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*Original Research Article*

# Chemical Composition, Antioxidant and Antimicrobial Activities of the Essential Oil of *Origanum majorana* Growing in Middle Atlas of Morocco

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ABSTRACT

The purpose of this work was to determine the chemical composition and assess the antibacterial and antioxidant activity of the essential oil of *Origanum majorana* grown in the Middle Atlas of Morocco. The yield of essential oil extracted by hydro-distillation is about 1 ± 0.05%.The chemical composition of the essential oil extracted from the Aerial parts of *Origanum majorana* was studied by gas chromatography coupled with mass spectrometry (GC/MS). The results of the GC-MS analysis of the volatile components of the EO revealed the presence of 21 components, representing 98.51% of the total essential oils (EOs). The main compounds were carvacrol (41.09%), terpinen-4-ol (28.08%), followed by p- Cis-Sabinene hydrate (21.12%), γ-Terpinene (2.7%), Spathulenol (1.5%) and thymol (1.32%). Antioxidant activity was determined by the DPPH and FRAP assays. The results demonstrated that our EOs exhibit a significant antiradical effect with an $IC_{50}$ value of 0.25 mg/mL compared to the pure reference antioxidant BHT with an $IC_{50}$ value of 0.11 mg/mL. The antibacterial effect of this essential oil was tested against five bacterial strains.*Staphylococcus aureus* was the most sensitive with a MIC value of 1.98 ± 0.01, followed by *Klebsiella pneumonia* with an MIC value of 2.01 ± 0.35ug/ml. The results show that *Origanum majorana* essential oil process antibacterial and antioxidant properties and it can be used as a natural food preservative and as an antimicrobial agent for the treatment of several infectious diseases caused by bacteria.

***Keywords*:** *Origanum majorana*, Essential oil, Chemical composition, Antibacterial activity, Antioxidant activity.

## Introduction

*Origanum majorana* (known as Merdadouch, Saataror or Zatertadlaoui, currently named sweet marjoram. *Origanum majorana* is a medicinal plant of the Lamiaceae family, a perennial herb of Origanum genus,[1] that has been used in traditional medicine for various diseases.[2]Thisplant is distributed around the Mediterranean regions, in particular, Morocco, Algeria, Egypt, Spain, and Portugal.[3] Traditionally, O. majorana L. has been used to treat gastrointestinal disorders, migraines, depression and as a diuretic.[4]

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In Moroccan traditional medicine, marjoram is used as an antipyretic and antidiabetic, as well as for allergies, fever, flu and high blood pressure.[5,6] Several studies have demonstrated that *Origanum majorana* L. is very rich in phytochemical substances such as carvacrol, thymol, tannins, hydroquinone, sitosterol, cis-sabinene hydrate, limonene, terpinene, camphene and flavonoids, which explain its biological activities.[7]

Pharmacological studies of methanol extracts and essential oils of O. majorana have shown significant antimicrobial activities against different pathogenic bacteria and fungi.[8,9] Antioxidant and cytotoxic capacities have also been reported.[10]

The present study focuses on the chemical composition, antioxidant, and antimicrobial activities of the essential oil of *O. majorana* grown in Beni–Mellal, located in the Middle Atlas region of Morocco. This study aims to provide further insights into the potential applications of this plant in the field of medicine and natural products research.

## Materials and Methods

*Plant material*

Aerial parts of *Origanum majorana* were collected in Morocco at the flowering stage (April 2020) from the region of Beni Mellal-Morocco(32° 20′ 22″ north, 6° 21′ 39″ west).A botanical identification

of *Origanum majorana* was made by a botanist and assigned the reference 20/04/16-2/BN before being placed in the herbarium of the biology department. The aerial parts were then dried in the shade for 7 days in a dry, ventilated area at 25°C.

*Extraction of essential oils*

The extraction method was performed using a Clevenger apparatus, according to the European Pharmacopoeia.[11]The distillation was performed by boiling 100 g of the plant material for 2 hours with 500 mL of distilled water in a 2 L flask.The EOs obtained were dried over anhydrous sodium sulfate and stored in an Eppendorf tube at 4°C before analysis. The oil yield was calculated based on the dried weight of the sample using the following equation (1):

$$REO(\%) = \frac{mEO}{mDM} \times 100 \qquad (1).$$

With: REO the essential oil yield in (%), mEO: the mass of the essential Oil in g and mDM: the mass of the dry plant material.[12]

