

Chemical Composition and Antioxidant Activity of *Origanum elongatum* Essential Oil

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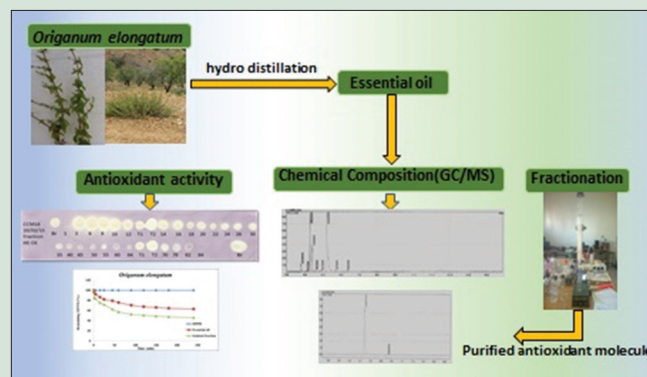
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ABSTRACT

Background: In recent years, antioxidants have become essential in nutrition because of their ability to protect the body against oxidative cell damage that can produce dangerous diseases. Medicinal plants are a major source of many bioactive molecules that act directly on the body. **Objective:** The main objective of our work is to highlight the antioxidant potential of the essential oil (EO) of *Origanum elongatum* Emb and Maire (endemic plant of Morocco), determine its composition, and carry out fractionation operations to isolate the molecule (s) responsible for this activity. **Materials and Methods:** The measurement of the antioxidant activity of the EO is carried out by trapping the free radical 2,2-diphenyl-1-picrylhydrazyl and the composition of the EO is determined using gas chromatography coupled with mass spectrometry. The fractionation of the EO was carried out by various chromatography techniques. **Results:** The results show that the EO has an interesting antioxidant activity with an EC_{50} in the order of 1.2 in comparison with a reference antioxidant, δ -tocopherol whose EC_{50} is 0.26. The EO of *O. elongatum* consists of 11 compounds, of which 3 are the majority: carvacrol (60.42%), para-cymene (13.9%), and γ -terpinene (9.4%). **Conclusion:** Fractionation of the oil made it possible to isolate a purified antioxidant molecule consisting mainly of carvacrol (95%). **Key words:** 2,2-diphenyl-1-picrylhydrazyl, antioxidant activity, essential oil composition, fractionation, *Origanum elongatum*

SUMMARY

- *Origanum elongatum* is an endemic species of Morocco. In this work, the antioxidant activity of his essential oil (EO) is evaluated using the 2,2-diphenyl-1-picrylhydrazyl test
- The analysis of the EO is carried out by the gas chromatography coupled with mass spectrometry
- Fractionation of the EO made it possible to isolate a purified antioxidant molecule.



Abbreviations Used: DPPH: 2,2-diphenyl-1-picrylhydrazyl; GC/MS: Gas chromatography coupled with mass spectrometry; EOs: Essential oils; TLC: Thin-layer chromatography; NIST: The National Institute of Standards and Technology; Ppm: Parts per million; OD: Optic density; CL: Column chromatography.

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DOI: 10.4103/pr.pr_157_18

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INTRODUCTION

The beneficial effect of antioxidants on human health has become an important topic giving rise to a lot of research worldwide. In recent decades, the interest in natural antioxidants has increased given their importance and their use in various fields of agriculture, medicine, food industry, etc.

In addition, antioxidants have become essential nutrients in nutrition; they are important in terms of their ability to protect against oxidative cell damage that can lead to diseases such as Alzheimer's, cancer, cardiovascular disease, and other chronic diseases.^[1]

However, the most used synthetic antioxidants, such as butyl hydroxyl anisole, butyl hydroxytoluene, propyl gallate, and tertiary butyl hydroquinone have been suspected to cause or promote undesirable side effects which are associated with their toxic effects.^[2] For these reasons, there are evident requirements for increasing application of natural antioxidants obtained from plant material.

Medicinal plants are used for their richness in nutrients and for their ability to promote human health and protect against a number of diseases and infections. These plants present an important source of secondary metabolites such as essential oils (EOs) and phenolic compounds that include flavonoids which present interesting biological activities.^[3-5]

In addition, plant extracts and particularly the EOs of many plant species are known for their significant antioxidant capabilities.^[6,7]

The EOs are a very heterogeneous group of natural products derived from aromatic plants and whose composition may be different between different species or varieties.^[8] In spite of their aroma, odor, and fragrance, some of these EOs are also widely used in cosmetics and nutrition.^[9]

Different species of *Origanum*, plants of *Lamiaceae* family, have been the subject of numerous scientific studies.^[6,10]

Many reports have shown that *Origanum vulgare* L. is one of the most widespread and known species of this family which EOs is particularly rich in mono- and sesquiterpenes.^[11]

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Cite this article as: Oualili H, Nmila R, Chibi F, Lasky M, Mricha A, Rchid H. Chemical composition and antioxidant activity of *Origanum elongatum* essential oil. Phcog Res 2019;11:283-9.

Chemical composition of the EOs of the genus *Origanum* is well documented. The major constituents are phenolic monoterpenes such as thymol and carvacrol. This oil also contains sesquiterpene, terpinene, flavonoids, and other compounds with variable relative proportion.^[7,10,12] By the way, these oils have been shown to possess antioxidant, antibacterial, antifungal, diaphoretic, carminative, antispasmodic, and analgesic activities.^[11]

Consequently, the EOs of this genus and its major constituents are considered a potential source of active compound interest.

