

Metabolic profiling and inhibitory properties of different parts of *Salsola vermiculata* against acetylcholinesterase and α -glucosidase

Saeed Mollaei¹, Poopak Farnia^{*2}, Jalaledin Ghanavi^{*2}, Mohammad Majidi³

¹ Department of Chemistry, Faculty of Sciences, Azarbaijan Shahid Madani University, Tabriz, Iran.

² Mycobacteriology Research Centre (MRC), National Research Institute of Tuberculosis and Lung Disease (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.

³ Department of Biotechnology, Faculty of Agriculture, Azarbaijan Shahid Madani University, Tabriz, Iran.

ABSTRACT: Herbal plants play significant roles in the treatment of diseases and development of novel drugs. *Salsola vermiculata* is an annual plant which is broadly distributed in the southwest Asia, and used for the treatment of stomach disorders. This present study aimed at identifying and comparing the metabolic profiles of different parts of *S. vermiculata* and evaluating the inhibitory potential of their extracts and fractions against acetylcholinesterase and α -glucosidase. LC-ESI-MS, GC, and GC-MS analytical methods were employed for metabolite profiling of the extracts, and their fractions. The inhibitory activities were determined by microplate reader-based colorimetric methods. 44 metabolites were identified in different parts of *S. vermiculata*. In roots, vanillic acid, rutin, salsoline, salsoline A, palmitic acid, oleic acid, linoleic acid, cumin aldehyde, and carvone; in seeds, vanillic acid, salsoline A, palmitic acid, oleic acid, linoleic acid, carvone, and β -caryophyllene; in leaves, gallic acid, vanillic acid, caffeic acid, rosmarinic acid, rutin, quercetin, limonene, and carvone, and in flowers, gallic acid, vanillic acid, cinnamic acid, rosmarinic acid, rutin, kaempferol, limonene, linalool, and carvone were reported as major components. According to the inhibitory activities results, the ethyl acetate fractions of leaves and the aqueous-acid fraction of roots displayed the highest inhibitory activity against acetylcholinesterase (IC₅₀: 17.24 μ g/mL), and α -glucosidase (IC₅₀: 62.37 μ g/mL), respectively. Finally, the leaves and roots of *S. vermiculata* are rich of phenolic and alkaloid compounds and the findings of this study describe them as a promising acetylcholinesterase and α -glucosidase inhibitors, and therefore, can be utilized for the development of new drugs.

KEYWORDS: Alkaloid, Biological activity, Fatty acids, Phenolic compounds, Terpenoids.

INTRODUCTION

Herbal medicines play a significant role in the treatment of diseases and the development of novel drugs. Therefore, searching for plants with specific biological activity will be an interesting field of knowledge. *Salsola* is a genus belonging to the family of Amaranthaceae, which is native to Asia, Europe, and Africa. This genus is widespread across hypersaline, semiarid, and arid areas [1-3]. Many plants in this genus are used to cure skin diseases, human heart ailments, influenza, and cough. In

addition, various species of the genus *Salsola* have displayed medicinal properties such as controlling obesity, diabetes, and Alzheimer's disease, functioning as anticancer, anti-inflammatory, antibacterial, antihypertensive, and antioxidant agents, CNC depressant activity, and being the cure for tape worm infestation [4-7].

A number of natural compounds with interesting biological properties have been previously isolated from different *Salsola* species. Shehab and Abu-Gharbieh [8]

*Corresponding authors (✉):

Poopak Farnia: p.farnia@sbmu.ac.ir

Jalaledin Ghanavi: j.ghanavi@sbmu.ac.ir

Received: 15 December 2021/ Revised: 14 April 2022

Accepted: 30 April 2022

studied the methanolic extract of *S. imbricata* and identified coumaric acid and quercitrin as the main detected compounds. Boulaaba et al. [9] concluded that the methanolic extracts of *S. kali* consist of seven phenolic compounds, and that the stems and leaves of *S. kali* have antioxidant and antimicrobial properties. In another study, the aerial part of *S. vermiculata* was shown to be more enriched in rutin and kaempferol derivatives versus root samples [10]. Hegnauer reported some isoquinoline alkaloids such as salsoline and salsolidin from *S. kali* L. [11]. Orekhoff and Proskurnina [12, 13] recognized salsoline and salsolidine from *S. arbuscula*. Furthermore, according to the findings of Tundis et al. [3], salsoline and salsolidine were the major alkaloids found in *S. soda*, *S. oppositifolia*, and *S. tragus* species, and these compounds were responsible for the anticholinesterase and antioxidant activities. *S. vermiculata* are annual plants that are broadly distributed in the southwest of Asia and belong to the Amaranthaceae family [3]. This plant is used for the treatment of stomach disorders [14]. Rasheed et al. [10] investigated the metabolite profile of *S. vermiculata* and identified several flavonoids, hydroxycinnamic acids, fatty acids, and alkaloids. In addition, the roots of *S. vermiculata* exhibited strong anti-acetylcholinesterase activity. Al-Tohamy et al. [15] discovered that *S. vermiculata* methanolic extract has strong antioxidant and antimicrobial properties. Due to the lack of data regarding the phytochemical composition and biological properties of different parts of *S. vermiculata*, the present research focused on phytochemical study and biological activities (α -glucosidase inhibitory and acetylcholinesterase enzyme activity) of different parts (roots, seeds, leaves, and flowers) of *S. vermiculata*.

MATERIALS AND METHODS

Chemicals

Solvents and chemicals used for the extraction and biological tests were purchased from Merck, German. All standards were developed from Sigma Aldrich, which was >95% pure. Also, the solvents used in the HPLC were grade HPLC.