*Chromatographic analysis (GC-MS)*

GC-MS was used to determine the composition of the essential oils (GC-MS).The volatile compounds were analyzed run on a Thermo Fischer capillary GC directly coupled to the MS system (model GC ULTRA S/N20062969; Polaris QS/N 210729), using an HP-5MS non-polar fused silica capillary column (60 m × 0.32 mm, 0.25 mm film thickness). The operating condition of the GC-MS oven temperature was kept as follows: The initial temperature for the analysis was 40 °Cfor 2 minutes, at a programed rate of 2 °C/min up to final temperature of 260 °C, with isotherm for 10 minutes and injector temperature of 250°C. Helium was used as the carrier gas with a flow rate of 1 mL/min. Essential oil was diluted in hexane. The volume of injected specimen was 1 µL (Fractionation ratio10:100) with the split injection technique, ionization energy 70 eV, in the electronic ionization mode, ion source temperature of 200°C, scan mass range of m/z 40–650, and interface line temperature of 300 °C. Component characterization was made by determination of their retention indices (RIs) relative to those of a homologous series of n-alkanes (C8–C20) and by matching their recorded mass spectra with those stored in the spectrometer database (NIST MS Library v. 2.0) and in the bibliography. [13]

*Antioxidant activity*

*Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity*

The ability of the essential oil to scavenge the DPPH radical was measured using the method described previously.[14, 15, 16]

A volume of 0.1mL of various concentrations of the essential oil or standard were added to 1.5 mL of the ethanoic solution containing 0.1 mmol of DPPH (2, 2-diphenyl-1 picrylhy-drazyl). The absorbance of the mixture was measured at517 nm by a spectrophotometer (Jasco V-530, France) after 30 min of incubation time at room temperature in the dark. The % inhibition ($IC_{50}$) was calculated by the following equation (2):

$$I(\%) = (1 - \left(\frac{As}{Ac}\right)) \times 100 \quad (2)$$

Where I (%) is the inhibition percentage, Ac and As are the absorbance values of the negative control and sample, respectively. Butylated hydroxyl toluene (BHT) served as positive control. The $IC_{50}$ values were calculated as the concentration of the sample causing 50% inhibition of the DPPH radical.

*Ferric Reducing Antioxidant Power (FRAP)*

The ferric reducing power of the tested oil was determined in accordance with the procedure of Oyaizu. [17]A volume of 200 mL of the essential oils was mixed with 500 mL of phosphate buffer (0.2 M, pH 6.6) and 500 ml of potassium ferricyanide [$K_3$ Fe $(CN)_6$] 1%. The obtained solution was incubated at 50 °C for 20 min. The mixture was acidified with 500 mL of trichloracetic acid (TCA) 10%, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 mL) was mixed with 500 mL of distilled water and 100 mL of $FeCl_3$ (0.1%), and the absorbance was measured at 700 nm using a spectrophotometer (Jascov-530). Quercetin was used as a standard. The results were expressed as $IC_{50}$ (mg/ml).

*Antibacterial activities of O. majorana essential oils*

*Bacteria species*

The antibacterial activity of *Origanum majorana* essential oil was tested against, Gram-positive bacteria *Staphylococcus aureus* (*S. aureus*) and Gram-negative bacteria included *Escherichia coli* (E. coli), *Pseudomonas aeruginosa* (P.aeruginosa), *Acinetobacter baumannii* (A. baumannii) and *Klebsiella pneumoniae.* All strains tested were isolated in a hospital environment from clinical patients in the resuscitation service (CHU, Morocco).

*Agar diffusion disc*

The antibacterial power of *Origanum majorana* EO was determined to use the agar disk diffusion procedure.[18] Each microorganism stock was suspended in Mueller-Hinton (MH) broth and incubated at 37°Cfor 18–24 h. The overnight cultures were diluted and adjusted to get a density of 1–5 × 106 CFU/ml (0.5 McFarland turbidity standards). The cultures were flood-inoculated onto the surface of MH agar plates. Sterile filter discs of Whatman paper N°3 impregnated with 15µL/disc of the EO and were delivered onto the inoculated agar MH. All plates were incubated for 18 h at 37°C. The antibacterial effect was tested by measuring the inhibition zones. The antibiotic standards used were imipenem, Vancomycin, Ceftizoxim, Cefaclor, and Amoxicillin. All the tests were performed in triplicate. The values of the inhibition diameter are given as mean± standard deviation.

