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Ethnopharmacological Study, Phytochemical Quality and Antibacterial Activity of the Essential Oils of *Chenopodium ambrosioides*, *Eucalyptus camaldulensis* and *Origanum majorana* from Taounate Region (Morocco)

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This study aims to collect the traditional uses of three medicinal plants, *Chenopodium ambrosioïdes, Eucalyptus camaldulensis* and *Origanum majorana,* in Taounate region of Morocco and to evaluate the phytochemistry and antimicrobial activity of their essential oils. The ethnobotanical survey showed that *Eucalyptus camaldulensis* is used to treat respiratory ailments (27%), followed by osteo-articular troubles (24%), neurological diseases (22%), genitourinary and also digestive tract ailments (11% for each), while only 2% use it against dermatological problems. *Origanum majorana* was recommended for cardiovascular diseases (31%), neurological diseases (25%), digestive tract diseases (25%) and genitourinary infections (14%), as well as metabolic troubles (5%). While *Chenopodium ambrosioides* is mainly consumed to treat neurological diseases (37%), digestive diseases (35%) and skin conditions (20%). After hydrodistillation, the essential oils were analyzed by gas chromatography and mass-spectrometry (GC/MS). The main component of *Chenopodium ambrosioides* essential oil is α-terpinene (80.74%). For *Eucalyptus camaldulensis* essential oil, 1,8-cineole (34.16%) and (-)-spathulenol (21.21%) are the main compounds. As for *Origanum majorana* essential oil, terpinene-4-ol (39.80%), α-phellandrene (15.15%) and carene (12.06%) represent the major components. The antibacterial activity of these essential oils indicates a stronger inhibitory effect on Gram-positive strains than Gram-negative strains; with minimum inhibitory concentrations varying between 5-40 µL/mL.

Keywords: ***Chenopodium ambrosioides*, *Eucalyptus camaldulensis*, *Origanum majorana*, Volatile compounds, Antibacterial activity.**

INTRODUCTION

Traditional knowledge about medicinal plants is frequently relied upon by people living in remote areas to cure a wide range of diseases. Moreover, plants offer shelter, food, fodder, timber and firewood; improve air quality, prevent soil erosion and assist in water recycling [1]. Many developing countries use medicinal plants and plant-based medications extensively in their health-care systems and many developed countries also value them as well [2,3]. World Health Organization (WHO) defines traditional medicine as knowledge and skills based on indigenous theories, beliefs and experiences of different cultures, used in the maintenance of good health and in the prevention of disease, diagnosis and treatment of mental and physical illnesses [4]. Thereby, over 80% of people in underdeveloped countries still use traditional herbal remedies for their daily healthcare needs [5-7].

In Morocco, the diverse ecological conditions have promoted the growth of over 42.000 plant species, which are classified into 150 families and 940 genera [8]. Moroccan phytotherapy has a long and illustrious history. Herbal medicine is an important element of Moroccan culture and it plays an important role in medicine, food preparation and perfumery composition. Taounate region is one of many Moroccan provinces that have a great potential such as herbal medicine source from which three medicinal plants have been selected for this study. *Chenopodium ambrosioides* (L.) is a wild species from tropical America, naturalized in the old World [9], which is used as an antirheumatic, analgesic [10], sedative and antipyretic [11], *etc*. It is also used as an herbal tea, to produce milk for breastfeeding women in Mexico and to improve blood flow. Moreover, the use of this plant, from the Amaranthaceae family, in the treatment of digestive disorders is very old and widespread [12]. *Eucalyptus camaldulensis*, from the Myrtaceae family, is a plantation species present in many places of the world, where it is reported as an anesthetic, antiseptic and astringent, besides being the popular remedy for colds and colic thanks to its red gum [13]. *Origanum majorana*, is an aromatic and medicinal plant, of the Lamiaceae family, distributed in different parts of the Mediterranean countries. This species is widely used in traditional medicine for the treatment of many diseases such as allergies, hypertension, respiratory infections, diabetes, stomach ache and as intestinal antispasmodics [14].

In the context of enhancing the three plants studied, this study has been conducted in the aim to collect all information about therapeutic and traditional applications of *Chenopodium ambrosioides*, *Eucalyptus camaldulensis* and *Origanum majorana* in Taounate region and to determine the chemical composition of their essential oils and to evaluate their antimicrobial activity.

