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Short Communication

Essential Oils of *Origanum compactum*: Antibacterial and Antioxidant Bioproducts

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ABSTRACT

Antibiotic resistance and oxidative stress are currently real threats to public health. Essential oils derived from aromatic plants are potential solutions to these health problems. In this context, the main objective of this study was to investigate the antioxidant and antibacterial properties of Moroccan oregano (*Origanum compactum*) essential oils (EOs). The EOs were extracted from the leaves and buds of the plant using the Clevenger apparatus. The oils obtained were characterized by GC-MS analysis. The EOs were also screened for antioxidants using the DPPH and TAC assays. Finally, the antibacterial activity of the EOs against some pathogenic microorganisms (*Escherichia coli*, *Staphylococcus aureus*, and *Bacillus subtilis*) was determined using the disk diffusion method. The GC-MS chromatographic analysis of the EOs derived from *O. compactum* (EOOC) revealed a high concentration of thymol and carvacrol as the predominant compounds in EOOC. The evaluation of EOOC's ability to scavenge DPPH radicals demonstrated a significant inhibition rate (IC_{50} = 0.235 mg/mL). Additionally, the measurement of total antioxidant capacity indicated the presence of a substantial amount of antioxidants in the oil (422.17 ± 22.53 mg EAA/g of EO). The antibacterial results displayed a higher efficacy of EOOC against the tested pathogenic bacteria, particularly *S. aureus* MIC = 0.751 µl/mL). From the experimental results obtained, the EO of Moroccan *Origanum compactum* contained eco-friendly antimicrobials and antioxidants and can constitute a promising agent to combat the issues of resistance to conventional drugs and ineffective drug usage.

Keywords: GC-MS, *Origanum compactum*, essential oil, antioxidant, antibacterial.

Introduction

Antibiotic resistance has emerged as a significant and pressing public health issue, leading to a crisis in numerous hospitals worldwide and contributing to the rise of nosocomial infections. Moreover, the challenges of antibiotic resistance have hindered global efforts in alternative drug research.[1] Consequently, there is an urgent need to explore new avenues for developing anti-infective agents. In this regard, essential oils (EOs) have gained attention as they have proven less prone to developing bacteria resistance, making them potential alternatives for addressing antibiotic resistance.[2] On the other hand, the harmful effects of oxidative stress on the human body have become a prominent health challenge. Synthetic antioxidants such as butylated hydroxytoluene (BHT) are commonly used in the food industry as antioxidant agents, but they can cause liver damage and be linked to cancer.[1,3] For this reason, there is considerable interest in using antioxidants and antibacterial agents of natural origin.[4]

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Origanum compactum is one of the most important medicinal plants in ethnobotany in Morocco and southern Spain.[5] *O. compactum* has a long history of traditional use for treating various pathologies. Its usage varies across different regions regarding the specific pathologies it targets, the parts of the plant utilised, and the methods of preparation employed. It has also been shown in various studies that its essential oil has important antibacterial, anti-leishmania, and antifungal activities.[1,5] Although oregano is a widely recognised plant, there is limited understanding regarding the antioxidant and antibacterial properties of the constituents found in the essential oil of *O. Compactum* from Taounate (Morocco), especially against the tested bacteria. Thus, this study aimed to investigate three key aspects: (i) identify the complete profile of active components present in the oil, (ii) evaluate the antioxidant capacity through *in vitro* testing, and (iii) assess the *in vitro* antibacterial activity of the essential oil derived from *O. compactum*.

Material and Methods

Plant material

The plant material used in this work includes the aerial part of *O. compactum* collected in the province of Taounate, region of Ain Madiouna, northern Morocco (34°30'0 "N; 4°33'0 "W) at the end of May 2022. The taxonomic identification of the plant was done in the laboratory of the National Agency for Aromatic and Medicinal Plants (ANPAM) of Taounate, and the reference DO15/05010 was assigned to it before being deposited in the agency's herbarium.

Extraction of essential oils

Essential oils were extracted from 100 g of *O. compactum* leaves and buds by hydrodistillation for 3 hours at a temperature of 120°C using a

Clevenger apparatus.[6] The oils obtained were dried at room temperature until the weight remained constant. Three replicates were performed, and the essential oils obtained were dried with anhydrous sodium sulfate and stored in the refrigerator at 4°C until use.

