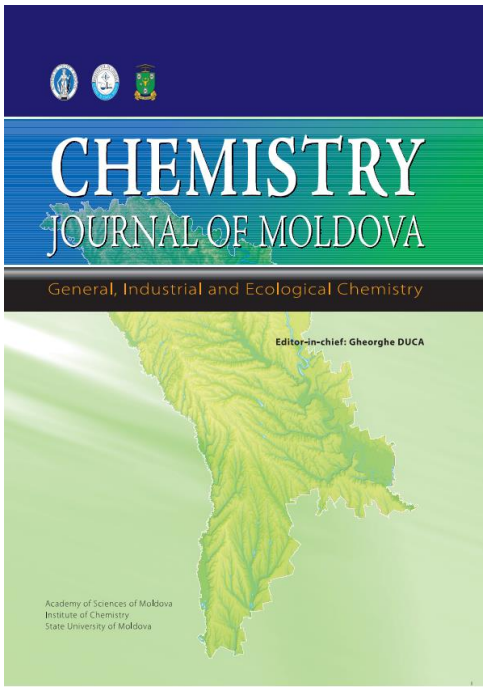

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Abstract. The main objectives of this study were to determine the chemical composition of the essential oil of *Marrubium deserti* de Noé (EOMD) from Bechar (Algeria), and to evaluate its physicochemical properties, antibacterial and antifungal activities. The yield of EOMD was 0.29±0.008%, with the main components being *α*-phellandrene (25.05%), *β*-pinene (14.05%), and *α*-pinene (12.83%). Both gram-negative and gram-positive bacteria were significantly inhibited by EOMD with inhibition zones ranging from 7.00 ±0.00 mm to 22±1.33 mm, and with minimum inhibitory concentrations (MICs) and minimum bactericidal concentration values ranging from 0.0022 to 0.014 v/v; likewise, intriguing antifungal activity against pathogen fungi was noticed with MICs and minimum fungicidal concentration values ranging from 0.00125 to 0.006 v/v. Furthermore, the studied essential oil demonstrated a total suppression of the sporulation and germination of spores at concentrations as from 0.002 v/v. These results emphasize the bactericidal and fungicidal characteristics of EOMD and their prospective usage as a substitute for synthetic bactericides and fungicides.

Keywords: chemical composition, essential oil, *Marrubium deserti* de Noé, antibacterial, antifungal.

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Introduction

The genus *Marrubium*, which belongs to the Lamiaceae family, is one of the genera that have piqued the interest of scientists on account of its promising chemical and biological features. In general, around forty species are declared, principally distributed in Asia, North Africa, and Europe [1]. In Algeria, the *Marrubium* genus contains seven species [2]. Among them, *Marrubium deserti* de Noé, commonly known as Djaada, is an endemic plant of the central and north Algerian Sahara [3]. It is usually utilized in folk medicine for remediating various ailments and diseases, including colds, fever, colics, cough, jaundice, hypertension, helminthiasis, nausea, and diabetes [4,5]. For external use, the plant is used in the treatment of allergic reactions as well as to cure scorpion stings [6]. On the other hand, there have been reported several studies on the biological properties of *Marrubium deserti* extracts, including antimicrobial [7], anti-inflammatory [8], antioxidant [9], antigenotoxic [6], analgesic [10] and antiviral activities [11]. Concerning the phytochemical content, plants of this genus primarily generate diterpenes, polyphenols, steroids, phenylpropanoids, and flavonoids, which have significant biological effects [12]. Essential oils are one of the intriguing natural bioactive substances [13], they have important biological activities, making them appropriate for use in various sectors including medical, pharmaceutical, cosmetics, agriculture, and environmental fields [14]. There are various published data on the chemical constituents of the essential oils of this genus, especially on the *M. vulgare* [15-17]. On the contrary, limited studies have been found on the identification of the chemical profile of the essential oil of *Marrubium deserti* de Noé [3], and to the best of our knowledge, chemical composition and antimicrobial activities employing *Marrubium deserti* essential oil from Bechar region have never been studied.