Plant materials

The plant was collected from its wild habitat in Azarshahr, Tabriz, Iran, in October 2017 according to the appropriate guidelines and licences for plant material.

The GPS coordinates were 45°10'E and 37°06'N, with an altitude of 1240m above sea level. The identification of the plant was done by Dr. Mostafa Ebadi from the Department of Biology, Faculty of Science, Azarbaijan Shahid Madani University. Also, a voucher specimen (ASMUH-10485) was deposited at the official Azarbaijan Shahid Madani University. The *S. vermiculata* parts were separated from each other, then dried at room temperature in darkness for 7 days and powdered.

Fractionation of crude extract

plant material (1 g) was extracted with methanol (10 mL, 80% v/v) at 25°C. After centrifugation, the supernatant was concentrated and suspended in distilled water (10 mL). The obtained extract was extracted with n-hexane (1:1 v/v). The n-hexane layer was distilled using the Clevenger apparatus, and two fractions, including volatile oils (fraction 1) and fixed oils (fraction 2), were achieved. Then, the aqueous phase was adjusted to pH=4.0 using HCl, and partitioned with ethyl acetate (1:1 v/v). The aqueous-acid phase (fraction 3), and ethyl acetate phase (fraction 4) were separated, and then they were dried under nitrogen at room temperature and transferred to the vials for further analysis (Fig. 1).

GC and GC-MS analysis of compounds

The analysis of volatile oils was performed by a GC-MS instrument (Agilent 6890 N GC-MS) equipped with a fused capillary column DB-5 (30 m × 0.25 mm, 0.25 μ m film thickness). The injector temperature was adjusted to 250 °C, and the oven temperature program was set at 70 °C (5 min) to 240 °C with a ramp-up of 5 °C/min and then held for 4 min. Nitrogen was used as a carrier gas (1.0 mL/min). The splitting ratio, ionization voltage, solvent delay, scan time, and mass range were 1:100, 70 eV, 2 min, 0.4 s, and 30-600 m/z, respectively. The identification of volatile oil components was done using Kovats Indices (KI), Wiley and NIST libraries, and previous literature. Electronic integration of FID peak regions without correction factors was used to calculate the % of each compound [16].

The analysis of fixed oils was performed using a GC instrument. After methylation of fatty acids according to our previous study [17], they were analyzed using a GC instrument, equipped with a capillary column ZB-1701 (60 m × 0.25 mm, 0.2 μ m film thickness). The injector temperature was adjusted to 260 °C, and the oven

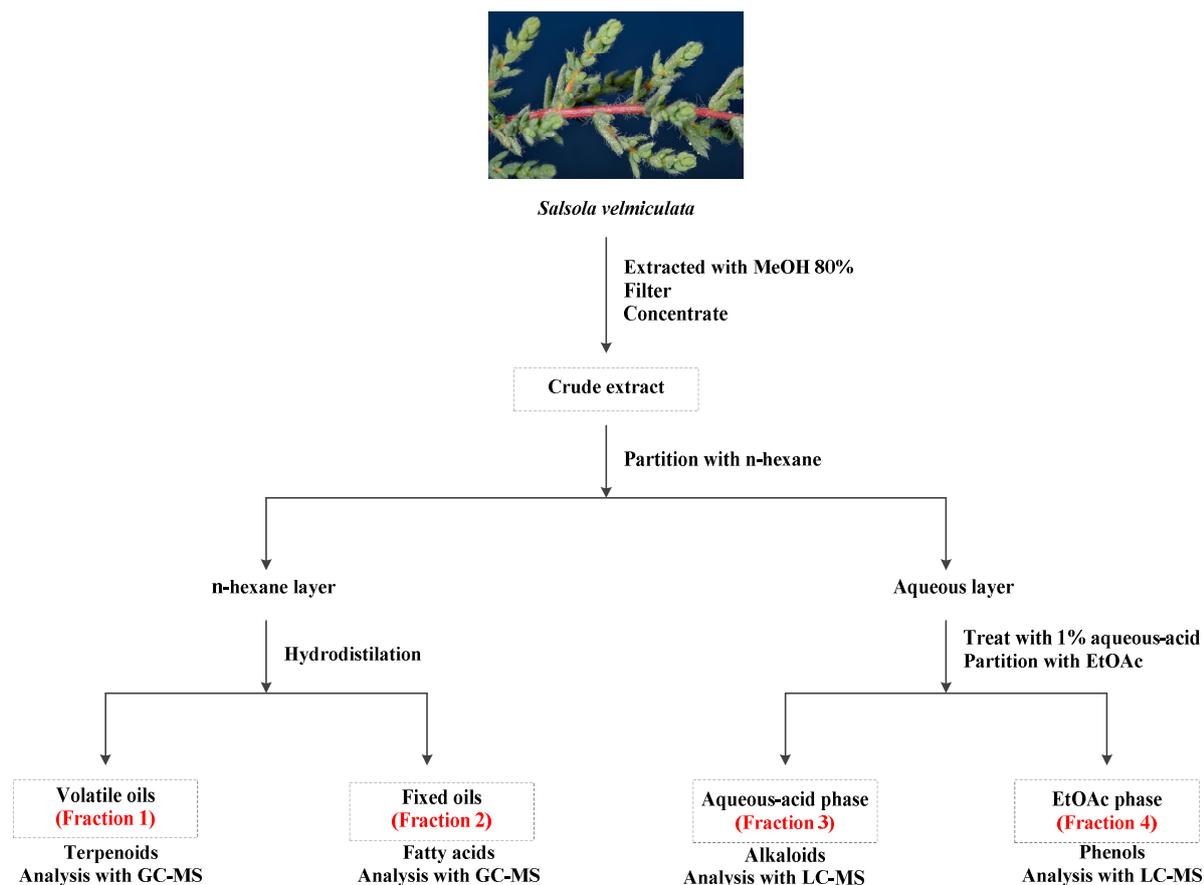


Figure 1. Schematic diagram of extraction and fractionation of *Salsola vermiculata* crude extract into different fractions.

temperature program was set at 80 °C to 120°C with a ramp-up of 20°C/min and then increased to 260°C by a ramp of 3°C/min and held for 4 min. Nitrogen was used as a carrier gas (1.1 mL/min). The injection volume and splitting ratio were 1 µL and 1:20, respectively.