Origanum elongatum an endemic species of Morocco, is widely used in local traditional medicine for treating vomiting, acute gastroenteritis, food poisoning, and respiratory problems.^[13] It remains however little explored.

In a previous work of screening biological properties among different species of Morocco, we demonstrated the presence of antioxidant activity in extracts of *O. elongatum*.^[14] The objective of this work is to study the antioxidant properties of *O. elongatum* EO and its composition in order to proceed to operations of fractionation and isolation of the substances responsible for this activity.

MATERIALS AND METHODS

Plant material

O. elongatum Emb and Maire was collected in June 2013 at “Targuist” in the Rif in Northern Morocco (34° 57’ North 4° 18’ West), a mountainous area of 994 m. Air-drying of plant (leaves and flowering tops) was performed in the shadow in a dry and ventilated room.

Preparation of the essential oil and fractions

Preparation of the essential oil

The EO is obtained by hydrodistillation in Clevenger-type apparatus: 250 g of flowered tops and leaves is extracted during 4 h. The obtained EO was dried over anhydrous sodium sulfate and stored at 4°C in the dark until required. The voucher specimens of plant material and EO are deposited at the Herbarium of the Department of Biology, Chouaib Doukkali University, Faculty of Sciences, El Jadida, Morocco.

Preparation of the fraction

The EO was fractionated by silica column chromatography (CL) and preparative thin-layer chromatography (TLC).

Chromatography on a silica column

The column used is an open column of large particle size silica (G 60, 0.063–0, 02 mm, Merck). It is glass, the diameter and length of the column changes according to the step of the fractionation. An amount of silica (50 g) was mixed with methanol (200 ml) and homogenized by ultrasound during 1 h. Several solvents combinations were tested to find the eluent which gives the best separation of the EO compounds. The extract was dissolved in the eluent (1/1; w/v) before being deposited. The elution rate was selected and kept constant during the fractionation.

The fractions obtained were concentrated with a rotary evaporator. The analysis and monitoring of the fractionation were carried out by TLC in two different ways: without migration (with the revelation by 2,2-diphenyl-1-picrylhydrazyl [DPPH]) and with migration (with reading under ultraviolet [UV] lamp at 260 and 366 nm initially and after revelation with DPPH or methanolic sulfuric acid (heated at 110°C for 10 min).

Preparative thin-layer chromatography

TLC plates were prepared with silica (Merck, Kiesel G, Type 60) having a thickness of 0.5 mm. After reactivation of the plate and deposition of

the extract on TLC plate in the form of a discontinuous line (the features of 1.5 cm), migration is performed with the same solvent used for CL. At the end of the migration, the plate is dried in the open air to protect from light. The edge of the plate (a zone of 1.5 cm) is revealed by DPPH to locate the zone containing the active product (s). The silica of the parallel zones corresponding to those which proved to be active was recovered and then eluted by different solvents (different polarities) and by the ultrasonic passage.

Antioxidant activity

Screening of antioxidant activity by spectrophotometry

The EO and the isolated fraction were solubilized in methanol (1 mg/ml) and 0.1 ml of the solution was mixed with 3.9 ml of methanolic DPPH (6.10^{-5} mol/l) previously prepared. The mixture was stirred and then the absorbance was measured at 517 nm at regular time intervals: 0 min, 3 min, 5 min, and every 15 min up to 120 min. Purple DPPH turned yellow in the presence of antioxidants.^[10]

Measurement of antioxidant activity

The antioxidant activity was measured by trapping free radical DPPH test described by Brand-Williams *et al.*^[15] with minor modifications. Each extract eight concentrations are prepared (0.2–2.5 mg/ml). 0.1 ml of each test solution is added to 3.9 ml of a solution of DPPH (Sigma Aldrich) previously prepared in methanol (6.10^{-5} mol/l). In parallel, a negative control was prepared by mixing 0.1 ml of methanol with 3.9 ml of the DPPH solution. The absorbance reading is made at 517 nm against a blank prepared for each concentration 1, 3, and 5 min then every 15 min until the reaction reached the plateau. The measurements are performed in triplicate. After stability of the reaction, the antioxidant activity was evaluated by determining the EC_{50} , the latter corresponds to the quantity of tested extract required to reduce by 50% the quantity of DPPH present in the reaction medium. The concentration of DPPH remaining was determined using the following formula:

$$\text{Abs } 517 \text{ nm} = a \times [\text{DPPH concentration}] + b.$$

Abs: Absorbance at 517 nm in the presence of extract

- Coefficient of the equation of the calibration curve of DPPH
- Slope of the equation of the calibration curve.

Analysis by gas chromatography coupled to mass spectrometry

The chemical composition of the EO and of the isolated fraction was analyzed by gas chromatography coupled with mass spectrometry (GC-MS) using a GC-MS-QP2016 with a capillary column (60 m × 0.25 mm, 0.25 μm film thickness). Helium is used as carrier gas. The oven temperature was maintained at 60°C for 6 min. Then, the temperature was programmed to increase at a rate of 8°C/min, then held constant at 260°C for 10 min. The temperatures of the injector and detector were 250°C and 220°C, respectively. The injection was in divided mode and the injected volume was 1 μl. Before injection, the sample was solubilized in hexane (1 ppm).

Peak identification was based on the chromatograms and mass spectra of each compound by comparing them with the NIST database.

RESULTS

Antioxidant activity

The DPPH test performed on the *O. elongatum* EO revealed a very interesting power to capture radicals, Table 1 shows the EC_{50} of *O. elongatum* EO compared to δ-tocopherol [Table 1].