The main goals of the present study were to determine the chemical composition and physicochemical properties of the essential oil of *Marrubium deserti* growing wild in the

Bechar region (South-West of Algeria), and assess its antibacterial and antifungal activities, for more discoveries about the possible nutritional and therapeutic uses of this species.

Experimental

Materials

Anhydrous sodium sulphate, ethanol (95%), potassium hydroxide was purchased from Sigma-Aldrich. All the solvents and reagents were of analytical grade.

All the aerial parts of *Marrubium deserti* were harvested from the mountain of the Bechar region (N 31° 42′ 00.0″, E 1° 58′ 00.0″, A 1206 m) during the flowering period (February 2023). The sample was dried in the shade for two weeks before the extraction of the essential oil. The voucher specimen (LB/23/UB-005) has been deposited in the Herbarium of the Laboratory of Biology Institute, University of Tahri Mohammed, Bechar.

Methods

Extraction procedure of essential oil

The aerial parts of *Marrubium deserti* were subjected to hydrodistillation for 4 hours using a Clevenger-type apparatus. After that, the extracted oil was dried over anhydrous sodium sulphate and kept in dark glass vials at 4°C. The average yield of essential oil was estimated by dividing the weight of the extracted oil by the weight of the plant's dry matter [18,19].

Physicochemical tests

The physical and chemical indices are determined by methods conforming to [20-22].

GC-MS analysis

The essential oil of *M.deserti* (EOMD) was analysed on a SHIMADZU GCMSQP2020 instrument fitted with a fused Rxi®-5ms capillary column (30 m×0.25 mm, 0.25 m film thickness, Phase: Cross bond® 5% diphenyl/95% dimethylpolysiloxane). Helium with a purity of 99.995% was used as the carrier gas with a flow rate of 1 mL/min. Temperatures in the injector and detector were kept constant at 250°C and 310°C, respectively. A volume of 0.5 (µL) of the sample was injected in split mode (1:10). The initial temperature of the column was set to 50°C for 2 minutes, then elevated to 310°C with an increase increment of 3°C/min, and finally maintained at 310°C for 2 minutes. The mass spectrometer conditions included an ionization voltage of 70eV, an ion source temperature of 200°C, and electronionization mass spectra were acquired over the mass range of 45-600 m/z.

Antimicrobial activity evaluation

Microbial strains

In the present study, eight strains of microorganisms were used, of which five were gram-positive and gram-negative strains *Enterococcus faecalis* (ATCC 49452), *Bacillus cereus* (ATCC 10876), *Bacillus subtilis* (ATCC 21332), *Salmonella tiphymurium* (ATCC 14028), and *Pseudomonas aeruginosa* (ATCC 27853), and three were fungal strains *Aspergillus flavus*, *Penicillium expansum*, and *Fusarium oxysporum f.sp. albedinis* .

Agar-disk diffusion method

The disk-diffusion method was used to evaluate the antibacterial efficacy of EO as formerly described by [23] with slight modifications. In the short, 100 (µL) of suspension containing 10^8 CFU/mL of bacterial strains were inoculated on Mueller-Hinton (MH) agar plates. Then, the sterile discs of paper (6 mm in diameter) impregnated with 1.5, 3, and 6 (µL) of EO were placed on the agar. Ceftazidime (30 µg /disc) was used as the positive control. The inoculated plates were incubated at 37°C for 24 h.

Determination of minimum inhibitory concentration

The direct contact method was used to determine the minimum inhibitory concentration (MIC) value of the EOMD [24]. The final concentrations of essential oil in the medium ranging from 1/100 to 1/7000(v/v). The incubation is done for 24 h at 37°C for bacteria, and 7 days at 25°C for fungi. The lowest concentration of essential oil that visibly prevented the growth of the studied strains was regarded as the MIC. The Eq.(1) is used to compute the fungal growth inhibition at various doses of EO.

$$PI = [(DT - D) \div DT] \times 100 \quad (1)$$

where, DT is the mean diameter of mycelial growth in the control;

D is the mean diameter of mycelial growth in the test [25].