LC-ESI-MS analysis of compounds

Fractions 3 and 4 were analyzed using liquid chromatography coupled to electrospray ionization mass spectrometry (LC-ESI-MS). The LC (Agilent 1200 Series HPLC system) system was equipped with a diode-array detector, a 20 µL loop, and a C₁₈ analytical column (250 mm × 0.46 mm, 5 µm). Separation of these fractions was performed by a gradient program run using solvent A (0.1% TFA in methanol) and solvent B (0.1% TFA in water, v/v). The solvent program was as follows: gradient elution from 20% A to 30% A, 0-10 min; gradient elution from 30% A to 60%, 10-30 for min; gradient elution from 60% A to 80% A, for 30-40 min; gradient elution from 80% A to 100% A, for 40-45 min; gradient elution from 100% A to 20% A, 45-52 min; isocratic elution of 20% A; post-time 6 min before the next injection. The flow rate

was maintained at 0.4 mL/min and the wavelength was adjusted at 275 and 254 nm, for fractions 3 and 4, respectively. The MS system included a Thermo Fisher Scientific (Bremen, Germany) ion trap mass spectrometer (model LCQ). Mass spectra were detected under the ESI negative ion mode the between m/z range of 50-1000 under a capillary voltage of -2.0 KV and a skimmer cone voltage of -20 V. The compounds were identified by comparing their retention times with those of the standards and mass spectra. Finally, quantification of all compounds was accomplished using the external standard method.

α-glucosidase inhibitory activity in vitro

The α-glucosidase inhibitory activity of the extracts was studied according to the Pistia-Brueggeman and Hollingsworth [18] method with some modifications. For 30 min at 37 °C, the extracts were incubated with 20 µL of enzyme solution containing α-glucosidase (0.5 U/mL) and 120 µL of phosphate buffer (0.1 M, pH =6.8). Then, 20 µL of p-nitrophenyl-α-D-glucopyranoside (5 mM, pH =6.8) was added, and incubated for 15 min at 37 °C. The

reaction was terminated by the addition of 80 μL of 0.2 M Na_2CO_3 solution. Finally, the absorbance was read at 405 nm and the inhibition (%) was calculated according to below equation:

$$\text{Inhibition (\%)} = \frac{A(\text{control}) - A(\text{sample})}{A(\text{control})} \times 100$$

***In vitro* acetylcholinesterase inhibitory assay**

The Ellman's method, with slight modifications, was utilized to evaluate the AChE inhibitory activity. Different amounts of the extracts were dissolved in phosphate buffer (0.1 M, pH =8), and then 25 μL of acetylthiocholine (ATCh) (1.0 mM) and 50 μL of 10 mM of DTNB in buffer were added, and the mixture was incubated for 15 min at 30 °C. Then, 50 μL of 0.3 U/mL AChE was added, to the initial mixture to start the reaction. Finally, absorbance was read at 412 nm, and the inhibition of ATCh activity (%) was calculated.

Statistical analysis

All tests were performed in triplicate. The analysis was done by SAS 9.2 using a completely randomized design (1-way ANOVA), and the mean comparisons were determined by Tukey's test ($p < 0.05$).

RESULTS

Metabolite profiling of crude methanolic extracts and fractions from different parts of *S. vermiculata* was studied, followed by acetylcholinesterase and α -glucosidase inhibitory activities.

Metabolite Profiling

In this study, 44 different metabolites were identified in different parts of *S. vermiculata* using LC-ESI-MS, GC, and GC-MS (Table 1, 2 and 3). The identified metabolites belonged to various classes, including phenolic compounds, alkaloids, fatty acid derivatives, and volatile oil compounds. Isolation and identification of alkaloids and phenolic compounds were done using LC-ESI-MS, whereas fatty acids and volatile oils were identified by GC and GC-MS.

In total, 14 phenolic compounds were detected in ethyl acetate fractions (Table 1) (Fig. 2). A total of ten phenolic acids were identified in the extracts, of which three phenolic acids including protocatechuic, cinnamic, and ferulic acids, were exclusively present in the ethyl acetate

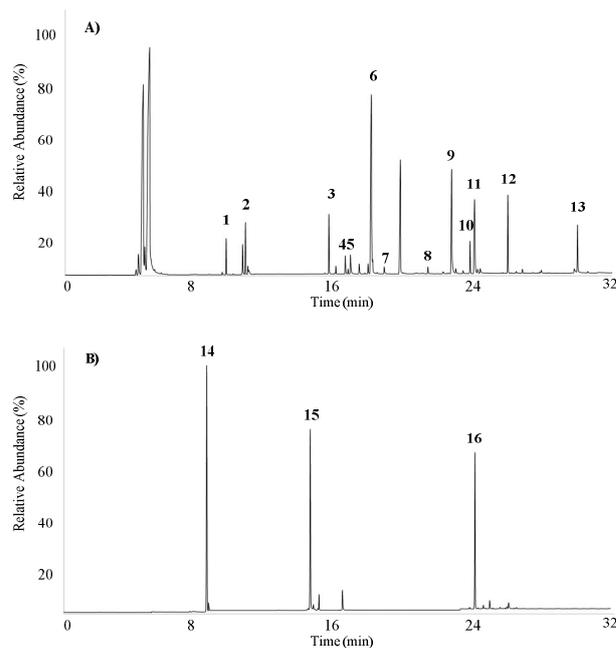


Figure 2. LC-MS chromatograms of A) Ethyl acetate fraction of leaves; B) Aqueous-acid fraction of roots. Peak identification: (1) gallic acid, (2) rutin, (3) protocatechuic acid, (4) cyanidin, (5) *p*-Hydroxybenzoic acid, (6) kaempferol, (7) vanillic acid, (8) quercetin, (9) rosmarinic acid, (10) *p* coumaric acid, (11) ferulic acid, (12) salicylic acid, (13) cinnamic acid, (14) salsoline A, (15) salsoline, and (16) salsolidine.