Gas Chromatographic and mass spectrometric analysis
The essential oil was analysed using an Agilent-Technologies 6890 N Network GC system equipped with a flame ionisation detector and HP-5MS capillary column (30 m x 0.25 mm, a film thickness of 0.25 µm; Agilent-Technologies, Little Falls, CA, USA). The injector and detector temperatures were set at 250 and 280°C, respectively. The column temperature was programmed from 35 to 250°C at a rate of 5°C/min, with the lower and upper temperatures held for 3 and 10 min, respectively. The flow rate of the carrier gas (helium) was 1.0 ml/min. A sample of 1.0 µL was injected using split mode (split ratio, 1:100). All quantifications were carried out using a built-in data-handling program provided by the manufacturer of the gas chromatograph. The composition was reported as a relative percentage of the total peak area. The constituents of the volatile oils were also identified by comparing their GC retention indices.

Antioxidant activity
DPPH Free Radical Scavenging Assay
The activity of Scavenging DPPH radicals by essential oils was measured as described previously,[7,8] with slight modifications. For the essential oil, 0.5 ml of various concentrations in methanol was added to 1.5 ml of a freshly prepared 0.004% methanol solution of DPPH. The blank was prepared by replacing the sample with methanol. After shaking, each mixture was incubated in the dark for 30 min at room temperature, and then the absorbance was measured at 517 nm. Ascorbic acid was used as a positive control. The percentage of inhibition of DPPH free radicals was calculated according to the formula:

$$\% \text{ Inhibition} = \frac{\text{Abs Control} - \text{Abs sample}}{\text{Abs Control}} \times 100$$

Abs control: Absorbance of the control (containing all reagents except the test compound).
Abs sample: Absorbance of the test compound
The IC_{50} is calculated graphically by linear regression of the graph.

Determination of total antioxidant capacity.
A volume of 75 µL of *O. compactum* EO studied was mixed in 3 ml of liquid reactive solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). After incubation at a temperature of 95 °C for 90 min, the spectrophotometer measured the optical density at 695 nm with a blank containing 75 µL of methanol instead of the EO.[9] The antioxidant capacity was expressed in milligrams of ascorbic acid equivalent per gram of EO (Mg AAE/g of EO) from the ascorbic acid standard curve plot.

Antimicrobial activity of EO
Microbial strains
The present study examined the effectiveness of *O. Compactum* EO in inhibiting the growth of three bacterial strains, namely *Escherichia coli* (ATB:57) B6N, *Staphylococcus aureus*, and *Bacillus subtilis*. All of which are known to be pathogenic and resistant to many antibiotics. Müller-Hinton broth (MHB) and Müller-Hinton agar (MHA) from VWR Chemicals were used as the growth media for bacterial cultivation.
After 18 to 24 hours of growth, colonies were obtained from a fresh culture and transferred to a 0.9% NaCl solution to create a microbial suspension. The optical density of the suspensions was assessed at 625 nm using a UV-Visible spectrophotometer and adjusted to a range of 0.08 to 0.1 nm, corresponding to suspensions containing 10^7 to 10^8 CFU/mL.

Determination of the zone of inhibition on solid substrates
To assess the antibacterial properties of *O. compactum* EO, the disk diffusion method was employed.[1] Petri dishes containing nutrient broth medium were prepared and inoculated with the tested bacterial strains. Following this, circular Whatman paper discs measuring 6 mm in diameter were impregnated with 20 µL of *O. compactum* EO and carefully positioned on the surface of the inoculated culture media. The Petri dishes containing the bacterial strains were placed in a dark incubator at 37°C. After a 24-hour incubation period, the diameter of inhibition zones and the corresponding percentage of inhibition were evaluated. This study utilised a negative control comprising 10 µL of 0.2% agar, while Ampcilin (0.02 mg/disc) was used as the positive control.
Determination of minimum inhibitory concentration in liquid medium
The microdilution method was used to determine the lowest inhibitory concentration of *O. compactum* EO against bacterial strains, according to the method reported previously.[1] After 18 hours of incubation at 30°C, the MIC was determined using the colourimetric method (TTC 0.2% (w/v)).[10-11]

Results and Discussion

Essential oils extracted from the aerial parts of *Origanum compactum* yielded 2.72 ± 0.32 % (mL per 100 g of dry matter). The yield obtained remains lower than that obtained by Allali *et al*.[12] The analytical and identification methods used in this work identified 14 distinct compounds, of which thymol (approx. 58%), carvacrol (approx. 22%) and gamma-terpinene (approx. 5%) are the major ones (Table 1). The other compounds were present in small amounts (Table 1). These results align with those of Belkamel *et al*.,[13] who reported a characteristic dominance of 4 compounds: gamma-terpinene, paracymene, thymol, and carvacrol in several samples of oregano oils. Close results were also observed by Rezouki *et al*.[5] Essential oils' yield, quantity, and chemical composition are all influenced by factors such as plant species, environmental conditions, extraction method, drying, and cultivation techniques.[1,5]

