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Research Article

ANTIMICROBIAL, ANTIOXIDANT ACTIVITY, AND CHEMICAL COMPOSITION OF *ORIGANUM COMPACTUM* BENTH FROM TAOUNATE PROVINCE, NORTH MOROCCO

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ABSTRACT

Objective: *Origanum compactum* is one of the most important medicinal species in terms of ethnobotany in Morocco. It attracts the attention of several research works; however, its chemical composition under local conditions is not well documented. Here, we aimed at determining essential oil chemical composition, antibacterial, and antioxidant activities of organic extracts of *O. compactum*.

Methods: Antimicrobial activities were assessed against four strains (*Escherichia coli*, *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans*). Antioxidant activities were estimated by radical 2,2-diphenyl-1-picrylhydrazyl scavenging activity. Polyphenols and flavonoids were determined. Chemical composition was screened using gas chromatography.

Results: Our results showed that yield extracts varied significantly among solvents and ranged from 10.30% (n-hexane) to 31.70% (methanolic). Methanolic extracts had the highest values of yield, polyphenols, and flavonoids, while n-hexane extracts showed the lowest values of yield and polyphenols. Regarding antimicrobial activities, *E. coli* showed the most important activity. Concerning antioxidant activities, n-hexane extracts had the most important activity. The phytochemical screening revealed 12 compounds. Among them, γ-Terpinene, o-Cymene, and carvacrol were the major compounds (around 73% of total chemical compounds).

Conclusion: It could be concluded that the studied plant might be a promising source of chemical compounds responsible for important biological activities.

Keywords: *Origanum compactum*, Antioxidant and antimicrobial activities, Chemical composition.

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INTRODUCTION

Morocco is one of the most interesting floristic areas in North Africa. The flora of Morocco is estimated to comprise 978 endemic taxa, which constitute more than half of North African endemic plants [1]. This endemic richness may be due to the presence of mixed and well-differentiated environments. *Origanum* is one of the genera that contain important endemic plant taxa in the country.

Origanum compactum Benth is belonging to the Lamiaceae family. It is one of the most important medicinal species in terms of ethnobotany in Morocco. It is considered a threatened species as it is heavily exploited. Its domestication remains the most efficient way to safeguard it for future generations. For this purpose, wide evaluation of the existing variability in all over the Moroccan territory is required.

The genus *Origanum* is a taxonomically complex group of aromatic plants that are used all over the world for their aromatic and medicinal properties and as a culinary herb [2]. According to the classification of Laghmouchi *et al*. [3], this genus is divided into 38 species, six subspecies, and 17 hybrids, arranged in three groups and ten sections. Geographical distribution of *Origanum* is wide, covering Canary and Azores Islands, Mediterranean basin, North Europe up to East Asia. However, the Mediterranean basin region is the most important area of this genus, and it is characterized by a large morphological and chemical diversity [4].

O. compactum Benth, known locally as "Zaatar," constitutes one of the most appreciated aromatic herbs, widely used in Moroccan folk medicine in the form of infusions and decoctions to treat bronco-pulmonary, gastric acidity, gastrointestinal diseases, and numerous infections [5]. Due to its pleasant flavor and spicy fragrance, this species is used as an aromatic ingredient of choice for flavoring some traditional dishes (barley soup, couscous, etc.).

Origanum species are used worldwide in traditional medicine for the treatment of several diseases such as diabetes and diarrhea [6-8]. *In vitro* studies reported a wide range of pharmacological properties of species belonging to this genus. They include antibacterial, anti-inflammatory, antioxidant, antitumor, antifungal, antiviral, and antileishmanial [9-12]. Several studies have reported that *Origanum* essential oils (EOs) contain numerous terpenoids and phenolic compounds such as carvacrol, thymol, γ-terminene, and p-cymene [2,13]. These compounds are linked to several biological properties such as anticancer, antimicrobial, and antioxidant activities [14-16].