fraction of flowers; one phenolic acid, caffeic acid, was only identified in the leaf extract; and gallic and salicylic acids, were observed in both the leaf and flower extracts. In addition, vanillic acid was identified in all extracts. Three flavonoids (i.e. rutin, kaempferol, and quercetin), and one anthocyanin (i.e. cyanidin) were detected in leaf and flower parts, followed by two flavonoids (i.e. rutin and kaempferol), one anthocyanin (i.e. cyanidin) and one flavonoid (i.e. kaempferol) in the seeds. Moreover, the maximum identified phenolic compounds belonged to rosmarinic acid, gallic acid, kaempferol, and rutin in the flowers. So far, several phenolic compounds have been reported from different species of *Salsola*. Shehab and Abu-Gharbieh [8] reported a number of phenolic compounds in a methanolic extract of *S. imbricata*, categorized as flavonoids, phenolic acids, and simple phenolic compounds, while coumaric acid and quercitrin were the main detected compounds. Boulaaba et al. [9] analyzed the methanolic extracts of *S. kali* and concluded that this plant contains seven phenolic compounds. In another study, the aerial part of *S. vermiculata* was shown to be more enriched in rutin and kaempferol derivatives than root samples. Therefore, ethyl acetate fractions of the

Table 1. Phenolic profiling of *Salsola vermiculata* parts using LC-ESI-MS.

Fraction	Subgroup	Compounds	Parts of plant			
			Root	Seed	Leaf	Flower
Ethyl acetate	Phenolic acids	Gallic acid	N.D	N.D	116.1	614.7
		Protocatechuic acid	N.D	N.D	N.D	254.1
		<i>p</i> -Hydroxybenzoic acid	N.D	46.8	53.9	43.6
		Vanillic acid	131.4	62.7	168.9	74.9
		Caffeic acid	N.D	N.D	167.3	N.D
		<i>p</i> -Coumaric acid	N.D	51.3	98.7	194.3
		Ferulic acid	N.D	N.D	N.D	28.4
		Cinnamic acid	N.D	N.D	N.D	412.9
		Rosmaric acid	N.D	49.7	254.9	647.8
		Salicylic acid	N.D	N.D	72.4	201.4
	Flavonoids	Rutin	108.5	N.D	429.7	448.8
		Quercetin	N.D	N.D	267.7	194.2
		Kaempferol	54.2	46.4	147.1	574.9
		Antocyanidine	Cyanidin	N.D	N.D	119.7

The amounts of phenolic compounds were expressed in $\mu\text{g/g}$ of dry weight.

Table 2. Alkaloids profiling of *Salsola vermiculata* parts using LC-ESI-MS.

Fraction	Subgroup	Compounds	Parts of plant			
			Root	Seed	Leaf	Flower
Aqueous-acid	Isoquinoline	Salsoline	29.4	N.D	N.D	N.D
		Salsolidine	12.7	N.D	N.D	N.D
		Salsoline A	49.8	19.9	N.D	N.D

The amounts of alkaloids were expressed in mg/g of dry weight.

leaves and flowers which are rich in phenolic compounds can be considered as appropriate candidates for some biological properties [19-22].

According to the analysis of the aqueous-acid fractions by LC-ESI-MS (Fig. 2), three alkaloids, including salsoline, salsoline A, and salsolidine were observed in the root extract, and only one alkaloid, salsoline A, was detected in the seed extract (Table 2). These compounds were previously isolated from different *Salsola* species. *S. collina* Pall. contains isoquinoline alkaloids such as salsoline A, and salsoline B [23]. In 1964, Hegnauer reported salsolin and salsolidin from the herb *S. kali* L. [11]. In another study, Orekhoff and Proskurnina [12, 13] identified salsoline and salsolidine from *S. arbuscula*. In addition, according to the report by Tundis et al. [3], salsoline and salsolidine were the main alkaloids found in *S. tragus*, *S. oppositifolia*, and *S. soda*, and these alkaloids were responsible for the anticholinesterase activities. As a result, the aqueous-acid fractions of roots and seeds can be considered as suitable candidates for some biological properties, such as antimalarial effects.

According to our results (Table 3), palmitic, stearic, linoleic, oleic, and linolenic acids were the dominating fatty acids in the root samples. A predominance of palmitic, linoleic, and oleic acids was observed in the fixed oils fraction of seeds. 9-Hexadecenoic, arachidic, and docosadienoic acids were detected exclusively in the seeds. Similarly, myristoleic, and lignoceric acids were only present in roots. Moreover, according to the obtained results, the fixed oil fraction of leaves contained only three fatty acids, including stearic, oleic and linolenic acids. Similarly, only two fatty acids, oleic and linolenic acid, were identified in the flower fraction. Therefore, it can be concluded that the composition of fatty acids significantly depends on the plant parts. Thanks to the unique properties of fatty acids, the parts possessing these compounds might be potentially applied as a promising source of biological agents.