Determination of minimum bactericidal/ fungicidal concentration

In order to determine the minimal bactericidal concentration (MBC), and the minimal fungicidal concentration (MFC), from the dishes that revealed no bacterial and fungi growth, were-inoculated on new MH and PDA agar. The MBC and MFC were designated as the lowest concentration that prevents the development of any microorganism [26,27].

Antifungal effect of EO on spore sporulation

The method mentioned by [28] was used to evaluate the sporulation, with some changes, utilizing the same dishes that were employed to calculate the antifungal activity of EOs on mycelial growth incubated at 25°C for 10 days, from each strain at different concentrations, four 5 (mm) diameter washers were taken and placed in tubes containing 1 (mL) of sterile distilled water. The percentage inhibition of sporulation (*Is%*) was determined *via* the Eq.(2).

$$Is\ \% = [(N_c - N_t) \div N_c] \times 100 \quad (2)$$

where, N_c is the number of spores estimated in control;
N_t is the number of spores estimated in the presence of EO.
Each test was carried out in triplicate.

Antifungal effect of EO on spore germination

The antifungal activity of EOMD on spore germination was performed using the method as described by [24], with slight modifications. In short, 100 (µL) of the fungal spore suspension (10^5 spores/mL) was inoculated on Petri dishes containing PDA medium, to which oils are inserted at the same concentrations as above mentioned. The plates were incubated for 18 h at 25°C. The percentage inhibition of germination (*Ig%*) was calculated using the Eq.(3).

$$Ig\% = [(N_c - N_t) \div N_c] \times 100 \quad (3)$$

where, N_c is the number of germinated spores in control;
N_t is the number of germinated spores in the presence of EO.

Statistical analysis

All experiments were carried out in triplicate. Microsoft Excel 2010 was used to express the values as the mean ± deviation.

Results and discussion

Physicochemical analysis of essential oil

Hydrodistillation of the total aerial parts of *Marrubium deserti* gave a strongly odorous yellow oil with a yield of 0.29±0.008%. It is high compared to that obtained from *Marrubium deserti* aerial parts of Meguibra, El-Oued, Algeria (0.15%) [3], and El Masrane, Djelfa, Algeria (0.02%) [29], however, this rate is lower than those of the *Marrubium deserti* oil of Saudi Arabia (0.41%) [30].

The results of the physicochemical properties of EOMD were illustrated in Table 1. For physical indices, our EO has a freezing temperature of -17°C±0.3, which is within the fixed interval of French standards NFT-222, which ranges from -15°C to -19°C [20], and it is miscible by 1 volume for 2 volumes of ethanol (95%). The refractive index is higher than the refractive index of water (1.3356). Indeed, [31] provides for essential oils a refractive index between 1.495 and 1.513 (1.495 for high-quality oils and 1.513 for lesser-quality oils). This index attained a value of 1.498±0.2 in this situation. As a result, it appears that the studied essential oil is of good quality. Additionally, according to [24], this index is mostly affected by the concentration of monoterpenes and oxygenated derivatives. A substantial monoterpene amount will give a high index. While the density (0.916±0.1) is lower than that of water. Regarding the optical rotation, the findings revealed a value (-), which means that this essential oil is laevorotatory.

For chemical indexes, an acid index less than 2 is proof of good conservation of the oil (low quantity of free acids) [32]. In the present study, this index (1.15±0.1 mg/KOH/g) is less than 2. For the ester index, a value of 51.45±0.4 mg/KOH/g was obtained. According to [33], a very high EO quality must have a higher ester index and a lower acid index than a low oil quality.

Table 1

Physicochemical indices of *M.deserti* EO.