*Determination of the minimum inhibitory concentration (MIC)*

The minimum inhibitory concentration (MIC) was performed using a microdilution assay in 96-well plates according to theexperimentof the National Committee for Clinical Laboratory Standards (NCCLS, 1999) with some modifications.[19]The different concentrations of essential oils were prepared in a suspension containing 0.2% agar in sterile distilled water in order to disperse the compounds without adding solvent or detergent. They were carried out by successive dilutions 1/2 ranging from 100 to 0.09 mg/mL. The concentrations obtained in the well plates ranged between 25 and 0.02 mg/mL. Bacterial suspensions were prepared in the same manner described previously and diluted in MH broth and plated in 96 well plates at a density of 1–5 × 106 CFU/mL. Finally, the plates were incubated at 37 for 18 h, bacterial growth was assessed visually by adding 20µL of 2,3,5-triphenyltetrazolium chloride (TTC) in aqueous solution, with additional incubation for1 hour. MIC was defined as the lowest concentration that does not produce a red color.[20]

Statistical analysis

The mean values and ±standard deviations were calculated by using Graph Pad Prism 5 (Microsoft Software). The results were compared by one-way ANOVA followed by Tukey-test, using the same software. Differences at $P < 0.05$ were considered significant.

## Results and Discussion

*Chemical composition of Origanum majorana essential oils*

The yield of *Origanum majorana* essential oils obtained by hydrodistillation of the dried aerial parts is 1 ± 0.05%. This yield is almost the same (0.97%) as that of marjoram collected in the Azzemour region (south-west Morocco),[21] but is lower than that of marjoram from Tunisia, whose yields vary between 1.7% and 1.85%.[9] These differences in terms of yield can be attributed to the geographical location as well as to the harvesting period and maturity status of the plant.[22, 23]

The results of the GC-MS analysis of the volatile components of the EOs revealed the presence of 21 components (Table 1, Figure 1) representing 98.51% of the total EO and summarized in Table 1. The main compounds were carvacrol (41.09%), terpinen-4-ol (28.08%), followed by p- Cis-Sabinene hydrate (21.12%), γ-Terpinene (2.7%), Spathulenol (1.5%) and Thymol (1.32%). These results are in line with previous studies.[23,24]

In fact, the chemical composition of *Origanum majorana* essential oil has been studied in several scientific research studies. Similar to our study, the main components of EOs are carvacrol, thymol, terpinen-4-

ol and Cis-sabinene hydrate. [23, 24]Carvacrol is one of the most abundant compounds in *Origanum majorana* essential oil, with concentrations ranging from 36.06% to 69.26%.[23, 24, 25]Thymol is another important component of marjoram essential oil, with concentrations ranging from 48.06% to 51.20%.[23, 24]

Fliou *et al*. conducted a comparative study of the chemical composition of *Origanum compactum* essential oil from different regions of Morocco.[24] They found that *Origanum compactum* essential oil from the Al Hoceïma region contains carvacrol and p-Cymene as the predominant constituents. Altitude was also found to affect the content and composition of *Origanum vulgare* essential oil.

Galeş et al. conducted a study to investigate the effects of altitude on the quantity and quality of volatile oil produced by leaves of *Origanum vulgare*.[22]They collected plant samples at different altitudes in Romania and found that altitude significantly influenced the composition of *Origanum vulgare* essential oil.

*Antioxidant activity of essential oil of Origanum majorana*

Essential oils (EOs) are a mixture of volatile compounds obtained from aromatic plants. [26]

Several EOs have antioxidant properties, and the use of EOs as natural antioxidants is a field of growing interest because some synthetic antioxidants such as BHA and BHT are suspected to be potentially harmful to human health.[27] As a part of an investigation of natural antioxidants from spice plants, the goal of this study was to assess antioxidant activities of *Origanum majorana* essential oil collected from Beni Mellal region in the Middle Atlas of Morocco. DPPH test and FRAP assay were used to assess antioxidant activities of the essential oil. DPPH is a stable free radical that can receive hydrogen or electron from an antioxidant to become a stable molecule.[28,29] Results in table 2 indicate that marjoram essential oil presents a significant antiradical effect with an $IC_{50}$ value of 0.25 mg/mL. This value was significantly ($p < 0.05$) lower than that of pure reference antioxidant BHT with an $IC_{50}$ value of 0.11 mg/mL. The ferric reducing power of OMEO was evaluated by the FRAP assay. The reducing power assay is used to evaluate the ability of an antioxidant to transform the $Fe^{3+}$ to $Fe^{2+}$. The results demonstrated that our EO exhibits a good reducing power activity with $IC_{50}$ values of 0.67 mg/mL, but it was still significantly ($p < 0.05$) less than that of the synthetic antioxidant quercetin with $IC_{50}$ value of 0.03 ± 0.006 mg/mL. This reducing power activity can be related to the presence of carvacrol and Terpinen-4-ol as major components in our essential oil.[30, 32]

