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Chemical composition, free radicals, pathogenic microbes, α-amylase and α-glucosidase suppressant proprieties of essential oil derived from Moroccan *Mentha pulegium* L. : *in silico* and *in vitro* approaches

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Abstract

Several bioactive phytochemicals found in herbal products, particularly in essential oils (EOs), are proved to be vital in the prevention of chronic illnesses including infectious and metabolic disorders. The aim of this study was, firstly to identify the phyto-chemical composition and to assess, *in vitro*, the antioxidant and antimicrobial properties of *Mentha pulegium* EO, and secondly, to evaluate its potential antidiabetic effects, *in silico*. GC-MS analysis was employed for the examination of EO phytochemical composition. The antioxidant capacity was evaluated by *in vitro* tests against free radicals. The antimicrobial efficacy against pathogenic bacteria as well as *Candida albicans* was evaluated qualitatively and quantitatively. By the use of molecular docking antidiabetic potential of pennyroyal EO was also tested. Pulegone (72.05%) was the major component of *M. pulegium* EO, followed by 8-hydroxy-*p*-menthan-3-one (5.97%) and imidazolidine (3.23%). Pennyroyal EO displayed a notable antioxidant potential, as assessed by FRAP and DPPH assays, marking an EC_{50} and IC_{50} values of 26.500 ± 0.200 mg/mL and 054.630 ± 1.350 mg/mL, correspondently. The examined EO also possessed a total antioxidant capacity of 52.610 ± 4.734 mg AAE/g EO. The findings of antimicrobial test showed a notable efficacy of *M. pulegium* EO against *S. aureus* (MIC = MBC = 3.058 mg/mL), *E. coli* (MIC = 6.076 mg/mL / MBC = 6.125 mg/mL) and *C. albicans* (MIC = MBC = 3.063 mg/mL). Regarding the antidiabetic potential, *in silico* analysis identified imidazolidine as the most active molecule against the α-glucosidase (PDB: 5NN8) and the α-amylase (PDB: 1B2Y) enzymes marking glide scores of -8.393 and -7.172 kcal/mol, correspondently. These findings suggest that the EO derived from Moroccan *M. pulegium* holds promise as a potent natural remedy against free radicals and resistant pathogenic microbes. Moreover, it shows potential as a promising solution for managing diabetes disorders.

Keywords: *Mentha pulegium*; Essential oil; Antimicrobial; Antioxidant; Diabetes; *in vitro*; *in silico*

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1. Introduction

For centuries, diabetes mellitus (DM) poses a significant problem for impoverished areas as well as for affluent nations. DM is a persistent metabolic condition distinguished by prolonged hyperglycemia resulting from compromised insulin production and/or malfunctions in insulin functionality. It disrupts the fat metabolism, carbohydrates,

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and proteins (Czech, 2017). According to the World Health Organization, there was a 3% rise in premature mortality due to diabetes from 2000 to 2019. Moreover, in 2019, the number of fatalities attributed directly to diabetes and kidney disease due to diabetes was 2 million (WHO, 2023).

A serious concern in modern times is oxidative stress, a state that has the potential to impact healthy cells and transform them into cancerous ones due to the accumulation of a significant quantity of reactive oxygen species (ROS) (EL Abdali et al., 2023). In parallel, the issue of antimicrobial resistance and the illnesses it causes poses a significant public health issue. This situation leads to a critical situation in numerous healthcare facilities worldwide and contributes to a high occurrence of nosocomial diseases (EL Moussaoui et al., 2021). Nevertheless, these health challenges can be mitigated through the utilization of medicinal plants and their derivatives which exhibit antioxidant and antimicrobial properties, and serve as promising alternatives to synthetic antioxidants and traditional antibiotics (El Abdali et al., 2023b; Wang et al., 2023).

The oxidative stress constitutes a significant risk factor for the development of type 2 diabetes, and the mechanisms underlying this relationship involve hormonal and inflammatory disorders that favor also insulin resistance (Fernández-Sánchez et al., 2011; Jaradat et al., 2024). In addition, diabetes, together with its correlated hyperglycemia, intensifies oxidative stress, consequently creating a favorable milieu for the progression of cancer (Giri et al., 2018). Moreover, uncontrolled hyperglycemia and tumor growth caused by oxidative stress can compromise the immune system's function and effectiveness according to recent investigations (Akash et al., 2020). Consequently, individuals with these conditions are more prone to being infection by a variety of opportunistic pathogens. The complex interconnections between oxidative stress, diabetes, and microbial infections emphasize the necessity of comprehensive health management strategies. This also underscores the significance of lifestyle modifications and therapeutic interventions that focus on connected pathways associated with these interrelated conditions.

In the context of these multifactorial diseases, the use of herbal remedies based on aromatic plants and their derivatives presents a complementary therapeutic approach. Numerous aromatic plants, known for their hypoglycemic and antioxidant properties, are incorporated into diets to help regulate blood sugar levels and reduce the risk of complications related to diabetes and also obesity (Paul et al., 2022). In addition to their implication in the treatment of microbial infections (El Abdali et al., 2021; EL Moussaoui et al., 2021), medicinal plants represent a highly effective reservoir of biologically active compounds that possess anti-free radicals and anticancer properties with greater efficacy for controlling oxidative stress and cancer therapy (Jain et al., 2016). Belonging to this panoply of medicinal plants, *Mentha pulegium* L. (Lamiaceae), commonly known as pennyroyal, is a prominent aromatic herb originating from North Africa, Europe, and the Middle East (Domingues and Santos, 2019). Extracts obtained from this herb, such as EOs, have exhibited properties including carminative, anti-inflammatory, antioxidant, antispasmodic, and antimicrobial effects (Bouyahya et al., 2017; Nickavar and Jabbareh, 2018). Nonetheless, there exists a scarcity of information and investigation concerning the biological activities of EO derived from Moroccan chemotypes of *M. pulegium*, particularly in relation to their impact on enzymes and specific proteins associated with diabetes and related disorders.

In light of the close correlations between diabetes, oxidative stress and also microbial infections, this study was carried out using a complementary therapeutic approach. Specifically, the objectives were to analyze the chemical composition of *M. pulegium* EO and assess its, *in vitro*, antioxidant and antimicrobial activities against three pathogenic microbial strains. Second, the study used molecular docking analysis to examine the inhibitory actions of the same EO on the α-glucosidase and α-amylase enzymes, in order to assess its antidiabetic activity *in silico*.

2. Materials & Methods

2.1. Plant material

In the research conducted, *Mentha pulegium* L. leaves collected in the Taounate (Kariat Ba Mohamed) area of Morocco in May 2022 were utilized as the plant material. The plant samples were identified by laboratory botanists through consultation of different botanical references and plant directories. Following this, the samples underwent a cleaning process prior to being left to air-dry in the shade for a duration of 15 days before commencing the extraction process.

2.2. EO extraction

A mass of 100 grams of dried *M. pulegium* leaves underwent hydrodistillation for a duration of 3 hours utilizing a Clevenger-type apparatus utilizing 800 mL of distilled water, following the established procedure as detailed in the European Pharmacopoeia (EDQM., 2004). The resulted EO was subsequently dehydrated using anhydrous sodium sulfate and afterwards conserved in dark conditions at temperatures of 4-5 °C until further testing and analysis. The EO yield was calculated as a percentage (*v/w*) based on the weight of the dried plant material (El Abdali et al., 2023a).

2.3. EO Chromatographic analysis:

The current study utilized GC-MS to accurately analyse and determine the various plant chemical phytocomponents present in the EO extracted from *M. pulegium*. Utilizing a GC Agilent-Technologies 6890 N Network gas-phase chromatograph (Little Falls, California, USA), the analysis was performed using an HP-5MS capillary column with the following specifications: 30.00 m × 0.250 mm × 0.250 µm of film thickness. The flame ionization detector (FID)

utilized in this research was set at a temperature of 260.0 °C. A 1.00 µL volume was injected using a split mode at 250.0 °C. During the chromatographic process, helium gas was utilized as the carrier and programmed at 1.00 mL/min in flow rate. The temperature of the column was set to increase at a rise of 0.5 °C/min, starting from 35 °C and reaching 250.0 °C. Kovats retention indices were meticulously employed to identify the EO components by comparing them to a homologous set of *n*-alkanes. Furthermore, the NIST MS Library (v. 2.0) was carefully utilized as a mass spectral database to improve the accuracy of compound identification (Adams, 2007; Aimad et al., 2022).

2.4. Essential oil's in vitro antioxidant properties

Three distinct assays were conducted to evaluate the antioxidant potential of the investigated *M. pulegium* EO in a laboratory: the reducing power (FRAP) test, the free DPPH radical scavenging test, and the total antioxidant capacity (TAC) test.

2.4.1. Scavenging free DPPH radical test

The DPPH assay was performed in accordance with the modified procedure outlined by (Moattar et al., 2016). A mixture comprising 0.1 mL of pennyroyal EO prepared in methanol at different concentrations (0.1–100 mg/mL) added to 0.75 mL of a 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution (0.1 mM) dissolved in methanol, was prepared. After being incubated during 30 minutes at room temperature, the solution's absorbance was noted at 517 nm and compared to that of a negative control containing methanol in lieu of the EO studied. The experiment was repeated using butylated hydroxytoluene (BHT) antioxidant serving as a standard reference. After that, the EO-induced DPPH radical inhibition rate was determined accordingly:

$$\text{DPPH inhibition (\%)} = [1-(A/A_0)] \times 100 \quad (1)$$

A and A_0 represent the observed absorbance values of the solution of DPPH when the sample is present and absent (as the negative control), respectively.

2.4.2. Ferric reducing assay

The ferric reducing antioxidant power (FRAP) assay followed a standard protocol as previously described (Moattar et al., 2016). This involved adding 0.5 mL of potassium ferricyanide solution [$K_3Fe(CN)_6$] at 1% and the same volume of phosphate buffer solution (0.2 M; pH = 6.6) to 0.1 mL of increasing concentrations of the studied EO, ranging from 0.1 to 25.0 mg/mL in methanol. After incubating the mixture during 20 minutes at a temperature of 50°C using a water bath, 0.5 mL of 10% trichloroacetic acid was added to acidify it. Subsequently, the resulting mixture was supplemented with 0.1 mL of 0.1% $FeCl_3$ and 0.50 mL of distilled water. The solution's absorbance at 700 was then noted after using a blank. The obtained results were analysed and expressed as the 50% effective concentration (EC_{50}), derived from the resulting graph, indicating the antioxidant concentration required to achieve 0.5 nm in absorbance. Additionally, conventional antioxidants such as BHT and quercetin were assessed using the same experimental procedure.

