Phytochemical analysis and evaluation of antimicrobial, antioxidant, and antidiabetic activities of essential oils from Moroccan medicinal plants: *Mentha suaveolens, Lavandula stoechas, and Ammi visnaga*

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ABSTRACT

Mentha suaveolens, Lavandula stoechas, and *Ammi visnaga* are widely used in Moroccan folk medicine against several pathological disorders, including diabetes and infectious diseases. This work was designed to determine the chemical profile of *M. suaveolens* (MSEO), *L. stoechas* (LSEO), and *A. visnaga* (AVEO) essential oils and assess their antimicrobial, antioxidant, and antidiabetic effects. The volatile components of LSEO, AVEO, and MSEO were analyzed using Gas Chromatography-Mass Spectrometry (GC–MS). The in vitro antidiabetic activity was assessed using α-amylase and α-glucosidase enzymes, while DPPH, FRAP, and β-carotene/linoleic acid methods were used to determine the antioxidant capacity. The antimicrobial activities were investigated using disc diffusion and broth-microdilution assays. GC-MS investigation revealed that the main components were fenchone (29.77 %) and camphor (24.9 %) for LSEO, and linalool (38.29 %) for AVEO, while MSEO was mainly represented by piperitenone oxide (74.55 %). The results of the antimicrobial evaluation showed that all examined essential oils (EOs) had noticeable antimicrobial activity against both bacteria and yeast, especially *Micrococcus luteus* and *Bacillus subtilis*. The MIC, MBC, and MFC values were ranged from 0.015 % to 0.5 %. The MBC/MIC and MFC/MIC ratios were less than or equal to 4.0 % (v/v), indicating their noticeable bactericidal and candidacidal efficacy. Moreover, the three EOs showed significant inhibitory effects on α-amylase and α-glucosidase ($p < 0.05$). It also exerted remarkable activity on FRAP, β-carotene, and DPPH radicals. These findings demonstrated that the tested plants have promising biological activities, validating their ethnomedicinal value and providing potential applications as natural drugs.

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1. Introduction

Despite the significant advancements in allopathic medicine, over 85 % of people in developing countries still rely on traditional medicine. Additionally, people in developed countries are increasingly drawn to alternative and complementary medicine [1]. Moreover, approximately 25 % of drugs currently used in modern medicine are derived from medicinal herbs [1]. Several studies have shown that natural products are rich in bioactive phytochemicals, especially essential oils, which play a crucial role in preventing chronic diseases such as infectious, metabolic, and vascular diseases [2]. These volatile oils have several pharmacological and biological properties, including anti-inflammatory, antimicrobial, antioxidant, anticancer, and antidiabetic actions [3]. Essential oils are also used to fight against microorganisms that cause dangerous infectious diseases due to their antibacterial, antifungal, and antiviral actions [2].

Infectious diseases remain the leading causes of morbidity and mortality worldwide [4]. The Coronavirus disease (Covid-19) pandemic is a prime example, which has seriously impacted lives and livelihoods worldwide [5,6]. Therefore, the scientific community is striving to control the resistance of microbial pathogens by discovering new antibiotics from natural products [7]. Additionally, oxidative stress triggered by reactive oxygen species or free radicals has been found to cause significant health complications especially in individuals with diabetes mellitus [8]. This latter is a highly prevalent metabolic disorder worldwide, particularly in Middle Eastern countries like Morocco. It carries a significant social and economic burden with serious implications for morbidity and mortality [9,10]. Accordingly, there is a great necessity to find alternative approaches to combat the increasing burden of diseases.

Since antiquity, people have explored drugs in nature to cure multiple illnesses. Indeed, many medical systems based on plants have been used in different civilizations to heal and prevent diseases, especially Ayurveda in India, traditional Chinese medicine, Unani medicine, and indigenous healing traditions [11]. Today, more than 85 % of people in developing countries still rely on traditional medicine and almost 25 % of modern drugs are derived from medicinal herbs [1,12].

Medicinal plants have been used traditionally for their therapeutic benefits to treat the health problems indicated above. Hence, the scientific evidence of these practices could validate the traditional uses associated with these herbs. Recently, there has been a great deal of scientific interest in investigating the bioactivities of volatile oils and their bioactive compounds. In fact, the antimicrobial properties of natural products can play a crucial role in developing new agents to combat antimicrobial resistance and prevent infectious diseases [13]. Also, the evaluation of the antioxidant power of plants can demonstrate their ability to neutralize dangerous free radicals, thus preventing oxidative stress, which is usually related to the development of diabetes [14]. Further, the growing incidence of diabetes worldwide motivates scientists to discover effective alternatives to managing diabetes [15]. Thus, evaluating the biological properties of medicinal plant extracts can lead to the discovery of innovative, secure, and potent bioactive compounds with antimicrobial, antioxidant, and antidiabetic behaviors.

In this context, we targeted three potential medicinal plants - *Mentha suaveolens, Ammi visnaga, and Lavandula stoechas* - based on our previous ethnobotanical and ethnopharmacological investigations including their traditional uses and beneficial therapeutic aspects [16].

Mentha suaveolens Ehrh. is a perennial, herbaceous, and aromatic herb belonging to the Lamiaceae family. It is native to Southern and Western Europe and is usually found in the humid regions of Morocco. It can reach a height of 90 cm and has a sickly-sweet fragrance. *M. suaveolens* has demonstrated promising antimicrobial and antioxidant activities [17,18].

Ammi visnaga (L.) Lam. is commonly known as Khella in Arab countries and Toothpick weed in the United Kingdom. It is a common annual or biennial herb belonging to the Apiaceae family. It grows wildly in Mediterranean areas of North Africa, Asia, and Europe. *A. visnaga* L. contains an erect, cylindrical, and tall branched stem that can reach a height of 120 cm. This plant is noted for its beneficial role as an antimicrobial, emmenagogue, diuretic, and in alleviating diabetes and headaches [19,20].

Lavandula stoechas L. is a widely distributed Mediterranean plant belonging to the Lamiaceae family. This species frequently grows at very high altitudes in Northern Morocco [21]. *L. stoechas* has already demonstrated considerable antimicrobial, antioxidant, anti-inflammatory, and anti-diabetic activities [22]. *L. stoechas* has already demonstrated considerable antimicrobial, antioxidant, anti-inflammatory, and antidiabetic activities [23].

Previously, the chemical profile and antibacterial properties of *Mentha suaveolens, Ammi visnaga,* and *Lavandula stoechas* EOs have been mainly examined [24–27]. Nevertheless, work on other significant biological activities of these three Moroccan medicinal plants' EOs is restricted. On this basis, the current investigation was designed not only to explore phytochemical compounds and antibacterial aspect of the selected EOs but also to analyze in vitro anticandidal, antioxidant, and anti-diabetic potentials of *Mentha suaveolens, Ammi visnaga*, and *Lavandula stoechas,* collected from Sidi Slimane Region, Northwest Morocco, for their possible uses in the agriculture, food, and pharmaceutical industries.