To the best of our knowledge, the chemical composition of *O. compactum* from Taounate Province (North Morocco) is not well documented. Hence, the originality of this work which had as goals, (i) to study biological activities of aerial parts from *O. compactum*, (ii) to investigate the chemical composition of this medicinal species, and (iii) to compare extracts from various solvents in terms of yield, polyphenols, flavonoids, and their biological activities.

METHODS

Plant material

The plant species has first been authenticated by the Botanist Professor A. Ennabili, Higher School of Technologies, Sidi Mohamed Ben Abdellah University, Fez, Morocco. The flowering tops (the aerial part) of *O. compactum* Benth were collected in May 2018 in the region of Bouadel (25 km from the Taounate Province) in North Morocco

(34°32′9″N 4°38′24″W). The plant was dried in a dark room to prevent the photo-oxidation, then crushed to a fine powder using an electric grinder. The obtained powder was subjected to extraction by maceration. Briefly, 25 g of powder were used for extraction using different solvents separately (methanol, ethanol, n-hexane, and ethyl acetate). The extraction period was 72 h. The extracts were then filtered (Whatman No. 1), and the filtrates obtained were concentrated by means of a rotary evaporator (Heidolph Collegiate, LV28798826, New Jersey, USA) at a temperature of 45°C.

The extraction of EOs was carried out by the hydro-distillation method using Clevenger-type apparatus. The obtained oils were dried by anhydrous sodium sulfate, weighed and stored at 4°C until use.

Chemical reagents

2,2-diphenyl-1-picrylhydrazyl (DPPH), aluminum trichloride colorimetric method ($AlCl_3$), sodium carbonate, Folin–Ciocalteu, methanol, ethyl acetate, ethanol, and n-hexane were used. These chemical reagents were purchased from Sigma-Aldrich (St. Louis, MO). All solvents and reagents used are of the highest purity.

Microbial strains

Extracts obtained by various solvents were tested for antibacterial activities against the following microbial strains: *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC29213, *Bacillus subtilis* ATCC 3366, and *Candida albicans* ATCC 10231.

Evaluation of total polyphenols content (TPC)

TPC was determined by Folin–Ciocalteu method [17]. Briefly, 100 µl of extracts (1 mg/ml) were added to 500 µl of 1:10 Folin–Ciocalteu reagent (prepared before use). After 4 min, 400 µl of sodium carbonate 7.5% (m/v: 75 mg/ml) were added. After 30 min of incubation at room temperature, the optical density was measured at 765 nm using spectrophotometer type VARIAN Cary 50 ultraviolet/visible. A standard curve was prepared from a solution of GALLIC acid (GA) (5 mg/ml) with concentrations ranging from 0 to 150 mg/ml. The results were reported in GA equivalents (GAE) per g of sample.

Determination of total flavonoids content (TFC)

TFC was determined by the aluminum trichloride colorimetric method ($AlCl_3$) [18,19], with a slight modification. Briefly, 250 µl of extracts (2 mg/ml) were added to 1.4 ml of deionized water, 50 µl of potassium acetate (1 M), 50 µl of $AlCl_3$ (10%, m/v), and 750 µl of absolute ethanol. After 30 min of incubation at room temperature, the absorbance at 415 nm was measured. Quercetin was used as the reference compound to produce the standard curve, and the results were expressed as gram of quercetin equivalents (QEq) per gram dry weight.

Antimicrobial activity

Antibacterial activities of the extracts were examined by disk diffusion method [20] with slight modifications. Briefly, bacterial strains were cultured overnight at 37°C and 30°C for fungal strains on Luria-Bertani broth. Then, an inoculum consisting of 0.5 McFarland was prepared. The bacterial inoculum (100 µl) was inoculated in Petri dishes containing a sterile Luria-Bertani agar medium. Sterile filter paper discs (5 mm of diameter) were deposited on medium and impregnated with 10 µl of extract solution (500 mg/ml of dimethyl sulfoxide [DMSO] to 2%). The control was performed with discs containing 10 µl of DMSO to 2%. Each experiment was performed in triplicate.