Scavenging of the free radical DPPH
Evaluation of the DPPH radical scavenging capacity of *Origanum compactum* essential oils showed a lower inhibition rate (IC_{50}= 235 µg/mL) compared to the positive control (ascorbic acid: IC_{50} = 2.558 µg mL) (Figure 1). These results are still very significant compared with other oregano plants or Lamiaceae species, whose antioxidant capacity was inferior to those obtained in this study.[1]

Total antioxidant capacity (TAC)
The measurement of the total antioxidant capacity revealed the presence of a significant amount of antioxidants in the EO studied (422.17 ± 22.53 mg AAE/g EO); this capacity is expressed in equivalents of ascorbic acid from a calibration curve ($y = 1.5554x + 0.087$; $R^2 = 0.9948$). This result is better than that of El Abdali et *al.*[1], which was around 173.90 mg AAE/g EO in the OE of the same plant.
Moreover, the work of Sbayou et *al.*[14] revealed that *O. compactum* EO exhibited higher antioxidant activity than mint and white wormwood. This activity could be attributed to the phenolic compounds in the studied EO at high concentrations. In the same context, other studies[1] have shown that oregano EO exhibited significant antioxidant activity with an IC_{50} = 0.690 mg/mL. In another study, *O. compactum* EO showed comparable antioxidant activity (IC_{50} = 2.00 mg/L) to vitamin C (IC_{50} = 1.90 mg/L) using the ABTS assay.[15]
Several studies on the antioxidant potencies of several compounds suggested that thymol and carvacrol were the most potent in terms of antioxidant activity compared to 1,8 cineole, camphor, borneol, terpinen-4-ol, and linalool.[16-17]

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Table 1: Phytochemical composition of *O. compactum* EO

Peak	RT (min)	Compounds	Molecular formula	Relative Percentage (%)
1	4.542	Beta-Myrcene	$C_{10}H_{16}$	0.68
2	5.157	P-Cymene	$C_{10}H_{16}$	0.54
3	5.285	Gamma-Terpinene	$C_{10}H_{16}$	5.35
4	5.438	Thymol	$C_{10}H_{14}O$	58.12
5	5.659	L-Linalool	$C_{10}H_{18}O$	2.86
6	6.341	Borneol	$C10H18O$	1.21
7	6.471	Carvacrol	$C_{10}H_{14}O$	22.26
8	6.762	Piperitenone	$C_{10}H_{14}O$	0.11
9	6.984	Pulegone	$C_{10}H_{16}O$	1.75
10	7.093	caryophyllene	$C_{15}H_{24}$	2.03
11	7.224	Caryophyllene oxide	$C_{15}H_{24}O$	0.24
12	7.845	Methyl linoleate	$C_{19}H_{34}O_2$	0.97
13	10.243	Ethyl linolenate	$C_{20}H_{34}O_2$	1.07
14	12.249	Ethyl α-linolénate	$C_{20}H_{34}O_2$	0.32
	Total			97.51

Table 2: Inhibition diameter (mm) of *O. compactum* EO against pathogenic bacterial strains

Bacterial strains	***E. coli***	***B. subtilis***	***S. aureus***
O. compactum EO	27 mm	100%	70 mm
Ampcilin	26 mm	100 %	33 mm

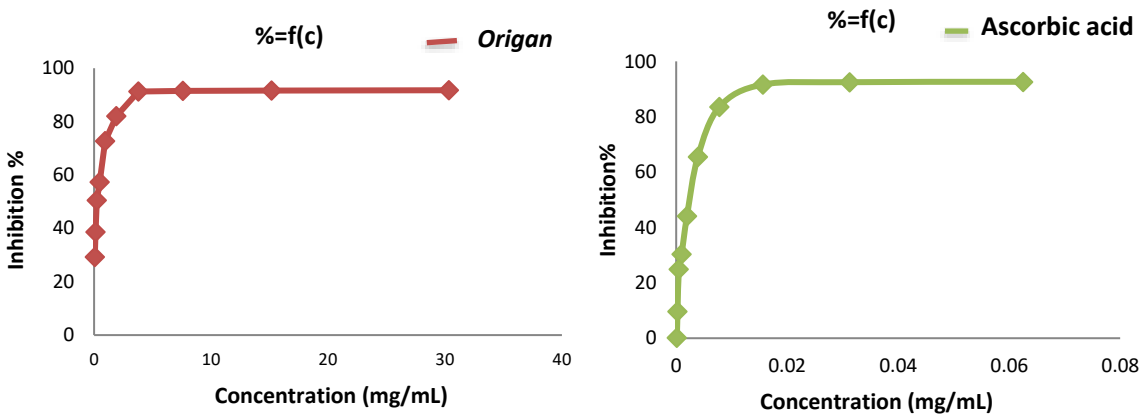


Figure 1: DPPH free radical scavenging activity of *O. compactum* EO and ascorbic acid.