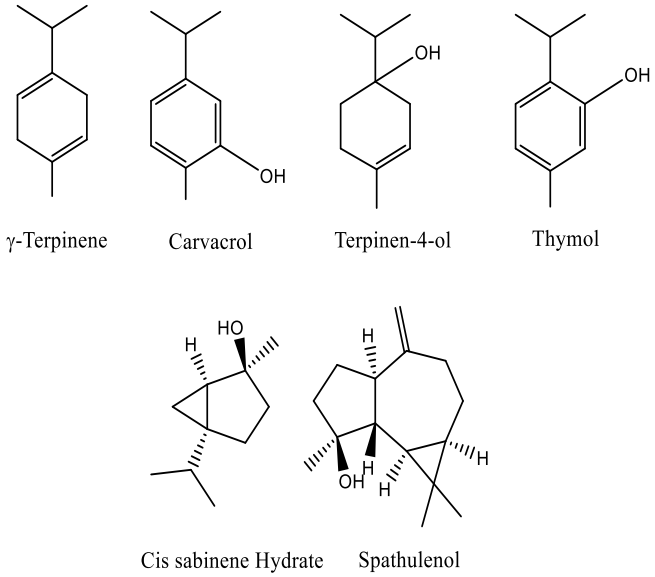


**Figure.1:** Structures of essential oils' major components

**Table 1:** Chemical composition of *Origanum majorana* essential oils from Beni Mellal(Middle Atlas of Morocco)

| Number | Compound | Percentage (%) | Retention time(min) |
|---|---|---|---|
| 1 | α –phellandrene | 0.02 | 8.44 |
| 2 | α-Pinene | 0.21 | 9.25 |
| 3 | Camphene | 0.05 | 10.11 |
| 4 | p-cymene | 0.04 | 10.17 |
| 5 | β-Myrcene | 0.06 | 10.48 |
| 6 | Linalool | 0.2 | 11.60 |
| 7 | Thymol | 1.32 | 14.23 |
| 8 | 2-Carene | 0.27 | 15.98 |
| 9 | z-citral | 0.5 | 17.01 |
| 10 | □□Terpinene | 0.17 | 17.80 |
| 11 | Carvacrol | 41.09 | 17.89 |
| 12 | Terpinen-4-ol | 28.08 | 18.32 |
| 13 | γ-Terpinene | 2.7 | 18.27 |
| 14 | Trans-sabinene hydrate | 0.09 | 20.91 |
| 15 | Cis-sabinene hydrate | 21.12 | 27.4 2 |
| 16 | Spathulenol | 1.5 | 29.46 |
| 17 | Sabinene hydrate | 0.46 | 31.74 |
| 18 | α-terpenylacetate | 0.56 | 32.31 |
| 19 | β-Ocimene | 0.76 | 33.40 |
| 20 | Bezynen | 0.04 | 35.12 |
| 21 | trans-caryophyllene | 0.13 | 37.10 |
| 22 | Other compounds | 1.19 | ------- |

The antioxidant activities of thymol, carvacrol and Terpinen-4-ol have been reported, using various testing systems.[30,31,32] However, it is difficult to attribute antioxidant power to the main compounds in EOs, because both minor and major constituents are involved in explaining biological activities of essential oils. [32]

Many studies have examined the antioxidant activity of EOs and extracts obtained from different parts of *Origanum majorana*. It should be noted that DPPH, FRAP, Beta-carotene, ABTS, HFRSA, superoxide quenching activity, and TAC assays were used to evaluate antioxidant activity. Erdogan and Ozkan investigated the antioxidant effect of OMEO obtained from the aerial parts using DPPH radical scavenging assay. [33] The results of this study indicate that the $IC_{50}$ value was 170 µg/mL. Hajlaoui *et al*.who showed that the $IC_{50}$ value was 62.66 µg/mL for DPPH radical scavenging assay,[9]also reported the anti-DPPH effect of the essential oil from the aerial part of *Origanum majorana*. Another research work reported the antioxidant effect of *O. majorana* essential oil using the DPPH assay and reducing power. As a result, the essential oil extract from leaves exerted a good anti-DPPH effect with $IC_{50}$ = 0.3 mg/mL, and an important reducing power with $IC_{50}$ = 0.4 mg/mL. [34]