Antioxidant activity (AA)

The ability to trap the radical DPPH was determined by the standard method with some modifications [21]. Briefly, 0.2 ml of different concentrations (20–480 µg/ml) of *O. compactum* extracts are added to 1.8 ml of methanolic solution DPPH (0.11 mM). After incubation, 30 min in darkness at room temperature (23±2°C), absorbance was read at 517 nm against a methanol blank and the solution of DPPH. The radical scavenging activity (also known AA) was calculated as follows:

$$\%(AA) = \left[\frac{(Abs_{control} - Abs_{sample})}{Abs_{control}}\right] \times 100$$

$Abs_{control}$ is the absorbance of the control reaction (containing all reagents, and except the test sample), and Abs_{sample} is the absorbance of extracts or reference. Ascorbic acid was used as a positive control and the concentration of extract that inhibits 50% (IC_{50}) of the radical DPPH was calculated from the percentage graph inhibition according to the concentration of extract using the exponential equation.

Phytochemical screening using gas chromatography-mass spectrometry

The analysis of EOs was made according to Talbaoui *et al*. [22]. It was carried out on a TRACE GC ULTRA equipped with non-polar VB5 (95% methyl polysiloxane, and 5% phenyl), capillary column (30 m×0.25 mm i.d. and 0.25 µm as a film thickness), directly coupled to a mass spectrometer (Polaris Q) (EI 70 eV). The temperatures of the injector and detector were set at 250 and 300°C, respectively. The oven temperature was programmed to increase from 40 to 180°C at 4°C/min, for 180–300°C at 20°C/min. The gas carrier was helium with a flow rate of 1 mL/min; the sample (1 µL) was injected with a splitless mode.

Statistical analysis

Each determination was achieved in triplicates. Quantitative differences were assessed by the general linear procedure followed by Duncan's test. Data statistical analyses were performed using the SPSS package version 13.0 for Windows. Values were expressed as means±standard deviations. Differences were considered significant at $p<0.05$. The correlation matrix was carried out on mean values.

RESULTS

Mean comparison of yield, TPC, and TFC

TPC in the extracts examined from the plant using the Folin–Ciocalteu reagent and expressed in GAE terms (the standard equation curve: Abs=0.010×(AG)+0.002; R^2=0.998). The values obtained for the concentration of TPC were expressed in mg GAE/g extract (Table 1). TPC in the extracts examined was ranged from 160.58 to 103.26 mg GAE/g extract.

Yield ranged from 31.70% dry matter (Mt-OH) as the highest value to 10.30 (n-hexane) as the lowest one. With regard to TPC and TFC, the greatest values were recorded in Mt-OH (160.58 mg GAE/g of extract) and 57.30 mg QEq/g of extract, respectively. However, the smallest values of TPC (103.26 mg GAE/g of extract) and TFC (24.08 mg QEq/g of extract) were displayed by extracts of n-hexane and ethyl-acetate, respectively.

Antimicrobial activity in the extracts

Fig. 1 shows the results obtained for antimicrobial activities (inhibition zone diameter). The four extracts tested showed significant differences among the four studied strains for each solvent. *E. coli* presented the most important antimicrobial activity for Et-OH, while the extract of

Table 1: Mean values of yield, TPC, and TFC for the various solvents used. In each column, mean values followed by the same letter are not significantly different at a probability level of 5%

Solvent	Yield (% DM)	TPC (mg Eq GA/g of extract)	TFC (mg QEq/g of extract)
Et-OH	22.67±1.81^b	117.56±2.74^c	24.97±2.56^c
Mt-OH	31.70±2.73^a	160.58±7.59^a	57.30±4.05^a
Eth-Acet	20.48±0.87^c	119.34±0.92^b	24.08±1.74^c
n-hexane	10.30±0.68^d	103.26±2.57^d	29.35±0.93^b

Values are expressed as mean±standard deviation (n=3). TFC: Total polyphenols content, TFC: Total flavonoids content, DM: Dry matter, QEq: Quercetin equivalents, Et-OH: Ethanol, Mt-OH: Methanol, EthAcet: Ethyl acetate. ^{a,b,c,d}Indicate statistical differences between different values in each column

EthAcet showed the lowest value for the same strain. *S. aureus* had the greatest activity in n-hexane and the lowest one was obtained in EthAcet. Concerning *B. subtilis*, the most important activity was presented by EthAcet and the smallest one was recorded in Et-OH extract. Regarding *C. albicans*, the score of activity was obtained in n-hexane extract, while EthAcet extract showed the lowest score of activity.

