vulgaris L. locally known as "Zâaytr" or "zaitra", is broadly utilized in Moroccan traditional medicine thanks to their antispasmodic, antitussive, carminative and diuretic properties.[10] This plant's E.Os have been used for thousands of years, especially in pharmaceuticals, natural therapies, alternative medicine, and preservation of food.[10,11,5] Recent studies have revealed that *Thymus* species exhibit significant antifungal, antibacterial, antioxidant, anti-viral, spasmolytic, antiparasitic activities.[12] Moreover, the main components of its leaves E.Os are carvacrol, geranial, γ-terpineol, linalool, and thymol.[13]
Vetiveria zizanioides (L.) Nash, native to India, is a perennial, bushy, and fragrant plant, 1 to 2 meter high, with an upright stem, narrow and long leaves with a developed abundant, complex and extensive root system.[14,15] Since ancient times, vetiver has been used in perfumery and traditional medicine due to the biological and aromatic properties of its root's essential oils.[16] Indeed, recent studies have shown that its root's extracts have diverse pharmacological properties especially, antifungal,[17] anti-inflammatory,[18] antioxidant, and anticancer[19]

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properties. The Vetiver EO can also be used to treat patients with dementia-related behaviors, in order to develop cognitive function and mental alertness.[20]
Currently, the uses of synthetic antioxidants and antibacterial drugs (antibiotics) have shown increasing limitations in foods and medical application due to their low efficacy and their excessive side effects. Therefore, the search for alternative antioxidants and antibacterial agents from natural products has been seriously increased. Therefore, the current work aims to establish the phytochemistry of the essential oils extracted from three Moroccan medicinal plants: *Origanum compactum, Thymus vulgaris* and *Vetiveria zizanoides*; and to investigate their antibacterial effects and antioxidant power.

Materiel and Methods

Plant material
The *T. vulgaris* and *Origanum compactum* aerial parts, and the roots of *V. zizanoides* were collected during May and June 2018 in the region of Taounate – Morocco (34° 32' 09" N, 4° 38' 24" W) (Figure 1). Plants authenticity was carried out by the botanist Pr. Amina BARI. The voucher samples, *O. compactum* (RO 001780121), *T. vulgaris* (RT 001270127) and *V. zizanoides* (RV001060132) were dropped at the herbarium of our laboratory.

Essential oils extraction
The plants parts previously described were subjected to hydrodistillation (Clevenger apparatus) for three hours as referred before.[21, 22] After calculating their yield, the collected EOs were kept protected from light at 4 °C until analysis and use.
The yield of volatile oils was calculated according to the next formula:

$$\mathbf{Oil}\left(\%\frac{\mathbf{v}}{\mathbf{w}}\right) = \frac{\text{Observed volume of oil (ml)}}{\text{Weight of the sample (g)}}\, x\ 100$$

Identification of essential oils components
T. vulgaris, *O. compactum* and *V. zizanoides* essential oils were analyzed through gas chromatography (GC) coupled with mass spectrometry (MS), and gas chromatography with flame ionization detection (GC-FID).

Gas chromatography-mass spectrometry (GC/MS) and Gas chromatography (GC/FID) analysis conditions
The EOs chemical composition was established using a Varian capillary column (TR5-CPSIL-5CB; 50 mm in length, 0.32 mm in diameter and 1.25 µm in film thickness). The column temperature went from 40 °C to 280 °C with an increase rate of 5 °C/min. The temperature of the injector was set at 260 °C and that of the detectors (FID) at 260 °C. The flow rate of the carrier gas (Helium) was 1 ml/min. 1 µl of oil diluted in hexane (10%) was injected in the column. The chemical constituents of oils were identified via the comparison with compounds collected by the Adams table[23] and the NIST-MS library.

Antimicrobial activity evaluation
Bacterial strains
The antibacterial activities of the three studied E.Os were evaluated against *Salmonella enterica* (S.E.) (ATCC 14028) and *Staphylococcus aureus* (S.A.) (ATCC 29213) by evaluating the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) according to Benkhaira *et al.* and Ouaritini *et al.*[24,25] Both strains were provided from a collection of food microorganisms in the laboratory. Bacterial strains were revived by subcultures in Luria-Bertani (LB) plates at 37°C during 24 h, before their use.