Antimicrobial activity

The results of the antibacterial activity of the studied EO evaluated qualitatively (disk diffusion method) and quantitatively (MIC) are listed in Tables 2 and 3, respectively.

The data obtained for inhibition zone diameter show that *O. compactum* EO exerted significant inhibitory activity against all bacterial strains tested, compared with the synthetic antibiotic (Ampcilin) (Table 2). In addition, MICs values ranged from 0.72 to 1.562 µL/mL for all bacteria (*E. coli*, *S. aureus* and *B. subtilis*). Therefore, we can conclude that low doses of oregano EO can inhibit bacterial growth of the tested strains. It is also interesting to note that the bacteria with the largest zones of inhibition in the diffusion method have the lowest MIC values. In another study,[1] *O. compactum* EO showed antibacterial activity against several bacteria strains, expressing zones of inhibition between 27.16 and 70.16 mm and MICs values between 0.06 and 1.25 mg/mL.

EOs are mixtures of several bioactive molecules, so their antimicrobial activity is linked to their composition and interactions. These interactions can be of three types: additive, synergistic, or antagonistic.[18] The *O. compactum* EO contains a significant amount of both thymol and carvacrol in varying proportions. When combined,

these components produce an additive effect. Carvacrol is a phenol widely studied for its inhibitory properties against many bacteria. Its isomer, thymol, has a significant antibacterial effect.[19] Moreover, several studies have demonstrated that the antimicrobial effect of thymol and carvacrol is linked to their ability to permeabilise and depolarise the plasma membrane.[19] In addition to its high carvacrol and thymol content, other molecules, such as p-cymene, give the studied EO a more advantageous antibacterial activity.

Conclusion

Various compounds, like carvacrol, thymol, and γ-terpinene, in the EO of Moroccan *O. compactum* may have been responsible for the significant antibacterial activity against the pathogenic bacteria tested in this study and claimed to be responsible for nosocomial infections. The EO of *O. compactum* also exhibited a strong antioxidant effect compared to many other essential oils. These results show that the EO extracted from Moroccan oregano may present a better alternative to the synthetic products used in antibiotics and antioxidants.

Conflict of Interest

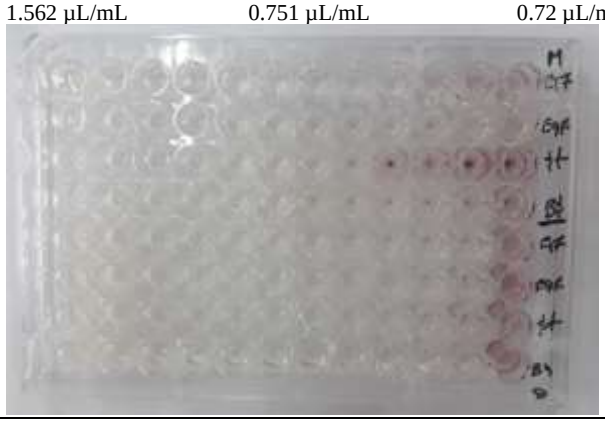
The authors declare no conflict of interest.

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.

Table 3: Minimum inhibitory concentration of *O. compactum* EO against the bacterial strains studied

Bacterial strains	***E. coli***	***S. aureus***	***B. subtilis***
Minimal inhibitory concentration (MIC)	1.562 µL/mL	0.751 µL/mL	0.72 µL/mL