The EO of *O. elongatum* has an antioxidant activity with an EC_{50} equal to 1.20 g of extract/g DPPH. This activity is very interesting in comparison

with δ -tocopherol; a reference molecule known as an antioxidant ($EC_{50} = 0.26$).

Chemical composition

Analysis of the oil by gas chromatography reveals a rich and varied chemical composition. Figure 1 presents the chromatogram of the EO of *O. elongatum* obtained by GC/MS.

Analysis of the chromatogram of the EO of *O. elongatum* obtained shows that the EO consists of 11 compounds, of which 3 are the majority: carvacrol, para-cymene, and γ -terpinene. All the compounds detected represent 91.5% of the EO, the chemical composition of *O. elongatum* EO obtained by GC/MS analysis is presented in Table 2.

Among the compounds detected is carvacrol, which represents the majority component of the EO of *O. elongatum* with a percentage of 60.42%, followed by para-cymene (13.90%) then γ -terpinene (9.49%) and the α -thujene (2.02%).

The *O. elongatum* belongs to the *Elongatispica*,^[16] which is characterized by species whose EOs contain carvacrol as the main compound (47.0%–79.0%), thymol in the most species (12.5%–17%), γ -terpinene (3.7%–10.4%), and para-cymene (5.2%–13.7%).^[17,18] Our results show that *O. elongatum* from Moroccan Rif has a composition comparable to other *Origanum* species except for Thymol which is present in very small quantities (0.6%). The high content of thymol and its isomer carvacrol is correlated with several biological activities.^[19,20]

The antioxidant activity of the EO is due to the presence of major compounds such as carvacrol, p-cymene, and γ -terpinene but can also be due to the presence of other compounds present in small amounts [Table 2]. These compounds can also act synergistically. Thymol and its isomer carvacrol are powerful scavengers DPPH; they are known as antioxidant molecules^[16] and they were described as the main components responsible for the antioxidant activity of *Origanum* EOs.^[7,21–23]

Fractionation of *Origanum elongatum* essential oil

Fractionation by column chromatography

The fractionation of *O. elongatum* EO on a silica column gave rise to 84 fractions. After concentration, twenty fractions deposited on a TLC plate in the presence of two positive controls; the δ -tocopherol and ascorbic acid. A revelation with DPPH was carried out in order to demonstrate the antioxidant activity registered of the deposited fractions. This activity is proportional to the degree of discoloration of deposits. Figure 2 presents the image of the silica plate with the EO fractions of *O. elongatum* after 15 min of reaction with DPPH.

Compared to deposit-positive controls, crude EO of *O. elongatum* and several fractions react positively to the test by inducing degradation of

DPPH. Furthermore, this activity is highly localized at the first fractions. A first combination of the fractions obtained is achieved by taking into account the antioxidant activity test [Figure 2] and depending on the composition of the different fractions. In this sense, a first analytical TLC was performed and analyzed under UV then revealed by methanolic sulfuric acid and heated at 110°C for 10 min. Figure 3 illustrates the chromatogram of the different fractions from the EO of *O. elongatum* after migration and visualization by sulfuric acid.

The result of these operations yielded eight fractions, which were analyzed by analytical TLC. These two plates are subjected to two types of revelation: by a DPPH to visualize the presence or absence of the antioxidant activity and the other by the methanolic sulfuric acid to preview on the composition of fractions. The chromatograms after development and revelation by DPPH (TLC1) and the methanolic sulfuric acid (TLC2) of the various grouped fractions of *O. elongatum* EO is presented in Figure 4.

The analysis of the two plates shows that the most predominant products localized by sulfuric acid (TLC2) have antioxidant activity (TLC1). Moreover, most of the antioxidant activity is found together at the III and IV fractions with a significant enrichment in compounds of these two fractions.

Fractionation by column chromatography and preparative thin-layer chromatography

The results of the first fractionation of *O. elongatum* oil allowed us to select the Fraction III [Figure 4] which proved to be rich in compounds

Table 1: EC_{50} of *Origanum elongatum* essential oil compared to δ -tocopherol

Tested product	EC_{50}
<i>Origanum elongatum</i>	1.20
δ -tocopherol	0.26

Table 2: Chemical composition of *Origanum elongatum* essential oil obtained by gas-chromatography/mass spectrometry analysis

Compounds	Retention time (min)	Percentage
α -thujene	3.800	2.02
β -myrcene	4.103	1.01
para-cymene	4.290	13.90
γ -terpinene	4.433	9.49
Linalool	4.596	1.09
terpinene-4-ol	4.903	0.60
Thymol	5.200	0.60
Carvacrol	5.228	60.42
β -caryophyllene	5.700	0.70
β -bisabolene	6.334	0.68
Caryophyllene oxide	11.515	1.02

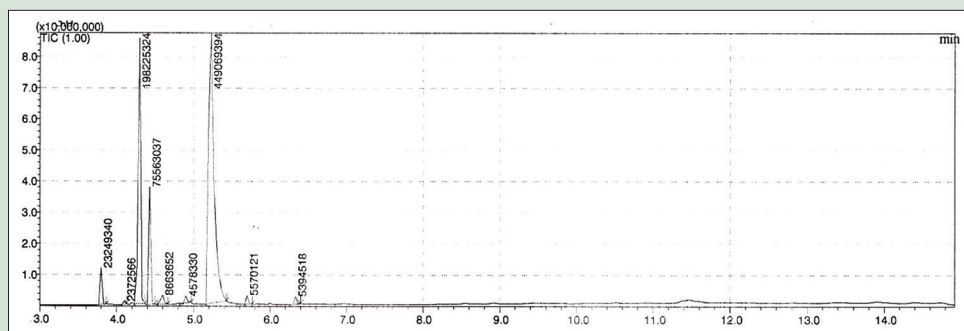


Figure 1: Chromatogram of the essential oil of *Origanum elongatum* obtained by gas chromatography combined with mass spectrometry

and whose antioxidant activity seems the most important. By following the same procedure as above, a second fractionation was performed on the Fraction III with minor modifications of the operating conditions (column diameter and elution rate).