Determination of minimum inhibitory concentration (MIC)
The MICs were determined using the microdilution test in a 96-well microplate as reported by Benkhaira *et al.*[24]
Oils dilution is made in a microplate filled by 20 µL of DMSO, then the concentration range is prepared in 96 wells.
100 µL of EO is added to the 1st well of each row, from which a twofold micro-dilution is made. Then 50 µL of Mueller Hinton broth, inoculated with 50 µL of a bacterial suspension previously prepared and adjusted to 10^6 $CFU.ml^{-1}$ is added. The final concentrations of EOs ranged from 8 % to 0.007% (v/v).
The twelfth well represented the growth control. The microplates were incubated at 37°C for one day. Then, we add 10 µL of resazurin to each well to indicate the bacterial growth.[26] After 2 hours of incubation at ambient temperature, the bacterial growth was detected by a change from purple to pink. Tests were performed in triplicate to minimize experimental error.
Determination of minimum bactericidal concentration (MBC)
The MBC was identified by inoculating 3 µL from each negative well on LB agar plates and incubating for 24 h at 37°C.[27]

Antioxidant activity
DPPH free radical-scavenging assay
The DPPH test was used to assess the antioxidant effect of EOs.[28] 100 µL of different concentrations of EOs dissolved in methanol were added to 750 µL of a 0.004% methanol solution of DPPH. After 30 min of incubation at room temperature, the optical density was measured against a blank at 517 nm using a UV spectrophotometer. The percentage (%) of antiradical activity was obtained as follows:

DPPH scavenging activity (AA in %) = [(Ac-At)/Ac] × 100.
With:
Ac: the absorbance of the control (without sample);
At: the absorbance of the test (with sample).
Butylated Hydroxytoluene (BHT) and ascorbic acid were considered as standards. The EO concentrations demonstrating 50% of inhibition (IC_{50}) were calculated by plotting the inhibition percentages against EO concentrations. The test was performed three times and IC_{50} values were determined as means ± SD.

Reducing ferric power assay (FRAP)
Reductive capacity was established by the transformation of Fe^{+3} to Fe^{+2} in the presence of the tested EOs.[28] EOs and standard (BHT and acid ascorbic) were combined with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 mL, 1%). The solution was incubated at 60 °C for 15 min. 500 µL of trichloroacetic acid (TCA) (10%) was added to the mixture, then this mixture was centrifuged for 10 min at 2000 rpm. Later, 500 µL of supernatant was supplemented to 500 µL of distilled water and 100 µL of $FeCl_3$ (0.1%). The wavelength set to measure the absorbance was 700 nm.
The E.O concentration indicating 50% of absorbance (EC_{50}) was determined by plotting absorbance (at 700 nm) against the corresponding sample concentration. The test was carried out in triplicate and EC_{50} values were considered as means ± SD for the expression of the results.

Statistical analysis
Data were presented as the average ± SD for three replicates for each sample. The IC_{50} values of DPPH and FRAP were designed by linear regression analysis using Microsoft Excel.

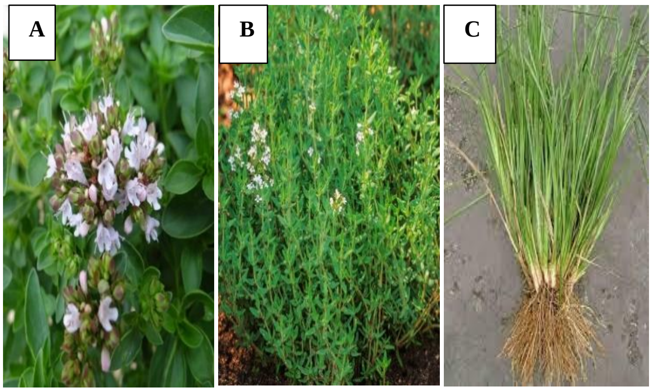

Figure 1: Moroccan *O. compactum* (A) *T. vulgaris* (B) *and V. zizanoides* (C) at flowering stage.