EXPERIMENTAL

Study area: The Province of Taounate is a predominantly rural subdivision of the Moroccan region of Fez-Meknes. It is bounded by Chefchaouen and Al-Hoceima provinces in the north, Fez region in the south, Taza province to the east and Sidi Kacem province to the west of Morocco.

An ethnopharmacological survey was conducted, for one month (April 2020), using the questionnaire form, submitted to about 100 people and completed by oral questioning, taking place over a period of about 15 min for each interview.

Extraction: In a Clevenger-type apparatus, the air-dried plants (100 g) were hydrodistilled in 1.5 L of water for 3 h.

Chemical composition analysis of essential oils: The essential oils analysis was performed using a Gas Chromatograph (Trace GC ULTRA, S/N 20062969, Thermo-Fischer, France) coupled with spectrometer (Polaris Q/S/N 210729, Thermo Fischer, France). Varian capillary column was utilized (TR5-CPSIL-5CB; with 50 m length, 0.32 mm of diameter and film thickness 1.25 µm. The temperature was programmed from 40 to 180 ºC at 4 ºC/min and from 180 ºC to 280 ºC at 20 ºC/min and then held at 280 ºC for 2 min. The gas vector (helium) was characterized by a flow rate of 1.4 mL/min and 1 µL of essential oil was injected at 220 ºC. The spectra were recorded in electron impact (EI) mode with an ionization energy of 70 eV. The identification of the essential oils compounds was carried out by comparing each one to those reported by Adams table and library of NIST-MS, on the basis on its specific retention index [15].

Determination of the minimum inhibitory concentration: In order to determine the minimum inhibitory concentration (MIC), the microdilution test in 96-well microplates was carried out as described by Benkhaira *et al.* [16]. Briefly, each well was filled with 50 µL of LB (Luria-Bertani) liquid medium, then 50 µL of the essential oils were added to each well of column 1. A series of two-fold dilutions of the essential oils have been done in the concentration range from 80 µL/mL to 0.156 µL/mL (v/v), then 50 µL of cell suspension containing 10^8 CFU/mL of bacteria corresponding to 0.5 McFarland standard was added to the wells. Positive controls were utilized as a reference without essential oil, while negative controls were used as blanks without inoculum. The plates were covered and incubated at 37 ºC for 24 h. After incubation, 10 µL of sterile resazurin was added to the plates. Readings of the results were done after 2 h of incubation. The reduction of resazurin to resorufin causes a colour change which indicates the absence (blue colour) or presence (pink colour) of cell viability. The minimum inhibitory concentration (MIC) is the lowest concentration at which no colour change was observed.

Microbial strains: The essential oils extracted from the selected plants were tested against two microorganisms of clinical importance (*Staphylococcus aureus* and *Salmonella enterica*). These microbial strains have been obtained from Laboratory of Microbial Biotechnology & Bioactive Molecules, Sciences and Technologies Faculty of Fez, Sidi Mohamed Ben Abdellah University, Fez, Morrocco.

Data processing: The data recorded on the raw cards were transferred to a database and processed by the IBM SPSS version 21, statistical processing software.

RESULTS AND DISCUSSION

Socio-demographic information

Gender: The results of this ethnobotanical study have shown that women and men are both concerned with traditional medicine. However, women are the major users of medicinal plants with a higher frequency of 63% than men (37%). This can be explained by the responsibility they have been given in caring for themselves, their husbands and their children. This result is consistent with those of previous ethnobotanical studies conducted in the Eastern High Atlas of Morocco, where women utilize medicinal plants more than men by 53% [17]. Moreover, in the prefecture of Agadir-Ida-Outanane, the women's use of such plants corresponds to 57.40% [18].

Age: In the Taounate region, people aged between 50 and 60 use the selected plants with high frequency of 27% and those who are over 60 use them with a slightly lower frequency of 23%. While the use of MAPs by the population under 20 years old don't exceed 3% (Fig. 1). The elderly population possesses a greater understanding of medicinal plants as a result of their accumulated experiences and the transmission of popular knowledge [19]. Other ethnobotanical studies have shown that adults over the age of 50 had the highest knowledge about medicinal plants [20,21].