2. Materials and methods

2.1. Reagents and apparatus

Potassium persulfate ($K_2S_2O_8$), 2,2-diphenyl-1-picrylhydrazyl (DPPH), linolenic acid, Tween 80, p-iodonitrotetrazoliumchloride (TTC), α-amylase, α-glucosidase, β-carotene, chloroform, methanol, 3,5 dinitrosalicylic acid (DNSA), trichloroacetic acid (TCA), ascorbic acid BHT butylated hydroxytoluene, potassium ferricyanide were purchased from Sigma-Aldrich, Saint-Quentin-Fallavier, France. Luria-Bertani, Mueller–Hinton and Sabouraud Agar, dimethyl sulfoxide (DMSO), Yeast extract-peptone-glucose (YPG), Tetracycline, and Clotrimazole were purchased from Biokar Diagnostics, Beauvais, France. All used chemicals were analytical grade.

Clevenger-type device (VWR, Radnor, USA), UV–visible spectrophotometer (Perkin Elmer, Shelton, CT USA), Spectrophotometry- ELISA Reader YR05127 (Kalstein, France) and Hewlett-Packard (HP6890) GC instrument (Santa Clara, CA, USA) coupled with an HP5973 MS and equipped with a 5 % phenylmethyl silicone HP-5MS capillary were used in this exploratory investigation.

2.2. Plant material and EOs extraction

The aerial parts of *M. suaveolens* Ehrh. *A. visnaga* (L.) Lam and *L. stoechas L.* were harvested from its wild habitat in the region of Sidi Slimane, Morocco (34° 15′ 35″ N, 5° 55′ 45″ W, June 2021) at flowering stage. The plant identification was carried out by botanists from University of Sidi Mohamed Ben Abdellah, Morocco (Voucher numbers: BLMUP 350-352). The samples were dried to a constant mass at 25 °C. EOs extraction was done by hydrodistillation using in a Clevenger-type apparatus for 3 h. The obtained oils were recovered and dried with Na_2SO_4, and then stored at 4 °C until experimental use.

2.3. GC-MS analysis of essential oils

The chemical analysis of MSEO, AVEO and LSEO was performed using gas chromatography (GC) (Trace GC-Ultra, S/N 20062969) coupled with mass spectrometry (MS) (Quadrapole, Polaris Q, S/N 210729) (GC/MS) (Benali et al., 2020). The materiel is equipped with non-polar HP-5MS capillary column (30 m, 0.32 mm × 0.25 μm). The temperature of injector and detector was established at 250 and 300 °C, respectively. The used column temperature increased from 50 °C for 5

min to 180 °C at 4 °C/min and from 180 to 300 with a 20 °C/min rate. Helium (He) was used as gas carrier with a 1.5 mL/min flow rate. The samples (diluted in hexane 1/10) of 0.5 µL were injected manually. The characterization of different compounds was conducted by comparing its retention index (RI) (processed based on homologous series of C_8–C_{24} alkanes) and its mass spectra (MS) fragmentation patterns to those reported in the literature [28,29]. EOs components were quantified via internal normalization of the total area of peaks revealed in each chromatogram. Moreover, MS of each compound was confirmed through reference data previously described in NIST2022 and PubChem libraries by computer matching.

2.4. Antimicrobial activity

2.4.1. Microbial strains and growth conditions
For the antimicrobial evaluation of the EOs, six microbial strains were used in the current investigation, including Gram-positive (Gram +) bacteria; *Micrococcus luteus* (clinical isolate) and *Bacillus subtilis* (ATCC 6633); Gram-negative (Gram -) bacteria: *Pseudomonas aeruginosa* (ATTC 27853), *Escherichia coli* (ATCC 25922), and *Salmonella enterica* (clinical isolate); and one yeast; *Candida albicans* (clinical isolate). All strains were brought from the Laboratory of Microbial Biotechnology (LBMB), at the Faculty of Sciences and Technologies (Morocco). Strains were incubated at 4 °C on an inclined Luria-Bertani (LB) agar medium. Prior to usage, bacteria were revived by subculturing them in LB at 37 °C for 20–24 h. Regarding yeast, revival was achieved by subculturing it on in Sabouraud agar (SA) plates at 25 °C for 48 h. Final inoculum concentrations of 10^6 CFU/mL for bacteria and around 10^4 CFU/mL for yeast were used for antimicrobial screening in accordance with the criteria of the National Committee for Clinical Laboratory Standards, United States [30].

2.4.2. Agar disc-diffusion assay
The antibacterial screening was first performed using the disc diffusion technique according to the method of Van et al. [31]. This technique has been applied as preliminary step to measure the inhibition diameters produced by the EOs around the disk. The culture suspension was seeded on LB agar medium for bacteria and Sabouraud agar for *Candida albicans*. Each of the sterile paper discs (6 mm-diameter) was soaked with 10 µL of pure EO before being put on an agar plate. Tetracycline (15 µg/disc) was the positive control for bacteria, while Clotrimazole (10 µg/disc) was used as reference for *Candida albicans*. Plates containing bacteria were incubated at 37 °C for 24 h, whereas plates containing yeast were incubated at 25 °C for 48 h. Then, the diameters of the appeared inhibition zones were expressed in millimeters, and the results were represented as the mean ± standard deviation for three independent measurements.

2.4.3. Determination of minimum inhibitory concentration (MIC)
The minimum inhibitory concentration (MIC) of the EOs was estimated using previously described method with minor changes [32]. In brief, in sterile 96-well plates, EO concentrations ranging from 8.0 % to 0.007 % (v/v) were diluted in Mueller–Hinton broth containing 2 % DMSO (two-folds dilution). Literature data have shown that the concentration of dimethyl sulfoxide (DMSO) up to 7.8 % had no significant influence on viable bacterial cell count [32]. Then, 10 µL of the previously prepared bacterial culture were put into each well. The prepared 96-well plates were then incubated overnight at 30–35 °C. Same methodology was used with the yeast after replacing the bacterial medium with peptone yeast extract broth and incubated for 48 h at 25 °C. Mueller–Hinton broth containing 5 % DMSO without microbial suspension was utilized as a standard growth. After incubation, p-iodonitrotetrazoliumchloride (TTC) 95 % was injected into all microtubes to determine bacterial growth (growth indicator). The MIC was the maximum sample dilution where the yellow-to-pink color shift was undetectable.