2.4.3. Total antioxidant capacity assay

A reagent solution consisting of 1 mL of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was prepared. To this solution, 25 µL of EO was added. The reaction mixture was then incubated during 90 min at 95°C. Subsequently, the absorbance at 695 nm was measured following the method described by (Maškovič et al., 2012). The total antioxidant capacity was determined utilizing a previously calibration curve prepared using ascorbic acid and expressed as mg of ascorbic acid equivalent relative to one gram of EO (mg AAE/g EO). The experimental protocol was triplicated for reliability.

2.5. Essential oil's antimicrobial properties

2.5.1. Microbial strains, growth medium and inoculums standardization

The Gram-negative *Escherichia coli* ATCC 25922, and the Gram-positive *Staphylococcus aureus* ATCC 29213, in addition to *Candida albicans* ATCC 10231, were used to assess the antimicrobial activity of pennyroyal EO. The examined pathogenic microbial strains were sourced from the laboratory of microbiology belonging to the medicine and pharmacy faculty located in Fez (Morocco). Numerous investigations have reported that the microbial strains under investigation are resistant to multiple drugs (EL Moussaoui et al., 2021; Mulani et al., 2019).

Bacterial strains were grown on Müller-Hinton Agar (MHA) and Müller-Hinton Broth (MHB), whereas *C. albicans* was cultured on Sabouraud agar (SB) (EL Abdali et al., 2023). The diverse microbial strains were standardized and inoculated according to the procedure outlined for bacterial and fungal strains (CLSI, 2018, 2008). Ampicillin (AMP) and fluconazole (FLU) were employed as standard antibiotic and antifungal, respectively. Standards underwent sterilization through filtration.

2.5.2. Disk diffusion method

The antimicrobial activity was examined qualitatively utilizing the disk diffusion technique, which is based on the Kirby-Bauer method with minor adaptations (Furtado and Medeiros, 1980; Kiehlbauch et al., 2000). This technique was employed in order to calculate, in mm, the diameter of the inhibition zone resulting from the EO's impact on the microbial strain. To achieve this, circular disks of Whatman number 1 paper, measuring 0.6 cm, were infused with EO (20.00 µL), FLU (5.00 mg/mL) and AMP (0.50 mg/mL). Subsequently, these disks were then positioned onto the surface of the agar culture medium's surface within a Petri dish that had been inoculated with standardized suspensions of bacterial strains (1-5 × 10^8 CFU/mL) and *C. albicans* strain (1–5 × 10^6 CFU/mL). Finally, the antimicrobial efficacy of the EO studied was evaluated following a 24-hour incubation period at 30°C concerning yeast and 37 °C in the case of bacteria.

2.5.3. Evaluation of the minimal inhibitory, bactericidal and fungicidal concentrations of EO

The microdilution method, as described previously, was employed to determine the minimum inhibitory concentration (MIC) (Agour et al., 2022; El Abdali et al., 2023a). Dimethyl sulfoxide (DMSO) was used to dilute both the EO and the standards. Initially, 50 µL of the culture medium was dispensed into all microplate wells. Subsequently, the first microplate wells were loaded with 50 µL of the solution containing EO and standards. Microdilution was then performed by transferring a volume of 50 µL from the initial well to the last, except the positive growth control. Following this, 50 µL of bacterial and fungal suspensions was inoculated into all wells except the negative growth control. After a 24-hour incubation period at 30°C for *C. albicans* and 37°C for the bacterial strains, microbial growth was indicated by a white microbial spot beneath the wells. Confirmation was conducted using the colorimetric method, with the addition of 10 µL of 2,3,5-triphenyltetrazolium chloride (TTC). The MICs values of the EO, FLU and AMP were determined as the lowest concentrations of the samples that stopped microbial growth.

Additionally, the minimum fungicidal (MFC) and bactericidal (MBC) concentrations were determined. Briefly, following a 24-hour incubation period, the inoculum from the three juxtaposed MIC wells was swabbed and transferred to evaluate the growth of microorganisms on the non-selective agar surface. The concentrations at which 99.9% of the initial inoculum was eliminated were identified as MBC and MFC (Balouiri et al., 2016).

2.6. Molecular docking of EO enzymatic inhibition activity

The current investigation focused on the application of computational techniques for analyzing the antidiabetic effect of *M. pulegium* EO. It entailed a thorough examination of the inhibition of α-amylase and α-glucosidase enzymes to determine its possible potential in managing diabetes.

2.6.1. Ligand preparation

For the ligand preparation, an exhaustive compilation of all phytocompounds found in *M. pulegium* EO via GC/MS from PUBCHEM was carried out meticulously in the Structure Data File (SDF) format. Following this, a comprehensive pretreatment phase was applied to these various ligands for the purpose of docking calculations utilizing the LigPrep tool within the Schrödinger Software program (v. 11.5). For this specific approach, the OPLS3 force field was employed, and a total of 32 stereoisomers for every ligand were generated, and the ionization states at pH 7.00 ± 2.00 were determined (El Abdali et al., 2023b; Lafraxo et al., 2022).

2.6.2. Target protein preparation

The preparation of proteins involved obtaining three-dimensional crystal structures of α-amylase (PDB ID: 1B2Y) and α-glucosidase (PDB ID: 5NN8) antidiabetic enzymes as documented by (Ouahabi et al., 2023). The structures of investigated enzymes were generated from the Protein Data Bank (PDB format), and meticulously constructed and improved utilizing the Protein Preparation Wizard integrated in Schrödinger-Maestro (v. 11.5). The optimization procedure was carried out through the addition of hydrogen atoms (H), finalizing bond orders, elimination of water molecules, determination of hydrogen bonds, adjustment of receptor atom's potential, and reduction of energy employing the OPLS3 force field (Amrati et al., 2023). The inception of the receptor grid was commenced by triggering the initiation of the creation module, in which a specific ligand atom was chosen, resulting in the creation of a default grid box. Subsequently, the ligands were connected to the grid box resulted from the studied protein utilizing the Standard Precision method.

2.6.3. In silico test

The implementation of the standard precision, flexible ligand docking protocol was conducted using the Glide module within the Schrödinger-Maestro software (v. 11.5). The procedure entailed the incorporation of penalties for non-cis/trans amide bonds. Specific parameters governing ligand atoms, including the partial charge cutoff in addition to the Van der Waals scaling factor, were carefully calibrated to 0.15 and 0.80, correspondently. Then, the resultant score, derived from energy-minimized conformations, was reported as a Glide score. The most favorable docking conformation for the studied ligand was determined as the one displaying the lowest Glide score value (Amrati et al., 2023). This rigorous computational methodology aimed to elucidate the potential molecular interactions between *M pulegium* EO compounds and α-amylase and α-glucosidase, offering valuable insights into their antidiabetic mechanisms of action.

2.7. Statistical Analysis

The software utilized for the calculation of mean values and standard deviations in this study was GraphPad Prism 8, developed by Microsoft (California, USA). Statistical comparison of data from all tests was conducted through ANOVA (one-way), followed by using a Tukey-test. Significance of differences was determined at a level of $p<0.05$.

3. Results & Discussion

3.1. Essential oil yield

The leaves of *M. pulegium* obtained from Kariat Ba Mohamed in Morocco yielded a clear yellow EO with a characteristic scent, amounting to 2.25% (*v/w*). It is widely recognized that various factors such as plant species, geographical location, and plant part used can impact the EO yield. Additionally, the time of collection, as well as the drying and extraction techniques employed, play a role (Ahmed et al., 2018; Rezouki et al., 2021). For example, studies by Aljaiyash et *al.*, Amalich et *al.*, and Allali et *al.* reported different yields of EO from *M. pulegium* leaves from various regions, with percentages of 1.78%, 5.20%, and 2.14%, respectively (Ahmed et al., 2018; Aimad et al., 2021; Amalich et al., 2024). The pennyroyal chemotypes described

in these studies are all collected from different geographic regions than the plant under study.

3.2. Essential oil' phytochemical composition

The EO derived from *M. pulegium* was subjected to a comprehensive analysis and characterization of its chemical composition using the GC-MS technique. The components identified are listed in Table 1. Results showed that a total of thirteen compounds were detected in the pennyroyal EO under investigation, collectively constituting 97.74% of the overall essence. The primary compounds found included pulegone (72.05%), 8-hydroxy-*p*-menthan-3-one (5.97%), imidazolidine (3.23%), and piperitenone (3.02%). Notably, the monoterpenes were the predominant constituents of the examined EO, making up 83.85% of the total composition. Other compounds were present at levels below 4%. In a recent study on the phytochemical composition of *M. pulegium* EO, five significant components were identified, with pulegone (68.11%), l-menthone (8.83%), limonene (2.90%), iso-pulegone (2.69%), and iso-menthone (1.48%) being the main components (Azadi et al., 2023). Furthermore, *M. pulegium* EO contains various elements such as pulegone, piperitenone, menthone, isomenthone, α-terpineol, and oxygenated monoterpenes. These phytochemicals exhibit varying concentrations, with pulegone ranging from 40.98% to 76.35%, piperitenone fluctuating between 3.82% to 27.8%, and menthone present in concentrations of 6.9% to 21.16% as indicated by several studies (Aimad et al., 2021; Bouyahya et al., 2017; Casiglia et al., 2017; Messaoudi et al., 2021).

These variations in compound amounts and composition of EO are likely influenced by various factors such as, selection criteria (young, mature, disease-free, parasite-free, etc.), method of leaf preservation prior to drying, developmental stage of the leaves, methods used for extraction, timing of harvest, seasonal changes, environmental conditions, and daily biological rhythms (El Abdali et al., 2023b; Justus et al., 2018). It is important to note that numerous compounds found in pennyroyal EO have bioactive properties. For instance, pulegone has been shown to exhibit diverse pharmacological effects such as antioxidant, antimicrobial, anti-feeding, antifungal, antiviral, and pesticide properties (Dhingra and Chopra, 2023). Likewise, piperitone provides improved treatment outcomes for people with diabetes, obesity, arthritis, metabolic syndrome, multiple myeloma, oral cancer, Alzheimer's disease, Parkinson's disease, breast cancer, stroke, heart disease, kidney disease, inflammatory disorders, and rhinopharyngitis (Tripathi et al., 2022). Menthone was also recognized for its numerous pharmacological attributes such as antifungal, antibacterial, antipruritic, anticancer, and analgesic effects, in addition to serving as an efficient fumigant (Kamatou et al., 2013). The complex chemical composition of *M. pulegium* EO highlights its potential as a rich source of bioactive substances, further emphasizing its importance in diverse applications within the fields of pharmacology and natural product research.