The analysis of volatile compounds in various parts of *S. vermiculata* showed significant variations in their types and percentages (Table 4). The main constituents of the volatile compounds in different parts were carvone,

Table 3. Fixed oils profiling of *Salsola vermiculata* parts using GC.

Fraction	Subgroup	Compounds	Parts of plant			
			Root	Seed	leaf	Flower
Fixed oils	Saturated	Myristic acid (C14:0)	0.5	1.2	N.D	N.D
		Palmitic acid (C16:0)	42.1	36.9	N.D	N.D
		Stearic acid (C18:0)	4.2	1.3	0.9	N.D
		Arachidic acid (C20:0)	N.D	0.6	N.D	N.D
		Lignoceric acid (C24:0)	0.7	N.D	N.D	N.D
	Unsaturated	Myristoleic acid (C14:1)	1.2	N.D	N.D	N.D
		9-Hexadecenoic acid (C16:1)	N.D	1.6	N.D	N.D
		Oleic acid (C18:1n9c)	16.7	13.3	7.4	2.4
		Linoleic acid (C18:2n6c)	22.2	17.6	N.D	N.D
		Linolenic acid (C18:3n6)	8.6	1.6	0.6	2.8
		Docosadienoic acid (C22:2)	N.D	0.8	N.D	N.D

The amounts of fatty acids were expressed as percentages.

Table 4. Volatile oils profiling of *Salsola vermiculata* parts using GC-MS.

Fraction	Subgroup	Compounds	Parts of plant			
			Root	Seed	leaf	Flower
Volatile oil	Monoterpenes	α -pinene	0.9	N.D	1.2	1.4
		Limonene	N.D	3.6	11.5	14.4
		1,8-cineole	0.7	0.6	0.9	0.7
	Oxygenated monoterpenes	Linalool	7.6	5.6	7.2	12.9
		Isoborneol	N.D	N.D	N.D	1.4
		Cumin aldehyde	12.9	5.9	5.2	8.7
		Carvone	52.6	59.9	48.6	51.7
	Phenylpropene	(E)-anethole	0.9	N.D	1.6	N.D
	Sesquiterpene	β -caryophyllene	9.4	14.3	4.4	5.0
		δ -cadinene	1.7	N.D	1.5	N.D
		Caryophyllene oxide	1.4	-	0.9	N.D
	Oxygenated sesquiterpene	T-cadinol	0.5	1.1	1.6	N.D
		T-muurolol	1.1	2.4	2.2	N.D
		α -muurolol	0.7	0.9	0.8	N.D
		Ar-turmerone	2.4	1.4	3.1	N.D
		hexahydrofarnesylacetone	3.9	N.D	4.2	N.D

The amounts of volatile oils were expressed as percentages.

cumin aldehyde, β -caryophyllene, linalool, hexahydrofarnesyl acetone, and Ar-turmerone at the roots. Carvone, β -caryophyllene, cumin aldehyde, linalool, limonene, and T-muurolol were the major constituents of the volatile compounds in the seeds. In the leaves, values were as follows: carvone, limonene, linalool, cumin aldehyde, β -caryophyllene, hexahydrofarnesyl acetone, and Ar-turmerone. Lastly, in the flowers, carvone (51.7%), limonene (14.4%), linalool (12.9%), and cumin aldehyde

(8.7%) were measured as the predominant volatile constituents (Table 2).

The volatile compounds were classified into five groups according to their chemical formula. Based on the results, the major percentage of the volatile compounds was related to oxygenated mono-terpenes. So, because of the biological activity of volatile oil [24], the parts possessing these compounds might be potentially applied as a promising source of biological agents.

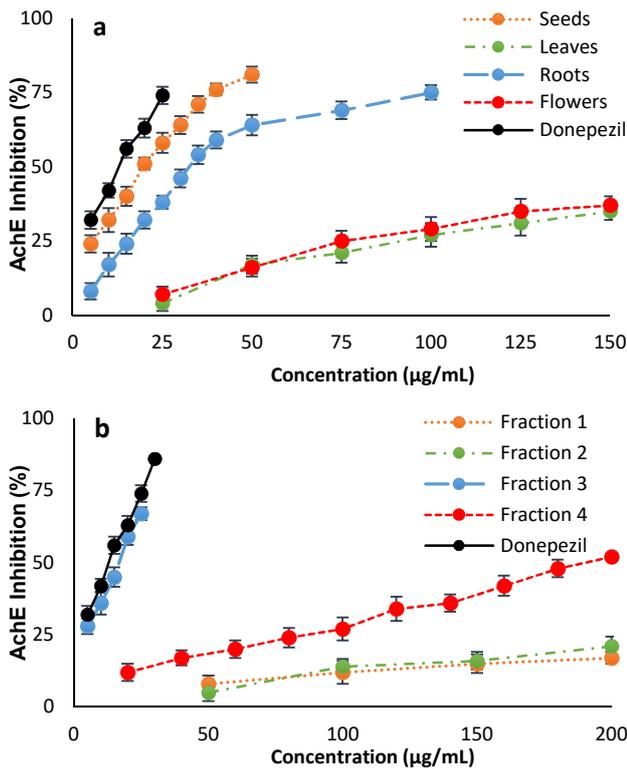


Figure 3. *In-vitro* anti-acetylcholinesterase activity of a) methanolic extracts of different parts of *S. vermiculata*; b) the different fractions of *S. vermiculata* roots.