Socio-economic level: In Taounate region, the majority of respondents are from a low socio-economic level with a frequency of 74%, while 15% of those belong to a medium socio-economic level and only 11% have a high socio-economic level (Fig. 2). Several previous studies have shown that people with low socio-economic level utilize medicinal plants more extensively [22,23].

Study's level: In the study region, the majority of interviewed persons who use the chosen plants are out of school, followed by those who have primary education with a frequency

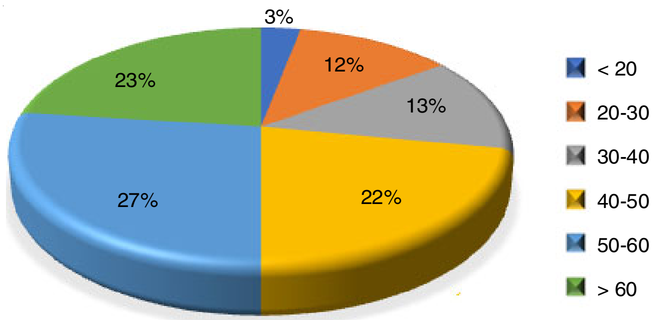

Fig. 1. Distribution of the age frequency of the studied plants users

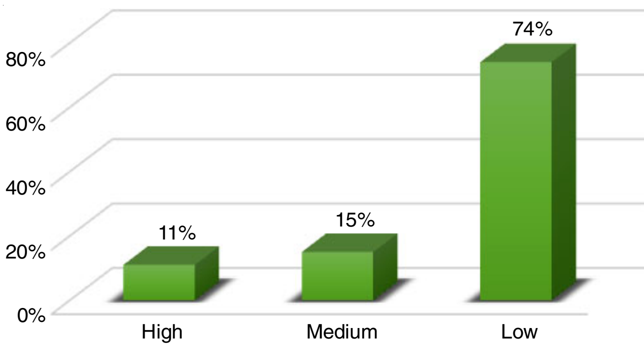

Fig. 2. Socio-economic frequency

of 24% while 15% have secondary education. Those with university education level have a percentage of 14% (Fig. 3). These results are consistent with the previous studies which showed that people who frequently utilize medicinal plants are illiterate [20,24].

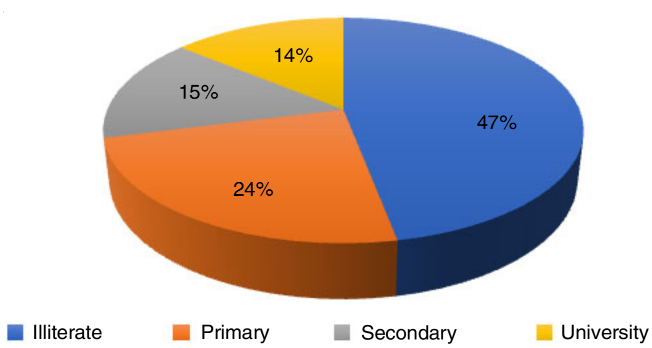

Fig. 3. Distribution of the study's level frequency

Type of medicine used by the population: Concerning the type of medicine used by the population, 72% of those interviewed use traditional medicine, while only 28% use modern medicine. These results are similar to those obtained in the Taounate region by Jeddi *et al.* [25], according to which 77% of the population turns to traditional medicine and only 23% use modern medicine.

Effectiveness of the studied plants: The results of the ethnobotanical survey revealed that the majority of people interviewed in the Taounate region were satisfied with the usage of the selected plants. Thus, 47% of them found that the selected medicinal plants can help them to improve their health state and 46% of them believe that these plants may provide a cure. While only 7% of them believe that these plants have negative side effects (Fig. 4).