Antioxidant activities of extracts

Results of antioxidant activities of different extracts of *O. compactum* are presented in Fig. 2. All extracts proved to be able to reduce radical DPPH. There was a significant decrease in this radical as a function of the concentration of extract. At 40 µg/ml, ascorbic acid showed a higher capacity to inhibit the radical DPPH (66.50%). Among our extracts, n-hexane was the most important regarding DPPH reduction. At the same concentration (40 µg/ml) inhibition percentages of n-hexane, Me-OH, Et-OH, and EthAcet were 60.00, 50.10, 46.15, and 20.25%, respectively. Antioxidant activities followed a pattern of dose-dependent type. In fact, once the concentration of extract increased, the percentage of inhibition was significantly increased. As compared to control (ascorbic acid), n-hexane presented the highest activity among all extracts, while the EthAcet extract found to have the smallest value of AA.

The IC_{50} value is defined as the concentration of an extract that inhibits 50% of the DPPH radical. The IC_{50} for each of the different extracts was determined. The n-hexane extract presented the highest antiradical activity (IC_{50}=35.50 µg/ml) followed by the Me-OH extract with an IC_{50} of in the range of 42.75 µg/ml. On the other hand, the lowest antiradical activity was expressed by the Et-OH extract and the extract ethyl acetate (IC_{50}=61.25 and 85.50 µg/ml, respectively).

Table 2: Chemical composition of essential oils of aerial parts from *Origanum compactum*

Number	Compound	RT (min)	Relative %
1.	3-Thujene	4.841	1.522
2.	α-Pinene	4.966	0.779
3.	Myrcene	6.635	2.486
4.	2-Carene	7.256	4.116
5.	o-Cymene	7.602	27.046
6.	γ-Terpinene	8.585	28.690
7.	β-Linalool	9.827	4.684
8.	4-Terpineol	11.848	0.457
9.	Carvacrol	15.937	17.120
10.	β-Caryophyllene	18.222	9.018
11.	β-Bisabolene	20.459	2.658
12.	Caryophyllene oxide	22.025	1.423

RT: Retention time

EO composition

The yield of EOs obtained by hydro-distillation was expressed as the percentage of plant dry weight (5.68%). The obtained chromatogram (Fig. 3) for EO was characterized by 12 chemical compounds (Table 2) accounting for 99.99% of the total chemical composition. The major ten compounds were γ-Terpinene (28.69%), o-Cymene (27.05%), carvacrol (17.12%), β-caryophyllene (9.02%), β-linalool (4.68%), 2-Carene (4.12%), β-Bisabolene (2.66%), myrcene (2.49%), 3-Thujene (1.52%), and caryophylene oxide (1.42%). Minor compounds (whose percentage was lesser than 1%) were 4-terpineol (0.46%) and α-Pinene (0.78%).

Correlations' study

The correlation matrix among studied parameters is presented in Table 3. Important correlations were found between some studied parameters. In this context, yield of extracts was positively and significantly associated with polyphenols (r=0.865***) and flavonoids (r=0.703**). In addition, polyphenols and flavonoids were positively linked (r=0.881***). The remaining correlations were, in general, low or insignificant.

DISCUSSION

Results of the yield of extracts, TPC, TFC, and antimicrobial activities were mainly under the type of solvents used for extraction in agreement with other authors [23].

TPC in extracts of *O. compactum* Benth depended on the extract type. This is due to the difference in terms of the polarity of solvents used in extraction. The high solubility of phenols in polar solvents gives the high concentration of these compounds in extracts obtained using polar solvents for extraction [23]. The TFC in the extracts ranged from 57.30 to 24.08 mg GAE/g extract, with the methanolic extract containing the highest TFC. The concentration of flavonoids in the extracts of the plant depends on the polarity of the solvents used in the extract preparation [23,24].