Physical indices	
Density D ^{20}	0.916 ±0.1
Refractive index n ^{20}	1.498 ±0.2
Optical rotation (°)	-10.5° ±0.4
Freezing point (°C)	-17°C ±0.3
Solubility in ethanol 95 %	1 v:2v
Chemical indices	
Acidity (mg/KOH/g)	1.15 ±0.1
Ester index (mg/KOH/g)	51.45±0.4

Chemical composition of M. deserti essential oil

Seventeen compounds accounting for 100% of the analysed essential oil were identified (Table 2 and Figure S1 see supplementary material). The main constituents were *α*-phellandrene (25.05%), *β*-pinene (14.05%), *α*-pinene (12.83%), limonene (9.78%), and *o*-cymene (9.19%) (Figures S2-S6, see supplementary material). Besides, other components were discovered in lower quantities, like myrcene (7.76%), *(Z)*-linalool oxide (5.80%), myristicin (4.85%), *(E)*-linalool oxide (2.75%), 4-vinyl-guaiacol (1.73%), and eugenol (1.35%).

The quantitative and qualitative essential oil compositions demonstrated in the current

investigation are very different from those published in the literature. As reported by [29], *Marrubium deserti* EO of Djelfa region (Eastern Algeria) was distinguished by the dominance of germacrene D (45.7%), *β*-bourbonene (4.0%) and *α*-terpinolene (3.9%). Another study showed that the major constituents of *Marrubium deserti* EO from Ghardaïa zone were 9-methyl-undecene (21.30%), *β*-cadinene (12.20%), and germacrène D (11.90%) [34]. However, [3] found that EOMD from a Meguibra area is characterized by high contents of tetracosane (31.11%), germacrene D (7.91%) and *δ*-cadinene (6.51%). Whereas, the principal compounds determined by Al Shammari, B.R for *Marrubium deserti* originating from Saudi Arabia were linalool (37.82%), cineole (12.04%), and borneol (11.07%) [30]. The existence of this variation in the chemical composition of EOMD from different regions might be related to numerous factors, including geographical location, harvest season, extraction techniques, nature of the soil, climatic conditions, and genetic differences [35]. Several studies have been documented on the composition of essential oils of various *Marrubium* species, and it has been generally proven that one of these components, *β*-caryophyllene, bicyclogermacrene, germacrene D spathulenol, and caryophyllene oxide, is the main chemical in these oils [1].

To the best of the authors' knowledge, *α*-phellandrene has never been recorded as a major constituent of essential oils of the species *Marrubium*, and it most likely represents a new chemotype typical to the region.

Table 2

Chemical composition of the essential oil from aerial parts of *Marrubium deserti*.

No.	*RT* (min)*	*Component*	*%*
1	8.88	*α*-Pinene	12.83
2	10.43	Sabinene	1.51
3	10.57	*β*-Pinene	14.05
4	11.19	Myrcene	7.76
5	11.73	*α*-phellandrene	25.05
6	12.61	*o*-Cymene	9.19
7	12.79	Limonene	9.78
8	14.75	*(Z)*-Linalool oxide	5.80
9	15.48	*(E)*-Linalool oxide	2.75
10	19.62	Terpinen-4-ol	0.29
11	24.21	Undecanal	0.89
12	25.79	4-Vinyl-guaiacol	1.73
13	27.72	Eugenol	1.35
14	30.40	Caryophyllene	0.79
15	34.53	Myristicin	4.85
16	34.66	*δ*-Cadinene	0.61
17	39.50	*β*-Eudesmol	0.77
Total		100	

*RT - retention time

Antibacterial activity of Marrubium deserti essential oil

The *in vitro* antibacterial effect of EOMD is demonstrated in Tables S1and S2. It can be seen that the zone of inhibition notably increased with increasing the volume of the essential oil for all the bacteria strains. Interestingly, bacteria were discovered to be resistant to the antibiotic used. *E. faecalis* was the most sensitive, being inhibited at MIC as weak as 0.0022 v/v with a maximum inhibition zone diameter of 22.00 ±1.33mm. This oil was shown to be bactericidal for all examined bacteria strains with MBC/MIC ratio ≤4 [36].

