Jundishapur J Nat Pharm Prod. In Press(In Press):e143185. https://doi.org/10.5812/jjnpp-143185.

Published online 2024 January 20. **Research Article**

Salvia mirzayanii and *Salvia hypoleuca* Essential Oils: Chemical Composition, Alpha-Glucosidase Inhibitory, and Synergistic Effects of Selected Components

Masoud Ebrahimi [#1], Houra Jazayeri Gharehbagh [#1], Farid Dabaghian [1], Somayeh Mojtabavi [2], Sedigheh Khademian [3], Mina Saeedi [4, 5, *], Mohsen Amini [6], Mohammad Ali Faramarzi [3] and Mahnaz Khanavi [1, 5, 7, **]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[2]Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[3]Department of Phytopharmaceuticals (Traditional pharmacy), School of Pharmacy, Shiraz University of Medicinal Sciences, Shiraz, Iran
[4]Medicinal Plants Research Center, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[5]Persian Medicine and Pharmacy Research Center, Tehran University of Medical Sciences, Tehran, Iran
[6]Department of Medicinal Chemistry, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran
[7]Faculty of Land and Food Systems, University of British Columbia, B.C., Canada

[*]*Corresponding author*: Tehran University of Medical Sciences, Tehran, Iran. Email: m-saeedi@tums.ac.ir
[**]*Corresponding author*: Tehran University of Medical Sciences, Tehran, Iran. Email: khanavim@tums.ac.ir
# These authors are contributed equally as the first author.

Received 2023 November 19; **Revised** 2023 December 19; **Accepted** 2023 December 26.

Abstract

Background: *Salvia* is one of the most important genera belonging to the Lamiaceae family that has been used in various applications in folk medicine and the pharmaceutical and food industries.
Objectives: This study investigated the chemical composition, antioxidant activity, and α-glucosidase inhibitory activity of *Salvia mirzayanii* Rech. f. and Esfand. and *Salvia hypoleuca* Benth. essential oils (EOs). Additionally, the enzyme inhibitory activity of the mixture of compounds was evaluated to find whether the synergistic effect is responsible for the desired activity or not.
Methods: The constituents of *S. mirzayanii* and *S. hypoleuca* EOs collected from Fars and Alborz provinces, Iran, were determined using gas chromatography-mass spectroscopy (GC-MS) analysis. They were evaluated for their α-glucosidase inhibitory activity by the determination of *para*-nitrophenol (*p*NP), derived from the enzymatic degradation of *para*-nitrophenol-D-glucopyranoside (*p*NPG) as the substrate. The activity of the selected components was also tested. Moreover, the antioxidant activity of EOs was evaluated based on the radical scavenging capability (2,2-Diphenyl-1-picrylhydrazyl [DPPH]) assay, and their total phenolic content (TPC) was determined using the Folin-Ciocalteu method in terms of gallic acid equivalent (GAE).
Results: In total, 66 compounds were detected in the *S. mirzayanii* and *S. hypoleuca* EOs. The results showed that *S. mirzayanii* EO had more potent antioxidant activity (half-maximal inhibitory concentration [IC_{50}] = 0.77 ± 0.00 mg/mL), higher TPC (78.26 ± 1.26 mg GAE/g EO), and a greater inhibitory effect toward α-glucosidase (IC_{50} = 55.15 ± 1.60 mg/mL) than *S. hypoleuca* EO. Furthermore, caryophyllene oxide (IC_{50} = 19.94 ± 0.26 mg/mL), α-pinene (IC_{50} = 17.59 ± 0.19 mg/mL), and linalool (IC_{50} = 38.00 ± 0.22 mg/mL) showed high levels of α-glucosidase inhibitory activity among the major constituents. In addition, the combination of linalool: 1,8-cineole: α-terpineol (40: 35: 25) inhibited this enzyme synergistically (combination index [CI] < 1).
Conclusions: The findings indicated that *S. mirzayanii* EO had a high potential for developing efficient anti-type 2 diabetes agents.

Keywords: Essential oil, α-Glucosidase, Lamiaceae, *Salvia* spp., Synergism

1. Background

Diabetes mellitus (DM) is a chronic metabolic disease in which hyperglycemia occurs due to the lack of insulin production or insensitivity of the related receptors (1). According to the estimations, there will be 700 million diabetic individuals globally by 2045 (2). Basically, delaying postprandial hyperglycemia in the gastrointestinal canal is one of the substantial therapeutic approaches for treating DM, particularly type 2, that is achieved by decreasing glucose absorption through the inhibition of carbohydrate-hydrolyzing

enzymes. Alpha-amylase and α-glucosidase are two primary enzymes located in the brush border of the intestinal villi and are responsible for the breakdown of carbohydrates. Alpha-amylase degrades long-chain carbohydrates; however, starch and disaccharides are converted to glucose by α-glucosidase, leading to an increase in plasma glucose levels and postprandial hyperglycemia. As α-glucosidase is an essential enzyme that catalyzes the final stage of carbohydrate digestion, its inhibitors have been at the center of attention. Acarbose, miglitol, and voglibose are substantial medications for decreasing blood sugar levels in diabetic patients that inhibit α-glucosidase inhibitors (3). However, they have caused gastrointestinal side effects, notably flatulence, diarrhea, abdominal pain, and pneumatosis intestinalis in patients with DM (4). In this respect, the design and development of synthetic (5-7) and natural α-glucosidase inhibitors (8, 9) have received great attention.

Hyperglycemia is associated with various metabolic disorders and oxidative stress. An increase in the level of free radicals in the long term brings up some concerns, such as cancer, cardiovascular, and neurological disorders. Additionally, oxidative stress itself has been linked to the development and progression of diabetes (10). As a result, it is critical to find compounds possessing both antioxidant properties and hypoglycemic effects.