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Results and Discussion

Yield of essential oils

The yield of essential oil obtained from *Origanum compactum* leaves by hydro distillation is 4.61% (Table 1). This result is more significant than that reported by Laghmouchi and his colleagues [4] for the same plant collected from Northern Morocco (1.22 to 4.24%) and by Benazzouz (2011)[29] for oregano collected in eastern Morocco (2%).
The distillation of *Thymus vulgaris* leaves provides an E.O yield of 3.64% (Table 1); which is higher than that obtained by Imelouane *et al.*[10] for this species growing in eastern Morocco. Distillation of the roots of *Vetiveria zizanioides* harvested in the region of Taounate provides 2.54% of essential oils (Table 1). This yield is greater than that indicated by Soidrou *et al.*[30] for the vetiver from Ndzuwani (Comores) and by Pripdeevech et *al*[31] in Thailand.
The extraction yield's variation can be explained by the climatic and topographic conditions of the collection areas as well as by the medicinal plant storage time.

Chemical composition of essential oils analyzed by CPG / SM.

Chemical compositions of *O. compactum*, *T. vulgaris* and *V. zizanoides* EOs were identified using GC-MS and GC-FID methods as listed in Table 2 and Figure 2.
GC-MS analysis of *O. compactum* essential oil enabled to identify ten constituents representing 99.92% of the total E.O. The main constituents are caryophellene (19.930%), carvacrol (15.359%), *α*-Terpineol (12.096%) and linalool (10.146%); while minor compounds such as α-phellandrene (4.739%) and *α*-Pinene (4.879%) were also identified (Figure 2a). These data are in concomitant with those found in other geographical areas. The predominance of caryophellene and carvacrol in the studied EO is quite consistent with the results obtained in other studies.[4, 32]
Concerning the analyzed *T. vulgaris* EO, 10 compounds corresponding to 93.282% were detected (Table 2); with caryophyllene (17.925 %), thymol (15.127 %) and 4-terpineol (11.669 %) as major compounds (Figure 2b). However, this chemical composition is different from that reported by Imelouane *et al.*[10] for the same species collected in eastern Morocco. Similar studies conducted in Spain, Romania and Italy, respectively, reported that the main compounds of *T. vulgaris* EO are terpinene and thymol.[33,34,35]
The Phytochemical analysis of *V. zizanoides* EO made it possible to identify 10 components, representing 99.96% of the total detected compounds, of which the main constituents are longiverbenone (27.314%), longipinocarvone (26.876%), Cedr-8-en -13-ol (26.260%) (Figure 2c). These results are close to those of [36] who showed Cedr-8-en-13-ol among the major components of vetiver EO.
Therefore, the constituents of the three studied EOs remain almost identical to those reported in other previous works and the main compounds remain globally the same (Figure 3), with slight variations in quantity mainly due to the storage duration, the geographical origin, and the harvest time of the studied plant.

Table 1: Comparison of the essential oils extraction yield of the three plants studied.

Species	Yield of studied essential oils	Literature	Reported Yield %
O. compactum	4.61 %	Laghmouchi *et al.* (2018)[4]	1.22-4.24%
		Benazzouz (2011)[29]	2%
T. vulgaris	3.64%	Imelouane *et al.* (2009)[10]	1 %
V. zizanioides	2.54%	Pripdeevech *et al.* (2006)[31]	0.06-0.27%
		Soidrou *et al.* (2020)[30]	1%