References

1. El Abdali Y, Mahraz AM, Beniaich G, Mssillou I, Chebaibi M, Bin Jardan YA, Lahkimi A, Nafidi HA, Aboul-Soud MAM, Bourhia M, Bouia, A. Essential oils of *Origanum compactum* Benth: Chemical characterisation, *in vitro*, in silico, antioxidant, and antibacterial activities. Open Chem. 2023; 21: 20220282.
2. El Abdali Y, Beniaich G, Mahraz AM, El Moussaoui A, Bin Jardan YA, Akhazzane M, Chebaibi M, Nafidi HA, Eloutassi N, Bourhia M, Bouia A. Antibacterial, Antioxidant, and in silico NADPH Oxidase Inhibition Studies of Essential Oils of *Lavandula dentata* against Foodborne Pathogens. Evidence-Based Complement Altern Med. 2023; 2023: 1-12.
3. Djeridane A, Yousfi M, Brunel JM, Stocker P. RETRACTED: Isolation and characterisation of a new steroid derivative as a powerful antioxidant from *Cleome arabica* in screening the in vitro antioxidant capacity of 18 Algerian medicinal plants. Food Chem Toxicol. 2010; 48: 2599-606.
4. Ngozi PO, Falodun A, Oluseyi D. Evaluation of the antioxidant activity of root extract of pepper fruit (*Dennetia tripetala*), and its potential for the inhibition of Lipid peroxidation. Afri J Trad Complem and Altern Med. 2014; 11(3): 221-227.
5. Rezouki S, Allali A, Louasté B, Eloutassi N, Fadli M. The Impact of the Harvesting Period and Drying Conditions on the Essential Oil Yield of *Rosmarinus Officinalis*, *Thymus Satureioides*, and *Origanum Compactum* from the Taza-Taounate Region. Asian J Agric Biol. 2021; 3: 202004251.
6. Falodun A, Siraj R, Choudhary MI. GC-MS analysis of insecticidal leaf essential oil of *Pyrenacantha staudtii* Hutch and Dalz (Icacinaceae). Trop J Pharm Res. 2019 ; 8(2) : 139-143.
7. *El Abdali Y, Allali A, Agour A, Mikou K, Lahkimi A, Eloutassi N, Bouia A. Phytochemical Screening, and in Vitro Antiradical and Immunostimulant Potential of Linum Usitatissimum L. Pharmacologyonline. 2021; 3: 602-614.*
8. Egharevba E, Chukwuemeke-Nwani P, Eboh U, Okoye E, Bolanle IO, Oseghale IO, Imieje VO, Erharuyi O, Falodun A. Antioxidant and Hypoglycaemic Potentials of the Leaf Extracts of *Stachytarphyta jamaicensis* (Verbenaceae). Trop J Nat Prod Res. 2019; 3(5): 170-174.
9. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 1999; 269: 337-341.
10. Chebaibi A, Marouf Z, Rhazi-Filali F, Fahim M, Ed-Dra A. Evaluation of the antimicrobial power of essential oils from seven medicinal plants harvested in Morocco. Phytothérapie. 2016; 14: 355-362.
11. Eloff J. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica. 1998; 64(8): 711-713.
12. Allali A, El abdali Y, Rezouki S, El moussaoui A, Bourhia M, Salamatullah AM, Alzahrani A, Alyahya HK, Albadr NA, Nafidi HA, Ouahmane L, Fadli M. Chemical composition and antifungal, insecticidal and repellent activity of essential oils from *Origanum compactum* Benth. Used in the Mediterranean diet. Front Plant Sci. 2022; 13: 798259.
13. Belkamel A, Bammi J, Belkamel A, Douira A. Study of the chemical composition of the essential oil of an Ibero-Moroccan endemic: *Origanum compactum* (Benth.). J Anim Plant Sci. 2013; 19(1): 2880-2887.
14. Sbayou H, Boumaza A, Hilali A, Amghar S. Antioxidant properties of *Artemisia herba-alba* Asso., *Mentha pulegium* L. and *Origanum compactum* Benth. Essential oils. J Mater Environ Sci. 2016; 7: 2908-2912.

15. Leyva-López N, Gutiérrez-Grijalva EP, Vazquez-Olivo G, Heredia JB. Essential oils of oregano: Biological activity beyond their antimicrobial properties. Molecules. 2017; 22(6): 989.
16. Cutillas AB, Carrasco A, Martinez-Gutierrez R, Tomas V, Tudela J. Thyme essential oils from Spain: Aromatic profile ascertained by GC–MS, and their antioxidant, anti-lipoxygenase and antimicrobial activities. J Food Drug Anal. 2018; 26: 529-544.
17. De Martinoa L, De Feoa V, Fratiannib F, Nazzaro F. Chemistry, Antioxidant, Antibacterial and Antifungal Activities of Volatile Oils and their Components. Nat Prod Commun. 2009; 4: 1741-1750.
18. Faleiro ML. The mode of antibacterial action of essential oils. In: Méndez-Vilas A (Eds.), Science Against Microbial Pathogens: Communicating Current Research and Technological Advances. Badajoz, Spain: Formatex Research Center; 2011. 1143-1156 p.
19. Xu J, Zhou F, Ji BP, Pei RS, Xu N. The antibacterial mechanism of carvacrol and thymol against *Escherichia coli*. Lett Appl Microbiol. 2008; 47: 174-179.