*Origanum majorana* is one of the most important aromatic plants that contain several compounds, such as phenolic acid, flavonoids, and terpenoids, which are known to possess antioxidant activities. These compounds can scavenge free radicals and play a major role in the protection of the human body. However, the antioxidant capacity of the plant may vary depending on different factors such as growing conditions, extraction methods, the part of the plant used,or even genetic factors.[35, 36]

*Antibacterial activity essential oil of Origanum majorana*

The antimicrobial activity of the essential oil of *Origanum majorana* grown in Beni Mellal was evaluated using the agar diffusion and minimal inhibitory concentration (MIC) methods against five bacteria strains (*S. aureus, P. aeruginosa , E. coli, A. Boumanii* and *K. pneumonia*) responsible for nosocomial infections isolated in a hospital environment from clinical patients in the resuscitation unit (University Hospital, Morocco). The results obtained are shown in Table 3.

Results revealed that the tested EOs showed a wide antibacterial spectrum, against tested bacteria species with inhibition zone diameters varying from 12 to 22 mm. It is interesting to note that these diameters were sometimes greater than those obtained with standard antibiotics used as controls. Gram-positive *S. aureus* and Gram-negative *K. pneumonia* were the most sensitive of the strains tested. However, the essential oil of marjoram showed a weak activity against the Gram-negative *A.boumannii* with an inhibition diameter value of 12± 0.1mm. The MIC values of the essential oils against the tested bacteria are summarized in table 3.It can be seen that the essential oils tested have significant antibacterial effect against *S.aureus* (MIC = 1.98 ug/mL) compared to the other strains. In contrast, our essential oil had low activity against A.boumannii, which was only inhibited at a high concentration (6.14 ug/mL).

The antimicrobial properties of EOs can be due to the high proportion of their major constituents, such as carvacrol (41,09%), and terpinene-4-ol (28.08%). However, many reports have suggested that minor components from the EOs such as γ-terpinene, and sabinene should be taken into consideration to explain the antimicrobial activity.[37,38]

Several studies have demonstrated the antibacterial efficacy of essential oil extracts from different parts of *Origanum majorana*. Chaves et al. have tested the antibacterial effect of *O. majorana* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* using standard antibiotics as positive controls. The author reported interesting results, in which *Escherichia coli* and *Pseudomonas aeruginosa* were more sensitive to our essential oil(MIC = 31.25 µg/mL) compared to *Staphylococcus aureus*.[39] Another study reported the antibacterial activity of *O. majorana* EO at the dose of 10 mL extracted from its aerial part against Gram-positive and Gram-positive bacteria. Results showed that the most sensitive bacteria were *Escherichia coli* and *Streptococcus aureus*, and the less sensitive one was *Pseudomonas aeruginosa* (MIC = 1/16). [40]

Some previous reports confirm that Gram-negative bacteria are less sensitive than Gram-positive bacteria to essential oils. [41, 42] Indeed, in the present work, *S. aureus* appears to be the most sensitive bacteria to *Origanum majorana* essential oil. This difference is possibly due to the presence of lipopolysaccharides in the outer membrane of Gram-negative bacteria making them resistant to external agents, such as antibiotics, detergents, and lipophilic compounds.[43,44]

## Conclusion

In this study, *Origanum majorana* essential oil from Morocco was investigated for its antibacterial and antioxidant activities. The results showed that *Origanum majorana* essential oil possesses antibacterial and antioxidant properties. It may be used as a natural food preservative and as an antimicrobial agent for the treatment of several infectious diseases caused by bacteria. Our findings justify the use of *Origanum majorana* in Moroccan traditional medicine; however, further studies are required to identify compounds responsible of antioxidant and antibacterial activities of the plant and to explore the mechanism of action.

## Conflict of Interest

The authors declare no conflict of interest.

**Table 2:** DPPH radical scavenging activity and ferric reducing power capacity of *Origanum majorana* essential oil

| | **DPPH $IC_{50}$(mg/ml)** | **FRAP $IC_{50}$(mg/ml)** |
|---|---|---|
| *Origanum majorana* EO | $0.25 \pm 0.01^a$ | $0.67 \pm 0.03^a$ |
| BHT | $0.11 \pm 0.002^b$ | - |
| Quercetin | - | $0.03 \pm 0.006^b$ |

Values are giving as mean ± SD (n=3). In each column, different letters are significantly different by the tukey-test (P<0.05),BHT: Butylated hydroxytoluene.