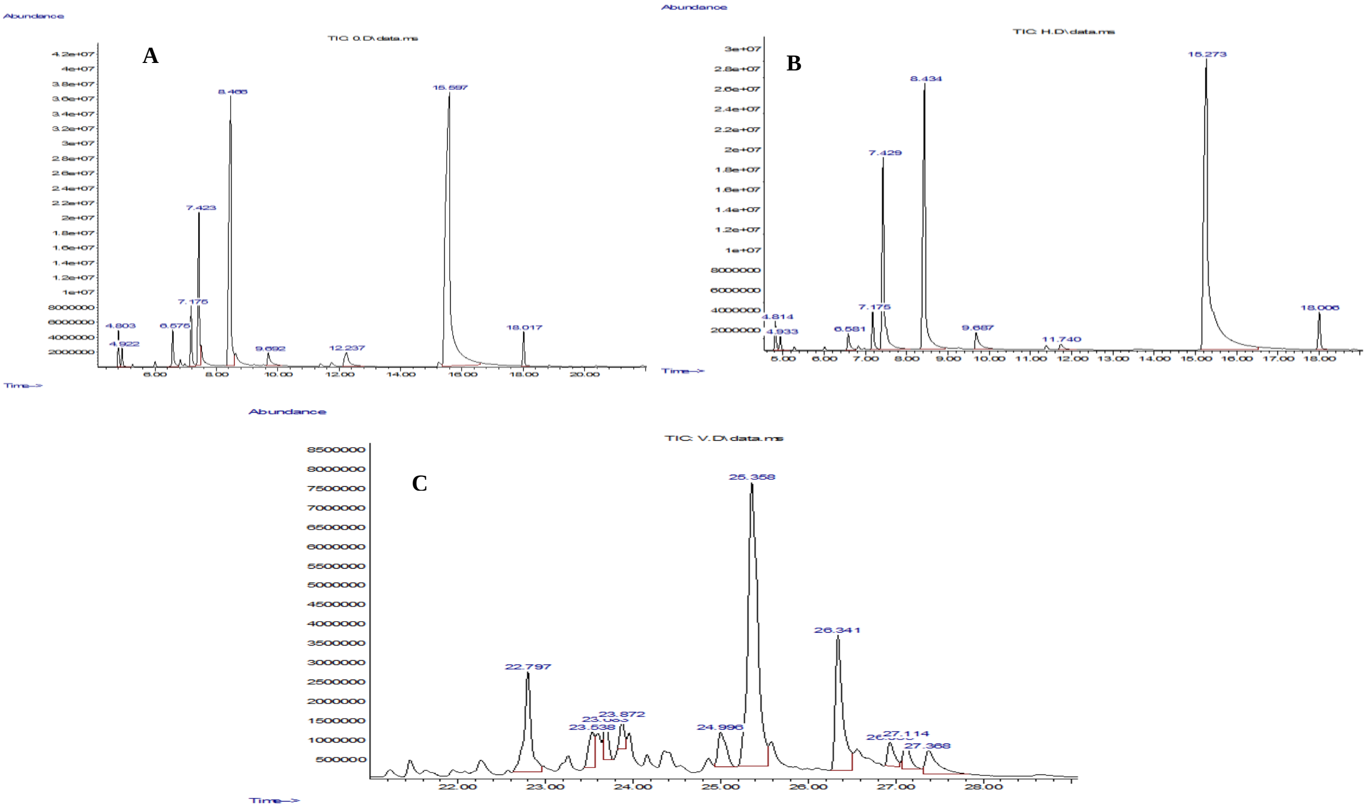

Figure 2: Chemical profiles of (a) *O. compactum*, (b) *T. vulgaris* and (c) *V. zizanoides* EOs using GC-MS and GC-FID

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Antimicrobial activities of the studied essential oils

The MIC and MBC values of the three studied plants EOs against *S. enterica* and *S. aureus* are shown in Table 5. All tested EOs have shown significant antibacterial effects. Indeed, a concentration of only 0.25% (v/v) of *O. compactum* EO was needed to impede the growth of *S. enterica* and *S. aureus*, while the MIC values of *T. vulgaris* and *V. zizanoides* EOs respectively ranged from 1% to 0.5% (v/v) against both tested strains. This is to note that the standard antibiotic tetracycline has shown respective MIC values (0.5 - 0.25% (v/v)).

The MBC values of the three tested EOs are quite similar to the MIC values obtained against both bacterial strains for *T. vulgaris* and *V. zizanoides* EOs; and two times higher with respect to these strains for the *O. compactum* EO (Table 3). In fact, the MBC values of the *O. compactum*, *T. vulgaris* and *V. zizanoides* EOs were respectively 0.5%, 1%, 1% against *S. enterica* and 0.5% (v/v) against *S. aureus*. Thus, these EOs could display a bactericidal effect on the studied bacteria.