The analysis of the fractions obtained (39 fractions) was also conducted using the same procedure and following the same steps: analytical TLC and revelation with DPPH and then with methanolic sulfuric acid.

This second fractionation on a column made it possible to isolate a fraction which presents the same profile as the Fraction II resulting from the first fractionation. To refine the separation and maximize purification of the active compounds, these two fractions were pooled (Fa) and fractionated on preparative TLC using the same migration solvent. After migration and in order to identify the active regions, the edge of the plate was revealed by the DPPH, the representation of the preparative TLC plate and the various fractions to be recovered (A, B, C, and D) is illustrated in Figure 5.

This preparative TLC fractionation [Figure 5] showed that the fractionated product is separated into four bands (A, B, C, and D) and that the activity is localized at band B [Figure 5]. The fractions thus separated were analyzed by analytical TLC and was revealed by DPPH and sulfuric acid. The chromatogram after development and revelation with DPPH and sulfuric acid of the four fractions resulting from fractionation by preparative TLC is presented in Figure 6.

The result obtained [Figure 6] shows that the antioxidant activity is localized at Fraction B (TLC + DPPH) and appears to be more important at this fraction compared with that revealed at the level of the crude EO. Reflecting an enrichment in the active(s) product(s).

In addition, the revelation with a sulfuric acid makes it possible to show that the fractionation leads to an important enrichment in compounds (TLC + H₂SO₄).

To evaluate the effectiveness of our various fractionation operations and followed isolation, crude extract (EO) and the fraction obtained (Fraction B) are subjected to an evaluation of their power to degrade free radical DPPH by a spectrophotometric method.

Figure 7 presents the DPPH reduction kinetics (%) in chronological order in the presence of EO of *O. elongatum* and the isolated fraction at 1 mg/ml.

In the absence of extract [Figure 7], the optic density of the DPPH (without control extract) remained stable during the 240 min test. The results of the spectrophotometric analysis in the presence of two extracts appear to confirm those previously obtained on a silica plate [Figure 6].

Comparing with DPPH alone, the presence of the *Origanum* crude EO extract in the reaction induced an 18% reduction in DPPH as early as the first 25 min. This reduction in DPPH tends to stabilize and reach a plateau. After 120 min, the amount of DPPH remaining in the reaction medium

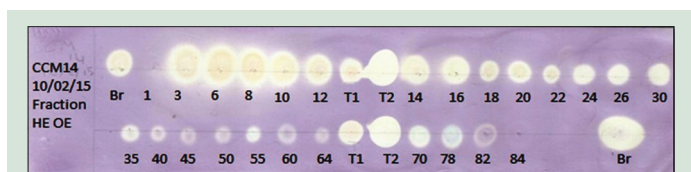


Figure 2: Image of the silica plate with the essential oil fractions of *Origanum elongatum* after 15 min reaction with DPPH. DPPH at $6 \cdot 10^{-5}$ M. Br: Crude essential oil, 1-84: Selected fractions. T1: δ -tocopherol; T2: Ascorbic acid. DPPH: 2,2-diphenyl-1-picrylhydrazyl

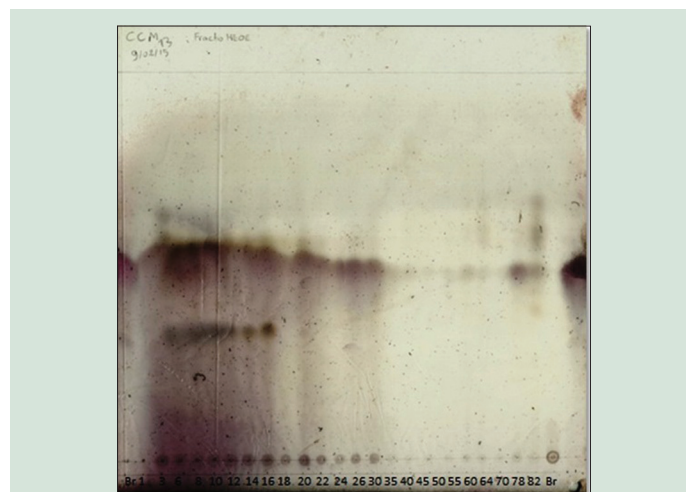


Figure 3: Chromatogram of the different fractions from the essential oil of *Origanum elongatum* after migration and visualization by sulfuric acid. Br: Crude essential oil, 1, 3, 6; 82: Fractions; Eluent: (hexane/diethyl ether/ethyl acetate, 60/35/5, v/v/v)