The high inhibitory effect of the studied EO can be attributed mainly to its high content of *α*-phellandrene (25.05%), *β*-pinene (14.05%), and *α*-pinene (12.83%). Indeed, these chemicals' antibacterial efficacy against a wide range of microbes has been frequently reported [37,38]. Even though the mechanism of antibacterial activity of these monoterpene hydrocarbon compounds is not completely understood, some authors assume that this is due to a disruption of the lipid part of bacterial plasma membranes, leading to changes in membrane permeability and intracellular substance seepage [39]. Furthermore, other minor constituents such as myrcene have also been identified as having antibacterial properties [40]. However, because essential oils include a variety of compounds, their antibacterial activity could also be related to the synergistic impact of the single constituents [41].

Antifungal Activity of Marrubium deserti essential oil

As shown in the Table S3, the EOMD demonstrated antifungal effectiveness against all fungi species tested. Concerning the fungal mycelial growth, the *F.oxysporum f.sp. albedinis* mycelia were totally inhibited at a low concentration of 1/800 (v/v). While those of *A. flavus* and *P. expansum* were completely inhibited at the same concentration of 1/200 (v/v). Therefore, *F. oxysporum f.sp. albedinis* was more sensitive than other studied molds to *M. deserti* essential oil. In addition, a fungicide concentration of 1/300 (v/v) was discovered for *F.oxysporum f.sp. albedinis*, while for both strains, *A. flavus* and *P. expansum*, it was 1/150(v/v).

On the other hand, the EOMD exhibited significant antifungal impacts on germination and sporulation spores. *A. flavus* and *P. expansum* were inhibited at the identical minimum sporulation inhibitory concentrations of 1/150 (v/v). *F.oxysporum f.sp. albedinis* was the most sensitive; it was inhibited from sporulating at a weak dose of 1/500 (v/v). Regarding the

inhibitory effect of spore germination, it was revealed that the essential oil of *M. deserti* was more active on *F. oxysporum f.sp. albedinis* spores, with an inhibitory effect of 1/500 (v/v), followed by *P. expansum* and *A. flavus*, which were inhibited to germinate at concentrations of 1/300 (v/v) and 1/150 (v/v), respectively.

In this investigation, the essential oil of *Marrubium deserti*, which has a substantial amount of *α*-phellandrene (25.05%), *β*-pinene (14.05%), and *α*-pinene (12.83%), was shown to be extremely potent against different fungi strains. As stated by authors, *α*-phellandrene and *α*-pinene was displayed a considerable antifungal activity [42,43]. This constituent may damage the fungal cell membrane's integrity, producing leakage of its components and potassium ions as well as an increase in total lipid level, extracellular pH, and penetrability of the membrane [44]. Besides, minor compounds, like eugenol is also recognized for their antifungal characteristics, and it should be noted as well that the antifungal activity of the essential oil of *Marrubium deserti* may be due to synergistic interactions between the essential oil molecules [45].

Conclusions

The current study is focused on the identification of the chemical profile and the physicochemical properties of essential oil from *Marrubium deserti* growing wild in Bechar (South -West Algeria). The 17 components were determined by the GC-MS analysis of this oil, and *α*-phellandrene was the most abundant component (25.05%). The physicochemical properties also confirmed the good quality of the studied oil. On the other hand, the obtained results demonstrate that the essential oil of *Marrubium deserti* possess high antibacterial activity against both gram-positive and gram-negative bacteria. Similarly, it proved that it is extremely efficient in inhibiting mycelial growth, sporulation, and spore germination of fungi. As a result, the EOMD can be employed as natural antibacterial and antifungal agents for the treatment of several illnesses, as a food preservative, or in the cosmetic and pharmaceutical industries.

Supplementary information

Supplementary data are available free of charge at http://cjm.ichem.md as PDF file.

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