The *O. compactum* EO has a stronger inhibitory activity compared to *Thymus vulgaris* and *V. zizanoides* EOs against the two tested strains with a low MIC value (0.125% (v/v)). This inhibitory effect is ascribed to various chemical compounds of these EOs.

Thus, oxygenated monoterpenes and hydrocarbon monoterpenes such as carvacrol, caryophyllene, Thymol, and Terpinene have been described to be responsible for the antimicrobial properties of many EOs.[26]

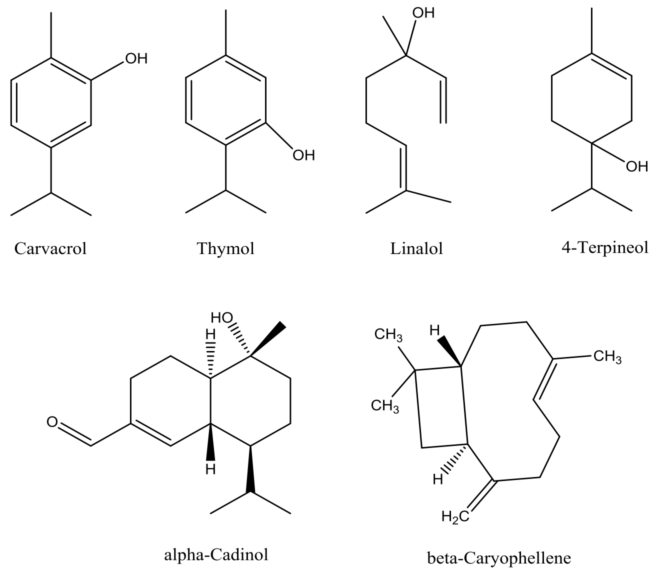

Figure 3: Major compounds identified in *O. compactum, T. vulgaris and V. ziznoides* essential oil

Table 2: Chemical composition of *O. compactum*, *T. vulgaris* and *V. zizanoides* EOs.

NO.	Compounds[a]	Chemical formula	RI[b]	%Relative peak area		
				O. compactum	*T. vulgaris*	*V. zizanoides*
1	α-Pinene	$C_{10}H_{16}$	939	4.879	4.890	-
2	β-Pinene	$C_{10}H_{16}$	980	6.392	6.521	-
3	2-Carene	C_{10}H16	1001	7.332	-	-
4	α-phellandrene	$C_{10}H_{16}$	1018	4.739	4.755	-
5	P-Cymene	$C_{10}H_{14}$	1026	7.483	7.343	-
6	γ-Terpinene	$C_{10}H_{16}$	1062	8.596	8.320	-
7	α–Terpinolene	$C_{10}H_{16}$	1088	-	7.110	-
8	Linalool	$C_{10}H_{18}O$	1098	10.146	**9.622**	-
9	4-Terpineol	$C_{10}H_{18}O$	1177	-	**11.669**	-
10	α–Terpineol	$C_{10}H_{18}O$	1189	**12.096**	-	-
11	Thymol	$C_{10}H_{14}O$	1290	-	**15.127**	-
12	Carvacrol	$C_{10}H_{14}O$	1298	**15.359**	-	-
13	Longipinocarvone	$C_{15}H_{22}O$	1398	-	-	26.876
14	β-Caryophellene	$C_{15}H_{24}$	1428	**17.930**	**17.925**	-
15	γ-Himachalene	$C_{15}H_{24}$	1479	-	-	23.656
16	β-Guaiene	$C_{15}H_{24}$	1490	-	-	23.440
17	D-4-ol-Germacrene	$C_{15}H_{26}O$	1511	-	-	22.630
18	γ-Gurjunen epoxide-(2)	$C_{15}H_{24}O$	1575	-	-	24.937
19	Cedr-8-en-13-ol	$C_{15}H_{24}O$	1668	-	-	26.260
20	Aromadendrene oxide-(1)	$C_{15}H_{24}O$	1702	-	-	25.201
21	α-Cadinol	$C_{15}H_{26}O$	1652	-	-	**27.314**
22	Longiverbenone	$C_{15}H_{22}O$	2148	-	-	**27.065**
23	Aromadendrene oxide-(2)	$C_{15}H_{24}O$	2299	-	-	23.824

[a] Compounds identified based on retention indices and mass spectra.
[b] Retention index on DB-5 MS column, experimentally determined using homologous series of C8–C24 alkanes.