Medicinal herbs have been used traditionally to treat diabetes, and researchers are currently focusing on the bioactive compounds derived from plant extracts or their essential oils (EOs) to develop more potent and safer medications. On the other hand, EOs are high in a variety of biochemicals that have a wide range of activities, such as antioxidant properties (11).

Salvia, the largest genus of the Lamiaceae family, possesses over 900 species of herbaceous aromatic plants and can be found all over the world, especially in Asia. There are 60 species of *Salvia* in Iran, and among them, 17 species are endemic. *Salvia* species have been used to treat a variety of diseases, such as colds, aches, infections, bronchitis, Alzheimer's disease, and hemorrhage (12, 13). Furthermore, extracts and EOs from various *Salvia* spp. have received great attention in modern medicine. The *Salvia sclarea* EOs from two different regions of Lebanon were tested for acute and subchronic anti-diabetic effects in alloxan-induced diabetic rats and indicated that the plant collected from Beirut reduced blood glucose levels by 51.70% and 52.00% in acute and subchronic tests (at the concentration of 200 mg/kg), respectively (14). In another study, the *S. officinalis* EO in the flowering stage was observed to have an α-glucosidase inhibitory activity (half-maximal inhibitory concentration [IC_{50}] = 22.24 ± 0.07 μg/mL, compared to acarbose as a standard [IC_{50} = 12.31 ± 0.05 μg/mL]) (15).

Salvia mirzayanii (known as "Moor-e-Talkh" in Persian) is a plant native to the southern parts of Iran. *Salvia hypoleuca* (known as "Boland Maryam-goli" in Persian) is a tall perennial plant that is widespread in Iran, in the northern and central provinces, particularly in Alborz, Tehran, and Mazandaran provinces. They have been used for years in the treatment of various diseases by local individuals living in the aforementioned regions.

2. Objectives

To the best of our knowledge, the α-glucosidase inhibitory activity of *S. mirzayanii* and *S. hypoleuca* EOs has not been reported in the literature, and this study aimed to identify the components of the EOs of the plants and evaluate their α-glucosidase inhibitory activity. An attempt was also made to study any synergistic or antagonistic interactions of selected main bioactive compounds on this enzyme.

3. Methods

3.1. Plant Material

The flowering aerial parts of the two *Salvia* species were collected from different parts of Iran (Fars and Alborz provinces), identified by a botanist, deposited in the Herbarium of the Faculty of Pharmacy, Tehran University of Medical Sciences, and dried in a dark place at room temperature.

3.2. Essential Oil Extraction

Essential oils were extracted by the hydrodistillation of each powdered dried plant (100 g) for 4 hours using the Clevenger apparatus (16). The water residue was removed by the anhydrous sodium sulfate, and EOs were stored in amber vials at refrigerator temperature (4°C).

3.3. Gas Chromatography-Mass Spectroscopy Analysis

Gas chromatography-mass spectroscopy (GC-MS) analysis was conducted on a 7890B Agilent® gas chromatograph, including a DB-5 column (60 cm, 0.25 μ) coupled with a 5977A Agilent® mass spectrometer. One μL of diluted samples (1:100, EO: ethyl acetate) was used for the corresponding analysis. The oven temperature was programmed to start at 40°C (held for 7 minutes), raised to 140°C by 10°C/minute, and then increased to 250°C at a rate of 3°C/minute, where it was kept for 7 minutes. Helium (99.99%) was used as a carrier gas (flow rate: 1 mL/min), and the ionization voltage of the detector was set at 70 eV. Normal alkanes (C_7 - C_{21}) were injected in the same

condition to compare calculated retention indices (RI) with references. For more precise identification, the mass spectra of each compound were compared to those in the National Institute of Standards and Technology (NIST) database (https://webbook.nist.gov/chemistry/ accessed 8 January 2022) and Dr. Adams' book (17).

3.4. Chemicals

Linalool, 1,8-cineole, α-terpineol, α-pinene, β-pinene, caryophyllene oxide, gallic acid, Folin-Ciocalteu reagent, α-glucosidase, *p*-nitrophenyl α-D-glucopyranoside, and buffers were obtained from Sigma-Aldrich (USA). Solvents and DPPH (2,2-diphenyl-1-picrylhydrazyl) were obtained from Merck (USA).

3.5. Alpha-Glucosidase Inhibitory Assay

The evaluation of α-glucosidase inhibitory activity of EOs and other samples was performed according to the literature (5). Briefly, all chemicals were pre-incubated in a water bath for 10 minutes at 37°C, and different concentrations of each sample were added to 155 μL of the 0.1 U/mL α-glucosidase solution prepared in a sodium phosphate buffer (50 mM). The mixture was then incubated for an additional 10 minutes at 37°C. Then, 25 μL of 4 mM *para*-nitrophenol-D-glucopyranoside (*p*NPG) solution was added to the mixture and re-incubated for 20 minutes at 37°C. The absorbance was read at 405 nm, each experiment was repeated three times, and acarbose was used as the positive control. Finally, based on the enzyme activity in the absence of the inhibitor (negative control), results were presented as inhibition percentage or IC_{50} values.

3.6. Antioxidant Activity: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) Assay

The DPPH assay was used to evaluate the anti-oxidative properties of the EOs with small modifications (18). For this purpose, 100 mg of each EO was diluted with methanol to achieve a concentration of 10 mg/mL. To obtain a 1 mg/mL concentration, 1 mL of the prepared solutions was diluted with 9 mL of methanol. Then, various concentrations were prepared using serial dilution (500, 250, 125, 62.5, and 31.25 μg/mL). Afterward, 2 mL of DPPH solution was added to 1 mL of each concentration of samples, and the mixtures were then placed in a dark room for 30 minutes. Methanol and quercetin were used as a blank and a reference, respectively. At the end, the absorbance of each sample was measured using a spectrophotometer at 517 nm. This procedure was replicated three times for each sample, and then IC_{50} values were calculated.