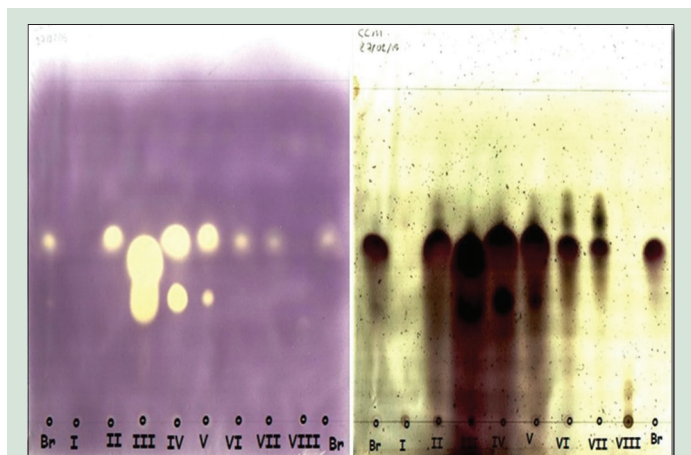


Figure 4: Chromatograms after development and revelation by DPPH (TLC1) and the methanolic sulfuric acid (TLC2) of the various grouped fractions of *Origanum elongatum* essential oil. Br: Crude essential oil; I to VIII: Grouped fractions. DPPH: 2,2-diphenyl-1-picrylhydrazyl; TLC: Thin-layer chromatography

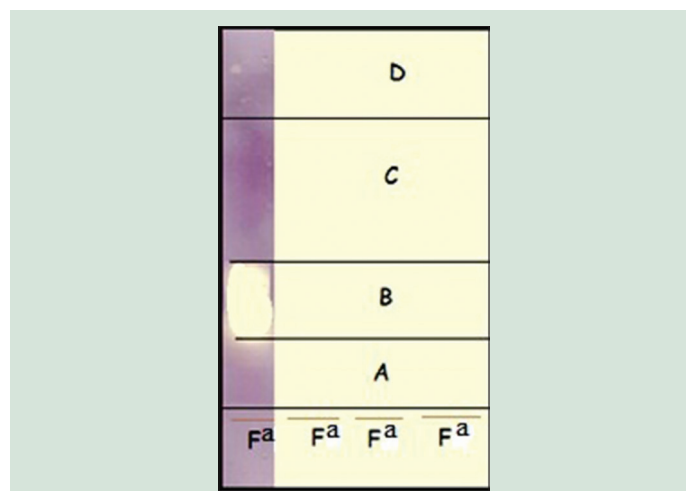


Figure 5: Representation of the preparative TLC plate and the various fractions to be recovered (A, B, C, and D). TLC: Thin-layer chromatography

represents 67.52%. As might be expected, the presence of Fraction B in the reaction medium also results in a reduction of DPPH. This reduction is important at the beginning because at 25 min reaction the percentage of DPPH reduced is in the order of 28.19%. After 90 min of reaction, the presence of Fraction B in the medium results in a reduction of 47.33% of DPPH. After 120 min of reaction, the amount of DPPH remaining in the medium is 50.68%.

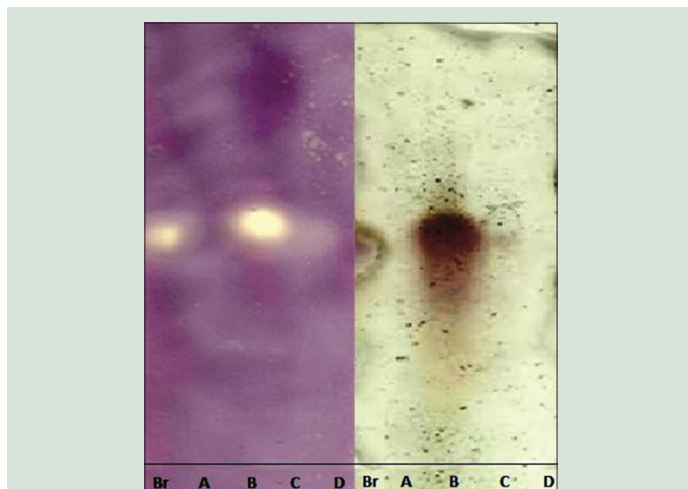


Figure 6: Chromatograms after development and revelation with DPPH and sulfuric acid of the four fractions resulting from fractionation by preparative TLC. Br: Crude essential oil; A, B, C, and D: Recovered fractions. DPPH: 2,2-diphenyl-1-picrylhydrazyl; TLC: Thin-layer chromatography

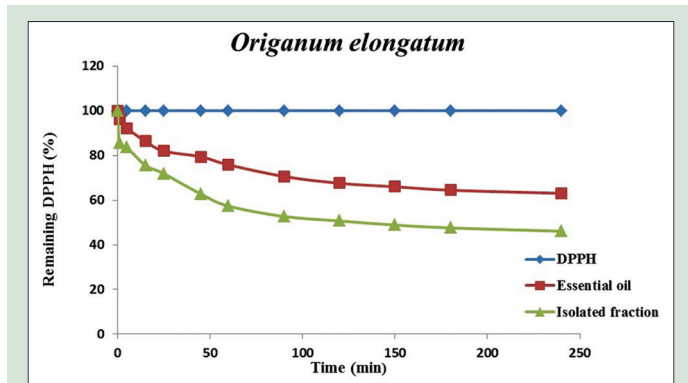


Figure 7: DPPH reduction kinetics (%) in chronological order in the presence of essential oil of *Origanum elongatum* and the isolated fraction at 1 mg/ml. DPPH at 6.10^{-5} M. DPPH: 2,2-diphenyl-1-picrylhydrazyl

Analysis of the active fraction isolated from the essential oil of *Origanum elongatum*

To determine the composition of the isolated active Fraction B of the EO, an analysis by GC/MS was performed. Figure 8 presents the chromatogram of the active fraction of *O. elongatum* EO obtained by GC/MS. Analysis of the chromatogram obtained shows that the isolated fraction is composed essentially of carvacrol (96.52%) together with the β -bisabolene with a very low proportion (1.48%), Table 3 presents the composition of the active fraction of *O. elongatum* EO obtained by CPG/MS.