3.3. In vitro essential oil' antioxidant activity

Oxidative stress is a potentially harmful effect of reactive species radicals (ROS) acting on living organism components and cells (Circu and Aw, 2010). The potential of a number of EOs to scavenge reactive species and counteract oxidative stress has been investigated (Leyva-López et al., 2017). The antioxidant potential of *M. pulegium* EO was examined in the current study using an *in vitro* multi-assay approach that included phosphomolybdenum TAC, FRAP, and DPPH inhibition assays. The collected results are displayed in Table 2 and Figure 1. Both pennyroyal EO and BHT demonstrated increased DPPH radical scavenging activity in depending on the dose, as illustrated in Figure 1. Additionally, data of Table 2 shows that *M. pulegium* EO's half maximum inhibitory DPPH radical concentration (IC_{50}) was 54.630 ± 1.350 mg/mL. Comparatively, ANOVA analysis data showed that the BHT's IC_{50} value (0.122 ± 0.021 mg/mL) was statistically ($p < 0.001$) lesser than that of the investigated EO's.

Table 1: Phytochemical composition of the *M. pulegium* EO.

N°	RT	MW	Compounds	Molecular formula	Content (%)
1	5.205	126	2-methyl-1,3-Cyclohexanedione	$C_7H_{10}O_2$	0.63
2	6.405	152	Isopulegone	$C_{10}H_{16}O$	2.21
3	6.779	208	2,2 Dicyclohexylpropane	$C_{15}H_{28}$	2.38
4	6.879	140	Dimethyldisopropenylsilane	$C_8H_{18}Si$	0.86
5	6.980	152	Pulegone	$C_{10}H_{16}O$	72.05
6	7.056	154	Menthone-D1	$C_{10}H_{18}O$	0.60
7	7.152	72	Imidazolidine	$C_3H_8N_2$	3.23
8	7.345	170	8-hydroxy-*p*-menthan-3-one	$C_{10}H_{18}O$	5.97
9	7.465	168	1-Ethyl-3-methyl-2-(2 methylpropylidene) Imidazolidine	$C_9H_{18}N_2$	1.75
10	7.767	150	Piperitenone	$C_{10}H_{14}O$	3.02
11	7.823	98	2-Cyclohexen-1-ol	$C_6H_{10}O$	1.45
12	8.899	166	Mint Furanone 2	$C_{10}H_{14}O_2$	1.21
13	9.236	154	3 –(3-thienyl)-2 propenoic acid	$C_7H_6O_2S$	2.38
			Total (%)		97.74

RT = Retention time; MW = Molecular weight.

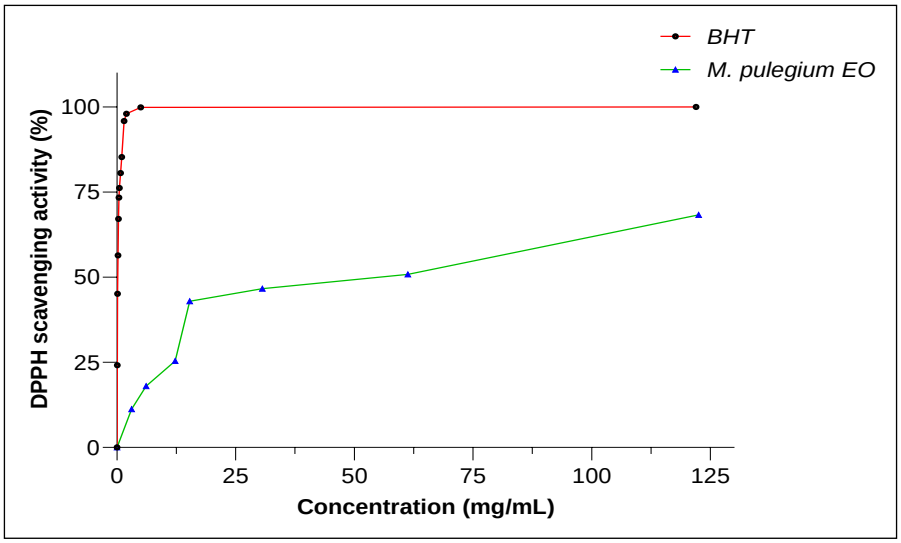

Figure 1: Anti-free DPPH radical scavenging activity of *M. pulegium* EO and BHT.

Table 2: Antioxidant activities of *M. pulegium* EO and standards (means ± SD).

	DPPH (IC_{50} mg/mL)	FRAP (EC_{50} mg/mL)	TAC (mg AAE/g EO)
Essential oil	54.630 ± 1.350[a]	26.500 ± 0.200[a]	52.610 ± 4.734[a]
BHT	0.122 ± 0.021[b]	0.362 ± 0.010[b]	48.530 ± 1.250[a]
Quercetin	-	0.032 ± 0.003[b]	29.470 ± 1.246[a]

Different letters in each column correspond to statistically different values ($p < 0.05$).

With an EC_{50} value of 26.500 ± 0.200 mg/mL (Table 2), the antioxidant propriety of the investigated pennyroyal EO was also assessed utilizing the FRAP test. This result indicates that EO had the potential to transform ferric iron (Fe^{3+}) to reduced ferrous iron (Fe^{2+}), but with an effectiveness statistically ($p<0.05$) compared to BHT and quercetin standards, which had EC_{50} values of 0.362 ± 0.010 mg/mL and 0.032 ± 0.003 mg/mL, correspondently.

By using the phosphomolybdenum assay, the pennyroyal EO's total antioxidant capacity (TAC) was also evaluated. In the presence of an antioxidant, molybdenum Mo (VI) present as molybdate ions at an acidic pH is reduced to molybdenum Mo (V), creating a green phosphate/Mo(V) complex (Prieto et al., 1999). In relation to the obtained results (Table 2), the TAC of *M. pulegium* EO in comparison to conventional antioxidants (BHT and quercetin) was 52.610 ± 4.734 mg/g, 48.530 ± 1.250 mg/g, and 29.470 ± 1.246 mg/g, correspondently, measured in ascorbic acid equivalents (mg AAE/g EO).

Our findings are consistent with previous studies that have also shown *M. pulegium* EO to possess antioxidant properties (Aimad et al., 2021; Bouyahya et al., 2017; Dehghani et al., 2018; Messaoudi et al., 2021). Used *in vitro* tests, a recent research found that the EO extracted from *M. pulegium* in a different location of Morocco has notable antioxidant activity, with an IC_{50} of 7.659 mg/mL in the DPPH scavenging assay and 583.066 mg AAE/g EO in the TAC assay (Aimad et al., 2021). In another work examining the antioxidant activity of the EO of a different *M. pulegium* chemotype, a higher DPPH scavenging IC_{50} value (1.027 mg/mL) was discovered (Dehghani et al., 2018). Additionally, Messaoudi et al. (2022) found a significant antiradical potential of the EO derived from the Algerian *M. pulegium* both in the DPPH scavenging test (IC_{50} = 7428.5 µg/mL) and ABTS test (25 682.7 µg/mL) (Messaoudi et al., 2021). Likewise, another EO of *M. pulegium* investigated recently, exhibited significant reducing and inhibitory capacities by marking IC_{50} values of 58.27 ±2.72 and 321.41 ± 2.53 µg/mL in the DPPH scavenging and FRAP tests, correspondently, when compared with ascorbic acid and Trolox (Bouyahya et al., 2017). It's important to note that antioxidant activity can vary depending on the specific composition of the EO, which can be influenced by several factors such as the plant's growth conditions, extraction techniques, and harvest time (Tit and Bungau, 2023).

EOs possess a variety of biological and chemical properties due to their composition of many volatile and semi-volatile phytocompounds possessing varying polarity and multiple functional groups. These attributes vary according to the test and method used (El Abdali et al., 2023b). The majority of investigations on the antioxidant capacity of pennyroyal EO have linked this activity to the EO's conjugated terpene components, such as piperitenone, menthone, and pulegone, which either act alone or in synergy to neutralize free radicals. A multitude of studies and experiments have revealed that these monoterpene compounds possess

significant antioxidant properties (Baccouri and Rajhi, 2021; Dhingra and Chopra, 2023; Wojtunik et al., 2014). In practice, it is expected that the many pathways by which EOs affect oxidative stress will determine, at least in part, their antioxidant qualities. Several mechanisms under investigation are: the capacity to scavenge free radicals, the regulation of anti-oxidant enzymes (e.g. superoxide dismutase), and the regulation of pro-oxidation (Gonzalez-Burgos and Gomez-Serranillos, 2012; Leyva-López et al., 2017). Furthermore, a recent study reported that, the amount of phenolic compounds, the phenol's reaction activity towards chain-carrying peroxyl radicals, as well the degree of stability of the phenoxyl radical generated in the reaction are the main factors that determine the antioxidative effectiveness of plant extracts (Ćavar et al., 2013).

Generally, EOs, including that of *M. pulegium*, contain various bioactive compounds. These phytochemicals can act as free radical scavengers, reducing agents, pro-oxidant inactivators, and radical species quenchers (Singh and Maurya, 2024). Additionally, some natural compounds, including EOs, may upregulate certain antioxidant enzymes or downregulate one or more enzymes involved in free radical generation (Amorati et al., 2013). Considering all of this, our findings as well as those of other research corroborate pennyroyal EO's ability to prevent and counteract the harmful effects of free radicals.

3.4. Essential oil' antimicrobial potential

The antimicrobial potential of *M. pulegium* EO was evaluated, *in vitro*, utilizing the microdilution method to determine the minimal inhibitory (MIC), fungicidal (MFC) and bactericidal (MBC) concentrations (Figure 2), as well the agar diffusion test, which examined qualitative results (Table 3). The present study's evaluated pathogenic microbial strains, namely: *C. albicans, E. coli* and *S. aureus*, which are considered community and hospital acquired infections and belong to the most prevalent pathogens to acquire multidrug resistance (Chouhan et al., 2017; EL Moussaoui et al., 2021; Mulani et al., 2019).