Antibacterial activity can be divided into three levels [25-27] as follows: Low activity (inhibition zone ≤12 mm), medium activity (12 mm<inhibition zone<20 mm), and high activity (inhibition zone ≥20 mm). The four extracts tested showed on the zones of inhibition

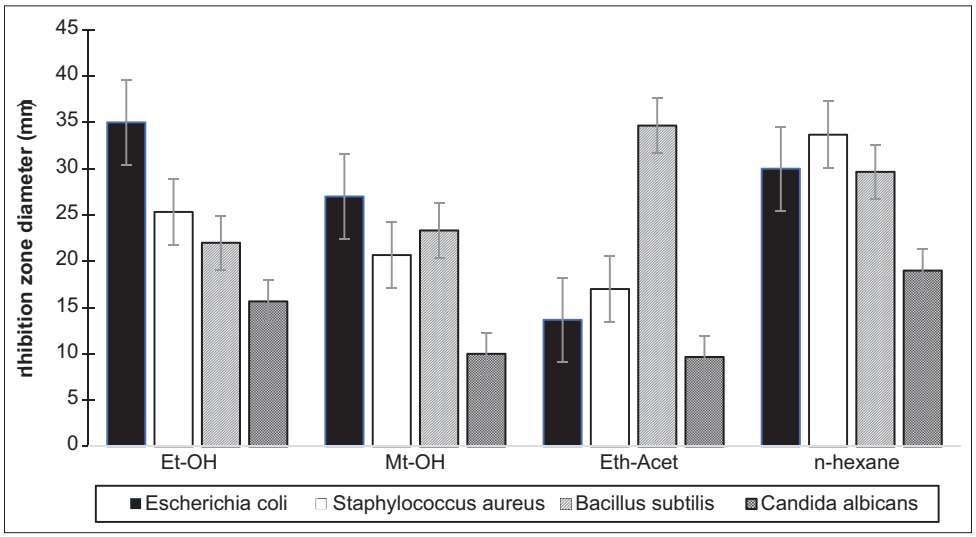

Fig. 1: Mean values of antimicrobial activity (inhibition zones diameter in mm) of aerial parts from *Origanum compactum* using four different solvents (Et-OH: Ethanol, Mt-OH: Methanol, EthAcet: Ethyl acetate, n-hexane). For each strain, mean values followed by the same letter are not significantly different at a probability level of 5%