**Table 3:** Antimicrobial activity of *Origanum majorana* EOs and antibiotic standards

| | **Essential oil** | | **Antibiotics** |
|---|---|---|---|
| **Bacterialspecies** | **DI (mm)** | **MIC (ug/ml)** | **DI (mm)** |
| *Staphylococcus aureus* | 22 ± 0.92 | 1.98 ± 0.01 | 17 (VAN), 12 (CFZ), 7(CEF) |
| *Pseudomonas aeruginosa* | 18± 1.15 | 2.49 ± 0.01 | 23 (IMP), 0 (CEF) ,12 (CIP) |
| *Escherichia coli* | 16± 0.1 | 3.51 ± 0.58 | 21 (SUL), 12 (CFZ), 0 (CEF) |
| *Acinetobacter baumannii* | 12± 0.1 | 6.14 ± 1,2 | 14 (IMP), 7.5 (OXA), 0 (CEF) |
| *Klebsiella pneumoniae* | 20 ± 1.2 | 2.01 ± 0.35 | 16 (IMP) ,17 (FLU), 12 (AMX) |

MIC: Minimum inhibitory concentration, DI: diameter of inhibition zone including disk diameter of 6 mm.IMP; Imipenem,VAC; Vancomycin, CFZ; Ceftizoxim, CEF; Cefaclor, AMX; Amoxicillin;SUL: Sulfamethoxazole ; OXA: Oxacillin ; FLU: Fluoroquinolone; CIP: Ciprofloxacin.

## 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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## References