The fractionation operations thus produced allow separation and purification of the active compounds. This can be seen by comparing the results of the GC/MS of Fraction B [Figure 8] to those of the crude EO [Figure 1]. The results of various CCM [Figure 6] show an enrichment in compounds and an increase in antioxidant activity.

DISCUSSION

Plant natural products have been shown to be excellent and reliable sources for the development of new drugs. Plant EOs including *Origanum* oil are known to possess a broad spectrum of activities.^[24] In the present study, *O. elongatum* Emb and Maire, an endemic species of Morocco, was investigated in order to evaluate the chemical composition and antioxidant properties of the hydrodistilled EO. It is according to the GC/MS analysis results, eleven compounds were identified in the EO without fractionation, with a dominance of monoterpenes among which carvacrol predominated (60.42%), followed by para-cymene (13.9%) then γ -terpinene (9.4%). Previous studies have also reported the presence of these three compounds in significant proportions in the EOs of Moroccan *O. elongatum*.^[17,25-27] Carvacrol and its isomer thymol are the two main phenols that constitute around 80% of the EO of *Origanum*.^[10] However, in our study, thymol was detected in lower amount (0.6%). A comparable result was reported by Benjilali *et al.*^[25] and by Ramzi *et al.*^[26] However, other authors have reported significant thymol values ranging from 13.6%^[17] to 62.4%.^[27]

Previous studies have been shown that composition of EO may be different between different species or varieties, related to a numerous of parameters such as local climatic, seasonal, cultivation, geological, and geographical factors.^[8,28]

Table 3: Composition of the active fraction of *Origanum elongatum* essential oil obtained by CPG/mass spectrometry

Compound	Retention time	Percentage
Carvacrol	5.212	96.52
β -bisabolene	6.300	1.48

GC-MS-QP2016

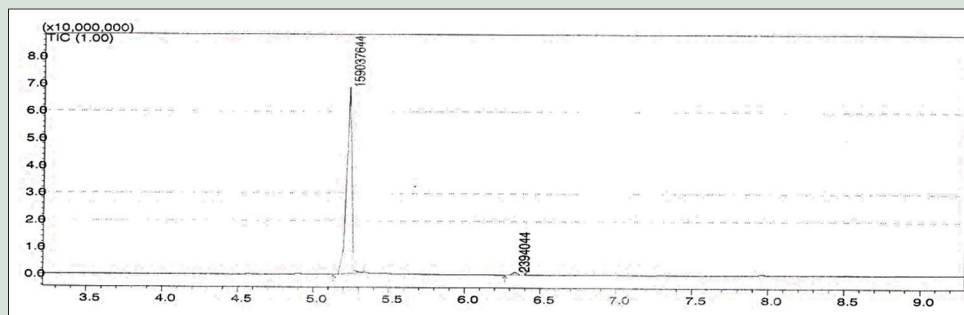


Figure 8: Chromatogram of the active fraction of *Origanum elongatum* essential oil obtained by gas chromatography associated with mass spectrometry

A recent study of 168 plants from Moroccan mountains has shown an important quantitative variability in the composition of EOs of endemic *O. elongatum* across its entire distribution area.^[27]

In the present work, the antioxidant properties of *O. elongatum* EO (without fractionation) and carvacrol its main constituent isolates after fractionation were studied and their potency was qualitatively and quantitatively assessed by TLC and EC₅₀ values.

There are different methods that are used in evaluating the activity of antioxidants.^[29] Among them, the DPPH method has been widely used in the determination of the antioxidant activity of single compounds as well as the different plant extracts.^[15]

To evaluate the antioxidant activity of the EO, we opted for the DPPH method. The DPPH method is not specific to any particular antioxidant component and allows testing both lipophilic and hydrophilic substances. DPPH is used to test the ability of compounds to act as free radical scavengers or hydrogen donors. Indeed, the DPPH method is fast, sufficiently sensitive, and requires small sample amounts and achieves reproducible results.^[30]

Our finding indicates that *O. elongatum* EO has obvious antioxidant properties and that carvacrol is a strong actor directly involved in this activity.

It is important to highlight that for the first time the antioxidant properties of EOs of Moroccan endemic *O. elongatum* has been studied in relation to their chemical composition.

In the works related to the biological activity of plant extracts, in particular, EOs, the effect is attributed to the major compound(s). However, the recorded effect remains depending on their concentrations, alone or mixed in the crude extract.^[31] In the plant material, the most prominence compound as antioxidant are phenolic and flavonoid compound.^[32] In this study, the carvacrol is the highest phenolic compound in *O. elongatum* EO. However, over 50% of *Origanum* species also contain thymol as the second abundant phenolic compound.^[23] These two phenols are known for their very important antioxidant properties.^[33]

Recently, the side effects of synthetic products used in pharmaceuticals and food industries have been scientifically approved which explains the increasing attention to natural resources.^[34] Various plant extracts including EOs are exploited and used as alternative remedies for treating many infections.^[19,35]

Currently, carvacrol is used in low concentrations as a flavoring and preservative ingredient.^[36,37] The carvacrol has several biological and pharmacological properties: antioxidant, antibacterial, antifungal, anticancer, anti-inflammatory, hepatoprotective, spasmolytic, and vasorelaxant.^[24,37,38] Evaluation of the antioxidant capacity of thymol, carvacrol, and γ -terpinene compared to that of the synthetic antioxidant Trolox showed that carvacrol and thymol have antioxidant activity similar to that of trolox.^[39] In addition, the antioxidant capacity of carvacrol was significantly higher than the same concentration of its isomer thymol.^[39,40]

CONCLUSION

The present study is focused on the EO of *O. elongatum* endemic species of Moroccan rif and its antioxidant properties.