The growth inhibition zone diameter (expressed in mm) exhibited by the studied EO was used to express the results that are displayed in Table 3. For all examined microbial strain, *M. pulegium* EO shown a significant growth inhibitory activity with various inhibition zone diameters. Gram (+) *S. aureus* bacteria showed the highest sensitivity to pennyroyal EO, measuring 22.00 ± 0.57 vs Gram (-) *E. coli*'s 15.66 ± 0.66 mm. Otherwise, the growth of the *C. albicans* fungal strain was stopped by 13.50 ± 0.28 mm after application of the investigated EO. The EO data are slightly lower than those of conventional antimicrobials.

Table 3: Inhibition zone diameter (expressed in mm) of *M. pulegium* EO tested on pathogenic microbial strains (means ± SEM).

	Inhibition Diameters (mm)		
	***E. coli* (ATCC 25922)**	***S. aureus* (ATCC 29213)**	***C. albicans* (ATCC 10231)**
Pennyroyal EO	15.66 ± 0.66[b]	22.00 ± 0.57[b]	13.50 ± 0.28[b]
Ampicillin	24.50 ± 0.76[a]	32.33 ± 1.48[a]	-
Streptomycin	-	9.61 ± 1.20[c]	-
Fluconazole	-	-	20.83 ± 1.30[a]

Inhibition zone includes disc diameter (6 mm); Different letters in each column correspond to statistically different values ($p<0.05$).

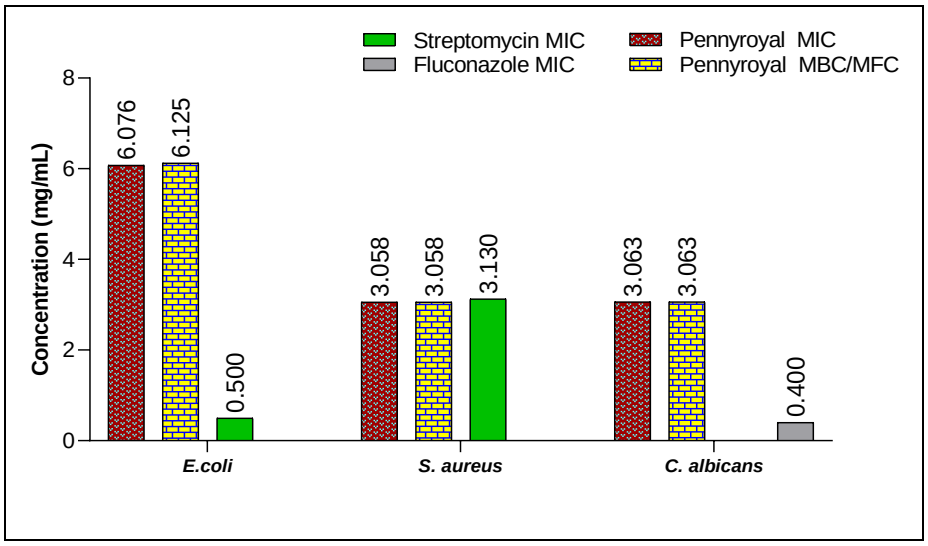

Figure 2: Minimal inhibitory (MIC) and minimal fungicidal/bactericidal concentrations (MFC/MBC) values (mg/mL) of EO extracted from *M. pulegium* against microbial strains.

The results of MIC, MBC and MFC evaluating the quantitative antimicrobial effect of *M. pulegium* EO are represented in Figure 2. The data obtained confirm the high sensitivity of *S. aureus* to the pennyroyal OE with a low MIC = MBC = 3.058 mg/mL vlaues, followed by *C. albicans* with the same MIC and MFC values of 3.063 mg/mL. The *E. coli* bacterial strain was the most resistant to the studied EO, with MIC and MBC values of 6.076 and 6.125 mg/mL, respectively. Streptomycin has recorded a MIC value higher than that of *M. pulegium* EO when tested against *S. aureus*.

Actually, *M. pulegium* EO has attract considerable interest to combat resistant Gram (+) and Gram (-) bacteria and pathogenic fungus (El Hassani, 2020). Our study findings are in agreement with those reported in recent research, when *M. pulegium* EO exhibited remarkable antibacterial action against ten bacterial strains, and marking highest inhibition zone diameters, especially against *P. mirabilis* (28 ± 1.32 mm) and *B. subtilis* (30 ± 1.43 mm). The same EO, against Gram (+) bacteria, showed significant activity when tested on *S. aureus* MBLA (MIC = MBC = 0.25% v/v) and *B. subtilis* 6633 (MIC = MBC = 1% *v/v*). While against Gram (-) bacteria, the EO had a remarkable antibacterial effect against *P. mirabilis* (MIC = 0.5% v/v) and *E. coli* K12 (MIC = 0.5% *v/v*) (Bouyahya et al., 2017). In another study, EO extracted using hydro distillation from wild *M. pulegium* collected from Algeria exhibited strong antibacterial activity against Gram (+) and Gram (-) pathogenic bacteria including *S. aureus, B. subtilis, P. aeruginosa*, and *E. coli.* The inhibition zone diameters ranged from 16.67 mm (*P. aeruginosa*) to 37.33 mm (*S. aureus*). The MIC values varied from 1 to 20 µL/mL, while the MBC values varied from 2 to 20 µL/mL. The same EO exhibited an antifungal effect against *C. albicans*, marking 11 mm in inhibition zone diameter (Messaoudi et al., 2021). Recent research on another Moroccan chemotype of pennyroyal, collected from the Ouazzane region, revealed that the extracted EO possessed antimicrobial activity against *B. subtilis, E. coli,* and *S. aureus*. The diameters of the inhibition zones ranged between 10.33 to 25 mm, while the MIC values varied from 0.704 µg/mL for *E. coli* to 2.812 µg/mL for *B. subtilis*. The same study found that the studied EO was effective against four different strains of fungus, namely *C. albicans, F. oxysporum, A. flavus,* and *A. niger*, with inhibition rates ranging from 23% for *C. albicans* to 100% for *A. niger* and MIC values between 11.25 and 22.50 µg/mL (Aimad et al., 2021).

Different antimicrobial reactions were observed by *M. pulegium* EO on the growth of the investigated fungal and bacterial strains in this research and other ones. This may allow for the diverse mechanisms of action of certain EO's compounds or the more efficient counteraction of the effects of *M. pulegium* oil by particular bacteria through their metabolism (Aimad et al., 2021). Strong antimicrobial activity against a variety of bacterial and fungal strains, including Gram (+) and Gram (-) bacteria and fungi like *C. albicans, F. oxysporum, A. flavus,* and *A. niger*, has been demonstrated for pulegone, which mainly present (72.05 %) in our studied EO (Amalich et al., 2016; Duru et al., 2004; Farhanghi et al., 2022). This phytochemical has been shown to cause damage to bacterial membranes and disrupt the structure of their polysaccharides and phospholipids, leading to bacterial death (Ez-Zriouli et al., 2022). Menthone, another potential component of pennyroyal EO, also demonstrated potent antibacterial and antifungal activities (Kamatou et al., 2013). This compound has exhibited significant antibacterial action against methicillin resistant *S. aureus* by altering bacterial membrane properties and integrity. This was achieved through the alteration of glycerophospholipids, glycolipids, and sphingolipids, which suggested a potential disruption in membrane composition and function, and thus contributing to the antibacterial effect of menthone against this bacterium (Zhao et al., 2023). Moreover, certain research has also stated that piperitone has antibacterial properties (Abdolpour et al., 2007; Božovic et al., 2015). In summary, the antibacterial properties of pennyroyal EO are influenced by the quantity, the synergistic/antagonistic effects, and the mechanisms of action of its constituents, as well as the susceptibility of the microorganisms tested (Ez-Zriouli et al., 2022).

Generally, the efficacy of EOs against pathogenic microbes may be related to the lipophilic nature of the monoterpenes they contain. These phytocompounds function by interfering with the cytoplasmic membrane of microorganisms, causing a reduction in its impermeability to protons and larger ions. Disruption of the membrane's integrity not only compromises its role as a barrier but also affects its function as an enzyme's platform and energy converter. Nevertheless, the specific mechanisms responsible for the antimicrobial effects of monoterpenes are still not well-defined (Stringaro et al., 2014). In addition, it was also reported that the antibacterial action of EOs including that of *M. pulegium* is believed to involve disruption of outer and inner bacterial membranes (Chouhan et al., 2017). All things considered, our findings and those of others support the antimicrobial activity of *M. pulegium* EO against a range of pathogenic bacteria and fungi, and it offers a viable avenue for the utilization of natural substitutes to address the issue of antibiotic resistance.

3.5. In silico molecular docking of α-amylase and α-glucosidase inhibition potentials by EO

Molecular modeling has emerged as a crucial tool in modern research activities, utilizing computer-aided drug design (CADD) technologies in order to examine and predicted the possible interactions of different components (ligands), stable or volatile, like EOs, with molecular targets associated with diverse biological activities (Mali et al., 2022). Molecular docking allows the computation of the affinity energy relative to ligand-protein complexes as well the

identification of active sites in three-dimensional structures, hence facilitating the *in silico* hypotheses' generation concerning the mechanism of action relative to a number of bioactive compounds (Mali et al., 2022; Yu and MacKerell, 2017).

The antidiabetic effects of EOs and extracts derived from various *Mentha* species have been observed and proved *in vitro* and *in vivo*. These effects were examined *in vitro* through the α-amylase and α-glucosidase enzyme's inhibition capacity (Abdellatief et al., 2017; Agawane et al., 2019; El Hachlafi et al., 2023; Gülçin et al., 2020; Saqib et al., 2022). The observed α-amylase and α-glucosidase inhibitory properties of *Mentha* plants are believed to be linked, in part, to the phytochemical compounds found in its EOs, such as pulegone and menthone, which are mostly found in the EO under investigation (Benabed et al., 2023; Revathi et al., 2022). However, the mechanisms through which these EOs and extracts regulate or inhibit the enzymatic activity associated with diabetes, as reported in these studies, remain unclear, rare, or under-researched. Therefore, the focus of our research is to computationally (*in silico*) analyse and elucidate the mechanisms of action of *M. pulegium* EO compounds in modulating the enzymatic activities related to this biological effect, aiming to validate and support the experimental findings reported about the antidiabetic propriety.

DM is associated with a modification in the synthesis of insulin by the pancreatic cells, leading to an abnormal glucose metabolism (Hosein Farzaei et al., 2015). The enzymes α-amylase and α-glucosidase play a crucial role in the degradation of carbohydrates and the absorption in the intestine. Consequently, the inhibition of these enzymes proves to be an effective therapeutic approach in the management and prevention of type 2 diabetes (Bouyahya et al., 2020).