3.7. Determination of Total Phenolic Content

The Folin-Ciocalteu assay was used with some modifications to determine the TPC of each EO (19). To achieve a concentration of 1 mg/mL, 10 mg of each EO was diluted with methanol to the final volume of 10 mL. After preparation, 1.5 mL of diluted Folin-Ciocalteu reagent (1 mL of reagent in distilled water up to 10 mL) was added to 1 mL of the prepared EO solution with a concentration of 1 mg/mL and kept in a dark place for 10 minutes. Following that, 1.5 mL of sodium bicarbonate solution (7.5% w/v in water) was added to the initial mixture, and the final solution was kept in a dark place for 30 minutes. Finally, the absorbance of the samples was measured using a spectrophotometer at 765 nm. Gallic acid was used as the reference standard to create a calibration curve. For each sample, these procedures were repeated three times.

4. Results

4.1. Essential Oils Extraction Yield

The EOs extraction yields were calculated as 0.84 %w/w and 0.44 %w/w for *S. mirzayanii* and *S. hypoleuca*, respectively (Table 1).

4.2. Chemical Composition of Essential Oils

Salvia mirzayanii and *S. hypoleuca* EOs were analyzed using GC-MS, leading to the identification of 54 and 34 components for each EO, respectively, reported in Table 2.

4.3. Alpha-Glucosidase Inhibitory Activity

The α-glucosidase inhibitory activity of *S. mirzayanii* and *S. hypoleuca* EOs and 6 selected standard compounds, including linalool, α-terpineol, 1,8-cineole, caryophyllene oxide, α-pinene, and β-pinene, was reported in Table 3, compared to acarbose as the reference drug. The components were selected based on their abundance in the EOs of different *Salvia* spp. (20). According to the data in Table 3, *S. mirzayanii* EO could inhibit the enzyme with an IC_{50} value of 55.15 ± 1.60 mg/mL, and that of *S. hypoleuca* showed no activity. Among the tested constituents, α-pinene (IC_{50} = 17.59 ± 0.19 mg/mL) had the lowest IC_{50} value followed by caryophyllene oxide (19.94 ± 0.26 mg/mL), linalool (IC_{50} = 38.00 ± 0.22 mg/mL), 1,8-cineole (IC_{50} = 47.95 ± 0.23 mg/mL), and α-terpineol (IC_{50} = 122.10 ± 0.29 mg/mL).

Table 1. Collection Sites and Essential Oils Yields of *Salvia mirzayanii* and *S. hypoleuca*

Species	Collection Site	Collection Date	Voucher No.	Longitude	Latitude	Altitude (m)	Yield (w/w%)
***Salvia mirzayanii* Rech. f. and Esfand.**	Mazayjan, Darab, Fars province, Iran	May 2021	7112-TEH	53.80° E	30.29° N	850	0.84
***Salvia hypoleuca* Benth.**	Gateh Deh, Taleghan, Alborz province, Iran	April 2021	7075-TEH	51.06° E	36.17° N	2150	0.44

Table 3. Alpha-Glucosidase Inhibitory Activity of *Salvia mirzayanii* and *S. hypoleuca* Essential Oils and Components

Sample	IC_{50} (mg/mL)
***S. mirzayanii* EO**	55.15 ± 1.60
***S. hypoleuca* EO**	Not active
Linalool	38.00 ± 0.22
1,8-Cineole	47.95 ± 0.23
α-Terpineol	122.10 ± 0.29
α-Pinene	17.59 ± 0.19
β-Pinene	nd [a]
Caryophyllene oxide	19.94 ± 0.26
Acarbose	0.10 ± 0.00

Abbreviations: IC_{50}: half-maximal inhibitory concentration; EO: essential oil.
[a] Not determined due to precipitation in buffer moiety.

4.4. Antioxidant Activity and Total Phenolic Content

Antioxidant capacity based on the DPPH assay and TPC of the EOs were measured, and the results are shown in Table 4. *Salvia mirzayanii* EO showed higher antioxidant activity (IC_{50} = 0.778 ± 0.00 mg/mL) than *S. hypoleuca* EO (IC_{50} > 1 mg/mL). As expected, *S. mirzayanii* showed higher TPC (mg gallic acid equivalent [GAE]/g EO) than that of *S. hypoleuca* (Table 4).

Table 4. Antioxidant Activity and TPC1 of *Salvia mirzayanii* and *S. hypoleuca* Essential Oils

Sample	IC_{50} (DPPH Assay, mg/mL)	TPC (mg GAE/g EO)
S. mirzayanii	0.77 ± 0.00	78.26 ± 1.26
S. hypoleuca	> 1	49.43 ± 1.13
Quercetin	0.25 ± 0.00	-

Abbreviations: IC_{50}, half-maximal inhibitory concentration; EO, essential oil; GAE, gallic acid equivalent; TPC, total phenolic content.

5. Discussion

In the present study, *S. mirzayanii* EO mainly contained monoterpenes (50.39%); however, sesquiterpenes accounted for 39.91% of the constituents. It also consisted of linalool (9.97%), α-terpineol (8.33%), α-terpinyl acetate (8.15%), 1,8-cineole (5.2%), and thymol (4.36%). In the *S. hypoleuca* EO, sesquiterpenes and monoterpenes accounted for 68.4% and 26.83%, respectively. Among different classes of compounds, sesquiterpene hydrocarbons were observed to be the most abundant components (45.76%). Moreover, it mainly included *trans*-β-caryophyllene (14.12%), caryophyllene oxide (12.17%), α-pinene (9.04%), germacrene D (8.69%), and β-pinene (8.47%). Comparing the EOs components (Figure 1) revealed that the amounts of monoterpenes and phenolic compounds in *S. mirzayanii* are higher than those of *S. hypoleuca*; however, it contained higher amounts of sesquiterpene hydrocarbons. It merits mentioning that sesquiterpene hydrocarbons constituted the larger portion of sesquiterpenes in both EOs (Figure 1).

