# Metabolic Profiling and Inhibitory Properties of Different Parts of Salsola Vermiculata Towards Acetylcholinesterase and α-glucosidase

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# Abstract

**Background:** Herbal and natural medicines play significant roles in treatment of diseases and development of novel drugs. *Salsola vermiculata* is an annual plant which is broadly distributed in southwest of Asia, and is used for treatment of stomach disorders.

**Results:** This present study aimed at identifying and comparing the metabolic profiles of different parts of *Salsola vermiculata* and to evaluate the inhibitory potential of their extracts and fractions towards 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 α-glucosidase and acetylcholinesterase inhibitory activities of the samples were determined by microplate colorimetric methods. Based on results, 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, rosmaric acid, rutin, quercetin, limonene, and carvone, and in flowers, gallic acid, vanillic acid, cinnamic acid, rosmaric acid, rutin, kaempferol, limonene, linalool, and carvone were recorded as the main components. According to the inhibitory activities results, the ethyl acetate fractions of leaves and the aqueous-acid fraction of roots displayed highest inhibitory activity towards acetylcholinesterase ($IC_{50}$: 17.24 μg/mL), and α-glucosidase ($IC_{50}$: 62.37 μg/mL), respectively.

**Conclusion:** Finally, the leaves and roots of *S. vermiculata* are rich of phenolic and alkaloids compounds and the findings of this study depict them as a promising acetylcholinesterase and α-glucosidase inhibitors, and therefore, can be utilized for the development of new drugs.


# Background

Herbal and natural medicines play significant roles in treatment of diseases and development of novel drugs. Therefore, the search for finding plants with specific biological activity would be an interesting field of knowledge. *Salsola* is a genus belonging to the family of Amaranthaceae in the major group of Angiosperms, which is native to Asia, Europe, and Africa. These plants are widespread across the hypersaline, semiarid, and arid areas [1–3]. Many plants of 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 the obesity, diabetes, and Alzheimer's disease, functioning as the 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] 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 the stems and leaves of *S. kali* had 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. arbziscula*. Furthermore, according to the findings of Tundis et al. [3], salsoline and salsolidine were the major alkaloids found in *S. soda*, *S. oppositofolia*, and *S. tragus* species, and these compounds were responsible for the anticholinesterase and antioxidant activities. Moreover, salsoline A and *Salsola vermiculata* is an annual plant which is broadly distributed in southwest of Asia and belongs to Amaranthaceae family [3]. This plant is used for treatment of stomach disorders [14]. Rasheed et al. [10] investigated the metabolite profile of *S. vermiculata*, and could identify 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] indicated high antioxidant and antimicrobial activities of *S. vermiculata* methanolic extract. Therefore, due to the lack of data regarding the phytochemical composition and biological properties of different parts of *Salsola vermiculata*, the present research focused on phytochemical study and biological activity (α-glucosidase inhibitory and acetylcholinesterase enzyme activity) of different parts (roots, seeds, leaves, and flowers) of *S. vermiculata*.

## Results And Discussion

Metabolite profiling of crude methanolic extracts and their fractions of different parts of *S. vermiculata* were investigated, and then their acetylcholinesterase and α-glucosidase inhibitory activities were studied.

# Metabolite Profiling

In this study, 44 various 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 oil compounds were identified by GC and GC-MS.

In total, 14 phenolic compounds were detected in ethyl acetate fractions (Table 1) (Fig. 1). A total of ten phenolic acids were identified in the extracts, of which three phenolic acids including protocatechuic acid, cinnamic acid, and ferulic acid were exclusively present in ethyl acetate fraction of flowers; one phenolic acid, caffeic acid was only identified in the leaves extract; and gallic acid and salicylic acid were observed in both leaves and flowers 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 leaves and flowers parts followed by two flavonoids (i.e. rutin and kaempferol), and one anthocyanin (i.e. cyanidin) in the seeds, and one flavonoid (i.e. kaempferol) in seeds extract. Moreover, the maximum identified phenolic compounds belonged to rosmaric 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 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 versus root samples. Therefore, ethyl acetate fractions of the leaves and flowers which are rich in phenolic compounds can be considered as appropriate candidates for some biological properties [16–19].