Table 4: Docking results of ligands (compounds of *M. pulegium* EO) in the targeted receptors: α-amylase enzyme (PDB: 1B2Y) and α-glucosidase enzyme (PDB: 5NN8).

Ligands	**α-amylase**			**α-glucosidase**		
	1B2Y receptor			5NN8 receptor		
	G. score (kcal/mol)	**G. emodel (kcal/mol)**	**G. energy (kcal/mol)**	**G. score (kcal/mol)**	**G. emodel (kcal/mol)**	**G. energy (kcal/mol)**
1-Ethyl-3-methyl-2-(2 methylpropylidene) Imidazolidine	-4.821	-38.012	-25.56	-5.448	-46.073	-29.624
2,2 Dicyclohexylpropane	-3.54	-22.865	-19.483	-2.93	-18.565	-15.625
2-Cyclohexen-1-ol	-4.343	-25.512	-19.549	-5.16	-29.019	-21.335
2-methyl-1,3-Cyclohexanedione	-3.739	-27.588	-21.991	-4.426	-30.704	-23.711
3-(3-thienyl)-2 propenoic acid	-4.36	-24.219	-18.4	-4.074	-20.896	-14.535
8-Hydroxy-p-menthan-3-one	-4.518	-35.884	-27.788	-4.875	-35.273	-25.344
Dimethylisopropylsilane	-1.908	-14.966	-13.321	-2.401	-17.417	-15.235
Imidazolidine	-7.172	-59.957	-28.484	-8.393	-79.719	-34.41
Isopulegone	-4.059	-24.677	-19.623	-3.82	-26.002	-20.644
Menthone	-4.373	-25.115	-19.259	-4.062	-22.569	-17.881
Mint Furanone	-4.423	-29.474	-22.408	-4.604	-26.567	-20.292
Piperitenone	-4.722	-26.507	-19.976	-4.155	-24.878	-19.333
Pulegone	-4.675	-26.514	-20.1	-3.941	-23.945	-18.975

Within this context, we conducted an assessment of the inhibitory potentials on α-amylase and α-glucosidase by *M. pulegium* EO using molecular docking approach. *In silico* evaluation of the antidiabetic activity revealed that imidazolidine, piperitenone, and pulegone, constituents of *M. pulegium* EO, exhibited significant inhibitory action against α-amylase (PDB: 1B2Y), with glide scores of -7.172, -4.722, and -4.675 kcal/mol, respectively (Table 4). Likewise, imidazolidine, 2-cyclohexen-1-ol, and 1-ethyl-3-methyl-2-(2 methylpropylidene) imidazolidine are the components of the same EO, which exhibit remarkable efficacy against α-glucosidase (PDB: 5NN8), achieving glide scores of -8.393, -5.160, and -5.422 kcal/mol, correspondingly.

The 2D and 3D viewers relative to the *M. pulegium* EO docked in the active site of α-amylase (PDB: 1B2Y) revealed that imidazolidine established 3 hydrogen bonds with ASP 197, ASP 300 and GLU 233 residues, and three salt bridges with the same residues and a pi-cation bond with residue TYR 62 (Figure 3A and 4A). Otherwise, piperitenone established a single hydrogen bond with the α-amylase enzyme's HIP 305 residue (Figure 3B and 4B), while pulegone also formed a single hydrogen bond in contact with the same enzymatic residue (Figure 3C and 4C).

Concerning the α-glucosidase (PDB: 5NN8), imidazolidine formed double hydrogen bonds in contact with residues ASP 518 and ASP 404, and the same time, two salt bridges with the same residues in the active site of the studied enzyme

(Figure 3D and 4D). Similarly, 2-cyclohexen-1-ol established two hydrogen bonds with residues ARG 600 and ASP 616 (Figure 3E and 4E). Additionally, 1-ethyl-3-methyl-2-(2 methylpropylidene) imidazolidine formed a single hydrogen bond when it interacted with the ASP 616 enzymatic residue and also established two salt bridges with the residues ASP 518 and ASP 616 (Figure 3F and 4F).

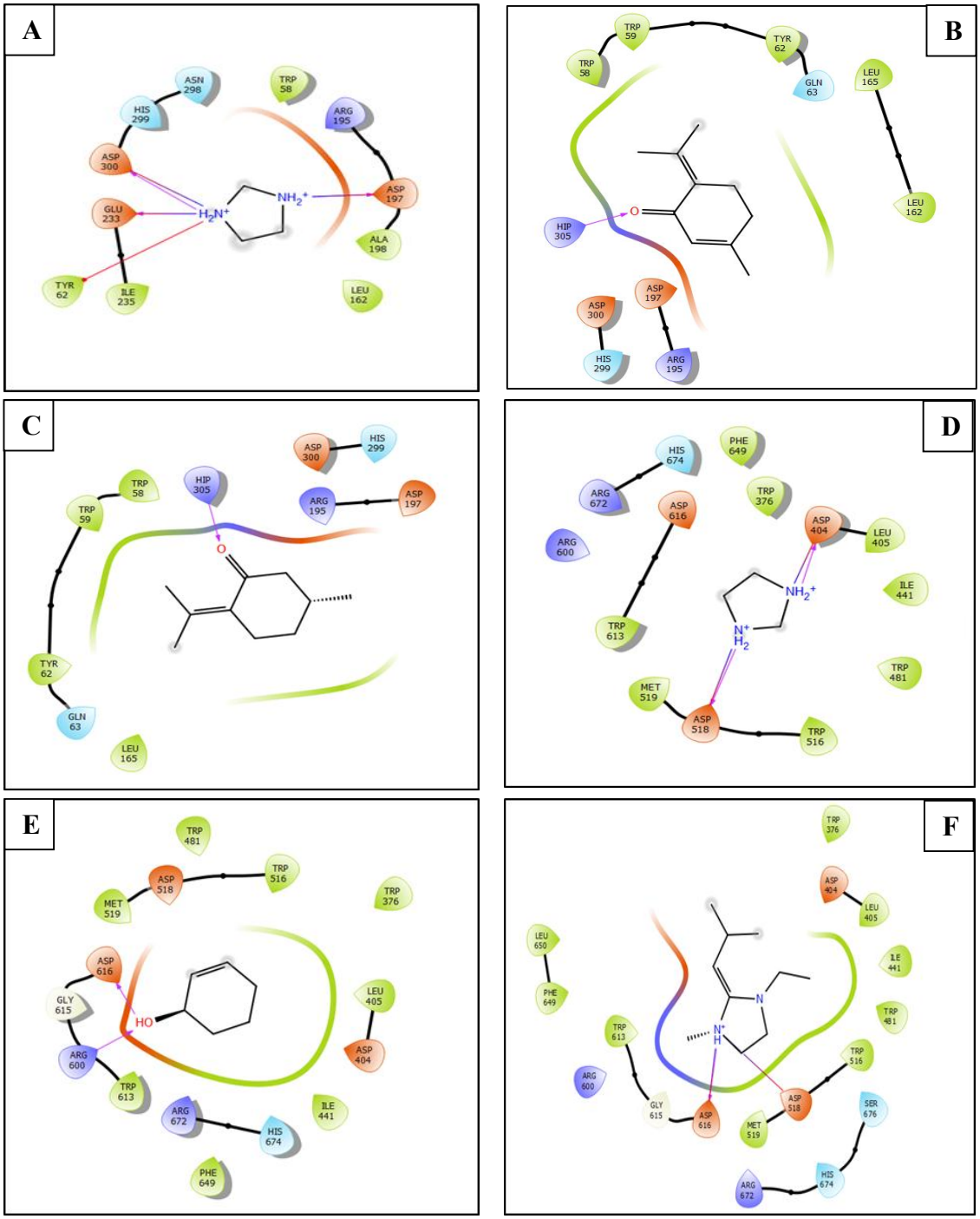

Figure 3: The 2D viewer of ligands interactions with the enzyme's active site. Respectively, A, D: Interactions of imidazolidine with the active site of α-amylase and α-glucosidase; B, C: Interactions of piperitenone and pulegone with the active site of α-amylase; E, F: Interactions of 2-cyclohexen-1-ol and 1-ethyl-3-methyl-2-(2 methylpropylidene) imidazolidine with the active site of α-glucosidase.