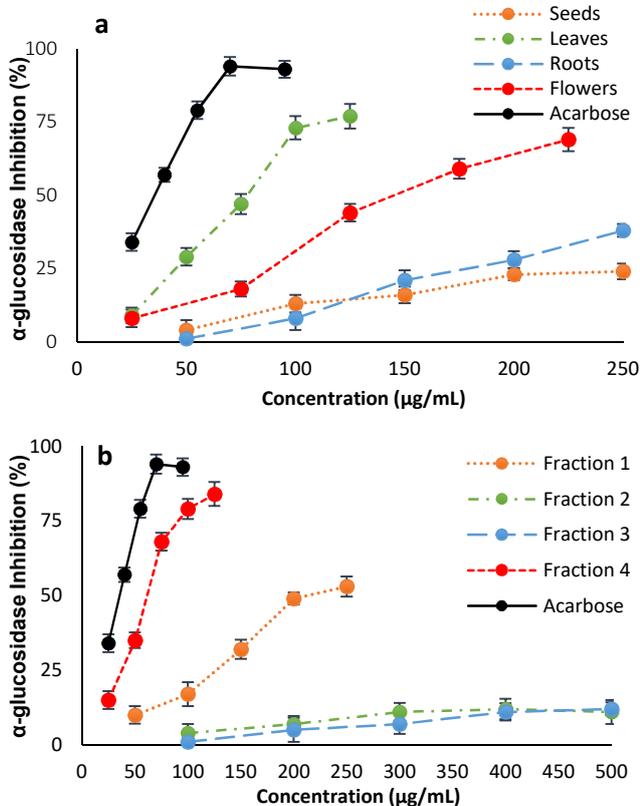


Figure 4. α -glucosidase inhibitory activity of a) methanolic extracts of different organs of *S. vermiculata*; b) the different fractions of *S. vermiculata* leaves

In vitro anti-acetylcholinesterase enzyme (anti-AChE) activity

To evaluate the potential of the *S. vermiculata* crude extracts as anti-Alzheimer’s disease drugs, their anti-AChE activities were tested. According to the results (Fig.3a), the cholinesterase inhibitory activity occurred in a dose-dependent manner, and the root extract with an IC_{50} of $19.21 \pm 1.23 \mu\text{g/mL}$ indicated the highest anti-AChE effects when compared to other parts. Moreover, the IC_{50} of the seed extract against AChE was $32.71 \pm 2.30 \mu\text{g/mL}$, and the leaf and flower extracts had no AChE inhibitory activity.

Since the crude extract of *S. vermiculata* roots displayed the highest AChE inhibitory activity, root fractions were also studied. Results indicated that the aqueous-acid fraction had the highest AChE inhibitory activity (Fig.3b), and its activity was almost equal to that of donepezil. Numerous authors have evaluated the activity of isoquinoline alkaloids such as berberine, anguinine, galantamine, and physostigmine on AChE [25, 26]. In addition, based on our phytochemical study, the aqueous-acid fraction of *S. vermiculata* roots contained isoquinoline alkaloids such as salsoline, salsolidine, and salsoline A. As a result, the high alkaloid content of this fraction could be responsible for its significant AChE inhibitory impact.

In-vitro α -glucosidase inhibitory activity

The α -glucosidase inhibitory activity of crude extracts of *S. vermiculata* parts was determined (Fig. 4a). The leaf extract had the highest inhibitory activity for the α -glucosidase enzyme ($IC_{50} = 78 \text{ g/mL}$), followed by the flowers ($IC_{50} = 138.64 \text{ g/mL}$). The crude extracts of the root and seed had no inhibitory activity against α -glucosidase. As a result, because crude extract of leaves had the highest α -glucosidase inhibitory activity, the activity of its fractions was also investigated (Fig. 4b). According to the findings, the fixed oil and volatile oil fractions exhibited no α -glucosidase inhibitory activity, whereas the IC_{50} values for the ethyl acetate and aqueous-acid fractions were 62.37 and $101.61 \mu\text{g/mL}$, respectively. It was confirmed that the ethyl acetate fraction had higher activity in comparison with other fractions. Şöhretoğlu et al [27] investigated the α -glucosidase inhibitory potential of several flavonoid compounds, including kaempferol and quercetin, and discovered that their IC_{50} values were 8.97 and $77.42 \mu\text{M}$,

respectively. Moradi-Afrapoli et al. [28] investigated the α -glucosidase inhibitory activities of phenolic compounds isolated from the methanolic extract of *Polygonum hyrcanicum*, and found quercetin to be particularly effective. In another study, the α -glucosidase inhibitory potential of quercetin, isoquercetin, and rutin was compared and their IC₅₀ values were reported as 0.017, 0.185, and 0.196 μ M, respectively, concluding that quercetin plays an important role in enzyme inhibition [29]. Results of the present research showed that the ethyl acetate fraction was rich in flavonoids such as rutin and quercetin (Table 2). Thus, the ethyl acetate fraction's moderate α -glucosidase inhibitory activity could be attributed to its high flavonoid content.

CONCLUSION

Phytochemical study of different parts indicated the presence of 44 various metabolites (14 phenolic compounds, 3 alkaloids, 11 fatty acids, and 16 volatile compounds). In roots, vanillic acid, rutin, salsoline, salsoline A, palmitic acid, oleic acid, linoleic acid, cuminaldehyde, and carvone, in seeds, vanillic acid, salsoline A, palmitic acid, oleic acid, linoleic acid, carvone, and β -caryophyllene, in leaves, gallic acid, vanillic acid, caffeic acid, rosmarinic acid, rutin, quercetin, limonene, and carvone, and in flowers, gallic acid, vanillic acid, cinnamic acid, rosmarinic acid, rutin, kaempferol, limonene, linalool, and carvone were recorded as the main identified components. Studying the biological activities of the fractions of different parts of *S. vermiculata* indicated the highest α -glucosidase inhibitory activities of ethyl acetate fractions of leaves; and the highest AChE inhibitory activity in the aqueous-acid fraction of roots. In conclusion, different parts and fractions of *S. vermiculata* are rich sources of bioactive compounds and the results of the present study could provide useful information to guide the application of *S. vermiculata* parts in food and pharmaceutical fields.