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

| Fraction | Subgroup | Compounds | Parts of plant | | | |
|---|---|---|---|---|---|---|
| | | | Roots | Seeds | Leaves | Flowers |
| **Ethyl acetate** | Phenolic acids | Gallic acid | N.D | N.D | 116.1 | 614.7 |
| | | Protocatechuic acid | N.D | N.D | N.D | 254.1 |
| | | *p*-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 |
| | | *p*-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 | 241.3 |
| The amounts of phenolic compounds were expressed in µg/g of dry weight | | | | | | |

According to the analysis of the aqueous-acid fractions by LC-ESI-MS (Fig. 1), three alkaloids including salsoline, salsoline A, and salsolidine were observed in roots extract, and only one alkaloid, salsoline A, was detected in the seeds 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 [20]. In 1964, Hegnauer reported salsolin and salsolidin from the herb of *S. kali* L. [11]. In another study, Orekhoff and Proskurnina [12, 13] identified salsoline and salsolidine from *S. arbziscula.* In addition,

according to the report of Tundis et al. [3], salsoline and salsolidine were the main alkaloids found in *S. tragus*, *S. oppositofolia*, 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 a suitable candidate for some biological properties such as antimalarial effects.

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

| Fraction | Subgroup | Compounds | Parts of plant | | | |
|---|---|---|---|---|---|---|
| | | | Roots | Seeds | Leaves | Flowers |
| **Aqueouse-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. | | | | | | |

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

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

| Fraction | Subgroup | Compounds | Parts of plant | | | |
|---|---|---|---|---|---|---|
| | | | Roots | Seeds | Leaves | Flowers |
| **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. | | | | | | |

The analysis of volatile compounds at 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, 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, limonene, linalool, and cumin aldehyde 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 monoterpenes. So, because of biological activity of volatile oil [21], the parts possessing these compounds might be potentially applied as a promising source of biological agents.

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

| Fraction | Subgroup | Compounds | Parts of plant | | | |
|---|---|---|---|---|---|---|
| | | | Roots | Seeds | Leaves | Flowers |
| **Volatile oil** | Monoterpenes | *α*-pinene | 0.9 | N.D | 1.2 | 1.4 |
| | | Limonene | N.D | 3.6 | 11.5 | 14.4 |
| | Oxygenated monoterpenes | 1,8-cineole | 0.7 | 0.6 | 0.9 | 0.7 |
| | | 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 |
| | Oxygenated sesquiterpene | Caryophyllene oxide | 1.4 | - | 0.9 | N.D |
| | | 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.

# 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. 2A), the cholinesterase inhibitory activity occurred in a dose-dependent manner, and the roots extract with $IC_{50}$ of 19.21 ± 1.23 µg/mL indicated the highest anti-AChE effects when compared to other parts. Moreover, the $IC_{50}$ of seeds extract against AChE was 32.71 ± 2.30 µg/mL, and the leaves and flowers 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. 2B), and its activity was almost equal to donepezil. The activity of isoquinoline alkaloids such as berberine, anguinine, galantamine, and physostigmine on AChE have been evaluated by numerous authors [22, 23]. 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. Therefore, the high AChE inhibition effect of this fraction could be attributed to its alkaloid content.

# In-vitro α-glucosidase inhibitory activity

The crude extracts of *S. vermiculata* parts were estimated for their *α*-glucosidase inhibitory activity (Fig. 3A). Results revealed that the inhibitory activity for *α*-glucosidase enzyme was maximum in the case of leaves extract ($IC_{50}$ = 78 µg/mL) followed by flowers ($IC_{50}$ = 138.64 µg/mL). The crude extracts of roots and seeds had no *α*-glucosidase inhibitory activity. Thus, crude extract of leaves had the highest *α*-glucosidase inhibitory activity, and hence the activity of its fractions was also investigated (Fig. 3B). Based on the results, the fixed oil and volatile oil fractions had no *α*-glucosidase inhibitory activity, whereas the $IC_{50}$ values of the ethyl acetate and aqueous-acid fractions were 62.37 and 101.61 µg/mL, respectively. It was confirmed that the ethyl acetate fraction had higher activity in comparison with other fractions. Şöhretoğlu et al [24] investigated the *α*-glycosidase inhibitory potential of some flavonoid compounds such as kaempferol and quercetin, and found their $IC_{50}$ values as 8.97 and 77.42 µM, respectively. Moradi-Afrapoli et al. [25] evaluated the *α*-glucosidase inhibitory activities of phenolic compounds isolated from the methanolic extract of *Polygonum hyrcanicum*, and reported that quercetin had interesting inhibitory activities. In another study, the *α*-glucosidase inhibitory potential of quercetin, isoquercetin, and rutin were compared and their $IC_{50}$ values were reported as 0.017, 0.185, and 0.196 µM, respectively, concluding that quercetin plays an important role in enzyme inhibition [26]. Results of the present research showed that the ethyl acetate fraction was rich in flavonoids such as rutin and quercetin (Table 2). Thus, the moderate *α*-glucosidase inhibitory activity of the ethyl acetate fraction could be attributed to its rich flavonoid content.