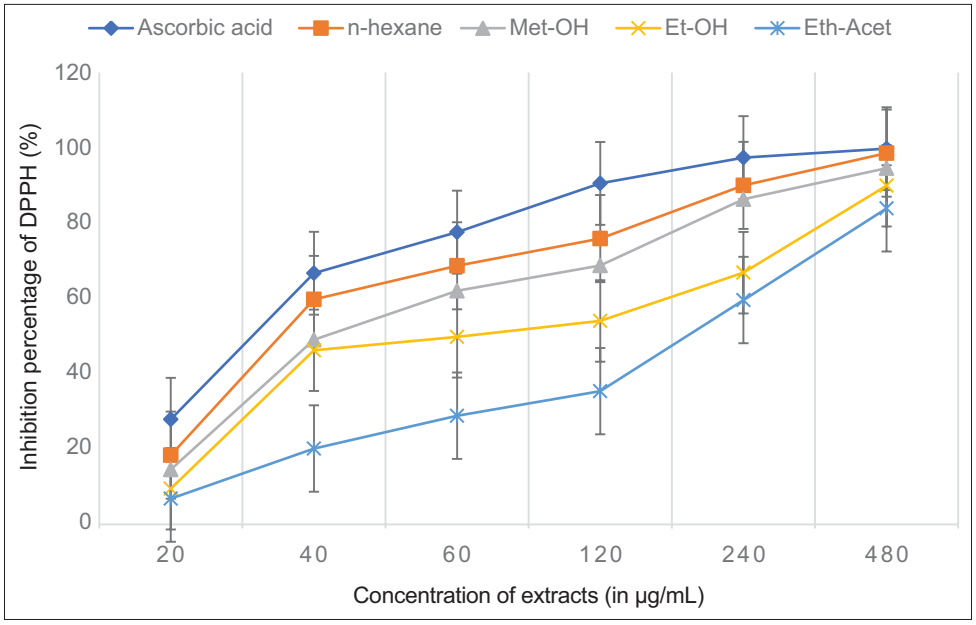

Fig. 2: Antioxidant activities expressed as inhibition percentage of 2,2-diphenyl-1-picrylhydrazyl of extracts obtained from aerial parts *Origanum compactum* Benth. Ascorbic acid was used as a control. Each point is a mean value of three replicates

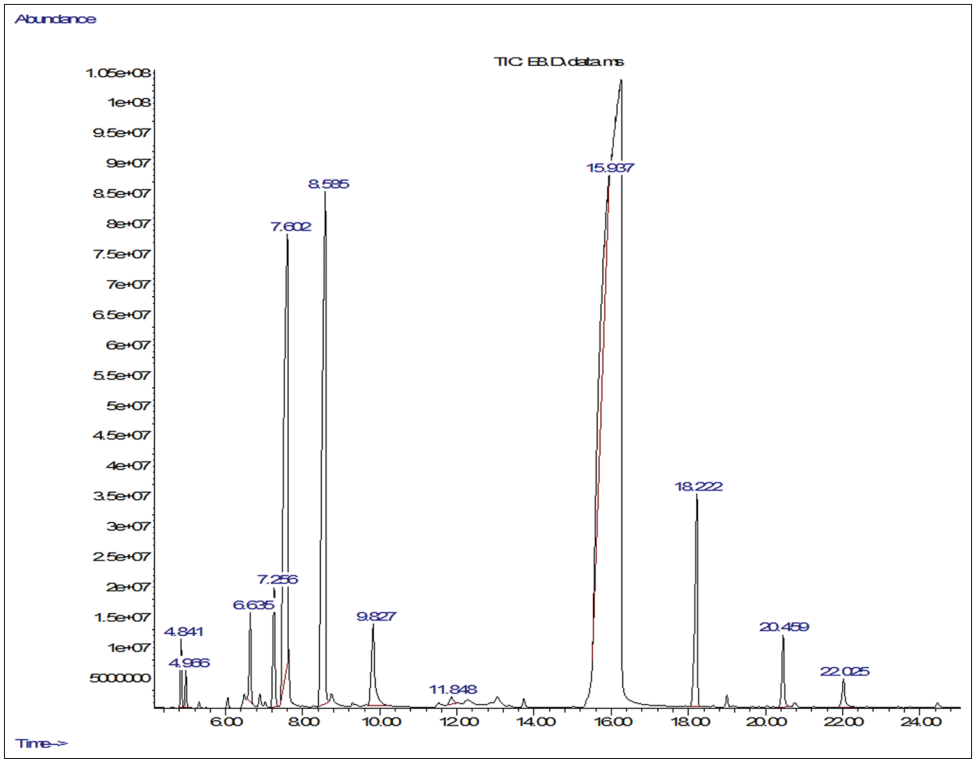

Fig. 3: Gas chromatography-mass spectrometry chromatogram of *Origanum compactum* Benth aerial parts essential oil

significant variability as well between extracts than between bacterial strains. This difference is related to the chemical composition of each extract and the nature of the bacterial strain tested. In this study, the bacteria Gram positive (*S. aureus* and *B. subtilis*) is more sensitive to extracts than Gram negative (*E. coli*) [28].

Indeed, the majority of antibacterial agents are more active against Gram-positive bacteria as against Gram-negative bacteria. The resistance of Gram-negative bacteria is attributed to the character hydrophilic of the membrane that blocks the penetration of hydrophobic molecules such as polyphenols [29]. The bacterial wall of *E. coli,* for example, is very rich in lipopolysaccharides that prevent molecules hydrophobic to cross the membrane [30-32].