As reported in Table 5, the components of two EOs were compared to those documented in the literature. In a study reported by Hasheminya and Dehghannya, EO constituents of *S. mirzayanii* collected from Kerman, Iran, were identified, indicating the presence of monoterpene hydrocarbons (5.48%), and the main compounds also belonged to the oxygenated monoterpenes (34.34%) (21). In another study, the EO components of *S. mirzayanii* from Lamerd, Fars province, Iran, were classified as sesquiterpene hydrocarbons (26.10%), oxygenated monoterpenes (18.40%), oxygenated sesquiterpenes (11.00%), and monoterpene hydrocarbons (1.00%) (22). Furthermore, the results from the analysis of *S. mirzayanii* EO collected from Jahrom in Fars province, Iran, are in line with the data of the current study as oxygenated monoterpenes (56.50%) and monoterpene hydrocarbons (7.00%) were observed to be the highest and lowest abundant components (23).

According to the study of Nickavar et al., sesquiterpene hydrocarbons (44.90%) were identified as the most abundant components in the *S. hypoleuca* EO, collected from Haraz, Tehran province, Iran, in comparison to the other compounds (oxygenated sesquiterpenes [28.20%], hydrocarbon monoterpenes [15.50%], and oxygenated monoterpenes [3.40%]) (24). The EO components of *S. hypoleuca* were collected from Mazandaran province, Iran, in three different vegetative, flowering, and fruiting stages. More specifically, in the flowering stage, sesquiterpene hydrocarbons and oxygenated

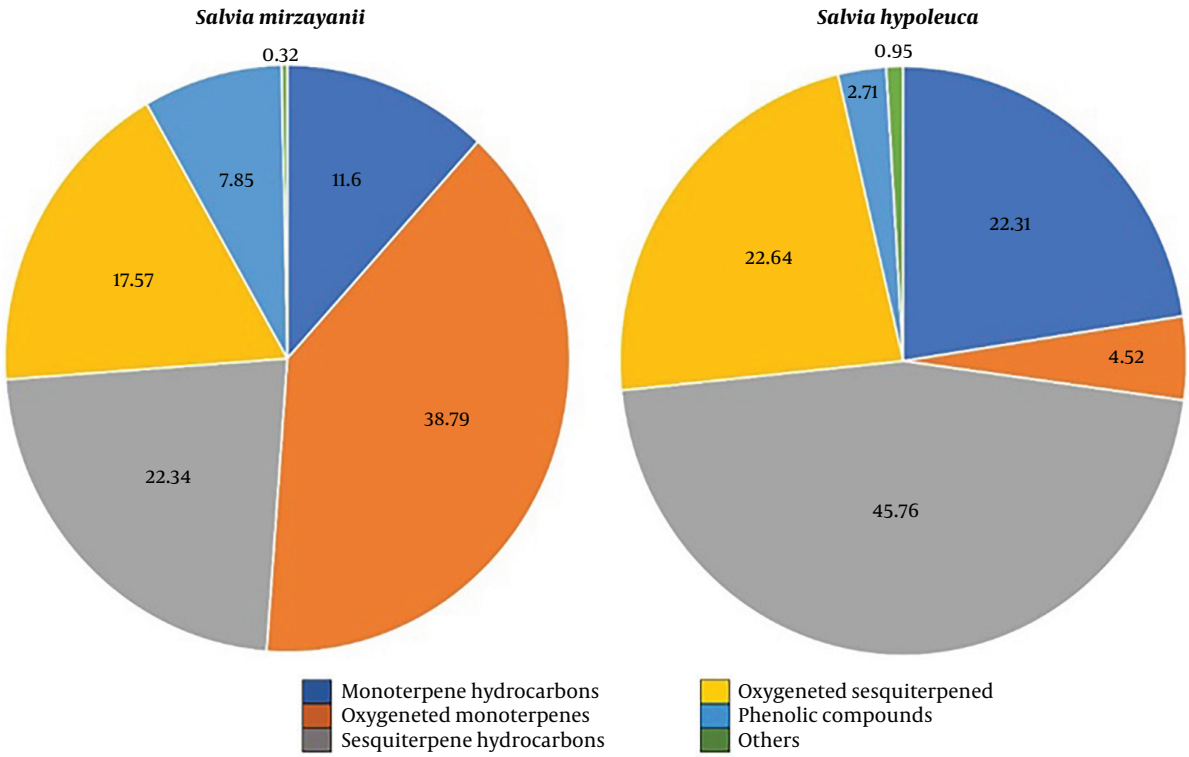

Figure 1. Classification of *Saliva mirzayanii* and *S. hypoleuca* EOs components

sesquiterpenes were the most abundant compounds, with 32.90% and 28.80%, respectively (25). In another study, the analysis of EOs components of 21 populations of *S. hypoleuca* collected from different sites of Alborz, Tehran, and Mazandaran provinces in Iran demonstrated that sesquiterpene hydrocarbons constituted 39.70%; nevertheless, oxygenated monoterpenes comprised 3.00% of the EO compounds of the sample collected from Alborz province, Chalos Road, Dizin, that are in alignment with two previous studies and the present study (26).