# Conclusion

In this investigation, the phytochemical profiles and biological activities of different parts and fractions of *S. vermiculata* were studied. 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, 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, rosmaric acid, rutin, quercetin, limonene, and carvone, and in flowers, gallic acid, vanillic acid, cinnamic acid, rosmaric acid, rutin, kaempferol, limonene, linalool, and carvone were recorded as the main identified components. Studding the biological activities of the fractions of different parts of *S. vermiculata* indicated the highest α-glucosidase inhibitory activates 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.

## Methods And Materials

# Plant materials

The plant was collected from its wild habitat in Azarshahr, Tabriz, Iran, during October 2017 according to the appropriate guidelines and licences for plant material. The GPS location details were the longitude of 45°10'E and latitude of 37°06'N, an altitude of 1240m above sea level. The identification of plant was done by Dr. Mostafa Ebadi from Department of Biology, Faculty of Science, Azarbaijan Shahid Madani University. Also, voucher specimen (ASMUH-10485) was deposited in 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

The fractionation of crude extract was done according to Fig. 4. Briefly, the plant (1 gram) was extracted with methanol (10 mL, 80%) at 25°C. After centrifugation, the supernatant was concentrated, and suspended in distillated water (10 mL). The obtained extract was extracted with n-hexane (1:l v/v). The n-hexane layer was distillated using Clevenger apparatus, and two fractions including volatile oil (fraction 1), and fixed oil (fraction 2) were achieved. Then, the aqueous phase was adjusted to pH = 4.0, and partitioned with ethyl acetate (1:l v/v). The aqueous-acid phase (fraction 3), and ethyl acetate phase (fraction 4) were separated, and then were dried under nitrogen at room temperature, and transferred to the vials.

# GC and GC-MS analysis of compounds

The analysis of volatile oils was performed by GC-MS instrument (Agilent 6890 N GC-MS) equipped with a fused capillary column DB-5 (30 m × 0.25 mm, 0.25 µm film thickness). The injector temperature was adjusted at 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 utilized as carrier gas (1.0 mL/min). Tche 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. The percent of each compound was determined by electronic integration of FID peak areas without any correction factors [27].

The analysis of fixed oil was performed using GC instrument. After methylation of fatty acids according to our previous study [28], they were analyzed using a GC instrument, equipped with a capillary column ZB-1701 (60 m × 0.25 mm, 0.2 µm film thickness). The injector temperature was adjusted at 260°C, and the oven 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 was held for 4 min. Nitrogen was utilized as 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

The fractions 3 and 4 were analyzed by liquid chromatography coupled to electrospray ionization mass spectrometry (LC-ESI-MS). The LC (Agilent 1200 Series HPLC system) system was equipped with diode-array detector, a 20 µl loop, and a $C_{18}$ analytical column (250 mm × 0.46 mm, 5 µm). Separation of these fractions were performed by a gradient program run using solvent A (0.1 % TFA in methanol) and solvent B (0.1 % TFA in water, v/v). solvent program was as follow: 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 for min; isocratic elution 20% A; post-time 6 min before the next injection. 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 ESI negative ion mode 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.

# In-vitro α-glucosidase inhibitory activity

The α-glucosidase inhibitory activity of the extracts were studied according to Pistia-Brueggeman and Hollingsworth [29] method with some modifications. 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) for 30 min at 37°C. 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 addition of 80 µl of 0.2 M $Na_2CO_3$ solution. Finally, the absorbance was read at 405 nm, the inhibition (%) was calculated.