2.4.4. Determination of MBC and MFC
The Minimum Bactericidal (MBC) and Minimum Fungicidal (MFC) values were evaluated by subculturing 10 µL from the MIC microtubes that did not show growth with the MIC test, in plates containing LB agar medium for bacteria and yeast extract-peptone-glucose (YPG) agar for the yeast, and incubating under suitable conditions for each microorganism. After incubation, the plates were inspected for visible growth. MBC or MFC was the lowest concentration at which no growth was observed. In addition, the MBC/MIC and MFC/MIC ratios were also calculated in order to elucidate the possible mode of action of the tested substance [33].

2.5. Antioxidant assays

2.5.1. DPPH radical-scavenging activity
The ability of EOs to scavenge the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical was determined according to the previous published assays [34], with some changes. In short, aliquots (100 µL) of different concentrations of EOs were added to 750 µL of a 0.004 % DPPH solution. The obtained solutions were incubated at 27 °C for 45 min. Absorbance was read at 517 nm using UV–visible spectrophotometer. The tests were conducted in three independent measurements (n = 3) and the percentage of scavenging ability was represented as the concentration of EOs exhibiting 50 % inhibition (IC_{50}). BHT and ascorbic acid were used as reference standards.

2.5.2. Linoleic acid/β-carotene bleaching assay
The bleaching test for β-carotene was carried out as described by Herrera-Calderon et al. [35]. First, we have prepared an emulsion containing, β-carotene/linoleic acid and Tween-80. A volume of 2 mL of this emulsion was mixed with 500 µL of various concentration of EOs and the optical density (DO) was then read at 470 nm against a blank (Methanol) and compared to standard antioxidants (BHT and ascorbic acid). All measurements were processed in triplicate. The antioxidant activity (AA) was examined in terms of the residual color percentage in accordance to the following formula [36]:

$$Residual\ Color(\%) = [1 - (OD(0) - OD(t))/(OD\ \ (0) - OD\ \ (t))] \times 100$$

Where OD (0) and OD (0) are the optical density of EOs and control at zero time, while OD (t) and OD(t) are the respective optical density of EOs samples and control after 2 h.

2.5.3. Ferric-reducing antioxidant power (FRAP) assay
Reductive ability of test oils was investigated according to the method by Tiji et al. [37] with minor changes. The reaction solution was prepared by mixing the EOs (1 mL) with the phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and the potassium ferricyanide (2.5 mL). Then, the EOs were incubated in a water bath at 50 °C for 25 min. A volume of 2.5 mL of 10 % trichloroacetic acid (TCA) was added to stop the reaction and then the mixture was centrifuged at 3000 r/min for 10 min. Afterwards, 2.5 mL of the supernatant was added to a reaction containing 2.5 mL of distilled water, and 0.5 mL of $FeCl_3$ (0.1 %). Absorbance was performed at 700 nm and compared against ascorbic acid and BHT, which were used as synthetic standards. The reducing power was expressed as IC_{50} values ± SD (µg/mL).

2.6. In vitro assessment of antidiabetic activity

2.6.1. In vitro inhibition of pancreatic α-amylase
The inhibitory potential of MSEO, AVEO and LSEO against pancreatic α-amylase was assessed as previously described by Laaraj et al. [38] with some modifications. In brief, a total of 0.2 mL of phosphated buffer (0.2 M; pH= 6.9) containing 0.2 mL of α-amylase enzymatic solution (13IU), and 0.1 mL of tested oils at various concentrations (0.062, 0.12, 0.25, 0.5 and 1 mg/mL) or acarbose (reference standard) was incubated

at 37 °C for 10 min. Next, 0.2 mL of 1 % starch previously dissolved in phosphate buffer (0.2 M) was added to the reacting mixture. Afterwards, the obtained mixture was incubated at 37 °C for 20 min. Then, 0.6 mL of 3,5 dinitrosalicylic acid (DNSA) was added and all test tubes were incubated in a water bath at 100 °C for 10 min. Finally, a volume of 1 mL of distilled water was added to the reaction mixture prior to the measurement of optical density at 540 nm.

2.6.2. In vitro inhibition of intestinal α-glucosidase

The α-glucosidase inhibitory activity of MSEO, AVEO and LSEO was determined by measuring the release of D-glucose from sucrose degradation according the method adopted by Boutahiri et al. [24]. Briefly, a volume of 0.02 mL of tested oils at different concentrations (0.062, 0.12, 0.25, 0.5 and 1 mg/mL) or acarbose solutions (0.45, 0.9, 2.25 and 4.5 mg/mL) was added to a reactive mixture, containing 0.1 mL of sucrose (50 Mm), 0.1 mL of α-glucosidase enzyme solution (10 IU), and 1 mL of phosphate buffer (50 Mm; pH = 7.5). The enzymatic mixture was incubated at 37 °C for 25 min. Then, the enzymatic reactions were immediately stopped by heating test tubes in a boiling water bath for 5 min. The released D-glucose was established by D-glucose oxidase technique using a commercially available kit (God-Pod, USA) and the optical density was processed at 500 nm.

Table 1
Chemical Composition of AVEO, MSEO, and LSEO (MS, RI identification).