ACKNOWLEDGEMENTS

We gratefully acknowledge financial support from Azarbaijan Shahid Madani University and Shahid Beheshti University of Medical Sciences.

REFERENCES

- [1] Botschantzev, V.P. 1969. Genus *Salsola* L. concise history of its development and dispersal. *Botan J*, 54(7):989-1001.
- [2] Kuhn, U. 1993. *Chenopodiaceae*. In the families and genera of vascular plants 2 (Kubitzki K, Rohwer JG, Bittrich V. eds.), Berlin Heidelberg etc, Springer, 258-281.
- [3] Tundis, R., Menichini, F., Conforti, F., Loizzo, M.R., Bonesi, M., Statti, G. and Menichini, F. 2009. A potential role of alkaloid extracts from *Salsola* species (*Chenopodiaceae*) in the treatment of Alzheimer's disease. *J Enz Inhibit Med Chem*, 24(3):818-824.
- [4] Hartwell JL. 1969. Plants used against cancer. A survey. *Lloydia*, 32(1):30-34.
- [5] Nikiforov, S.B., Semenov, A.A. and Syrchina, A.I. 2002. Effect of an aqueous extract of the above-ground part of *Salsola collina* on the cholesterol distribution between lipoprotein fractions in the blood serum of rabbit with experimental cholelithiasis. *Pharm Chem J*, 36:544-545.
- [6] Loizzo, M.R., Tundis, R., Statti, G.A., Passalacqua, N.G., Peruzzi, L. and Menichini, F. 2007. In vitro angiotensin converting enzyme inhibiting activity of *Salsola oppositifolia* Desf., *Salsola soda* L. and *Salsola tragus* L. *Nat Prod Res*, 21(9):846-851.
- [7] Hanif, Z., Ali, H.H., Rasool, G., Tanveer, A. and Chauhan, B.S. 2018. Genus *Salsola*: its benefits, uses, environmental perspectives and future aspects-a review. *J Rangel Sci*, 8(3):315-328.
- [8] Shehab, N.G. and Abu-Gharbieh, E. 2014. Phenolic profiling and evaluation of contraceptive effect of the ethanolic extract of *Salsola imbricata* Forssk. in male albino rats. *Evidence-Based Compl Alter Med*, 2014:695291.
- [9] Boulaaba, M., Medini, F., Hajlaoui, H., Mkadmini, K., Falleh, H., Ksouri, R. and Abdelly, C. 2019. Biological activities and phytochemical analysis of phenolic extracts from *Salsola kali* L. Role of endogenous factors in the selection of the best plant extracts. *South African J Bot*, 123:193-199.
- [10] Rasheed, D.M., El Zalabani, S.M., Koheil, M.A., El-Hefnawy, H.M. and Farag, M.A. 2013. Metabolite profiling driven analysis of *Salsola* species and their anti-acetylcholinesterase potential. *Nat Prod Res*, 27(24):2320-2327.
- [11] Hegnauer, R. 1964. *Chemotaxonomie der Pflanzen*. Bd. III, Birkhauser Verlag Basel und Stuttgart.
- [12] Orekhoff, A. and Proskurnina, N. 1933. Über die Alkalodie von *Salsola Richteri*. *Berichte der Deutschen Chem Gesellschaft (A and B Series)*, 66(6):841-843.

- [13] Orekhoff, A. and Proskurnina, N. 1934. Über die Alkaloide von *Salsola Richteri*, II. Mitteil.: Die Konstitution des Salsolins. Chem. Berichte – Chem Europe, 67:878-884.
- [14] Al-Oudat, M. and Qadir, M. 2011. The halophytic flora of Syria. International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria.
- [15] Al-Tohamy, R., Ali, S.S., Saad-Allah, K., Fareed, M., Ali, A., El-Badry, A. and Rupani, P.F. 2018. Phytochemical analysis and assessment of antioxidant and antimicrobial activities of some medicinal plant species from Egyptian flora. J Appl Biomed, 16(4):289-300.
- [16] Hazrati, S., Ebadi, M.T., Mollaei, S. and Khurizadeh, S. 2019. Evaluation of volatile and phenolic compounds, and antioxidant activity of different parts of *Ferulago angulata* (schlecht.) Boiss. Ind Crop Prod, 140:111589.
- [17] Ebadi-Nahari, M., Farnia, P., Nikzat, S. and Mollaei S. 2018. A chemotaxonomic evaluation of some *Scabiosa* L. species in Iran. Biochem Syst Ecol, 81:33-36.
- [18] Pistia-Brueggeman, G. and Hollingsworth, R.I. 2001. A preparation and screening strategy for glycosidase inhibitors. Tetrahedron, 57(42):8773-8778.
- [19] Hirai, I., Okuno, M., Katsuma, R., Aria, N., Tachibana, M. and Yamamoto, Y. 2010. Characterisation of anti-*Staphylococcus aureus* activity of quercetin. Inter J Food Sci Technol, 45:1250–1254.
- [20] Lee, K.A., Moon, S.H., Kim, K.T., Mendonca, A.F. and Paik, H.D. 2010. Antimicrobial effects of various flavonoids on *Escherichia coli* O157:H7 cell growth and lipopolysaccharide production. Food Sci Biotechnol, 19:257-261.
- [21] Calderón-Montaño, J.M., Burgos-Morón, E., Pérez-Guerrero, C. and López-Lázaro, M. 2011. A review on the dietary flavonoid kaempferol. Med Chem, 11:298-344.
- [22] Nadeem, M., Imran, M., Aslam Gondal, T., Imran, A., Shahbaz, M., Muhammad Amir, R. and Martins, N. 2019. Therapeutic potential of rosmarinic acid: A comprehensive review. Appl Sci, 9(15):3139.
- [23] Xiang, Y., Li, Y.B., Zhang, J., Li, P. and Yao, Y.Z. 2007. A new alkaloid from *Salsola collina*. Yao Xue Xue Bao, 42:618-620.
- [24] Gannoun, S., Mahfoudhi, A., Flamini, G., Helal, A.N. and Mighri, Z. 2016. Chemical composition and antimicrobial activities of Tunisian *Salsola vermiculata* L. J Chem Pharm Res, 8(4):1087-1092.
- [25] Pässler, U. and Knölker, H.J. 2011. The pyrrolo [2, 1-a] isoquinoline alkaloids. The alkaloids: Chem Biol, 70:79-151.
- [26] Konrath, E.L., Passos, C.D.S., Klein-Júnior, L.C. and Henriques, A.T. 2013. Alkaloids as a source of potential anticholinesterase inhibitors for the treatment of Alzheimer's disease. J Pharm Pharm, 65(12):1701-1725.
- [27] Şöhretoğlu, D., Sari, S., Barut, B. and Özel, A. 2018. Discovery of potent α -glucosidase inhibitor flavonols: insights into mechanism of action through inhibition kinetics and docking simulations. Bioorg Chem, 79:257–264.
- [28] Moradi-Afrapoli, F., Asghari, B., Saeidnia, S., Ajani, Y., Mirjani, M., Malmir, M. and Yassa, N. 2012. In vitro α -glucosidase inhibitory activity of phenolic constituents from aerial parts of *Polygonum hyrcanicum*. DARU J Pharm Sci, 20(1):37.
- [29] Li, Y.Q., Zhou, F.C., Gao, F., Bian, J.S. and Shan, F. 2009. Comparative evaluation of quercetin, isoquercetin and rutin as inhibitors of α -glucosidase. J Agri Food Chem, 57(24):11463-11468.