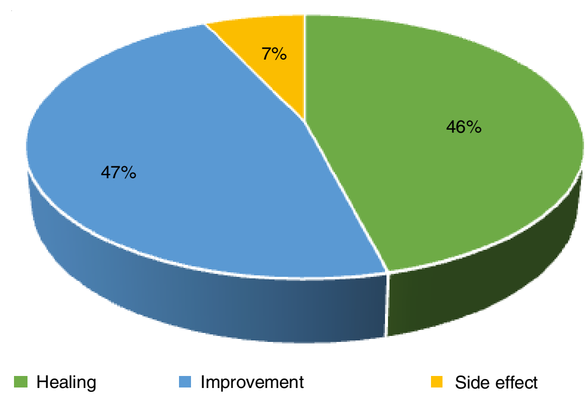

Fig. 4. Plants effectiveness

Parts used: Medicinal plants can be utilized in their entirety or in part. According to the results of current survey, leaves are the most commonly used parts by questioned people for *Eucalyptus camaldulensis*, *Chenopodium ambrosioides* and *Origanum majorana* with respective percentages of 88%, 88%, 47%; while the entire plant is used by almost 44 % of interviewed people for *O. majorana and* by 12 % of them for *C. ambrosioids*. Concerning the other parts, *E. camaldulensis* bark and roots are the least utilized with a frequency of 6% for each one; while *O. majorana* flowers are used by about 9% of people (Fig. 5). The quantity of chemical compounds in leaves, as well as the simplicity and speed of their harvesting, justify their frequent usage [26]. These results are in agreement with other studies carried out in the Taza province by Kabbach *et al.* [27], in the Guercif province by Benali *et al.* [28], in Sidi Kacem

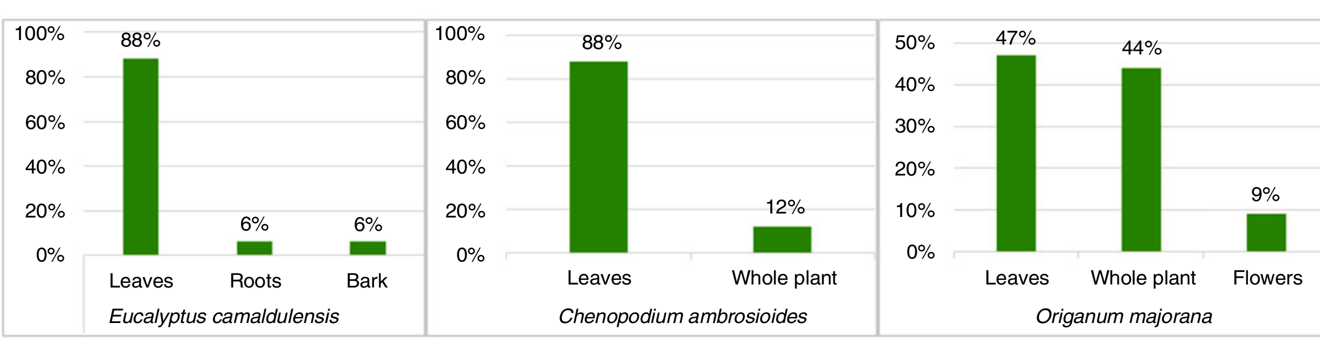

Fig. 5. Distribution of used parts of the plants

province by Fatima-Zahra *et al.* [29] and in Rabat-Salé-Kénitra region (Morocco) by El-Hachlafi *et al.* [30], which mentioned that leaves are the most used part of medicinal plants.

Preparation methods: For the three studied medicinal plants, many preparation methods have been utilized. The most frequent preparation methods for *E. camaldulensis* are decoction (48%), followed by cooked 25%, then infusion (23 %) and finally poultice (only 4%). As for *O. majorana*, decoction is the most frequent preparation method (40%), followed by infusion (30%), cooked (12%), poultice (8%) and 10% for the other methods, while the poultice preparation method was used by 100% of questioned people for *C. ambrosioides* (Fig. 6). According to the local people, the high percentage of medicinal plant decoction usage might be explained by the fact that heat reduces the plant toxicity [31].

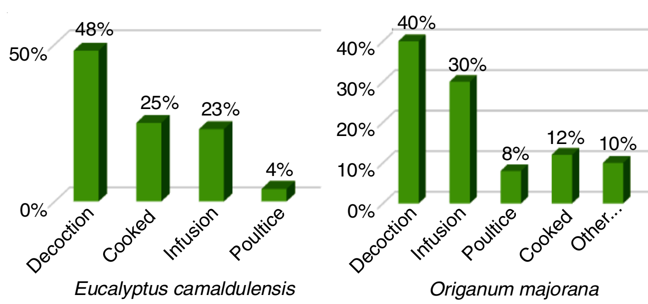

Fig. 6. Distribution of users of the plants studied according to the preparation methods

Phytotherapeutic use of the selected plants: In the study area, people use the selected plants to fight against several diseases. Hence, 28% of them use *E. camaldulensis* to treat the respiratory ailments, followed by osteo-articular troubles (24%), neurological diseases (22%), genitourinary and also digestive tract ailments (11% for each), while only 2% use it against dermatological problems (Fig. 7). These results are in agreement with those of another ethnobotanical study carried out in southwest of Morocco, which reported that *Eucalyptus* plant is mainly used to treat respiratory diseases [32].