The results show an EO of rich and varied composition whose main compounds are carvacrol, para-cymene, and γ -terpinene. The evaluation of the antioxidant activity shows that the oregano EO possesses antioxidant properties and a capacity to reduce the free radical DPPH interesting (EC₅₀ = 1.20) in comparison with δ -tocopherol (EC₅₀ = 0.26). The fractionation of the EO allowed to isolate a rather purified fraction consisting mainly of carvacrol. This fraction also proved to possess the power to reduce DPPH comparable to that observed in crude EO.

Our results show that the EO of *O. elongatum* has a real antioxidant power that would be largely due to its major compound, carvacrol, and the data presented here are supportive of the fact that this species could be a good candidate for potential use of its EO and antioxidant properties in various fields related to human health and nutrition.

Acknowledgements

The authors are immensely grateful to Mr. Mifdal Mohamed for his linguistic support.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest

REFERENCES

- Dinstel RR, Cascio J, Koukel S. The anti-oxidant level of Alaska's wild berries: High, higher and highest. *Int J Circumpolar Health* 2013;72:796-802.
- Maestri DM, Nepote V, Lamarque AL, Zygadlo JA. Natural products antioxidants. In: Imperato F, editor. *Phytochemistry: Advances in Research*. Research Signpost: Kerala, India; 2006. p. 105-35.
- Aneb M, Talbaoui A, Bouyahya A, Boury HE, Amzazi S, Benjouad A, *et al.* *In vitro* cytotoxic effects and antibacterial activity of Moroccan medicinal plants *Aristolochia longa* and *Lavandula multifida*. *Euro J Med Plant* 2016;6:1-13.
- Bouyahya A, Dakka N, Talbaoui A, Et-Touys A, El-Boury H, Abrini J, *et al.* Correlation between phenological changes, chemical composition and biological activities of the essential oil from Moroccan endemic Oregano (*Origanum compactum* Benth). *Ind Crops Prod* 2017;108:729-37.
- Mosbah H, Louati H, Boujbiha MA, Chahdoura H, Snoussi M, Flamini G, *et al.* Phytochemical characterization, antioxidant, antimicrobial and pharmacological activities of *Feijoa sellowiana* leaves growing in Tunisia. *Ind Crops Prod* 2018;112:521-31.
- Stanojević LP, Stanojević JS, Cvetković DJ, Ilić DP. Antioxidant activity of oregano essential oil (*Origanum vulgare* L.). *Biol Nyssana* 2016;7:131-9.
- Han F, Ma G, Yang M, Yan L, Xiong W, Shu JC, *et al.* Chemical composition and antioxidant activities of essential oils from different parts of the oregano. *Biomed Biotechnol* 2017;18:79-5.
- Vazirian M, Mohammadi M, Farzaei MH, Amin G, Amanzadeh Y. Chemical composition and antioxidant activity of *Origanum vulgare* subsp. *vulgare* essential oil from Iran. *Res J Pharmacogn* 2015;2:41-6.
- Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils – A review. *Food Chem Toxicol* 2008;46:446-75.
- Bouhaddouda N, Aouadi S, Labiod R. Evaluation of chemical composition and biological activities of essential oil and methanolic extract of *Origanum vulgare* L. ssp. *glandulosum* (DESF) letsvaart from Algeria. *Int J Pharmacogn Phytochem Res* 2016;8:104-12.
- De Falco E, Mancini E, Roscigno G, Mignola E, Tagliatalata-Scafati O, Senatore F, *et al.* Chemical composition and biological activity of essential oils of *Origanum vulgare* L. subsp. *vulgare* L. under different growth conditions. *Molecules* 2013;18:14948-60.
- Ozkan G, Baydar H, Erbas S. The influence of harvest time on essential oil composition, phenolic constituents and antioxidant properties of Turkish oregano (*Origanum onites* L.). *J Sci Food Agric* 2010;90:205-9.
- Bellakhdar J. La Pharmacopée marocaine traditionnelle. Médecine Arabe Ancienne et Savoirs Populaires. Paris: Ibis Press; 1997. p. 766.
- Oualili H, Rchid H, Nmila R. Phytochemical screening and antioxidant activity of: *Origanum elongatum* and *Cupressus atlantica* two endemic plants of Morocco. *Int J Pharmacogn Phytochem Res* 2018;10:196-4.
- Brand-Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. *LWT Food Sci Technol* 1995;28:25-30.
- letsvaart JH. A Taxonomic Revision of the Genus *Origanum* (Labiatae). Le Hague: Leiden Botanical Series 4, Leiden University Press; 1980. p. 153.
- Figuerdo G, Cabassu P, Chalchat JC, Pasquier B. Studies of mediterranean oregano populations VIII: Chemical composition of essential oils of oregano of various origins. *J Essent Oil Res* 2006;21:134-5.
- Aboukhalid K, Lamiri A, Agacka-Moldoch M, Doroszewska T, Douaik A, Bakha M, *et al.* Chemical polymorphism of *Origanum compactum* grown in all natural habitats in Morocco. *Chem Biodivers* 2016;13:1126-39.
- Du E, Gan L, Li Z, Wang W, Liu D, Guo Y. *In vitro* antibacterial activity of thymol