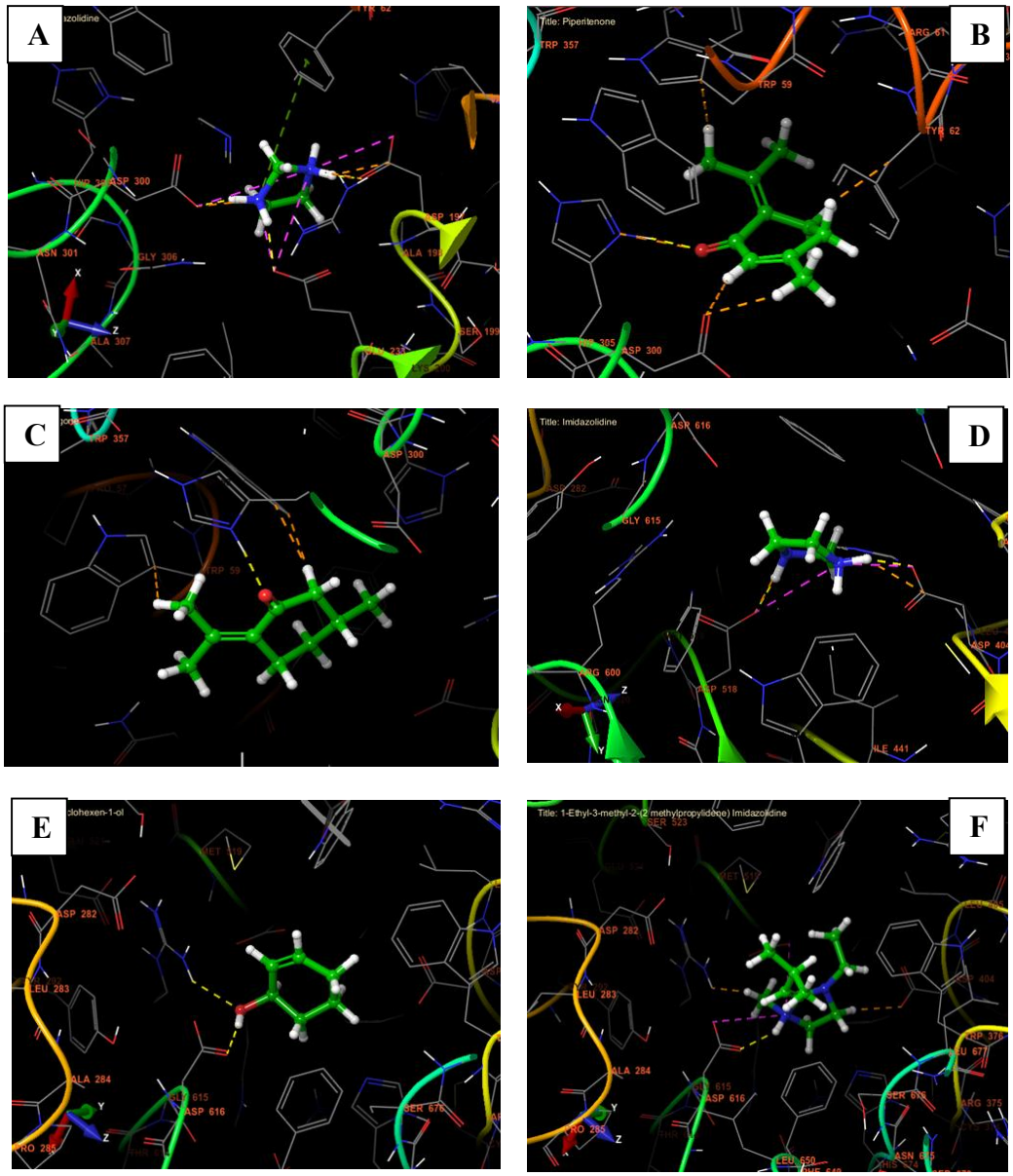

Figure 4: The 3D of ligands interactions with the enzyme's active site. Respectively, A, D: Interactions of imidazolidine with the active site of α-amylase and α-glucosidase; B, C: Interactions of piperitenone and pulegone with the active site of α-amylase; E, F: Interactions of 2-cyclohexen-1-ol and 1-ethyl-3-methyl-2-(2 methylpropylidene) imidazolidine with the active site of α-glucosidase.

The *in silico* data obtained provide a comprehensive explanation and validation of the experimental antidiabetic properties associated with certain phytochemical compounds found in EOs derived from *Mentha* species, as previously reported. Additionally, these findings offer valuable insights into the potential mechanisms responsible for the modulation of antidiabetic enzymatic activity by specific compounds present in *M. pulegium* EO. In fact, the management and regulation of blood sugar levels in individuals with type 2 DM and those at the borderline can be enhanced through the inhibition of α-amylase and α-glucosidase enzymes, which play a crucial role in carbohydrate digestion. This action has the potential to significantly reduce the surge in blood glucose levels post meals. The biological impacts that can be influenced by plant-derived remedies and specialized diets in addressing diabetes are currently gaining fresh scrutiny. Within the realm of botanical medicine, a variety of oral natural agents and their derivatives have shown remarkable hypoglycemic proprieties, offering minimal to no adverse secondary effects (Bungau et al., 2023). This was observed in this study for *M. pulegium* EO, which showed promising *in silico* results as an anti-diabetic agent, by inhibiting the enzymes involved in this metabolic disorder. It is important to note that these current findings need to be validated and confirmed by *in vivo* and *in vitro* studies as part of further research.

4. Conclusion

In conclusion, the obtained outcomes establish that the essential oil extracted from Moroccan *Mentha pulegium* exhibits notable antioxidant and antimicrobial characteristics. These findings emphasize the potential efficacy of this EO as a natural and effective therapeutic agent in alleviating the impact of free radicals and addressing the growing issue of microbial resistance to pathogenic microorganisms. According to computational studies, the examined pennyroyal EO could also serve as a natural inhibitor of α-amylase and α-glucosidase enzymes. Hence, it may offer advantages in managing diabetic disorders; nevertheless, further validation through *in vivo* and *in vitro* experiments is crucial to verifying the results. The observed bioactivities are partly linked to the existence of monoterpene phytochemicals in the EO. The diverse advantages of *Mentha pulegium* EO, as demonstrated in this research, lay a strong groundwork for additional research and utilization of its practical applications in combating microbial infections and managing diabetes.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data availability statement

Data will be available upon request from the corresponding

author.

References

Abdellatief, S.A., Beheiry, R.R., El-Mandrawy, S.A.M., 2017. Peppermint essential oil alleviates hyperglycemia caused by streptozotocin- nicotinamide-induced type 2 diabetes in rats. Biomed. Pharmacother. 95, 990–999. https://doi.org/10.1016/J.BIOPHA.2017.09.020

Abdolpour, F., Shahverdi, A.R., Rafii, F., Fazeli, M.R., Amini, M., 2007. Effects of Piperitone on the Antimicrobial Activity of Nitrofurantoin and on Nitrofurantoin Metabolism by *Enterobacter cloacae*. Pharm. Biol. 45, 230–234. https://doi.org/10.1080/13880200701213161

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatograpy/Mass Spectrometry., 4th ed, Illinois USA: Allured Publishing Corporation, Carol Stream. Allured Pub. Corp, Carol Stream, Ill, USA.

Agawane, S.B., Gupta, V.S., Kulkarni, M.J., Bhattacharya, A.K., Koratkar, S.S., 2019. Chemo-biological evaluation of antidiabetic activity of *Mentha arvensis* L. and its role in inhibition of advanced glycation end products. J. Ayurveda Integr. Med. 10, 166–170. https://doi.org/10.1016/J.JAIM.2017.07.003

Agour, A., Mssillou, I., Mechchate, H., Es-Safi, I., Allali, A., El Barnossi, A., Al Kamaly, O., Alshawwa, S.Z., El Moussaoui, A., Bari, A., Lyoussi, B., Derwich, E., 2022. *Brocchia cinerea* (Delile) Vis. Essential Oil Antimicrobial Activity and Crop Protection against Cowpea Weevil *Callosobruchus maculatus* (Fab.). Plants 11, 1–13. https://doi.org/10.3390/plants11050583

Ahmed, A., Ayoub, K., Chaima, A.J., Hanaa, L., Abdelaziz, C., 2018. Effect of drying methods on yield, chemical composition and bioactivities of essential oil obtained from Moroccan *Mentha pulegium* L. Biocatal. Agric. Biotechnol. 16, 638–643. https://doi.org/10.1016/J.BCAB.2018.10.016

Aimad, A., Sanae, R., Anas, F., Abdelfattah, E.M., Bourhia, M., Mohammad Salamatullah, A., Alzahrani, A., Alyahya, H.K., Albadr, N.A., Abdelkrim, A., El Barnossi, A., Noureddine, E., 2021. Chemical Characterization and Antioxidant, Antimicrobial, and Insecticidal Properties of Essential Oil from *Mentha pulegium* L. Evidence-Based Complement. Altern. Med. 2021, 1108133. https://doi. org/10.1155/2021/1108133

Aimad, A., Youness, E.A., Sanae, R., El Moussaoui, A., Bourhia, M., Salamatullah, A.M., Alzahrani, A., Alyahya, H.K., A. Albadr, N., Nafidi, H.A., Ouahmane, L., Mohamed, F., 2022. Chemical Composition and Antifungal, Insecticidal and Repellent Activity of Essential Oils From *Origanum compactum* Benth. Used in the Mediterranean Diet. Front. Plant Sci. 13, 1–10. https://doi.org/10.3389/fpls.2022.798259

Akash, M.S.H., Rehman, K., Fiayyaz, F., Sabir, S., Khurshid, M., 2020. Diabetes-associated infections: development of antimicrobial resistance and possible treatment strategies. Arch. Microbiol. 2020 2025 202, 953–965. https://doi.org/10.1007/S00203-020-01818-X

Amalich, S., Zerkani, H., Cherrat, A., Soro, N.K., Bourakhouadar, M., Mahjoubi, M., EL Hilali, F., Zair, T., 2016. Study on *Mentha pulegium* L. from M'rirt (Morocco): Antibacterial and antifungal activities of a pulegone-rich essential oil. J. Chem. Pharm. Res. 8, 363–370.

Amalich, S., Zerkani, H., Tagnaout, I., Ali, C., Kamal, F., Zair, T., 2024. Chemical composition of the essential oil and isolation of two main constituents of *Mentha pulegium* L. Vegetos 37, 82–93. https://doi.org/10.1007/S42535-023-00572-X/METRICS

Amorati, R., Foti, M.C., Valgimigli, L., 2013. Antioxidant activity of essential oils. J. Agric. Food Chem. 61, 10835–10847.https://doi.org/10.1021/JF403496K/ASSET/ IMAGES/MEDIUM/JF-2013-03496K0016.GIF

Amrati, F.E.Z., Elmadbouh, O.H.M., Chebaibi, M., Soufi, B., Conte, R., Slighoua, M., Saleh, A., Al Kamaly, O., Drioiche, A., Zair, T., Edderkaoui, M., Bousta, D., 2023. Evaluation of the toxicity of *Caralluma europaea* (C.E) extracts and their effects on apoptosis and

chemoresistance in pancreatic cancer cells. J. Biomol. Struct. Dyn. 41, 8517–8534. https://doi.org/10.1080/07391102.2022. 213 5595

Azadi, S., Osanloo, M., Zarenezhad, E., Farjam, M., Jalali, A., Ghanbariasad, A., 2023. Nano-scaled emulsion and nanogel containing *Mentha pulegium* essential oil: cytotoxicity on human melanoma cells and effects on apoptosis regulator genes. BMC Complement. Med. Ther. 23. https://doi.org/10.1186/S12906-023-03834-Y

Baccouri, B., Rajhi, I., 2021. Potential antioxidant activity of terpenes, in: Terpenes Terpenoids-Recent Adv. pp. 53–62.