All extracts proved to be able to reduce radical DPPH. There was a significant decrease in this radical as a function of the concentration of extract. We interpret this phenomenon by the transfer of single electrons that are located in the outer orbit of DPPH, and after reaching a given concentration, the antioxidant will react completely with the radical, and when we increase concentration, AA will stay constant since this is accompanied by the saturation of electron layers of the radical.

Table 3: Correlation coefficients among the studied traits

	Yield	Polyphenols	Flavonoids	*E. coli*	*S. aureus*	*B. subtilis*	*C. albicans*
Yield		0.895***	0.703**	−0.066	−0.652**	−0.377	−0.609*
Polyphenols			0.881***	−0.077	−0.464	−0.413	−0.574
Flavonoids				0.081	−0.179	−0.346	−0.314
E. coli					0.585	−0.714**	0.438
S. aureus						−0.153	0.778**
B. subtilis							−0.081
C. albicans							

Matrix correlation was carried out on mean values for each trait. *, ** and ***Indicate significance at 0.05, 0.01, and 0.001 levels of probability, respectively.
E. coli: *Escherichia coli*, *S. aureus*: *Staphylococcus aureus*, *B. subtilis*: *Bacillus subtilis*, and *C. albicans*: *Candida albicans*

The IC_{50} for each of the different extracts was determined. IC_{50} is defined as the concentration of the substrate that causes 50% inhibition of DPPH [33,34]. As stated in the results' section, there were significant differences in terms of IC_{50} values between solvents used for extraction. Our results were in agreement with those of El Babili *et al*. [35] who have shown that organic extracts *O. compactum* were found to be active and showed remarkable AA toward the radical DPPH [36]. From these results, it seems to be thymol and carvacrol that are excellent reductants in these assays. Our results were in agreement with several other findings [37-39]. However, these authors demonstrated that linalool-rich EOs exhibited a weak reducing power.

It has been proven that EO's yield correlates with harvest period, extraction method, and phenological stage [40,41]. These authors observed similar trends for EO's yield (5.7% [40] and 5.6% [41]).

EO was characterized by 12 chemical compounds as highlighted in the results' section accounting for 99.99% of the total composition. These outcomes were in line with those reported by other authors [2,10]. In fact, similar chemical composition was reported for the species harvested from Ouezzane Province (Northwest Morocco) [40]. However, EO's chemical composition depends on the pedoclimatic conditions, phenological stages, harvest time, and extraction method, as demonstrated by other authors [2,40,42-44].

CONCLUSION

The results of our study suggest the importance of *O. compactum* Benth from Morocco for its use in pharmacy and herbal medicine. The highest concentration phenolic compounds were obtained using solvents of increasing polarity. The methanolic extract had the greatest value of phenolic compounds and flavonoids.

The significant linear correlation between values of the concentration of phenolic compounds and AA indicated that these compounds are responsible for the observed AA. Results obtained for antimicrobial activity are significant; they could be attributed to higher quantity of phenolic compounds. Further *in vivo* studies would be needed to better understand the mechanism action of bioactive molecules, their therapeutic dose as well as their action site at the cell level. It would allow us to prepare pharmaceutical products of great therapeutic interest and having a natural origin. Several industries are now looking for sources of new natural and safe agents by determining antioxidant, antimicrobial, and antioxidant activities of *O. compactum* Benth species. EO extracted from this plant might be considered as an excellent source of natural compounds.

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AUTHORS' CONTRIBUTIONS

Author contributions: Ahmed Zeroual and Noureddine Eloutassi conceived and designed the experiments. Ahmed Zeroual and Noureddine Eloutassi, performed the experiments and the acquisition, analysis, and interpretation of data. Ahmed Zeroual, Mahdi Chaouch, and Abdellah Chaqroune wrote the paper. Abdellah Chaqroune supervised the work.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest regarding the publication of this paper.

DATA AVAILABILITY

The data used to support the findings of this study are included in the article and are available from the corresponding author on request.

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