- and carvacrol and their effects on broiler chickens challenged with *Clostridium perfringens*. *J Anim Sci Biotechnol* 2015;6:58.
20. Koparal AT, Zeytinoglu M. Effects of carvacrol on a human non-small cell lung cancer (NSCLC) cell line, A549. *Cytotechnology* 2003;43:149-54.
 21. Juliani HR, Simon JE. Antioxidant Activity of Basil. Trends New Crop New Uses. ASHS Press, Alexandria, VA; 2002. p. 575-9.
 22. Özkan A, Erdoğan A. A comparative evaluation of antioxidant and anticancer activity of essential oil from *Origanum onites* (Lamiaceae) and its two major phenolic components. *Turk J Biol* 2011;35:735-42.
 23. Kulisic T, Radonic A, Katalinic V, Milos M. Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chem* 2004;85:633-40.
 24. Baser KH. Biological and pharmacological activities of carvacrol and carvacrol bearing essential oils. *Curr Pharm Des* 2008;14:3106-19.
 25. Benjilali B, Richard HM, Baritoux O. Etude des huiles essentielles de deux espèces d'Origan du Maroc: *Origanum compactum* Benth et *Origanum elongatum* Emb et Maire. *Lebensm Wiss Technol* 1986;19:22-6.
 26. Ramzi H, Ismaili MR, Aberchane M, Zaanoun S. Chemical characterization and acaricidal activity of *Thymus satureioides* C. & B. and *Origanum elongatum* E. & M. (Lamiaceae) essential oils against *Varroa destructor* Anderson & Trueman (Acari: Varroidae). *Ind Crops Prod* 2017;108:201-7.
 27. Bakha M, El Mtili N, Machon N, Aboukhalid K, Amchra FZ, Khiraoui A, *et al.* Intraspecific chemical variability of essential oils of Moroccan endemic *Origanum elongatum* L. (Lamiaceae) from its whole natural habitats. *Arabian J Chem* 2018. <https://doi.org/10.1016/j.arabjc.2018.08.015>.
 28. Perry NB, Anderson RE, Brennan NJ, Douglas MH, Heaney AJ, McGimpsey JA, *et al.* Essential oils from dalmatian sage (*Salvia officinalis* L.): Variations among individuals, plant parts, seasons, and sites. *J Agric Food Chem* 1999;47:2048-54.
 29. Koleva II, van Beek TA, Linssen JP, de Groot A, Evstatieva LN. Screening of plant extracts for antioxidant activity: A comparative study on three testing methods. *Phytochem Anal* 2002;13:8-17.
 30. Molyneux P. The use of stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. *Songklanakarin J Sci Technol* 2004;26:211-9.
 31. Ipek E, Zeytinoglu H, Okay S, Tuylu BA, Kurkcuoglu M, Baser KH. Genotoxicity and antigenotoxicity of *Origanum* oil and carvacrol evaluated by Ames Salmonella/microsomal test. *Food Chem* 2005;93:551-6.
 32. Yusuf MI, Wahyuni, Susanty S, Ruslan, Fawwaz M. Antioxidant and antidiabetic potential of galing stem extract (*Cayratia trifolia* Domin). *Pharmacogn J* 2018;10:686-9.
 33. Aazza S, Lyoussi B, Miguel MG. Antioxidant and antiacetylcholinesterase activities of some commercial essential oils and their major compounds. *Molecules* 2011;16:7672-90.
 34. Pokorný J. Are natural antioxidants better - and safer - Than synthetic antioxidants? *Eur J Lipid Sci Technol* 2007;109:629-42.
 35. Suntres ZE, Coccimiglio J, Alipour M. The bioactivity and toxicological actions of carvacrol. *Crit Rev Food Sci Nutr* 2015;55:304-18.
 36. Losso JN, Shahidi F, Bagchi D. Anti-Angiogenic Functional and Medicinal Foods, Nutraceuical Science and Technology 6. Taylor and Francis, Boca Raton, FL: CRC Press; 2007. p. 611-2.
 37. Baydar H, Sağdıç O, Özkan G, Karadoğan T. Antibacterial activity and composition of essential oils from *Origanum*, *Thymbra* and *Satureja* species with commercial importance in Turkey. *Food Control* 2004;15:169-72.
 38. Baranauskaitė J, Kubiliene A, Marksa M, Petrikaite V, Vitkevičius K, Baranauskas A, *et al.* The influence of different oregano species on the antioxidant activity determined using HPLC postcolumn DPPH method and anticancer activity of carvacrol and rosmarinic acid. *Biomed Res Int* 2017;2017:1681392.
 39. Undeğer U, Başaran A, Degen GH, Başaran N. Antioxidant activities of major thyme ingredients and lack of (oxidative) DNA damage in V79 Chinese hamster lung fibroblast cells at low levels of carvacrol and thymol. *Food Chem Toxicol* 2009;47:2037-43.
 40. Guimaraes AG, Xavier MA, de Santana MT, Camargo EA, Santos CA, Brito FA, *et al.* Carvacrol attenuates mechanical hypernociception and inflammatory response. *Naunyn Schmiedebergs Arch Pharmacol* 2012;385:253-63.