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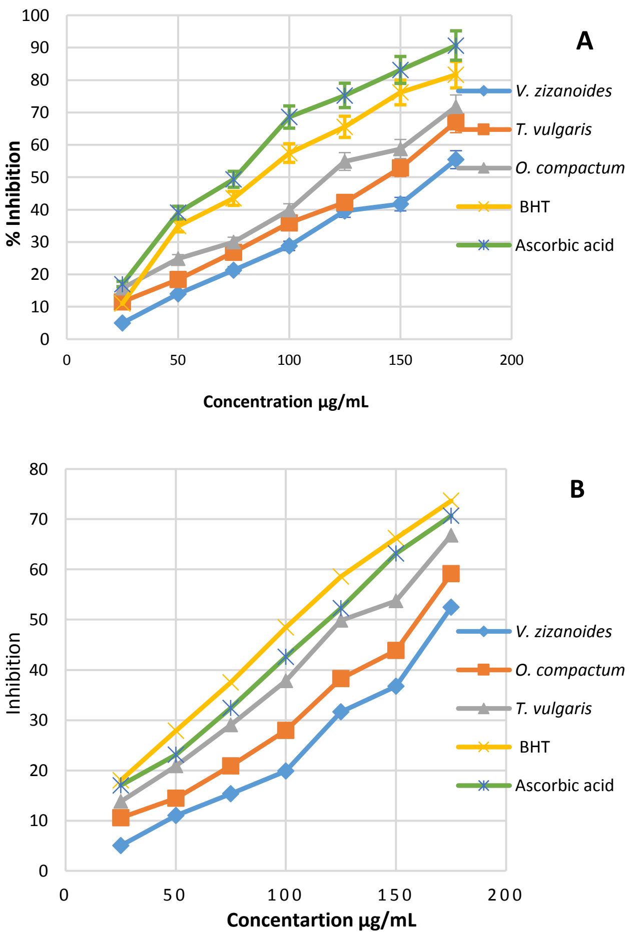

Figure 4: Antioxidant activities of *O. compactum*, *T. vulgaris* and *V. zizanoides* EOs using DPPH (A) and FRAP (B) assays.

Indeed, Sarrazin *et al*.[37] and Chraibi *et al*. [26] showed that Thymol can modify the outer layer, while the carvacrol compound is able to destroy the stability of the cell plasma membrane; act on proton exchange and produce reduction of the ATP pool, thus reducing the trans-membrane pH gradient, leading to cell layer rupture and subsequent escape of cytoplasmic contents, resulting in apoptosis. Furthermore, terpineols are recognized to have greater efficacy (Membrane permeability changes) and a wider range of antimicrobial activities.[26]
The significance of the hydroxyl group has been long-established, and its relative position on the phenolic ring does not seem to intensely affect the antibacterial action grade.[38, 39] However, the activity of EOs depends not only on the main constituents, but also on the synergy between minor compounds.[26] Therefore, since the main component is not necessarily responsible for the entire antibacterial effect of an EO; consideration of the antibacterial efficacy of some minority constituents should be taken into account.[40]