No.[a]	Compounds[b]	Molecular formula	RI[c]	RI lit[d]	% Relative peak area		
					AVEO	MSEO	LSEO
1	Allyl isovalerate	$C_8H_{14}O$	910	915	1.1	-	-
2	α-Thujene	$C_{10}H_{16}$	921	923	1.04	-	-
3	α-Pinene	$C_{10}H_{16}$	943	939	1.44	-	-
4	Camphene	$C_{10}H_{16}$	952	953	2.33	7.16	-
5	Butyric acid	$C_4H_8O_2$	957	970	15.01	-	-
6	β-Pinene	$C_{10}H_{16}$	965	979	3.11	-	-
7	β-Thujene	$C_{10}H_{16}$	968	968	2.16	-	-
8	β-Myrcene	$C_{10}H_{16}$	989	991	2.87	-	-
9	δ0.4-carene	$C_{10}H_{16}$	997	1001	0.3	-	-
10	Isobutyl isovalerate	$C_9H_{18}O_2$	1011	1005	1.44	-	-
11	Isopentyl isobutyrate	$C_9H_{18}O_2$	1017	1013	4.92	-	-
12	Butyl 2-methylbutanoate	$C_9H_{18}O_2$	1020	1013	tr	-	-
13	o-Cymene	$C_{10}H_{16}$	1026	1029	0.37	2.91	-
14	Eucalyptol	$C_{10}H_{18}O$	1030	1033	0.15	-	4.73
15	D-Limonene	$C_{10}H_{16}$	1031	1031	3.31	-	6.56
16	β-Phellandrene	$C_{10}H_{16}$	1035	1031	0.3	-	-
17	β-Ocimene	$C_{10}H_{16}$	1048	1050	5.07	-	-
18	Linalool oxide	$C_{10}H_{18}O_2$	1075	1078	2.18	-	-
19	Linalool	$C_{10}H_{18}O$	1082	1098	38.24	10.36	-
20	Fenchone	$C_{10}H_{16}O$	1088	1087	-	29.77	-
21	Camphor	$C_{10}H_{16}O$	1138	1143	-	24.9	-
22	Terpinen-4-ol	$C_{10}H_{18}O$	1176	1177	-	-	1.38
23	Isopinocampheol	$C_{10}H_{18}O$	1180	1178	-	-	2.16
24	α-Terpineol	$C_{10}H_{18}O$	1184	1185	0.7	-	-
25	Pulegone	$C_{10}H_{16}O$	1212	1209	2.11	-	-
26	Geraniol	$C_{10}H_{18}O$	1239	1255	0.1	-	-
27	Bornyl acetate	$C_{12}H_{20}O_2$	1277	1285	-	5.3	-
29	2-Undecanone	$C_{11}H_{22}O$	1290	1291	0.74	-	-
29	Carvacrol	$C_{10}H_{14}O$	1298	1298	-	4.9	-
30	Myrtenyl acetate	$C_{12}H_{18}O_2$	1314	1307	-	5.51	-
31	Piperitenone oxide	$C_{10}H_{14}O_2$	1366	1363	-	-	74.55
32	Copaene	$C_{15}H_{24}$	1370	1376	1.18	-	-
33	Benzyl isovalerate	$C_{12}H_{16}O_2$	1394	1382	0.78	-	-
34	Lavandulyl isobutyrate	$C_{14}H_{24}O_2$	1416	1418	1.2	-	-
35	trans-Caryophyllene	$C_{15}H_{24}$	1421	1428	-	-	1.32
36	β-Farnesene	$C_{15}H_{24}$	1451	1458	3.29	-	-
37	Germacrene D	$C_{15}H_{24}$	1500	1480	0.68	-	3.52
38	β-Himachalene	$C_{15}H_{24}$	1503	1499	-	-	1.4
39	Lavandulyl 2-methylbutanoate	$C_{16}H_{28}O$	1504	1495	2.09	-	-
40	γ-cadinene	$C_{15}H_{24}$	1513	1512	-	1.28	-
41	δ-cadinene	$C_{15}H_{24}$	1519	1524	-	1.88	-
42	Citronellyl butyrate	$C_{14}H_{26}O_2$	1526	1529	0.48	-	-
43	Cubebol	$C_{15}H_{26}O$	1584	1588	-	2.68	-
44	α-copaene-8-ol	$C_{15}H_{24}O$	1587	1595	-	1.43	-
45	Viridiflorol	$C_{15}H_{26}O$	1589	1590	-	2.39	-
46	Cinerolon	$C_{10}H_{14}O_2$	1641	1641	-	-	2.32
47	(E)-Atlantone	$C_{15}H_{22}O$	1698	1703	-	-	0.94
	Total identified %				98.69	96.17	98.88
	Monoterpene hydrocarbons				22.34	10.07	7.94
	Oxygenated monoterpenes				46.68	80.33	83.76
	Sesquiterpene hydrocarbons				5.15	7.27	6.24
	Oxygenated Sesquiterpenes				-	2.39	-
	Other				24.47	-	0.94
	Yield (%, v/w)				1.12	3.05	1.48

[a] In order of elution on HP-5 ms, [b] Compounds revealed based on RI and MS.

[c] Retention index calculated from alkanes series on HP-5 MS capillary column (C8–C24).

[d] Retention index from literature [39,40].

2.7. Statistical analysis

All tests were carried out in three independent measurements and the results are represented as mean ± standard deviations (SD). The data were analyzed using GraphPad prism 3.02 and XLSTAT statistics software version 2016 and the means were compared based on one-way analysis of variance ANOVA, using Tukey test. A p-value of < 0.05 was considered statistically significant.

3. Results

3.1. Chemical composition

The essential oil yields (v/w) for AVEO, LSEO, and MSEO were 1.12 %, 3.05 %, and 1.48 %, respectively. The chemical analyses of AVEO, LSEO, and MSEO, including the percentage of each compound, elution order, molecular formula, and retention index are summarized in Table 1. A total of 47 compounds were identified in the three investigated oils. Thirty, thirteen, and ten constituents were identified in AVEO, MSEO, and LSEO, respectively, representing 98.69 %, 96.17 %, and 98.88 % of these oils.

LSEO and MSEO were characterized by a high amount of oxygenated monoterpenes with 83.76 % and 80.33 %, respectively. The major identified components of LSEO were fenchone (29.77 %), camphor (24.9 %), and linalool (10.36 %), while piperitenone oxide (74.55 %) was the main component of MSEO. Monoterpenes were the most dominant compounds in AVEO, accounting for 46.68 % oxygenated monoterpenes and 22.34 % monoterpene hydrocarbons. In addition, the major bioactive compounds detected in AVEO were linalool (38.29 %), butyric acid (15.01 %), and β-ocimene (5.07 %).

3.2. Antibacterial activity

The disc-diffusion assay was used to examine the antimicrobial activity of MSEO, AVEO, and LSEO. As shown in Table 2, MSEO exhibited the highest antibacterial activity, with inhibition zones ranging from 15.0 ± 1.29–31.0 ± 2.4 mm, followed by LSEO with inhibition zones of 14.0 ± 1.9–28.5 ± 1.4 mm, and AVEO with inhibition zones of 11.0 ± 1.2–26.0 ± 0.9 mm, respectively. In general, Gram⁺ bacteria such as *B. subtilis* and *M. luteus* showed higher susceptibility to the EOs. Although some Gram - bacteria such as *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 exhibited high susceptibility to LSEO and MSEO, respectively. Similarly, in the antifungal activity test against *C. albicans*, MSEO showed the highest zone of inhibition (28.0 ± 0.5 mm), followed by LSEO (25.3 ± 0.1 mm) and AVEO (16.0 ± 1.5 mm). However, the reference antibiotics for bacteria and yeast (positive control) remained the most effective agents.

Results are expressed as means ± SD, of three independent

measurements; ND: not determined. [a] Final bacterial density was around 10^6 CFU/mL and about 10^4 CFU/mL for yeast. [b] Diameter of inhibition zone including disc diameter of 6 mm (10 μL of oil/disc).

The MIC and MBC results are presented in Table 3. The MIC and MBC values of AVEO and MSEO against Gram + bacteria (*B. subtilis* and *M. luteus*) ranged between 0.015 % and 0.25 % (v/v), and against Gram – bacteria, (including *E. coli, S. enterica,* and *P. aeruginosa*) ranged between 0.031 % and 0.5 % (v/v), confirming the results of the disc-diffusion test and supporting the claim that Gram + bacteria are more sensitive to the tested EOs than Gram - bacteria. The MIC and MBC values of AVEO, LSEO, and MSEO against the yeast (*Candida albicans*) varied between 0.125 % and 0.625 % (v/v). All EOs showed wide-spectrum and diverse antimicrobial activity with MBC/MIC ratios less than or equal to 4.0 % (v/v). The MBC/MIC and MFC/MIC ratios for all examined EOs were less than or equal to 4.0 %.