O. majorana has been mainly used by respondents to treat cardiovascular diseases (31%), neurological diseases (25%), digestive tract diseases (25%) and genitourinary infections (14%), as well as metabolic troubles (5%) (Fig. 7). A previous ethnobotanical study carried out in Settat province (Morocco) by Tahri *et al.* [33] indicated that *O. majorana* is used to treat the respiratory diseases, headache and gastric disorders.

C. ambrosioides is mainly consumed to treat neurological diseases (37%), skin conditions (35%), digestive diseases (20%) and other diseases (8%) (Fig. 7). Another study conducted in Mechrâa Bel Ksiri (western region of Morocco), has shown that *C. ambrosioides* is used to treat the gastrointestinal ailments, typhoid, dysentery for children and adults [34]. In addition, other studies have shown that medicinal plants from Morocco are frequently utilized in traditional pharmacopeia to treat various diseases [27,35-38]. Indeed, several species are used in the traditional treatment of a variety of illnesses, such as *Thymus algeriensis*, *Ammi visnaga*, *Myrtus communis* especially in the treatment of digestive, respiratory, genito-urinary and dermatological infections [22]. Furthermore, *Rosmarinus officinalis*, *Origanum compactum* and *Artemisia herba-alba* are especially used in the treatment of metabolic, digestive and respiratory infections [28]. Moreover, a previous study carried out in Rabat-Salé-Kénitra region by El-Hachlafi *et al.* [30] mentioned that the Lamiaceae, Apiaceae and Asteraceae families were the most cited to treat chronic diseases. Another previous survey conducted in Fez city (Morocco) by Benkhaira *et al.* [39] reported that for fighting against COVID-19, 49 species belonging to 28 botanical families are used with a dominance of Lamiaceae family, *Thymus vulgaris* L., *Syzygium aromaticum* L. and *Eucalyptus globulus* [39].

Yield of essential oils: The essential oil of *O. majorana* has a yield of 2.6%, which is higher than the yield of the essential oil of the same species collected from Tunisia (1.4%) as reported by Ezzeddine *et al.* [40]. For *E. camaldulensis* from the studied region, its essential oils yield is about 1.8%, which is a little higher than that obtained in Rabat (1.4%) as reported by Hmiri *et al.* [41]. Concerning the essential oil of *C.ambrosioides,* its yield is around 1.1 %.

Chemical composition of the studied essential oils

Chemical composition of *Chenopodium ambrosioides* essential oil: Gas chromatography-mass spectroscopy (GC-MS) analysis of *C. ambrosioides* essential oil enabled to identify five compounds with a percentage of 96.71%. α-Terpinene was the predominant compound (80.74%), followed by tetrahydrofuran-5-on-2-methanol (8.26%) and thymol (3.93%) (Table-1). There were also minor compounds such as 3-carene (2.12%) and γ-terpinene (1.66%). Another study reported that the essential oil of *C. ambrosioides* from the region of Salé (Morocco) contains α-terpinene (35.15%), terpinolene (22.82%) and *p*-cymene (12.26%) as major compounds [42], while the