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

## Abbreviations

AChE
Acetylcholinesterase enzyme
ATCh
Acetylthiocholine
GC
Gas chromatography
GC-MS
Gas chromatography-mass spectrometry
LC-ESI-MS
Liquid Chromatography Electrospray Ionization Tandem Mass Spectrometric

# Declarations

### Ethics approval and consent to participate

Not applicable.

### Consent for publication

Not applicable.

### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.


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### Authors' contributions

Saeed Mollaei: Conception and design of study, Writing-review & editing; Poopak Farnia: Conceptualization, Supervision, Project administration, Funding acquisition, Writing-original draft; Jalaledin Ghanavi: Investigation, Validation, Funding acquisition.


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### Research involving plants

The authors confirm that all the experimental methods and plants complied with relevant institutional, national, and international guidelines and legislation.

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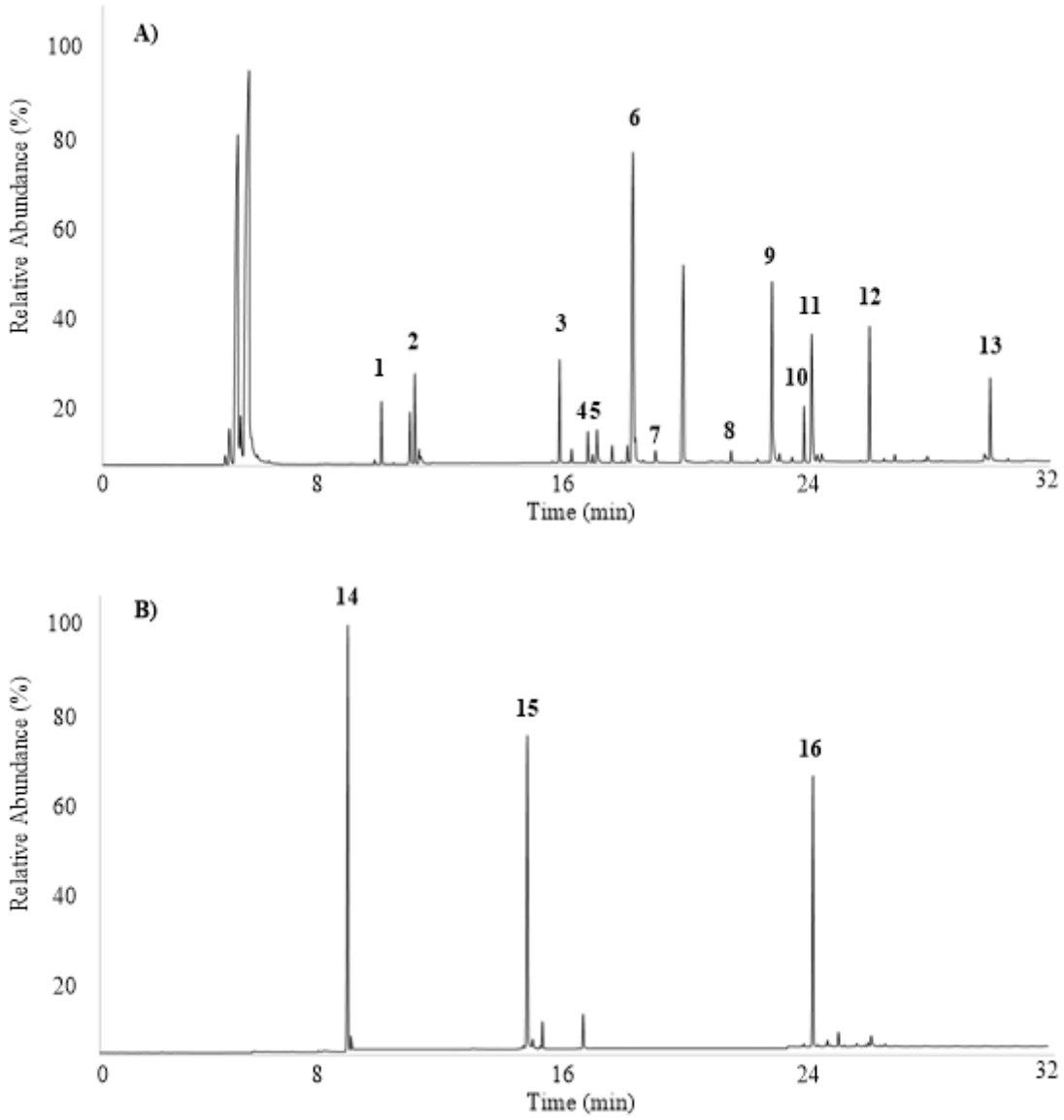
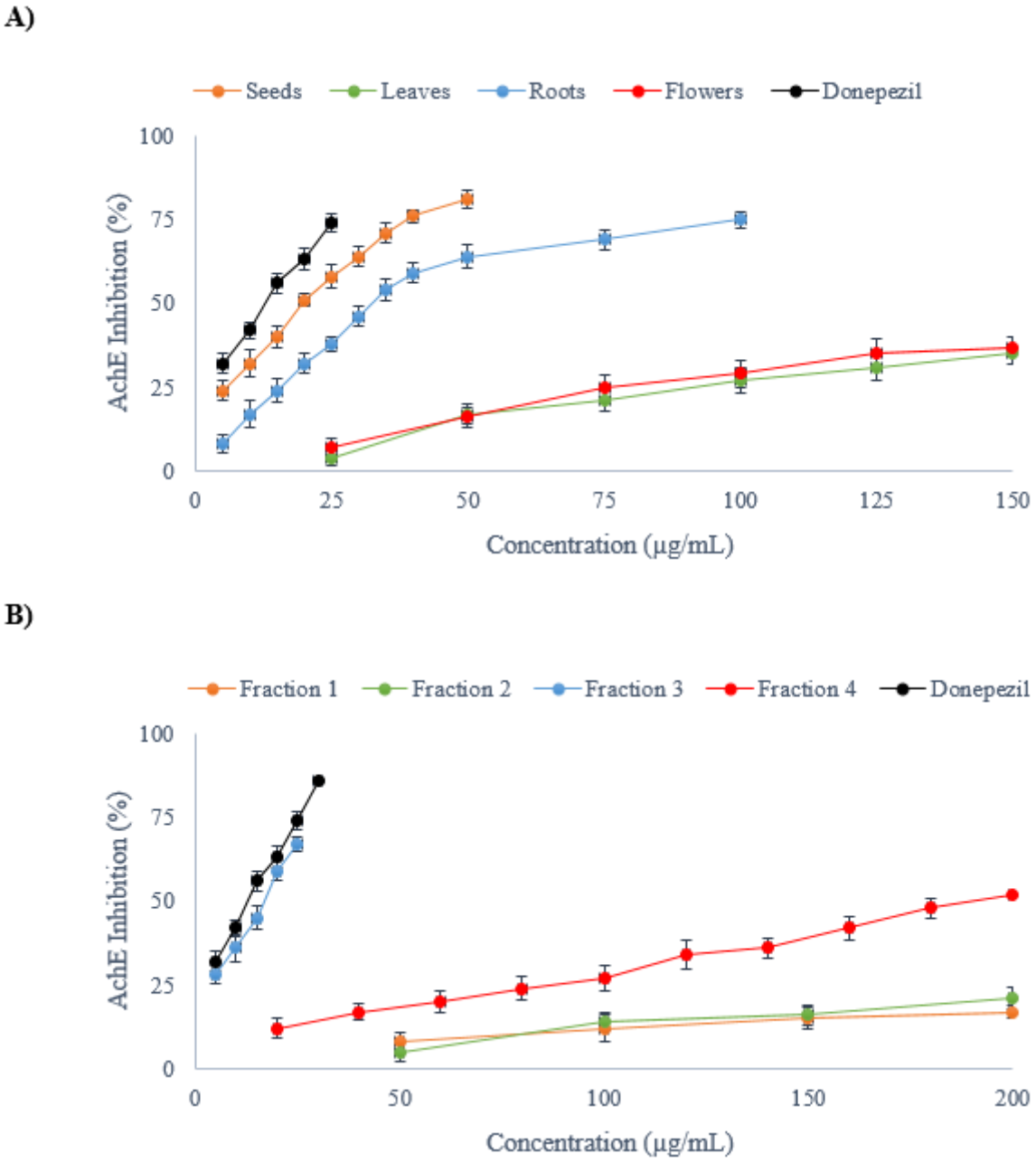
# Figures