3.3. Antioxidant activity

In this study, three complementary in vitro methods, including DPPH radical scavenging, ferric reductive power, and β-carotene bleaching test, were used to determine the antioxidant ability of AVEO, MSEO, and LSEO. As shown in Figs. 1, 2, and 3, the IC_{50} values indicated significant differences in the antioxidant effect of AVEO, MSEO, LSEO, and standard antioxidants BHT and ascorbic acid ($p < 0.05$). The results of the DPPH assay showed that LSEO, MSEO, and AVEO are able to scavenge the free radical DPPH with IC_{50} values of 163.46 ± 5.66 μg/mL, 215.59 ± 5.28 μg/mL, and 221.92 ± 1.2 μg/mL, respectively.

In the case of assessing the reductive potential of ferric ion (Fe3 +) to ferrous Fe2 + by tested EOs, a significant antioxidant effect was noticed with IC_{50} values of 144.38 ± 6.10 μg/mL, 192.27 ± 8.78 μg/mL, and 348.28 ± 5.34 μg/mL for MSEO, LSEO, and AVEO, respectively, in a concentration-dependent manner. For the β-carotene bleaching inhibition method, LSEO, MSEO, and AVEO showed respective IC_{50} values of 143.94 ± 2.05 μg/mL, 168.50 ± 3.20 μg/mL, and 284.29 ± 2.94 μg/mL. Despite their highest antioxidant potency, the tested EOs still less effective than the standard antioxidants ascorbic acid and BHT ($p < 0.05$).

3.4. Antidiabetic activity

The antidiabetic effects of essential oils from *M. suaveolens* (MS), *A. visnaga* (AV), and *L. stoechas* (LS) were evaluated through their inhibitory actions against α-amylase and α-glucosidase enzymatic activities. The studied EOs repressed the activities of both tested enzymes in a dose-dependent manner (Fig. 4). The findings are represented as IC_{50} values given in Table 4. The EOs were able to restrain the enzymes' activities at small doses compared with the standard agent used (Acarbose). There were no significant differences between the inhibitory activities of the studied EOs and acarbose ($p < 0.05$).

MSEO, AVEO, and LSEO exhibited significant inhibition against α-amylase, with IC_{50} values of 3.51 ± 0.04 mg/mL, 3.37 ± 0.04 mg/mL, and 3.00 ± 0.04 mg/mL, respectively. As for α-glucosidase inhibition, a significant effect was also recorded for the three tested EOs with IC_{50} values of 2.58 ± 0.04 mg/mL, 2.74 ± 0.01 mg/mL, and 3.02 ± 0.01 mg/mL for MSEO, LSEO, and AVEO, respectively.

4. Discussion

In this study, we investigated the potential of essential oils from A. visnaga, L. stoechas, and M. suaveolens as natural sources of antibacterial, anticandidal, antioxidant, and antidiabetic agents. We harvested the aerial parts of these plants from their wild habitats in the Sidi Slimane region of North-West Morocco, based on ethnomedicinal practices revealed by our laboratory [16]. As indicated by GC-MS analysis, AVEO, LSEO and MSEO showed the presence of various bioactive compounds mainly belonging to oxygenated monoterpenes class. These molecules

Table 2
The inhibitory diameters of AVEO, LSEO, and MSEO.

Microorganisms[a]	Diameter of Inhibition zone (mm ± SD)[b]				
	AVEO	LSEO	MSEO	Tetracycline	Clotrimazole
B. subtilis ATCC 6633	26.0 ± 0.9	24.0 ± 0.3	31.0 ± 2.4	28.01 ± 0.3	ND
M. luteus (Clinical isolate)	22.4 ± 0.1	27.0 ± 0.5	19.0 ± 1.0	33.0 ± 0.5	ND
E. coli ATCC 25922	20.0 ± 0.5	28.5 ± 1.4	15.0 ± 1.3	24.6 ± 2.3	ND
S. enterica (Clinical isolate)	15.0 ± 2.7	25.0 ± 3.3	16.0 ± 2.1	30.6 ± 1.4	ND
P. aeruginosa ATCC 27853	11.0 ± 1.2	14.0 ± 1.9	19.4 ± 0.5	17.2 ± 1.0	ND
C. albicans (Clinical isolate).	16.0 ± 1.5	25.3 ± 0.1	28.0 ± 0.5	ND	22.5 ± 0.5

Table 3
Antimicrobial activity of AVEO, LSEO, and MSEO using MIC, MBC and MBC/MIC, MBC/MFC assays.

| | Gram-Positive Bacteria | | | | | | Gram-Negative Bacteria | | | | | | | | | Yeast | | |
| | B. subtilis | | | M. luteus | | | S. enterica | | | E. coli | | | P. aeruginosa | | | C. albicans | | |
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MFC	MFC/MIC
AVEO	0.125	0.125	1.0	0.125	0.125	1.0	0.0625	0.0625	1.0	0.0625	0.0625	1.0	0.25	0.25	1.0	0.125	0.25	2.0
LSEO	0.015	0.031	2.0	0.25	0.25	1.0	0.125	0.125	1.0	0.125	0.125	1.0	0.5	0.5	1.0	0.125	0.125	0.5
MSEO	0.0625	0.125	2.0	0.0625	0.125	2.0	0.031	0.125	4.0	0.25	0.25	1.0	0.125	0.125	1.0	0.625	0.625	1.0
Tetracline	0.5	0.5	1.0	2.0	2.0	1.0	2.0	4.0	2.0	4.0	4.0	1.0	0.5	1.0	2.0	ND	ND	ND
Clotrizole	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	1.0	2.0	2.0