It is critical to identify efficient compounds in inducing desired biological activity. It is important to answer the question of whether major compounds are responsible for the biological activity (27) or whether a synergistic effect occurs. In this regard, the synergistic interactions of three major constituents of *S. mirzayanii* and *S. hypoleuca* EOs were investigated, as reported in Table 6. These combinations were provided based on their natural proportions in *Salvia* spp. EOs. The mixture of linalool, α-terpineol, and 1,8-cineole (40:35:25) exhibited α-glucosidase inhibitory activity with percentage inhibition of 72.5 ± 0.8; nonetheless, the mixture of caryophyllene oxide, α-pinene, and β-pinene (50:25:25) demonstrated a weaker inhibition (14.0 ± 1.8%).

The combination effect of components can be characterized by the combination index (CI), which is defined as:

$$CI = \frac{c_1}{c_{x,1}} + \frac{c_2}{c_{x,2}} + \frac{c_3}{c_{x,3}} + \ldots + \frac{c_n}{c_{x,n}}$$

Where $C_{x,n}$ is the concentration of the compound$_n$ alone that inhibits the enzyme (x%). C_n is the concentration of the compound$_n$ in combination with other compounds which inhibits the enzyme (x%). When the CI is equal to, less than, or greater than 1, the combination effect would be additive, synergistic, or antagonistic, respectively (28). According to the calculated CI values, the constituents

Table 5. Comparison of Major Components of Studied *Salvia* spp. to Those Reported in the Literature

Species	Major Compounds			Ref.
	In this Study	Literature	Region	
S. mirzayanii	Linalool (9.97%), α-Terpineol (8.33%), α-Terpinyl acetate (8.15%), 1,8-Cineole (5.20%), Thymol (4.36%)	1,8-Cineole (11.54%), Spathulenol (10.34%), α-Terpinyl acetate (10.32%), Bicyclogermacrene (6.34%), γ-Cadinene (5.67%), and Linalool (4.23%)	Kerman province, Iran	(21)
		γ-Cadinene (12.5%), Caryophyllene oxide (8.50%), Bicyclogermacrene (7.70%), α-Terpinyl acetate (6.70%), Linalool (3.6%), 1,8-Cineole (2.60%)	Lamerd, Fars province, Iran	(22)
		α-Terpinyl acetate (19.7%), Linalyl acetate (13.50%), Eudesm-7(11)-en-4-ol (9.10%), Linalool (7.40%), 1,8-Cineole (6.00%), δ-Cadinene (4.80), Bicyclogermacrene (4.70%), α-Terpineol (4.10%)	Jahrom, Fars province, Iran	(23)
S. hypoleuca	Trans-β-caryophyllene (14.12%), caryophyllene oxide (12.17%), α-pinene (9.04%), germacrene D (8.69%), β-pinene (8.47%)	Bicyclogermacrene (15.30%), trans- β-Caryophyllene (14.60%), Viridiflorol (13.30%), Spathulenol (12.50%), δ-Elemene (7.70%), β-Pinene (7.20%), α-Pinene (5.90%)	Haraz, Mazandaran province, Iran	(24)
		Caryophyllene oxide (21.30%), Bicyclogermacrene (10.30%), trans-β-Caryophyllene (13.00%), β-Pinene (9.80%), α-Pinene (9.70%)	Mazandaran province, Iran	(25)
		α-pinene (20.60%), β-pinene (19.70%), bicyclogermacrene (16.10%), trans-β-caryophyllene (11.50%), germacrene D (8.60%)	Alborz province, Iran	(26)

Table 6. Alpha-Glucosidase Inhibitory Activity of Mixtures of Essential Oils Components

Sample	Inhibition %	CI
Linalool: α-Terpineol: 1,8-Cineole (40: 35: 25) [a]	72.50 ± 0.80	0.09
Caryophyllene oxide: α-Pinene: β-Pinene (50: 25: 25) [a]	14.00 ± 1.80	40.62

Abbreviation: CI, combination index.
[a] Same as the ratio in the essential oils

of the first mixture seem to interact with one another synergistically (CI $<$ 1); in the meantime, the components of the second mixture have an antagonistic impact on one another (CI $>$ 1).

The *S. mirzayanii* EO was also observed to have higher antioxidant activity than the *S. hypoleuca* EO. To the best of our knowledge, no study reported the DPPH radical scavenging capacity of *S. mirzayanii* EO. However, in a study conducted in 2016, the antioxidant activity of *S. hypoleuca* EO at different growth stages was assessed by DPPH assay. The EO at the flowering stage showed the best radical scavenging capacity with IC_{50} = 25 mg/mL among the two other stages (positive control: Butylated hydroxytoluene [BHT] with an IC_{50} value of 0.4 mg/mL) (25). Various compounds, such as 1,8-cineole, α-pinene, camphor (29), carvacrol, and thymol (30), were reported to have antioxidant activity. Because the *S. mirzayanii* EO contains substantially more of the compounds than that of *S. hypoleuca*, it has shown greater antioxidant activity. Additionally, the results of the present study showed that *S. mirzayanii* EO has a higher phenolic content (TPC) (78.3 ± 1.3 mg GAE/g EO) than *S. hypoleuca* EO (49.4 ± 1.1 mg GAE/g EO) (Table 4). Total phenolic content and the antioxidant activity based on the DPPH assay were observed to be directly related, and the higher TPC of *S. mirzayanii* EO resulted in a lower DPPH IC_{50} value that can be explained by the fact that phenolic compounds neutralize free radicals by transferring the hydrogen of their hydroxyl group to them (31).

5.1. Conclusions

This study was designed to develop natural compounds for the treatment of type 2 diabetes based on their α-glucosidase inhibitory and antioxidant activities. In this study, *S. mirzayanii* and *S. hypoleuca* EOs were selected, leading to the desired activity of *S. mirzayanii*. Moreover, the evaluation of α-glucosidase inhibitory of selected pure components that have been identified in the corresponding EOs revealed that caryophyllene oxide, α-pinene, and linalool were more potent than others. It should be noted that the enzyme inhibitory activity of *S. mirzayanii* was observed to be induced through a synergistic effect. It seems that the *S. mirzayanii* EO can be considered a promising lead for the discovery of new natural anti-diabetic agents or supplements.