Balouiri, M., Sadiki, M., Ibnsouda, S.K., 2016. Methods for in vitro evaluating antimicrobial activity: A review. J. Pharm. Anal. 6, 71–79. https://doi.org/10.1016/J.JPHA.2015.11. 005

Benabed, K.H., Boussoussa, H., Khacheba, I., bekhaoua, A., Douadji, F.Z., Daïdi, S., Djaâfour, S., Yousfi, M., 2023. Alpha-amylase Inhibitory Activity of Extracts from Algerian *Calamintha nepeta* (L.). Curr. Enzym. Inhib. 19, 136–141.https://doi.org/10. 2174/ 157340801966623033 1084001

Bouyahya, A., Et-Touys, A., Bakri, Y., Talbaui, A., Fellah, H., Abrini, J., Dakka, N., 2017. Chemical composition of *Mentha pulegium* and *Rosmarinus officinalis* essential oils and their antileishmanial, antibacterial and antioxidant activities. Microb. Pathog. 111, 41–49. https://doi.org/10. 1016/J.MICPATH.2017.08.015

Bouyahya, A., Lagrouh, F., El Omari, N., Bourais, I., El Jemli, M., Marmouzi, I., Salhi, N., Faouzi, M.E.A., Belmehdi, O., Dakka, N., Bakri, Y., 2020. Essential oils of *Mentha viridis* rich phenolic compounds show important antioxidant, antidiabetic, dermatoprotective, antidermatophyte and antibacterial properties. Biocatal. Agric. Biotechnol. 23, 101471. https://doi.org/10.1016/J.BCAB.2019.101471

Božovic, M., Pirolli, A., Ragno, R., 2015. *Mentha suaveolens* Ehrh. (Lamiaceae) Essential Oil and Its Main Constituent Piperitenone Oxide: Biological Activities and Chemistry. Mol. 2015, Vol. 20, Pages 8605-8633 20, 8605–8633. https://doi.org/10.3390/MOLECULES20058605

Bungau, S.G., Vesa, C.M., Bustea, C., Purza, A.L., Tit, D.M., Brisc, M.C., Radu, A.F., 2023. Antioxidant and Hypoglycemic Potential of Essential Oils in Diabetes Mellitus and Its Complications. Int. J. Mol. Sci. 2023, Vol. 24, Page 16501 24, 16501. https://doi. org/10 .3390/ IJMS242216501

Casiglia, S., Bruno, M., Fontana, G., Senatore, F., 2017. Chemical Composition of the Essential Oil of *Mentha pulegium* Growing Wild in Sicily and its Activity on Microorganisms Affecting Historical Art Crafts. Nat. Prod. Commun. 12, 1311–1315. https://doi.org/https:/ /doi.org/10.1177/1934578X1701200840

Ćavar, S., Vidic, D., Maksimović, M., 2013. Volatile constituents, phenolic compounds, and antioxidant activity of *Calamintha glandulosa* (Req.) Bentham. J. Sci. Food Agric. 93, 1758–1764. https://doi.org/10.1002/JSFA.5967

Chouhan, S., Sharma, K., Guleria, S., 2017. Antimicrobial Activity of Some Essential Oils—Present Status and Future Perspectives. Medicines 4, 58. https://doi. org/10.3390/medicines4030058

Circu, M.L., Aw, T.Y., 2010. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radic. Biol. Med. 48, 749–762. https://doi.org/10.1016/J.FREERADBIOMED. 2009.12.022

CLSI, 2008. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi Approved Standard-Second Edition, 2nd ed. Clinical and Laboratory Standards Institute, Wayn, PA.

CLSI, 2018. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 11th ed. Clinical and Laboratory Standards Institute, Wayne, PA.

Czech, M.P., 2017. Insulin action and resistance in obesity and type 2 diabetes. Nat. Med. 2017 237 23, 804–814. https://doi.org/10.1038/nm.4350

Dehghani, N., Afsharmanesh, M., Salarmoini, M., Ebrahimnejad, H., 2018. Characterization of pennyroyal (*Mentha pulegium*) essential oil as an herbal, antibacterial, and antioxidant substance. Comp. Clin. Path. 27, 1575–1581. https://doi.org/10.1007/S00580-018-2776-4/ METRICS

Dhingra, A.K., Chopra, B., 2023. Pulegone: An Emerging Oxygenated Cyclic Monoterpene Ketone Scaffold Delineating Synthesis, Chemical Reactivity, and Biological potential. Recent Adv. anti-infective drug Discov. 18, 16–28. https://doi.org/10.2174/277243441 8666221018090507

Domingues, P., Santos, L., 2019. Essential oil of pennyroyal (*Mentha pulegium*): Composition and applications as alternatives to pesticides—New tendencies. Ind. Crops Prod. 139, 111534. https://doi.org/10.1016/J. INDCROP.2019.1115 34

Duru, M.E., Öztürk, M., Uğur, A., Ceylan, Ö., 2004. The constituents of essential oil and in vitro antimicrobial activity of *Micromeria cilicica* from Turkey. J. Ethnopharmacol. 94, 43–48. https://doi.org/10.1016/ J.JEP.2004.03.053

EDQM., 2004. European pharmacopoeia., 5th ed. ed. Council of Europe., Strasbourg, France.

El Abdali, Y., Allali, A., Agour, A., Mikou, K., Lahkimi, A., Eloutassi, N., Bouia, A., 2021. Phytochemical Screening, and in Vitro Antiradical and Immunostimulant Potential of *Linum Usitatissimum* L. Pharmacologyonline 3, 602–614.

El Abdali, Y., Beniaich, G., Mahraz, A.M., El Moussaoui, A., Bin Jardan, Y.A., Akhazzane, M., Chebaibi, M., Nafidi, H.-A., Eloutassi, N., Bourhia, M., Bouia, A., 2023a. Antibacterial, Antioxidant, and in silico NADPH Oxidase Inhibition Studies of Essential Oils of *Lavandula dentata* against Foodborne Pathogens. Evidence-Based Complement. Altern. Med. 2023, 1–12. https://doi.org/10.1155/2023/9766002

El Abdali, Y., Mahraz, A.M., Beniaich, G., Mssillou, I., Chebaibi, M., Jardan, Y.A. Bin, Lahkimi, A., Nafidi, H.-A., Aboul-Soud, M.A.M., Bourhia, M., Bouia, A., 2023b. Essential oils of *Origanum compactum* Benth: Chemical characterization, in vitro, in silico, antioxidant, and antibacterial activities. Open Chem. 21, 20220282. https://doi.org/10.1515/CHEM-2022-0282

EL Abdali, Y., Saghrouchni, H., Kara, M., Mssillou, I., Allali, A., Jardan, Y.A. Bin, Kafkas, N.E., El-Assri, E.-M., Nafidi, H.-A., Bourhia, M., Almaary, K.S., Eloutassi, N., Bouia, A., 2023. Exploring the Bioactive Compounds in Some Apple Vinegar Samples and Their Biological Activities. Plants 2023, Vol. 12, Page 3850 12, 3850. https://doi.org/10.3390/PLANTS12223850

El Hachlafi, N., Benkhaira, N., Al-Mijalli, S.H., Mrabti, H.N., Abdnim, R., Abdallah, E.M., Jeddi, M., Bnouham, M., Lee, L.H., Ardianto, C., Ming, L.C., Bouyahya, A., Fikri-Benbrahim, K., 2023. Phytochemical analysis and evaluation of antimicrobial, antioxidant, and antidiabetic activities of essential oils from Moroccan medicinal plants: *Mentha suaveolens, Lavandula stoechas*, and *Ammi visnaga*. Biomed. Pharmacother. 164, 114937. https://doi.org/10.1016/J.BIOPHA.2023.114937

El Hassani, F.Z., 2020. Characterization, activities, and ethnobotanical uses of *Mentha* species in Morocco. Heliyon 6, e05480. https://doi.org/10.1016/J.HELIYON. 2020.E05480

EL Moussaoui, A., Bourhia, M., Jawhari, F.Z., Salamatullah, Ahmad Mohammad Ullah, R., Bari, Ahmed, Mahmood, H.M., Sohaib, M., Serhii, B., Rozhenko, A., Aboul-Soud, M.A.M., Ezzeldin, E., Mostafa, G.A.E., Bousta, D., Bari, Amina, 2021. Chemical Profiling, Antioxidant, and Antimicrobial Activity against Drug-Resistant Microbes of Essential Oil from *Withania frutescens* L. Appl. Sci. 11, 5168. https://doi.org/https://doi.org/10.3390/app 111151 68

Ez-Zriouli, R., El Yacoubi, H., Imtara, H., El-Hessni, A., Mesfioui, A., Tarayrah, M., Mothana, R.A., Noman, O.M., Mouhsine, F., Rochdi, A., 2022. Chemical composition and antimicrobial activity of essential oils from *Mentha pulegium* and *Rosmarinus officinalis* against multidrug-resistant microbes and their acute toxicity study. Open Chem. 20, 694–702. https://doi.org/10.1515/CHEM-2022-0185/MACHINEREADABLECITATION/RIS

Farhanghi, A., Aliakbarlu, J., Tajik, H., Mortazavi, N., Manafi, L., Jalilzadeh-Amin, G., 2022. Antibacterial interactions of pulegone and 1,8-cineole with monolaurin ornisin against *Staphylococcus aureus*. Food Sci. Nutr. 10, 2659–2666. https://doi.org/10.1002/FSN3.2870

Fernández-Sánchez, A., Madrigal-Santillán, E., Bautista, M., Esquivel-Soto, J., Morales-González, Á., Esquivel-Chirino, C., Durante-Montiel, I., Sánchez-Rivera, G., Valadez-Vega, C., Morales-González, J.A., 2011. Inflammation, Oxidative Stress, and Obesity. Int. J. Mol. Sci. 2011, Vol. 12, Pages 3117-3132 12, 3117–3132. https://doi.org/10.3390/IJMS12053117

Furtado, G.L., Medeiros, A.A., 1980. Single-disk diffusion testing (Kirby-Bauer) of susceptibility of *Proteus mirabilis* to chloramphenicol: significance of the intermediate category. J. Clin. Microbiol. 12, 550–553. https://doi.org/10.1128/JCM.12.4.550-553.1980

Giri, B., Dey, S., Das, T., Sarkar, M., Banerjee, J., Dash, S.K., 2018. Chronic hyperglycemia mediated physiological alteration and metabolic distortion leads to organ dysfunction, infection, cancer progression and other pathophysiological consequences: An update on glucose toxicity. Biomed. Pharmacother. 107, 306–328. https://doi.org/10.1016/J.BIOPHA.2018.07.157

Gonzalez-Burgos, E., Gomez-Serranillos, M.P., 2012. Terpene Compounds in Nature: A Review of Their Potential Antioxidant Activity. Curr. Med. Chem. 19, 5319–5341. https://doi.org/10.2174/092986712803833335

Gülçin, İ., Gören, A.C., Taslimi, P., Alwasel, S.H., Kılıc, O., Bursal, E., 2020. Anticholinergic, antidiabetic and antioxidant activities of Anatolian pennyroyal (*Mentha pulegium*)-analysis of its polyphenol contents by LC-MS/MS. Biocatal. Agric. Biotechnol. 23, 101441. https://doi.org/10.1016/J. BCAB.2019.101441