1. Postu PA, Gorgan DL, Cioanca O, Russ M, Mikkat S, Glocker MO, Hritcu L. Memory-Enhancing Effects of *Origanum majorana* Essential Oil in an Alzheimer's Amyloid beta1-42 Rat Model: A Molecular and Behavioral Study. *Antioxidants*. 2020; 9(10):919.
2. Bina F, Rahimi R. Sweet Marjoram. J Evid Based Complementary Altern Med. 2016; 1(22): 175-185.
3. Prerna , Vasudeva N. Origanum majorana L. Phyto-pharmacological review. Indian J Nat Prod Resour. 2015; 6: 261-267.
4. El-Ashmawy IM, el-Nahas AF, Salama OM. Protective effect of volatile oil, alcoholic and aqueous extracts of Origanum majorana on lead acetate toxicity in mice. Basic ClinPharmacolToxicol. 2005 Oct;97(4):238-43. doi: 10.1111/j.1742-7843.2005.pto_136.x. PMID: 16176560.
5. Bellakhdar J, Claisse R , Fleurentin J, Younos C. Repertory of standard herbal drugs in the Moroccan pharmacopoeia. J Ethnopharmacol. 1991 ; 35: 123–143.
6. Tahraoui, A , El-Hilaly J, Israili ZH, Lyoussi B. Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south- eastern Morocco (Errachidia province). J. Ethnopharmacol. 2007; 110: 105–117.
7. Bouyahya A, Chamkhi I, Benali T, Guaouguaou FE, Balahbib A, El Omari N, Taha D, Belmehdi O, Ghokhan Z, El Menyiy N. Traditional use, phytochemistry, toxicology, and pharmacology of Origanum majorana L. J Ethnopharmacol. 2021; 30;265:113318.
8. Busatta C, Vidal RS, Popiolski AS, Mossi AJ, Dariva C, Rodrigues MR, Corazza FC, Corazza ML, Vladimir Oliveira J, Cansian RL. Application of Origanum majorana L. essential oil as an antimicrobial agent in sausage. Food Microbiol. 2008; 25(1):207-11.
9. Hajlaoui H, Mighri H, Aouni M, Gharsallah N, Kadri A. Chemical composition and in vitro evaluation of antioxidant, antimicrobial, cytotoxicity and anti-acetylcholinesterase properties of Tunisian Origanum majorana L. essential oil. MicrobPathog. 2016; 95:86-94.
10. Hussain A I, Anwar F, Rasheed S, Nigam PS, Janneh O, Sarker SD. Composition, antioxidant and chemotherapeutic properties of the essential oils from two Origanum species growing in Pakistan.Rev. bras. farmacogn. 2011; 21, 943–952.
11. EuropeanPharmacopoeia (1975) Volume 3. Maisonneuve, Sainte-Ruffine.
12. Jilali SBE, Rachid I, Ghada B, Tarik M, Sanae R, Abderrazzak K. Effect of Isolation Techniques on the Quantity, Quality, and Antimicrobial Activity of Lavanduladentata Essential Oils. Trop J Nat Prod Res. 2023; 7(4):2713-2717
13. Adams R P. 2007. Identification of essential oil components by gas chromatography/ mass spectrometry, 4th Edition. Allured Publ., Carol Stream, IL
14. Odion EE, Falodun A, Adelusi SA. Total flavonoid, Total Phenolic and antioxidant potential of root bark extract and fractions of from Cola rostrata (Sterculiaceae) K. Schum. University of Benin . J Sci Tech .2013; 1 (2): 38 - 42.
15. Okolie NP, Falodun A, Davids O. Evaluation of the antioxidant activity of root extract of pepper fruit (Dennetiatripetala), and it's potential for the inhibition of lipid peroxidation. Afr J Tradit Complement Altern Med. 2014;11(3):221-7
16. Egharevba E, Chukwuemeke-Nwani P, Eboh U, Okoye E, Bolanle IO, Oseghale IO, Imieje VO, Erharuyi O, FalodunA.Antioxidant and Hypoglycaemic Potentials of the Leaf Extracts of Stachytarphytajamaicensis (Verbenaceae). Trop J Nat Prod Res. 2019; 3(5):170-174.
17. Oyaizu, M. Studies on products of browning reaction. Antioxidative activities of products of browning reaction prepared from glucosamine. Jpn J Nutrit Diet. 1986; 44: 307–315.
18. Furtado GL, Medeiros AA. Single-disk diffusion testing (Kirby-Bauer) of susceptibility of Proteus mirabilis to chloramphenicol: significance of the intermediate category. J ClinMicrobiol. 1980 ;12(4):550-3
19. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests. Approved standard M2-A4. Wayne, Pa: National Committee for Clinical Laboratory Standards; 1999.
20. Remmal A, Bouchikhi T, Rhayour K. Improved method for the determination of antimicrobial activity of essential oils in agar medium. J Essent Oil Res.1993; 5:179–184.
21. Amor G, Caputo L, La Storia A, De Feo V, Mauriello G, Fechtali T. Chemical Composition and Antimicrobial Activity of Artemisia herba-alba and Origanum majorana Essential Oils from Morocco. Molecules. 2019; 6;24(22):4021.
22. Gales RC, Preotu A, Necula A, Gille E, Toma C. Altitudinal variations of morphology, distribution and secretion of glandular hairs in Orıganum vulgare L. leaves. StudiaUniv VG SSV. 2010; 20:59-62.
23. Hammam M, El-Sayed S, El-Sayed A, Mohamed W. Biochemical Studies On Antioxidant and Antimicrobial Activity Of Some Medicinal Plants Essential Oils. Menoufia J Agric Biotechnology. 2023; 2(8): 1-20.
24. Fliou J, Riffi O, Amechrouq A, Elhourri M, Ghouati Y. Comparative Study Of the Chemical Composition Of The Essential Oil Of Origanum Compactum From The Seven Regions Of Morocco And Their Antimicrobial Activity . J microb biotech food sci. 2020; 1(10): 42-48.
25. Yasar S, Nizamlıoğlu NM, Gücüş MO, Bildik Dal AE, Akgül K. *Origanum majorana* L. Essential Oil-Coated Paper Acts as an Antimicrobial and Antioxidant Agent against Meat Spoilage. ACS Omega. 2022 ;7(10):9033-9043.
26. Boureghida S, Slougui N, Fadel N, Hazmoune H, Bicha S. Chemical Composition and Antibacterial Activity of the Essential Oil from Erinaceaanthyllis Link (Fabaceae). Trop J Nat Prod Res. 2023; *7*(4): 2696–2700.
27. Amorati R, Foti MC, Valgimigli L. Antioxidant activity of essential oils. J Agric Food Chem. 2013 ; 20, 61(46):10835-47.
28. Al-Saadi HK, Awad HA, Saltan ZS, Hasoon B A, Abdulwahab A I, Al-azawi K F, Al-Rubaii B A. Antioxidant and Antibacterial Activities of Allium sativum Ethanol Extract and Silver Nanoparticles Trop J Nat Prod Res. 2023; 7(6): 3105-3110.
29. El Atki,Y., Aouam, I., El Kamari, F., Taroq, A., Lyoussi, B., Oumokhtar, B., Abdellaoui,A. Phytochemistry,