Antioxidant activity
The antioxidant ability of Moroccan *O. compactum*, *T. vulgaris* and *V. zizanoides* oils was measured using DPPH and FRAP tests compared with standards, BHT and ascorbic acid.
The results of DPPH and FRAP assays are reported in Figure 4 A and B. The radical scavenging potential of the tested EOs indicated a concentration-dependent action. *O. compactum* EOs displayed the strongest scavenging effect with an IC_{50} of 93.10±1.3 µg/mL, followed by *T. vulgaris* and *V. zizanoides* EOs: IC_{50}=98.95± 1.16 and 141.5± 2.41µg/mL, respectively. As regard the Ferric reducing power test, EOs of *T. vulgaris*, *O. compactum* and *V. zizanoides* showed interesting capacity to reduce ferric solution with respective IC_{50} values of 131.79± 2.64, 158.03±4.19 and 184.8±1.02 µg/mL (Table 4). Furthermore, the synthetic antioxidant BHT and ascorbic acid showed low IC_{50} values, especially with DPPH method.
The DPPH assay is based on scavenging the free radical DPPH by antiradical compounds, inducing a discoloration. This technique is simple and rapid to implement, and it performed at room temperature to prevent the risk of thermal degradation of the tested bioactive compounds.[28] In this context, we have chosen the DPPH test to determine the radical scavenging ability of *O. compactum*, *T. vulgaris*, and *V. zizanoides* EOs. The studied essential oils exhibited promising inhibition potential of DPPH free radical in a doses-dependent manner. This effect may be due to the raised rate of phenolic compounds found in volatile content.
The ferric reducing power test indicates the capacity of a given sample to reduce ferocyanidin potassium solution. This test allows to indicate the aptitude of a compound to produce an electron, and therefore its effect to protect the body from lipids peroxidation, making the intermediates of the electron respiration chain more stable.[24] This reduction is associated with an increase in the optical density at 700 nm. Figure 4B presents the increase of ferric reducing potential in various concentrations of studied EOs.
Many studies have investigated the antioxidant properties of the *O. compactum*, *T. vulgaris* and *V. zizanoides* EOs.[41,42,43] Indeed, Bouyahya *et al*.[28] indicated that *O. compactum* EOs exhibits interesting antiradical and ferric reducing power effects at different phenological phases (Vegetative, flowering, and post-flowering periods). For the *T. vulgaris* EOs, it has been found that EOs extracted from an Italian species displays significant antioxidant activity, scavenging the radical DPPH and reducing ferric iron.[44] The difference between the obtained data is certainly due to the chemical fluctuation, which depends to several factors, including areas of the plant collection, phenological stage, genotype, plants part used, and storage and extraction conditions.[45,46,47]

Conclusion

The E.Os of Thyme, Oregano and Vetiver collected from Taounate region showed a difference in antibacterial and antioxidant effects, and the chemical analysis. The thyme E.O is characterized by Caryophyllene, Thymol, and 4-Terpineol, while oregano E.O is rich with Caryophellene, Carvacrol and α-Terpineol, and vetiver E.O is dominated by Longiverbenone, Longipinocarvone and Cedr-8-en-13-ol. A significant capacity of Oregano E.O to reduce the DPPH was detected, while the strongest reducing power was displayed by Thyme EO. The best inhibitory activity against *S. aureus* and *S. enterica* strains was exhibited by *O. compactum* EO. These significant effects make the studied E.Os very useful as a food preservative agent at an industrial level.

Table 3: Antimicrobial activities of the studied essential oils

Strains	***O. compactum***		***T. vulgaris***		***V. zizanoides***		**Tetracycline** [c]	
	MIC[a]	**MBC**[b]	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
S. enterica	0.250	0.5	1	1	1	1	0.5	0.5
S. aureus	0.250	0.5	0.5	0.5	0.5	0.5	0.25	0.062

[a] MIC: Minimal inhibitory concentration (as % (v/ v).
[b] MBC: Minimal bactericidal concentration (as% (v/ v)). Final bacterial density was around 10^6 CFU/mL.
[C] Standard antibiotic.

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Table 4: IC_{50} (µg/mL) of *O. compactum*, *T. vulgaris* and *V. zizanoides* EOs, BHT, and ascorbic acid by DPPH and FRAP methods.

	DPPH IC_{50} (µg/mL)	FRAP IC_{50} (µg/mL)
O. compactum	93.10 ± 1.3	158.03 ± 4.19
T. vulgaris	98.95 ± 1.16	131.79 ± 2.64
V. zizanoides	141.5 ± 2.41	184.8 ± 1.02
Ascorbic acid	23.14 ± 0.5	119.01 ± 0.7
BHT	33.08 ± 1.21	107.46 ± 1.5

*The experiment was carried out in three repetitions, and the results are expressed as mean ± SD.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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