Acknowledgments

The authors would like to express their gratitude to all those who helped with this study.

Footnotes

Authors' Contribution: M. E. and H. J. G. prepared essential oils, contributed to the identification of chemical components of EOs, and wrote the manuscript. F. D. designed the project and wrote the manuscript. S. M. conducted the biological assay. S. K. contributed to the collection and identification of plants. M. S. supervised different steps and wrote the manuscript. M. A. performed GC analysis. M. A. F. supervised the biological assay. M. K. supervised all steps of the project. All the authors read and approved the submitted article.

Conflict of Interests: The authors declared no conflict of interest in this study.

Data Availability: The dataset presented in the study is available on request from the corresponding author during submission or after publication.

Ethical Approval: This study is approved under the ethical code of IR.TUMS.BLC.1401.003.

Funding/Support: This work was financially supported by the Tehran University of Medical Sciences under grant No. 1401-3-104-63710.

References

1. Khursheed R, Singh SK, Wadhwa S, Kapoor B, Gulati M, Kumar R, et al. Treatment strategies against diabetes: Success so far and challenges ahead. *Eur J Pharmacol*. 2019;**862**:172625. [PubMed ID: 31449807]. https://doi.org/10.1016/j.ejphar.2019.172625.
2. Aschner P, Karuranga S, James S, Simmons D, Basit A, Shaw JE, et al. The International Diabetes Federation's guide for diabetes epidemiological studies. *Diabetes Res Clin Pract*. 2021;**172**:108630. [PubMed ID: 33347900]. https://doi.org/10.1016/j.diabres.2020.108630.
3. Dash RP, Babu RJ, Srinivas NR. Reappraisal and perspectives of clinical drug-drug interaction potential of alpha-glucosidase inhibitors such as acarbose, voglibose and miglitol in the treatment of type 2 diabetes mellitus. *Xenobiotica*. 2018;**48**(1):89–108. [PubMed ID: 28010166]. https://doi.org/10.1080/00498254.2016.1275063.
4. Chen Y, Xiao Y, Lian G, Yi J, Liu X. Pneumatosis intestinalis associated with alpha-glucosidase inhibitors: a pharmacovigilance study of the FDA adverse event reporting system from 2004 to 2022. *Expert Opin Drug Saf*. 2023:1–10. [PubMed ID: 37929311]. https://doi.org/10.1080/14740338.2023.2278708.
5. Iraji A, Shareghi-Brojeni D, Mojtabavi S, Faramarzi MA, Akbarzadeh T, Saeedi M. Cyanoacetohydrazide linked to 1,2,3-triazole derivatives: a new class of alpha-glucosidase inhibitors. *Sci Rep*. 2022;**12**(1):8647. [PubMed ID: 35606520]. [PubMed Central ID: PMC9125976]. https://doi.org/10.1038/s41598-022-11771-y.
6. Sohrabi M, Binaeizadeh MR, Iraji A, Larijani B, Saeedi M, Mahdavi M. A review on alpha-glucosidase inhibitory activity of first row transition metal complexes: a futuristic strategy for treatment of type 2 diabetes. *RSC Adv*. 2022;**12**(19):12011–52. [PubMed ID: 35481063]. [PubMed Central ID: PMC9020348]. https://doi.org/10.1039/d2ra00067a.
7. Shareghi-Boroujeni D, Iraji A, Mojtabavi S, Faramarzi MA, Akbarzadeh T, Saeedi M. Synthesis, in vitro evaluation, and molecular docking studies of novel hydrazineylideneindolinone linked to phenoxymethyl-1,2,3-triazole derivatives as potential alpha-glucosidase inhibitors. *Bioorg Chem*. 2021;**111**:104869. [PubMed ID: 33839583]. https://doi.org/10.1016/j.bioorg.2021.104869.
8. Fattaheian-Dehkordi S, Hojjatifard R, Saeedi M, Khanavi M. A Review on Antidiabetic Activity of Centaurea spp.: A New Approach for Developing Herbal Remedies. *Evid Based Complement Alternat Med*. 2021;**2021**:5587938. [PubMed ID: 34285703]. [PubMed Central ID: PMC8275385]. https://doi.org/10.1155/2021/5587938.
9. Tousif MI, Tauseef S, Nabeelah S, Sharmeen J, Zengin G, Legoabe L, et al. Phenolics from endophytic fungi as natural α-glucosidase inhibitors: A comprehensive review. *J Mol Struct*. 2023;**1291**:135852. https://doi.org/10.1016/j.molstruc.2023.135852.
10. Rahimi-Madiseh M, Malekpour-Tehrani A, Bahmani M, Rafieian-Kopaei M. The research and development on the antioxidants in prevention of diabetic complications. *Asian Pac J Trop Med*. 2016;**9**(9):825–31. [PubMed ID: 27633293]. https://doi.org/10.1016/j.apjtm.2016.07.001.
11. Hou T, Sana SS, Li H, Xing Y, Nanda A, Netala VR, et al. Essential oils and its antibacterial, antifungal and anti-oxidant activity applications: A review. *Food Bioscience*. 2022;**47**:101716. https://doi.org/10.1016/j.fbio.2022.101716.
12. Jassbi AR, Zare S, Firuzi O, Xiao J. Bioactive phytochemicals from shoots and roots of Salvia species. *Phytochem Rev*. 2015;**15**(5):829–67. https://doi.org/10.1007/s11101-015-9427-z.
13. Askari SF, Avan R, Tayarani-Najaran Z, Sahebkar A, Eghbali S. Iranian Salvia species: A phytochemical and pharmacological update. *Phytochemistry*. 2021;**183**:112619. [PubMed ID: 33373790]. https://doi.org/10.1016/j.phytochem.2020.112619.
14. Raafat K, Habib J. Phytochemical Compositions and Antidiabetic Potentials of Salvia sclarea L. Essential Oils. *J Oleo Sci*. 2018;**67**(8):1015–25. [PubMed ID: 30012894]. https://doi.org/10.5650/jos.ess17187.
15. Assaggaf HM, Naceiri Mrabti H, Rajab BS, Attar AA, Alyamani RA, Hamed M, et al. Chemical Analysis and Investigation of Biological Effects of Salvia officinalis Essential Oils at Three Phenological Stages. *Molecules*. 2022;**27**(16). [PubMed ID: 36014393]. [PubMed Central ID: PMC9415112]. https://doi.org/10.3390/molecules27165157.
16. Dabaghian F, Azadi A, Zarshenas MM. Design, reformulation, and standardization of a traditional-based memory enhancer herbal preparation originated from Persian medicine. *J Med Plants*. 2022;**21**(82):93–110. https://doi.org/10.52547/jmp.21.82.93.
17. Adams RP. *Identification of essential oil components by gas chromatography/mass spectrometry*. 5th ed. Gruver, TX USA: Texensis Publishing; 2017.
18. Smeriglio A, Denaro M, Barreca D, Calderaro A, Bisignano C, Ginestra G, et al. In Vitro Evaluation of the Antioxidant, Cytoprotective, and Antimicrobial Properties of Essential Oil from Pistacia vera L. Variety Bronte Hull. *Int J Mol Sci*. 2017;**18**(6). [PubMed ID: 28587291]. [PubMed Central ID: PMC5486035]. https://doi.org/10.3390/ijms18061212.
19. Ahmed AF, Attia FA, Liu Z, Li C, Wei J, Kang W. Antioxidant activity and total phenolic content of essential oils and extracts of sweet basil (Ocimum basilicum L.) plants. *Food Sci Hum Wellness*. 2019;**8**(3):299–305. https://doi.org/10.1016/j.fshw.2019.07.004.
20. Gharehbagh HJ, Ebrahimi M, Dabaghian F, Mojtabavi S, Hariri R, Saeedi M, et al. Chemical composition, cholinesterase, and alpha-glucosidase inhibitory activity of the essential oils of some Iranian native Salvia species. *BMC Complement Med Ther*. 2023;**23**(1):184. [PubMed ID: 37270541]. [PubMed Central ID: PMC10239571]. https://doi.org/10.1186/s12906-023-04004-w.
21. Hasheminya SM, Dehghannya J. Development and characterization of novel edible films based on Cordia dichotoma gum incorporated with Salvia mirzayanii essential oil nanoemulsion. *Carbohydr Polym*. 2021;**257**:117606. [PubMed ID: 33541639]. https://doi.org/10.1016/j.carbpol.2020.117606.