antioxidant and antibacterial activities of two Moroccan Teucriumpolium L. subspecies: Preventive approach against nosocomial infections, Arab J Chem. 2020;13 (2):3866-3874
30. Yanishleiva NV, Marinova EM, Gordon MH, Reneva VG, Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. Food Chem.1999; 64: 59–66.
31. Ruberto G, Baratta MT. Antioxidant activity of selected essential oil components in lipid model systems. Food Chem. 2000; 69: 167-174.
32. Wang W, Wu N, Zu YG, Fu YJ. Antioxidative activity of Rosmarinus officinalis L.615 essential oil compared to its main components. Food Chem. 2008; 108: 1019-1022.
33. Erdogan A, Ozkan A. Investigatıon of antioxıdative, cytotoxic, membrane damaging and membrane-protective effects of the essentıal oil of Origanum majorana and its oxygenated monoterpene component linalool in human-derived Hep G2 cell line. Iran J Pharm Res. 2017; 16(24): 24-34
34. Khadhri A, Bouali I, Aouadhi C, Lagel MC, Masson E, Pizzi A, Determination of phenolic compounds by MALDI–TOF and essential oil composition by GC–MS during three development stages of Origanum majorana L. Biomed Chromatogr. 2019; 33: e4665.
35. Makrane H, El Messaoudi M, Melhaoui A, El Mzibri M, Benbacer L, Aziz M. Cytotoxicity of the aqueous extract and organic fractions from Origanum majorana on human breast cell line MDA-MB-231 and human colon cell line HT-29. AdvPharmacol Sci. 2018; 23 :3297193.
36. Taamalli A, Arr´aez-Rom´an D, Abaza L, Iswaldi I, Fern´andez-Guti´errez A, Zarrouk M, Segura-Carretero A. LC-MS-based metabolite profiling of methanolic extracts from the medicinal and aromatic species Menthapulegium and Origanum majorana : phenolic compounds in Menthapulegium and Origanum majorana. Phytochem Anal. 2015; 26:320–330.
37. Andrews RE, Parks LW, Spence KD. Some effects of Douglas fir terpenesoncertain microorganisms. App EnvMicrobiol. 1980 ; 40: 301-304.
38. Cimanga K, Kambu K, Tona L, Apers S, De Bruyne T, Hermans N, Totte J Pieters L, Vlietinck AJ. Correlation between chemical composition and antibacterialactivity of essential oils of some aromatic medicinal plants growing in the Democratic Republic of Congo. J Ethnopharmacol 2002; 79: 213-220.635
39. Chaves RDSB, Martins RL, Rodrigues ABL, Rabelo ÉM, Farias ALF, Brandão LB, Santos LL, Galardo AKR, de Almeida SSMDS. Evaluation of larvicidal potential against larvae of Aedesaegypti (Linnaeus, 1762) and of the antimicrobial activity of essential oil obtained from the leaves of Origanum majorana L. PLoS One. 2020;15(7):e0235740.
40. Omara ST, El-Moez SIA, Mohamed AM. Antibacterial effect of Origanum majorana L.(Marjoram) and Rosmarinus officinalis L.(Rosemary) essential oils on food borne pathogens isolated from raw minced meat in Egypt. Global Vet. 2014; 13: 1056–1064.
41. El Kamari F, Taroq A, Atki Y E, Aouam I, Lyoussi B, Abdellaoui A. Chemical composition of essential oils from Vitex Agnus-castus L. growing in morocco and its in vitro antibacterial activity against clinical bacteria responsible for nosocomial infections. *Asian J Pharm Clin Res*. 2018;11: 365-368.
42. El Atki Y, Aouam I, El Kamari F, Taroq A, Nayme K, Timinouni M, Lyoussi B, Abdellaoui A. Antibacterial activity of cinnamon essential oils and their synergistic potential with antibiotics. J Adv Pharm Technol Res. 2019; 10(2):63-67.
43. Djenane D, Yanguela J, Montanes L, Djerbal M, Roncales P. Antimicrobial activity of Pistacialentiscus and Saturejamontana essential oils against Listeria monocytogenes CECT 935 using laboratory media: efficacy and synergistic potential in minced beef. Food Control. 2011; 2(7): 1046-1053.660
44. Gupta SM, Arif M, Ahmed Z. Antimicrobial activity in leaf, seed extract and seed oil of Jatropha curcas L. Plant J App Nat Sci. 2011; 3: 102-105