MIC: Minimum inhibitory concentration in % (v/v), MBC: Minimum bactericidal concentration in % (v/v). MFC: Minimum fungicidal concentration in % (v/v). Tetracycline and Clotrimazole: used as standard drugs ND: not determined.
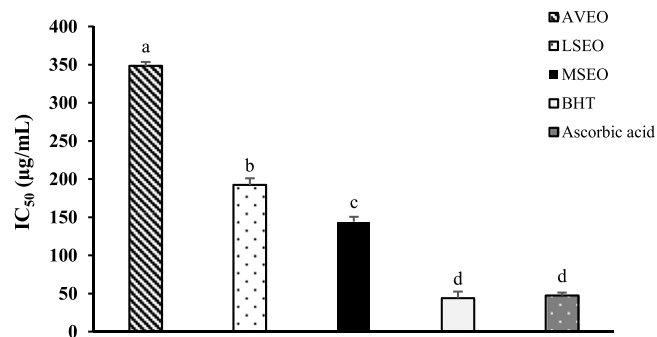
Fig. 1. Ferric reductive power of AVEO, LSEO and MSEO compared to standard antioxidants (BHT and ascorbic acid). Data with the same letter in the same test present non-significant difference by Tukey's multiple range test (ANOVA, $p < 0.05$). The results are expressed as means $\pm$ SD of three independent measurements.
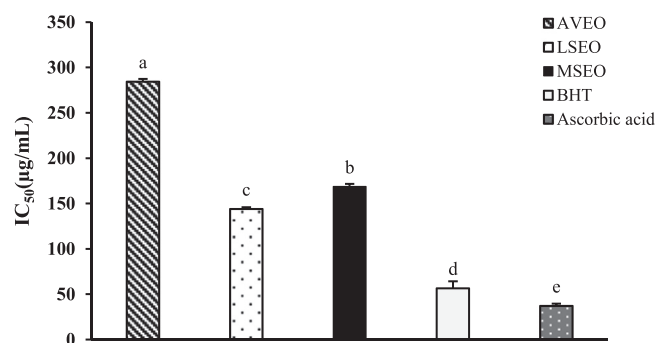
Fig. 2. Half inhibition concentration (IC_{50}) of AVEO, LSEO and MSEO compared to standard antioxidants (BHT and ascorbic acid). Data with the same letter in the same test present non-significant difference by Tukey's multiple range test (ANOVA, $p < 0.05$). The results are expressed as means $\pm$ SD of three independent measurements.
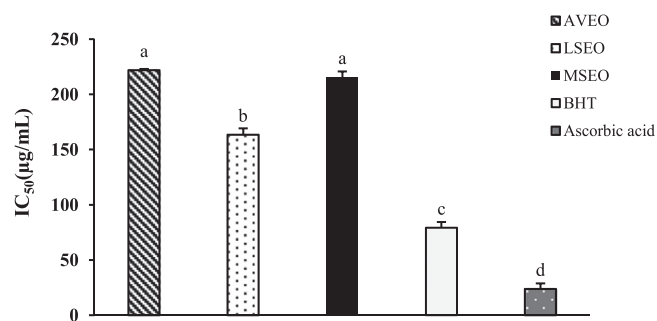

Fig. 3. DPPH radical scavenging ability of AVEO, LSEO and MSEO compared to standard antioxidants (BHT and ascorbic acid). Data with the same letter in the same test present non-significant difference by Tukey's multiple range test (ANOVA, $p < 0.05$). The results are expressed as means $\pm$ SD of three independent measurements.

are could be highly possible attributing to the health-promoting effects of the studied EOs. LSEO are dominated by fenchone and camphor and MSEO are mainly represented by piperitenone oxide. These findings are in congruent with other studies carried out on MSEO and LSEO in Morocco. Indeed, Bouyahya et al. [34] found that LSEO collected from Ouezzane region are rich in fenchone and camphor. However, another study by Cherrat et al. [41] reported that the major components of LSEO are cubenol, 10 s,11 s-Himachala-3(12),4-diene, methyl eugenol and δ-cadinene. Previous studies carried out in other areas worldwide indicated the presence of other chemotypes in LSEO such as p-Cymene and
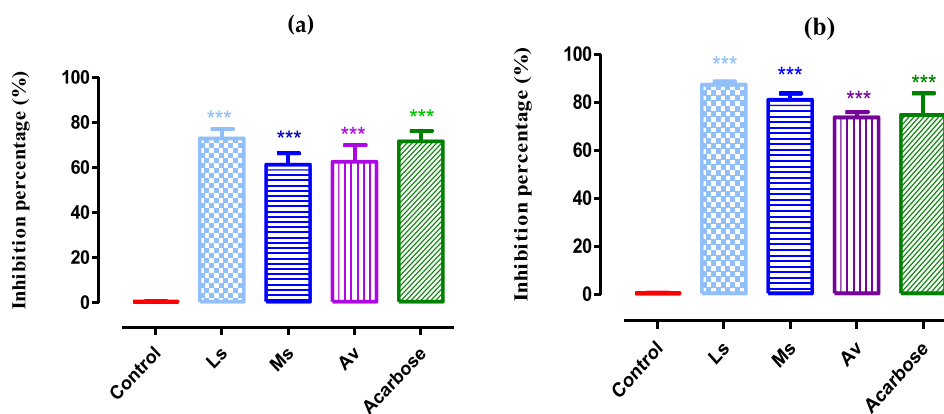
Fig. 4. **(a)**: *In vitro* inhibitory effect of *M. suaveolens* (MS), *A. visnaga* (AV), *L. stoechas* (LS) and acarbose against α-amylase enzyme in mg/mL; **(b):** Inhibitory effect of *M. suaveolens* (MS), *A. visnaga* (AV) and *L. stoechas* (LS) against α-glucosidase enzyme in mg/mL. The values are the means ± SEM (n = 3). **$p < 0.01$, ***$p < 0.001$ as function of the control.

Table 4
Half maximal inhibitory concentration (IC$_{50}$ in mg/mL) values of MSEO, AVEO and LSEO, and Acarbose towards α-amylase and α-glucosidase enzymatic activities.

Samples	α-Amylase	α-Glucosidase
MSEO	3.51 ± 0.04[a]	2.74 ± 0.01[a]
AVEO	3.37 ± 0.04[a]	3.02 ± 0.01[a]
LSEO	3.00 ± 0.008[a]	2.58 ± 0.04[a]
Acarbose	3.06 ± 0.03[a]	2.81 ± 0.02[a]

Values are expressed as standard error of the mean ± SEM (n = 3); [a] The data with the same letter in the same assay indicates a non significant difference by Tukey's multiple range test (ANOVA, $p < 0.05$).

eucalyptol [25,42]. For MSEO, Benali and his colleagues [43] have also identified piperitenone oxide as the main compound in MSEO collected from Taza region. Of note, other investigations indicated that pulegone, menthone and carveol as the major compounds [27,44].

On the other hand, the chemical profile of AVEO, growing wild in North-West of Morocco using GC-MS analysis, has yet to be elucidated. In spite of that, studies carried out in other areas have revealed a wide range of volatile compounds. In Tunisia, Khadhri et al. [45] have identified linalool, isoamyl 2-methyl-butyrate and isopentyl-isovalerate as dominate compounds of AVEO. Moreover, 2-methylbutyrate, linalool, limonene and isoamyl-isovalerate constituted the major molecules of AVEO originating from Algeria [46]. In another study conducted by Talaat et al. [47] on *A. visnaga* cultivated in Egypt, thymol, fenchyl acetate, 2,2-dimethyl butanoic acid, isobutyl isobutyrate, α-isophorone and linalool were the most abundant compounds. Furthermore, *cis*-pinene hydrate, methyl octadecanoate γ-terpinene and (*E*)-*β*-ocimene represented the major components of Iranian AVEO. This difference is mainly explained by the variability of ecological conditions between the geographical origin of plants. It is worth noting that Khalil et al. [48] showed that the chemical composition of AVEO is variable between several regions.