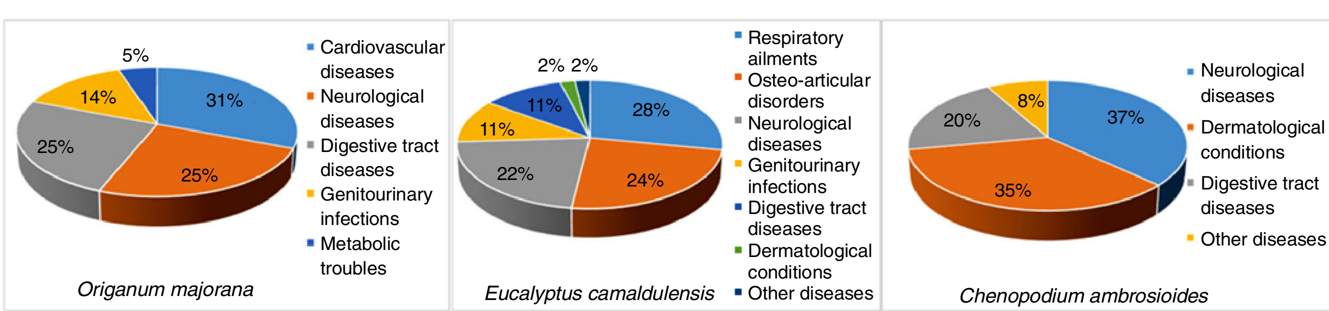

Fig. 7. Phytoterapeutic use of the studied plants

TABLE-1
CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *Chenopodium ambrosioides*

Compounds	TR (min)	Area (%)
Tetrahydrofuran-5-on-2-methanol	4.78	8.26
α-Terpinene	15.56	80.74
γ-Terpinene	16.78	1.66
3-carene	22.56	2.12
Thymol	24.04	3.93

essential oid isolated from *C. ambrosioides* from Agadir region contains 46.80% of α-terpinene, 17.42% of terpinolene and 10.81% of *p*-cymene [43]. Furthermore, in Cameron, the seeds' essential oil contained α-terpinene 73.55% and *p*-cymene 20.05% [43].

Chemical composition of *E. camaldulensis* essential oil: The essential oil of *E. camaldulensis* from Taounate region contains 17 compounds at a percentage of 93.02% with 1,8-cineole (34.16%), (-)-spathulenol (21.21%) and α-pinene (6.73%) as major compounds (Table-2). Minor compounds are 5-oxotetrahydrofuran-2-carboxylic acid (3.41%), α-phellandrene (3.83%), *p*-menth-1-ene-4-ol (2.09%), propanal, 2-methyl-3-phenyl (3.24%), phellandral (2.54%), aristolene (3.30%) and α-guajene (5.51%). These results are concordant with those of previous research carried out in Cameroon and in Mozambique, which mentioned that 1,8-cineole is the major component of *Eucalyptus camaldulensis* essential oil [44,45].

Chemical composition of *O. majorana* essential oils: GC-MS analysis of the essential oil of *O. majorana*, enabled to identify 10 compounds at a percentage of 97.04%. The major constituents are terpinen-4-ol (39.80%), followed by α-phellandrene (15.15%), carene (12.06%), linalyle acetate (9.21%) and γ-terpinene (6.46%). While *p*-cymene (3.04%), α-thujene (2.50%), sabinene (1.90%) and α-pinene (2.97%) are present as minor compounds (Table-3). These results are in accordance with previous studies conducted in Morocco, which revealed that the major components of *O. majorana* essential oils are 4-terpinene (28.96%), γ-terpinene (18.57%) and α-terpinene (12.72%) [46].

Antibacterial activity: In order to evaluate the antibacterial activity of the studied essential oils, their minimum inhibitory concentration (MIC) values have been determined. Table-4 shows the antibacterial activity of the essential oils of the studied plants (*E. camaldulensis*, *C. ambrosioides* and *O. majorana*) on the growth of two tested bacteria (*Staphylococcus aureus* and *Salmonella enterica*). The minimum inhibitory concentrations (MIC) range between 20-40 and 5-20 µL/mL, respectively for the Gram-negative and Gram-positive bacteria. The essential oil of *E. camaldulensis* showed the highest activity against *S. aureus* (MIC = 5 µL/mL) and a moderate activity against *S. enterica* (20 µL/mL). A previous study carried out by Musa *et al.* [47] reported that the MIC of the

TABLE-2
CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *Eucalyptus camaldulensis*

Compounds	TR (min)	Area (%)
Acide 5-oxotetrahydrofuranne-2-carboxylique	4.77	3.41
3-Carene	12.67	0.83
α-Phellandrene	15.05	3.83
1,8-Cineole	15.98	34.16
γ-Terpinene	16.79	0.58
p-Menth-1-ene-4-ol	20.67	2.09
α-Pinene	21.17	6.73
Propanal, 2-methyl-3-phenyl-	22.64	3.24
Phellandral	23.65	2.54
p-Cymen-	24.06	1.02
Carvacrol	24.36	1.70
γ-Gurjunene	27.94	0.88
Aristolene	28.56	3.30
α-Guajene	29.47	5.51
Aromadendrene, dehydro-	29.7	0.52
(-)-Spathulenol	31.71	21.21
Isolongifolene-9-one	35.25	1.47