Hosein Farzaei, M., Rahimi, R., Farzaei, F., Abdollahi, M., 2015. Traditional medicinal herbs for the management of diabetes and its complications: An evidence-based review. Int. J. Pharmacol. 11, 874–887. https://doi.org/10.3 923/IJP.2015.874.887

Jain, S., Dwivedi, J., Jain, P.K., Satpathy, S., Patra, A., 2016. Medicinal Plants for Treatment of Cancer: A Brief Review. Pharmacogn. J. 8, 87–102. https://doi.org/10.5530/ pj.2016.2.1

Jaradat, N., Dwikat, M., Amer, J., Ghanim, M., Hawash, M., Hussein, F., Issa, L., Ishtawe, S., Salah, S., Nasser, S., 2024. Total phenolic contents, cytotoxic, free radicals, porcine pancreatic α-amylase, and lipase suppressant activities of *Artemisia dracunculus* plant from Palestine. Front. Pharmacol. 15, 1351743. https://doi.org/10.3389/FPHAR.2024.1351743/BIBTEX

Justus, B., de Almeida, V.P., Gonçalves, M.M., da Silva Fardin de Assunção, D.P., Borsato, D.M., Arana, A.F.M., Maia, B.H.L.N.S., de Paula, J. de F.P., Budel, J.M., Farago, P.V., 2018. Chemical composition and biological activities of the essential oil and anatomical markers of *Lavandula dentata* L. Cultivated in Brazil. Brazilian Arch. Biol. Technol. 61, e18180111. https://doi.org/10.1590/1678-4324-2018180111

Kamatou, G.P.P., Vermaak, I., Viljoen, A.M., Lawrence, B.M., 2013. Menthol: A simple monoterpene with remarkable biological properties. Phytochemistry 96, 15–25. https://doi.org/10.1016/J.PHYTOCHEM.2013.08.005

Kiehlbauch, J.A., Hannett, G.E., Salfinger, M., Archinal, W., Monserrat, C., Carlyn, C., 2000. Use of the National Committee for Clinical Laboratory Standards guidelines for disk diffusion susceptibility testing in

New York State Laboratories. J. Clin. Microbiol. 38, 3341–3348. https://doi.org/10.1128 /JCM.38.9.3341-3348.2000

Lafraxo, S., El Moussaoui, A., A Bin Jardan, Y., El Barnossi, A., Chebaibi, M., Baammi, S., Ait Akka, A., Chebbac, K., Akhazzane, M., Chelouati, T., Nafidi, H.A., Farid, K., Bourhia, M., Bari, A., 2022. GC-MS Profiling, in Vitro Antioxidant, Antimicrobial, and in Silico NADPH Oxidase Inhibition Studies of Essential Oil of *Juniperus thurifera* Bark. Evidence-based Complement. Altern. Med. 2022. https://doi.org/10.1155/2022/6305672

Leyva-López, N., Gutiérrez-Grijalva, E.P., Vazquez-Olivo, G., Heredia, J.B., 2017. Essential oils of oregano: Biological activity beyond their antimicrobial properties. Molecules 22. https://doi.org/10.3390/molecules22060989

Mali, S.N., Tambe, S., Pratap, A.P., Cruz, J.N., 2022. Molecular Modeling Approaches to Investigate Essential Oils (Volatile Compounds) Interacting with Molecular Targets BT - Essential Oils: Applications and Trends in Food Science and Technology, in: Santana de Oliveira, M. (Ed.), . Springer International Publishing, Cham, pp. 417–442. https://doi.org/10.1007/978-3-030-99476-1_18

Maškovič, P.Z., Manojlovič, N.T., Mandič, A.I., Mišan, A.Ç., Milovanovic, I.L., Radojkovič, M.M., Cvijovič, M.S., Solujič, S.R., 2012. Phytochemical screening and biological activity of extracts of plant species *Halacsya sendtneri* (Boiss.) Dörfl. Hem. Ind. 66, 43–51. https://doi.org/10.2298/HEMIND110828068M

Messaoudi, M., Rebiai, A., Sawicka, B., Atanassova, M., Ouakouak, H., Larkem, I., Egbuna, C., Awuchi, C.G., Boubekeur, S., Ferhat, M.A., Begaa, S., Benchikha, N., 2021. Effect of Extraction Methods on Polyphenols, Flavonoids, Mineral Elements, and Biological Activities of Essential Oil and Extracts of *Mentha pulegium* L. Mol. 2022, Vol. 27, Page 11 27, 11. https://doi.org/10.3390/MOLECULES27010011

Moattar, F.S., Sariri, R., Yaghmaee, P., Giahi, M., 2016. Enzymatic and non-enzymatic antioxidants of *Calamintha officinalis* moench extracts. J. Appl. Biotechnol. Reports 3, 489–494.

Mulani, M.S., Kamble, E.E., Kumkar, S.N., Tawre, M.S., Pardesi, K.R., 2019. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review. Front. Microbiol. 10, 539. https://doi.org/10.3389/ FMICB.2019.00539/BIBTEX

Nickavar, B., Jabbareh, F., 2018. Analysis of the Essential Oil from *Mentha pulegium* and Identification of its Antioxidant Constituents. J. Essent. Oil Bear. Plants 21, 223–229. https://doi.org/10.1080/0972060X.2018.1433073

Ouahabi, S., Loukili, E.H., Daoudi, N.E., Chebaibi, M., Ramdani, Mohamed, Rahhou, I., Bnouham, M., Fauconnier, M.L., Hammouti, B., Rhazi, L., Ayerdi Gotor, A., Dépeint, F., Ramdani, Mohammed, 2023. Study of the Phytochemical Composition, Antioxidant Properties, and In Vitro Anti-Diabetic Efficacy of *Gracilaria bursa-pastoris* Extracts. Mar. Drugs 2023, Vol. 21, Page 372 21, 372. https://doi.org/10.3390/MD21070372

Paul, A.K., Jahan, R., Paul, A., Mahboob, T., Bondhon, T.A., Jannat, K., Hasan, A., Nissapatorn, V., Wilairatana, P., De Lourdes Pereira, M., Wiart, C., Rahmatullah, M., 2022. The Role of Medicinal and Aromatic Plants against Obesity and Arthritis: A Review. Nutrients 14. https://doi.org/10.3390/NU14050985

Prieto, P., Pineda, M., Aguilar, M., 1999. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Anal. Biochem. 269, 337–341. https://doi.org/10.1006/ABIO.1999.4019

Revathi, M. (M), Padmalochana, K. (K), Sankaranarayanan, C. (C), Nalini, S. (S), Ramya, P. (P), 2022. Antidiabetic Activities of Menthone on Insulin Resistant in 3T3-L1 Adipocytes. Int. J. Health Sci. (Qassim). 6, 9961–9971. https://doi.org/10.53730/IJHS.V6NS1.7360

Rezouki, S., Allali, A., Louasté, B., Eloutassi, N., Fadli, M., 2021. The Impact of the Harvesting Period and Drying Conditions on the Essential Oil Yield of *Rosmarinus Officinalis, Thymus Satureioides* and *Origanum Compactum* from the Taza-Taounate Region. Asian J. Agric. Biol. 2021, 1–7. https://doi.org/10.35495/ajab.2020.04.251

Saqib, S., Ullah, F., Naeem, M., Younas, M., Ayaz, A., Ali, S., Zaman, W., 2022. *Mentha*: Nutritional and Health Attributes to Treat Various Ailments Including Cardiovascular Diseases. Mol. 2022, Vol. 27, Page 6728 27, 6728. https://doi.org/10.3390/MOLECULES27196728

Singh, B.K., Maurya, A., 2024. Antioxidant Activity of Essential Oils: A Mechanistic Approach. Plant Essent. Oils 59–76. https://doi.org/10.1007/978-981-99-4370-8_3

Stringaro, A., Vavala, E., Colone, M., Pepi, F., Mignogna, G., Garzoli, S., Cecchetti, S., Ragno, R., Angiolella, L., 2014. Effects of *Mentha suaveolens* essential oil alone or in combination with other drugs in *Candida albicans*. Evidence-based Complement. Altern. Med. 2014. https://doi.org/10.1155/2014/125904

Tit, D.M., Bungau, S.G., 2023. Antioxidant Activity of Essential Oils. Antioxidants 2023, Vol. 12, Page 383 12, 383. https://doi.org/10.3390 /ANTIOX12020383

Tripathi, A.K., Ray, A.K., Mishra, S.K., 2022. Molecular and pharmacological aspects of piperine as a potential molecule for disease prevention and management: evidence from clinical trials. Beni-Suef Univ. J. Basic Appl. Sci. 2022 111 11, 1–24. https://doi.org/10.1186/S43088-022-00196-1

Wang, X., He, L., Huang, Z., Zhao, Q., Fan, J., Tian, Y., Huang, A., 2023. Isolation, identification and characterization of a novel antimicrobial peptide from *Moringa oleifera* seeds based on affinity adsorption. Food Chem. 398, 133923. https://doi.org/10.1016/J.FOODCHEM.2022.133923

Wojtunik, K.A., Ciesla, L.M., Waksmundzka-Hajnos, M., 2014. Model studies on the antioxidant activity of common terpenoid constituents of essential oils by means of the 2,2-Diphenyl-1-picrylhydrazyl method. J. Agric. Food Chem. 62, 9088–9094. https://doi.org/10.1021/JF502857S/ SUPPLFILE/ JF502857S_SI_001.PDF

World Health Organization (WHO), 2023. Diabetes [WWW Document]. URL https://www.who.int/news-room/fact-sheets/detail/diabetes (accessed 3.26.24).

Yu, W., MacKerell, A.D., 2017. Computer-Aided Drug Design Methods BT - Antibiotics: Methods and Protocols, in: Sass, P. (Ed.), . Springer New York, New York, NY, pp. 85–106. https://doi.org/10.1007/978-1-4939-6634-9_5

Zhao, W., Yang, C., Zhang, N., Peng, Y., Ma, Y., Gu, K., Liu, Xia, Liu, Xiaohui, Liu, Xijian, Liu, Y., Li, S., Zhao, L., 2023. Menthone Exerts its Antimicrobial Activity Against Methicillin Resistant *Staphylococcus aureus* by Affecting Cell Membrane Properties and Lipid Profile. Drug Des. Devel. Ther. 17, 219. https://doi.org/10.2147/DDDT.S384716