22. Asadollahi M, Firuzi O, Heidary Jamebozorgi F, Alizadeh M, Jassbi AR. Ethnopharmacological studies, chemical composition, antibacterial and cytotoxic activities of essential oils of eleven Salvia in Iran. *J Herb Med*. 2019;**17-18**:100250. https://doi.org/10.1016/j.hermed.2018.11.006.
23. Ghasemi E, Sharafzadeh S, Amiri B, Alizadeh A, Bazrafshan F. Variation in Essential Oil Constituents and Antimicrobial Activity of the Flowering Aerial Parts of Salvia mirzayanii Rech. & Esfand. Ecotypes as a Folkloric Herbal Remedy in Southwestern Iran. *J Essent Oil-Bear Plants*. 2020;**23**(1):51–64. https://doi.org/10.1080/0972060x.2020.1727367.
24. Nickavar B, Mojab F, Asgarpanah J. Volatile composition of the essential oil of Benth. *Int J Aromather*. 2005;**15**(1):51–3. https://doi.org/10.1016/j.ijat.2004.09.010.
25. Habib K, Giti B, Ali M. Short Communication: Chemical composition and antioxidant activity of essential oil from Salvia hypoleuca at different growth stages. *Nus Biosci*. 1970;**8**(2). https://doi.org/10.13057/nusbiosci/n080203.
26. Sonboli A, Salehi P, Gharehnaghadeh S. Chemical variability in the essential oil composition of Salvia hypoleuca, an endemic species from Iran. *J Essent Oil Res*. 2016;**28**(5):421–7. https://doi.org/10.1080/10412905.2016.1150214.
27. Majouli K, Besbes Hlila M, Hamdi A, Flamini G, Ben Jannet H, Kenani A. Antioxidant activity and α-glucosidase inhibition by essential oils from Hertia cheirifolia (L.). *Ind Crops Prod*. 2016;**82**:23–8. https://doi.org/10.1016/j.indcrop.2015.12.015.
28. Long F, Yang H, Xu Y, Hao H, Li P. A strategy for the identification of combinatorial bioactive compounds contributing to the holistic effect of herbal medicines. *Sci Rep*. 2015;**5**:12361. [PubMed ID: 26198093]. [PubMed Central ID: PMC4510521]. https://doi.org/10.1038/srep12361.
29. Ruberto G, Baratta MT. Antioxidant activity of selected essential oil components in two lipid model systems. *Food Chemistry*. 2000;**69**(2):167–74. https://doi.org/10.1016/s0308-8146(99)00247-2.
30. Tenore GC, Ciampaglia R, Arnold NA, Piozzi F, Napolitano F, Rigano D, et al. Antimicrobial and antioxidant properties of the essential oil of Salvia lanigera from Cyprus. *Food Chem Toxicol*. 2011;**49**(1):238–43. [PubMed ID: 20977923]. https://doi.org/10.1016/j.fct.2010.10.022.
31. Foti MC. Antioxidant properties of phenols. *J Pharm Pharmacol*. 2007;**59**(12):1673–85. [PubMed ID: 18053330]. https://doi.org/10.1211/jpp.59.12.0010.