TABLE-3
CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *Origanum majorana*

Compounds	TR (min)	Area (%)
Linalyleacetate	1.566	9.21
Terpinen-4-ol	1.640	39.80
α-Pinene	1.830	2.97
α-Thujene	8.850	2.50
α-Terpinene	10.146	3.95
γ-Terpinene	11.443	6.46
Sabinene	11.681	1.90
Carene	12.631	12.06
p-Phellandrene	14.892	15.15
p-Cymene	15.346	3.04

TABLE-4
MINIMUM INHIBITORY CONCENTRATION OF ESSENTIAL OILS

Concentration (µL/mL)	*Chenopodium ambrosioides*		*Eucalyptus camaldulensis*		*Origanum majorana*	
	Staphylococcus aureus	*Salmonella enterica*	*Staphylococcus aureus*	*Salmonella enterica*	*Staphylococcus aureus*	*Salmonella enterica*
80	–	–	–	–	–	–
40	–	–	–	–	–	–
20	–	+	–	–	–	+
10	+	+	–	+	+	+
5	+	+	–	+	+	+
2.5	+	+	+	+	+	+
1.25	+	+	+	+	+	+
0.625	+	+	+	+	+	+

+ Microbial growth, – No Microbial growth essential oil isolated from *E. camaldulensis* against a Gram-positive bacteria (*S. aureus)* is 12.5 mg/mL, while that against a Gram-negative bacteria (*E. coli)* is 50 mg/mL [47].

The essential oils of *C. ambrosioides* and *O. majorana*, have showed a lowest activity against *S. enterica*, with a MIC value of 40 µL/mL and a moderate activity against *S. aureus* with a MIC of 20 µL/mL. Another work conducted by Santiago *et al.* [48] mentioned that the MIC value of the essential oil isolated from *C. ambrosioides* against *S. aureus* is 62.5 µg/mL and against *E. coli* is 125 µg/mL [48]. Moreover, in another study, the MIC value of the essential oil isolated from *O. majorana* against *S. aureus* is 0.782 mg/mL and 0.920 mg/mL against *S. choleraensius* [49]. Additionally, it was observed that *S. choleraensius* (Gram-negative) was more resistant to the essential oils tested than *S. aureus* (Gram-positive).

This resistance is related to the composition of their cell wall, because the antibacterial activity of essential oils could be explained by the molecular interaction of their components' functional groups with bacteria walls. The resistance of Gram-negative bacteria could be explained by the constraints of diffusion through their outer membrane caused by the existence of a hydrophilic barrier [50]. The essential oils extracted from the three selected plants have shown significant antibacterial activities against the tested bacteria, which are due to their major compounds. These results are consistent with earlier research indicating that Gram-positive bacteria are more susceptible to the essential oils than Gram-negative bacteria [51-53]. Thus, due to the therapeutic virtues as well as the diversity of their essential oils' compositions, these plants could be considered as a source of bioactive molecules for the development of new drugs.

Conclusion

This study aimed to conduct an ethnobotanical investigation of three medicinal plants, with the objective of gathering information regarding the types of users and the many applications of these plants within the people of the Taounate region in Morocco. The findings of this ethnobotanical study demonstrated that the local population still prefers to treat their daily ailments using medicinal plants and that the leaves constitute the most commonly utilized part for the three selected plants. The most frequent preparation methods are decoction for both of *Eucalyptus camaldulensis* (48%) and *Origanum majorana* (40%), while the poultice is the only preparation mode used for *Chenopodium ambrosioides* (100%). The use of medicinal plants is more frequent among married than single persons. This study also enabled to have information on the chemical composition and the antibacterial activities of the essential oils of *C. ambrosioides*, *E. camaldulensis* and *O. majorana*, which have shown respective chemotypes of α-terpinene, 1,8-cineole and terpinen-4-ol. Concerning the antibacterial activities, the minimum inhibitory concentrations are respectively between 20-40 and 5-20 µL/mL for *S. enterica* and *S. aureus*. However, further studies should be carried out to evaluate the efficacy of essential oils extracted from the three selected plants against other bacterial strains, as well as their antioxidant and antifungal activities.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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