It is prudent to state that the chemical composition of essential oils is subject to qualitative and quantitative variability, which is influenced by the plant part and its phenological stage. Several investigations have dealt with the variability in chemical profile of AVEO, LSEO and MSEO [34,43,47]. They suggested that this fluctuation may be ascribed to the difference in biotypes and geographic origins, especially to the differences in the plant growth environment such as soil nature, solar radiation, and climatic conditions [34]. These environmental factors may affect the expression of key enzymes, leading to the regulation of biosynthesis, metabolism, and secretion of volatile secondary metabolites [49,50].

The present study was conducted in accordance with the current global trend of valuing natural products and medicinal plants. As the use of natural antimicrobial agents extracted from bioactive plants becomes more widespread [51], particularly in food and medicinal applications, the medical plant-related trade is expanding significantly each year. Natural resources have fewer adverse effects than synthetic pharmaceuticals, are eco-friendly and readily available, making them a popular alternative [52,53].

The current study supports the effectiveness of some EOS as potent antimicrobials. Using the disc-diffusion method, it was found that the essential oils of *A. visnaga*, *L. stoechas*, and *M. suaveolens* have remarkable antimicrobial activity against Gram-positive and Gram-negative bacteria and yeast, with high zones of inhibition. The microorganisms tested were *M. luteus* (19.0 ± 1.0–27.0 ± 0.5 mm), *B. subtilis* (24.0 ± 0.3–31.0 ± 2.4 mm), *P. aeruginosa* (11.0 ± 1.2–19.4 ± 0.5 mm), *E. coli* (15.0 ± 1.3–28.5 ± 1.4 mm), *S. enterica* (15.0 ± 2.7–25.0 ± 3.3 mm), and *C. albicans* (16.0 ± 1.5–28.0 ± 0.5 mm), with significant activity when compared to reference antibiotics (Table 2).

The inhibition zone of 14 millimeters or more, as measured by the disc diffusion test, is deemed to have remarkable antimicrobial activity [34]. Our findings are also in agreement with previous investigations that reported that the EOs extracted from fresh aerial part of *A. visnaga* showed promising antibacterial activity against *Klebsiella pneumoniae*, *E. coli* and *P. aeruginosa* [48]. LSEO was tested against four different bacteria, *Proteus mirabilis* and *Enterococcus faecalis* were the most susceptible bacteria using disc-diffusion test [46]. MSEO was also represented noticeable antibacterial activity against *Staphylococcus aureus*, P. *aeruginosa, E. coli,* and *K. pneumonia* [54]. Little is known about our EOs against *C. ablicans*, however, MSEO showed promising anti-candida activity [55].

However, the actions of EOs and monoterpenes on the bacterial plasma membrane have been extensively studied. These natural compounds have been found to exhibit significant antimicrobial activity by disrupting the integrity and function of the bacterial plasma membrane and several studies have demonstrated the membrane-targeting effects of EOs and monoterpenes. For example, carvacrol, a monoterpenic phenol found in oregano oil, has been shown to disrupt the bacterial plasma membrane by inducing depolarization and permeabilization [56]. Similarly, thymol, another monoterpenic phenol present in thyme oil, has been reported to cause membrane damage and leakage of intracellular components [57]. Linalool, a monoterpene alcohol found in many EOs, has also been shown to exert antimicrobial effects through membrane disruption. It can increase the permeability of the bacterial and candidal plasma membrane, leading to leakage of ions and cellular constituents [58,59]. Additionally, various other monoterpenes have been investigated for their membrane-targeting properties. For instance, eugenol, a major component of clove oil, can disrupt the plasma

membrane by interacting with lipid bilayers and altering membrane fluidity [60]. These studies collectively suggest that monoterpenes exert their antimicrobial effects by perturbing the bacterial plasma membrane. The disruption of membrane integrity ultimately leads to cell death or inhibition of bacterial growth.

The MIC, MBC, and MFC values of AVEO, LSEO, and MSEO in the current study were very low compared to the reference antibiotics (Table 3), indicating that these EOs are highly effective antimicrobial agents that can be used in food industries and pharmaceutical formulations after careful toxicological and pharmacological evaluations in vitro and in vivo. The MBC/MIC and MFC/MIC ratios for all tested EOs were less than or equal to four. These ratios reflect the nature of the tested EOs, which were found to be bactericidal, as substances with MBC/MIC and MFC/MIC ratios of four or below are considered bactericidal or fungicidal, whereas those with MBC/MIC and MFC/MIC ratios of more than four are considered bacteriostatic or fungistatic [61]. Interestingly, according to MIC, MBC and MFC values, the examined EOs showed wide-spectrum antibacterial activity against both of the Gram-positive and the Gram-negative bacteria (Table 3). Due to the nature of the outer membrane of Gram-negative bacteria, which protects them from the lethal effects of antibacterial molecules, it is considered the most difficult-to-treat bacteria, causing global resistance problems [62].

The possible modes of antimicrobial action of EOs can implicate the disintegration of the phospholipid bilayer or the outer membrane of bacteria, modification of the fatty acid composition, rise in membrane flexibility generating a leakage of potassium ions and protons, interference with glucose absorption, and inhibition of enzymes or cell disruption [13]. It was elucidated that terpenes interfere with the membrane proteins and phospholipids, causing a cellular respiratory chain repression, disturbance in oxidative phosphorylation, inhibition of nucleic acid production, and a deficit in metabolites. Other investigations supposed that the cell membrane may be a main target for bioactive composites to inactivate the microbial cell [63]. Indeed, the interference between bioactive compounds and bacteria makes the Gram$^+$ cell wall denser, leading to cell lysis. Other compounds can interact with the negatively charged outer layer of the Gram$^-$ strain, thus simplifying the penetration of the compound into the intracellular space, leading to disruption [64].

As a result, we recommend further studies on these essential oils in the future, particularly to assess their efficacy against drug-resistant Gram-negative bacteria. Additionally, the tested essential oils have shown promising antifungal activity in the current study, and we recommend considering their use in possible combinations or formulations to address the growing problem of candidiasis. Given the increasing resistance of Candida species to conventional antifungal drugs such as azoles and echinocandins, this is becoming an increasingly serious problem in clinical settings worldwide [65].