Table 2. Chemical Composition of *Salvia mirzayanii* and *S. hypoleuca* Essential Oils

Number	Compounds	RI [a]	RI [b]	% Relative Peak Area	Class	
				S. mirzayanii	*S. hypoleuca*	
1	α-Pinene	934	934	3.74	9.04	MH
2	Camphene	952	952	-	1.63	MH
3	Sabinene	973	973	0.35	2.06	MH
4	β-Pinene	981	981	3.25	8.47	MH
5	β-Myrcene	986	986	1.31	-	MH
6	α-Terpinene	1018	1015	-	0.38	MH
7	Limonene	1032	1029	1.13	0.42	MH
8	1,8-Cineole	1037	1034	5.20	1.14	OM
9	cis-β-Ocimene	1043	1040	0.62	-	MH
10	γ-Terpinene	1060	1057	0.65	0.31	MH
11	Terpinolene	1090	1088	0.55	-	MH
12	Linalool	1099	1097	9.97	1.64	OM
13	trans-Pinocarveol	1151	1146	-	0.29	OM
14	δ-Terpineol	1179	1170	0.49	-	OM
15	Borneol	1185	1176	-	0.60	OM
16	Terpinen-4-ol	1191	1184	-	0.62	OM
17	α-Terpineol	1205	1198	8.33	0.23	OM
18	Myrtenol	1209	1202	0.14	-	OM
19	Nerol	1228	1221	0.89	-	OM
20	Geraniol	1249	1246	2.28	-	OM
21	Linalyl acetate	1254	1249	1.43	-	OM
22	Decanol	1270	1269	0.32	-	Alcohol
23	Thymol	1290	1286	4.36	1.53	PC
24	Carvacrol	1296	1287	2.89	1.18	PC
25	δ-Elemene	1346	1338	-	3.20	SH
26	α-Terpinyl acetate	1353	1344	8.15	-	OM
27	Eugenol	1359	1351	0.60	-	PC
28	Neryl acetate	1371	1362	1.91	-	OM
29	α-Copaene	1389	1377	0.72	1.77	SH
30	β-Bourbonene	1403	1388	-	1.60	SH
31	β-Elemene	1404	1391	1.27	-	SH
32	α-Gurjunene	1428	1413	1.33	-	SH
33	trans-β-Caryophyllene	1441	1430	1.22	14.12	SH
34	β-Gurjunene	1451	1436	-	0.39	SH
35	α-Guaiene	1459	1441	1.15	-	SH
36	Aromandendrene	1460	1445	1.22	-	SH
37	cis-Muurola-3,5-diene	1467	1450	0.37	-	SH
38	α-Humulene	1476	1462	0.39	1.91	SH
39	9-epi-Caryophyllene	1482	1466	-	3.93	SH
40	Cadina-1(6),4-diene	1488	1477	0.47	-	SH
41	γ-Muurolene	1493	1479	1.05	0.92	SH
42	Germacrene D	1501	1485	0.47	8.69	SH
43	β-Selinene	1503	1490	0.93	0.68	SH

Continued on next page

Table 2. Chemical Composition of *Salvia mirzayanii* and *S. hypoleuca* Essential Oils (Continued)

44	δ-Selinene	1509	1493	1.11	-	SH
45	α-Muurolene	1517	1500	2.85	-	SH
46	Bicyclogermacrene	1521	1500	2.19	7.46	SH
47	γ-Cadinene	1528	1514	1.29	1.09	SH
48	δ-Cadinene	1532	1523	3.67	-	SH
49	Liguloxide	1549	1536	0.33	-	OS
50	α-Cadinene	1551	1539	0.64	-	SH
51	Ledol	1590	1574	0.35	-	OS
52	Germacrene D-4-ol	1596	1576	2.19	-	OS
53	Spathulenol	1597	1578	3.17	6.11	OS
54	Caryophyllene oxide	1605	1584	0.40	12.17	OS
55	Viridiflorol	1613	1593	0.51	0.39	OS
56	α-epi-7-epi-5-Eudesmol	1625	1608	0.29	-	OS
57	1,10-di-epi-Cubenol	1638	1619	0.30	-	OS
58	epi-Cubenol	1644	1627	0.75	-	OS
59	Isospathulenol	1645	1629	-	1.48	OS
60	Eremoligenol	1650	1631	0.39	-	OS
61	epi-α -Muurolol	1660	1642	0.93	-	OS
62	β-Eudesmol	1674	1651	1.32	-	OS
63	α-Cadinol	1676	1654	3.77	0.59	OS
64	Shyobunol	1717	1691	2.87	-	OS
65	Sclareoloxide	1915	1906	-	1.90	OS
66	Phytol	2100	2089	-	0.95	OD
Monoterpenes	50.39	26.83				
Monoterpene hydrocarbons	11.60	22.31	-			
Oxygenated monoterpenes	38.79	4.52	-			
Sesquiterpenes	39.91	68.40				
Sesquiterpene hydrocarbons	22.34	45.76	-			
Oxygenated sesquiterpenes	17.57	22.64	-			
Alcohol	0.32	-	-			
Phenolic compounds	7.85	2.71	-			
Oxygenated diterpene	-	0.95				
Total identified	98.47	98.89	-			

Abbreviations: MH, monoterpene hydrocarbon; OM, oxygenated monoterpene; SH, sesquiterpene hydrocarbon; OS, oxygenated sesquiterpene; PC, phenolic compound; OD, oxygenated diterpene.

[a] Retention index (calculated)

[b] Retention index from literature