مطالعه متابولیتی و خواص بازدارندگی اندام های مختلف *Salsola vermiculata* علیه استیل کولین استراز و آلفاگلوکوزیداز

سعید ملانی^۱، پوپک فرنیاء*^۲، جلال الدین غنوی*^۳، محمد مجیدی^۴

^۱گروه شیمی، دانشکده علوم پایه، دانشگاه شهید مدنی آذربایجان، تبریز، ایران.

^۲مرکز تحقیقات میکوباکتریولوژی، پژوهشکده سل و بیماری های ریوی، مرکز آموزشی، پژوهشی و درمانی سل و بیماری های ریوی بیمارستان دکتر مسیح

دانشوری، دانشگاه علوم پزشکی بهشتی، تهران، ایران.

^۳گروه بیوتکنولوژی، دانشکده کشاورزی، دانشگاه شهید مدنی آذربایجان، تبریز، ایران.

*نویسندگان مسئول: p.farnia@sbm.ac.ir و j.ghanavi@sbm.ac.ir

چکیده

گیاهان داروئی نقش مهمی در درمان بیماری ها و تولید داروهای جدید دارند. *Salsola vermiculata* گیاهی یک ساله است که به طور گسترده در جنوب غربی آسیا پراکنده بوده و برای درمان ناراحتی های معده استفاده می شود. این مطالعه با هدف شناسایی و مقایسه پروفایل متابولیتی اندام های مختلف *S. vermiculata* و ارزیابی پتانسیل بازدارندگی عصاره ها و فراکسیون های آن نسبت به استیل کولین استراز و آلفا-گلوکوزیداز انجام شد. روش های آنالیز GC-ESI-MS، GC و GC-MS برای مطالعه پروفایل متابولیتی عصاره ها و فراکسیون های آن ها استفاده شد. فعالیت های بازدارندگی با روش های طیف سنجی تعیین شد. بر اساس نتایج، ۴۴ متابولیت در اندام های مختلف *S. vermiculata* شناسایی شدند. در ریشه ها وانیلیک اسید، روتین، سالسولین، سالسولین A، پالمیتیک اسید، اولئیک اسید، لینولئیک اسید، کومین آلدهید و کاروون، در دانه ها وانیلیک اسید، کافئیک اسید، رزمارینیک اسید، روتین، کوئرستین، لیمونن و در گل ها گالیک اسید، وانیلیک اسید، سینامیک اسید، رزمارینیک اسید، روتین، کائمفرول، لیمونن، لینالول و کاروون به عنوان ترکیبات اصلی شناسایی شدند. با توجه به نتایج فعالیت های بازدارنده، فرکشن های اتیل-استاتی برگ و فرکشن آبی-اسیدی ریشه ها به ترتیب بیشترین فعالیت بازدارندگی را نسبت به استیل کولین استراز (با IC₅₀ برابر با ۱۷/۲۴ میکروگرم بر میلی لیتر) و آلفاگلوکوزیداز (با IC₅₀ برابر با ۶۲/۳۷ میکروگرم بر میلی لیتر) نشان دادند. در نهایت، برگ و ریشه *S. vermiculata* سرشار از ترکیبات فنلی و آلکالوئیدی است و یافته های این مطالعه، آن ها را به عنوان مهارکننده های مناسب استیل کولین استراز و آلفاگلوکوزیداز معرفی می کند و بنابراین می توان از آنها برای تولید داروهای جدید استفاده کرد.

کلمات کلیدی: آلکالوئید، اسیدهای چرب، ترپنوئیدها، فعالیت های بیولوژیکی، ترکیبات فنولی