The antioxidant properties of AVEO, MSEO, and LSEO were assessed using various in vitro assays. These methods employ different mechanisms to measure antioxidant activity: the β-carotene bleaching test estimates an antioxidant's ability to prevent lipid peroxidation in both initiation and propagation phases, the FRAP method determines ferric reductive capacity, and the DPPH assay evaluates free radical scavenging ability. This approach allows us to obtain complementary results and a comprehensive understanding of the antioxidant potential of these essential oils [66]. As anticipated, AVEO, MSEO, and LSEO exhibit promising antioxidant properties, indicating their potential use as natural antioxidants. Our findings align with those of previous investigations by various research teams that have demonstrated the antioxidant properties of these plant oils across different antioxidant-based assays [43,67,68]. The significant antioxidant activities of AVEO, MSEO, and LSEO may be attributed in part to their high content of phenolic compounds. It is well known that certain monoterpenes and sesquiterpenes possess remarkable inhibitory effects on oxidation [69,70]. We have identified a high percentage of oxygenated

monoterpenes, such as fenchone, camphor, linalool, piperitenone oxide, and β-ocimene, in LSEO, AVEO, and MSEO. Interestingly, the bioactive components of these essential oils could act alone or in synergy as natural antioxidant agents, providing a protective role for human health against many oxidative stress-related diseases.

Some studies suggested that EOs can directly interfere with the reactive radicals to ruin them by accepting or donating electrons to eradicate the unpaired state of the radical, or they can indirectly reduce the formation of free radicals by repressing the actions or productions of free or by improving the activities and generations of other antioxidant enzymes [14]. Moreover, it was reported that EOs can obstruct lipid oxidation by blocking continuous hydrogen extraction, and inhibiting singlet oxygen production and transition metal ion catalyst binding [14].

Despite several reports on the phytochemistry and antimicrobial effects of EOs obtained from *M. suaveolens*, *A. visnaga*, and *L. stoechas*, there is limited information on their antidiabetic properties. Thus, we evaluated the selected essential oils' antidiabetic effects through their potent inhibitory actions against α-amylase and α-glucosidase enzymatic activities. These enzymes play a crucial role in gastrointestinal starch digestion and carbohydrate hydrolysis into glucose reabsorption. Inhibiting these enzymes can reduce postprandial hyperglycemia, preventing the absorption of dietary starch. Therefore, α-amylase and α-glucosidase inhibition could play a key role in diabetes mellitus therapy [71].

Based on our findings, MSEO, LSEO and AVEO exerted notable antidiabetic actions against the two tested enzymes. Indeed, Al-Mijalli et al. [72] reported that MSEO repressed the activities of α-amylase and α-glucosidase enzymes at small concentrations compared to acarbose (standard drug) with an IC$_{50}$ value equal to 94.30 ± 0.06 μg/mL and 141.16 ± 0.21 μg/mL, for inhibiting the activity of α-amylase and α-glucosidase, respectively. Previous studies have indicated that *A. visnaga* displays important antihyperglycemic properties. In fact, the administration of an aqueous extract of *A. visnaga* demonstrated remarkable hypoglycemic activity on both normal and streptozotocin diabetic rats. Furthermore, a decoction of *A. visnaga* fruits reduced glycaemia levels by 51 % in normal rats, compared to tolbutamide, an oral hypoglycemic drug [47].

A recent study also evaluated the inhibitory potency of *Lavandula angustifolia* essential oil on the α-glucosidase enzyme. It was found that the studied essential oil was able to inhibit this enzyme with an IC$_{50}$ of 609.44 μg/mL, compared to acarbose with an IC$_{50}$ of 526.5 μg/mL [73]. It was also shown that the ingestion of small concentrations of *L. stoechas* hydroalcoholic extract at a dose of 50–150 mg/kg, b.w. to diabetic mice considerably minimized hyperglycemia in a dose-dependent way [74].

The present study has established that all essential oils display strong inhibitory effects against α-amylase and α-glucosidase enzymes. This significant antidiabetic action of the studied essential oils is likely due to their bioactive constituents, specifically the major ones. Moreover, monoterpenes compounds have been shown to enhance insulin release from pancreatic β-cells, diminish cellular oxidative stress, and moderate enzymes, proteins, and pathways that could lead to many pathological events. Thus, monoterpenes molecules appear to be promising agents to treat metabolic disorders such as diabetes.

Indeed, Sebai et al. [75] suggested that D-fenchone could be responsible for the protective effects of Lavender essential oil against diabetes induced by alloxan treatment in rats, as it represents the main compound of Lavender oil. Also, a study revealed that camphor has a hypoglycemic activity. The intra-gastric administration of camphor to hyperglycemic rats for three weeks reduced their blood glucose, triglyceride, and total cholesterol levels.

A recent study has shown that linalool exerts beneficial effects on glucose and lipid metabolism. It can also decrease the risk of developing diabetes vascular complications by improving antioxidant defense systems and anti-glycation properties [76]. Furthermore, Kumar et al. [77] found that supplementation of butyric acid (at a dose of 500 mg/kg body

weight/day) significantly improved the moderated activity of enzymes during diabetes and reduced lipid peroxidation in hyperglycemic rats. Indeed, little discussion about the mechanisms of the in vitro antidiabetic actions of EOs; however, a few molecular docking studies showed that some volatile compounds can bind strongly to the active sites of α-amylase and α-glucosidase to block their activity [15]. Therefore, our EOs can erase carbohydrate absorption, slowdown glucose digestion, and consequently, diminish blood sugar levels.

The findings of the current study are highly significant and suggest that essential oils may be able to combat diabetes. However, more research is needed to support this assumption.

5. Conclusion

This study has demonstrated that the three investigated Moroccan aromatic plants exhibit promising biological properties, including antioxidant, antibacterial, anticandidal, and antidiabetic activities. GC-MS analysis revealed that these effects may be attributed to various chemical compounds found in AVEO, MSEO, and LSEO. These findings demonstrate the ethnomedicinal value of these plants worldwide and suggest their potential applications as a source for developing natural antibacterial, anticandidal, antioxidant, and antidiabetic agents. However, further toxicological investigations are strongly required to verify the safety of these oils. Additionally, data on pharmacodynamics and pharmacokinetics are needed to understand the mechanisms behind the reported activities.

CRediT authorship contribution statement

Conceptualization, C.A.,N.E.H and K.F-B.; Data curation, N.E.H, N.B; Formal analysis, H.N.M, AB, K.F-B, N.E.H.; E.M. A; Funding acquisition, L.H.L., C.A., L.C.M.; Investigation, N.H, RA, N.B, M.J; Methodology, H. N.M., N.E.H.; M.B.; A.B., Resources, L-H.L.,N.E.H, H.N.B, N.B; Software, N.E.H, N.B.; R.A, MJ; Supervision, K.F-B., C.A.; Validation N.E.H, K.F-B, H.N.M, AB; Visualization, C.A., L.C.M., A.B., and S.H.A.M.; Writing—original draft, C.A., L.C.M., N.H, N.B, and E.M.A; Writing—review and editing, K.F-B, H.N.M, L-H.L.,A.B.; S.H.A.M. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

None.

Data availability

Data will be made available on request.

Acknowledgement

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.114937.

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