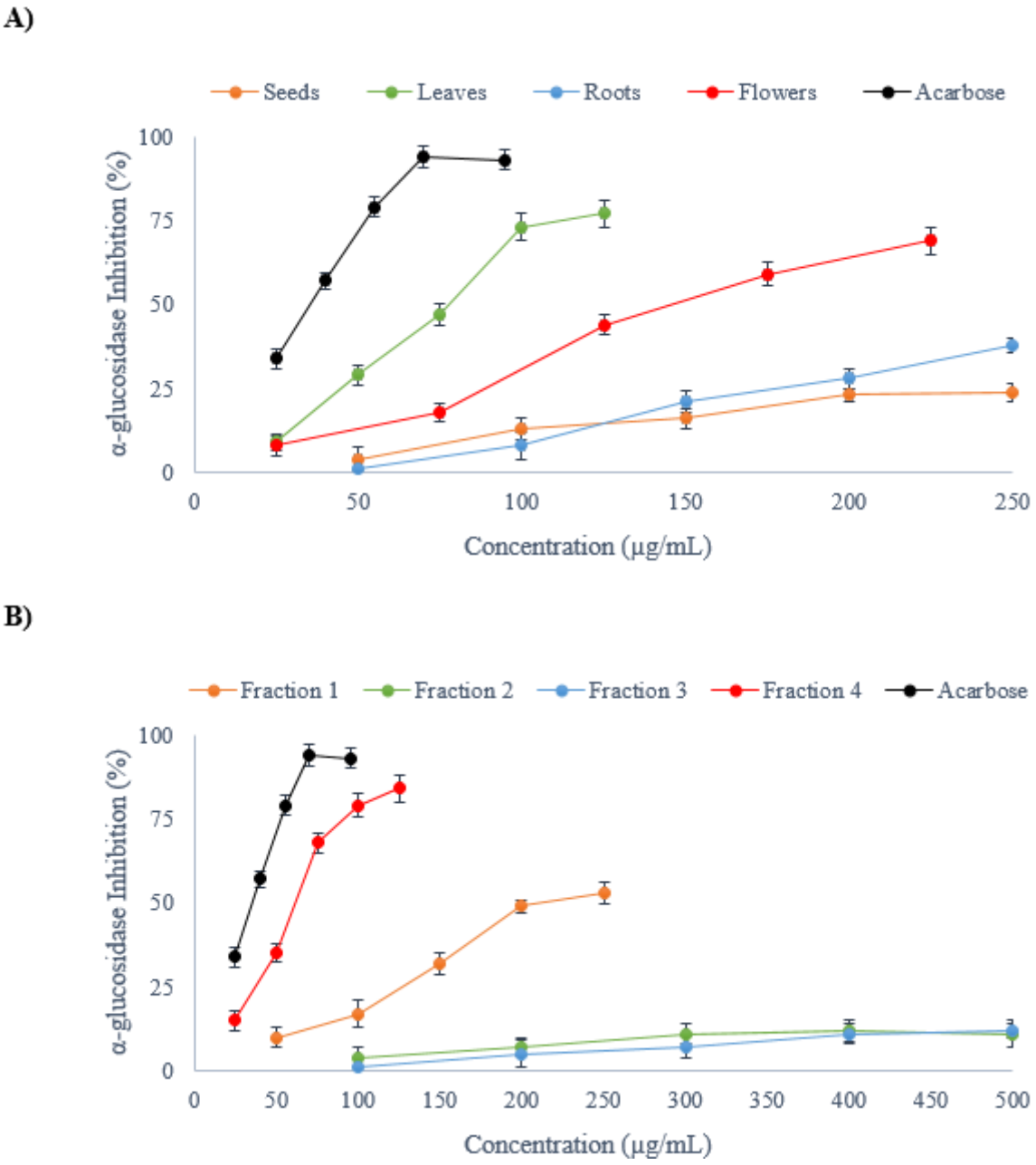

## Figure 1

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.



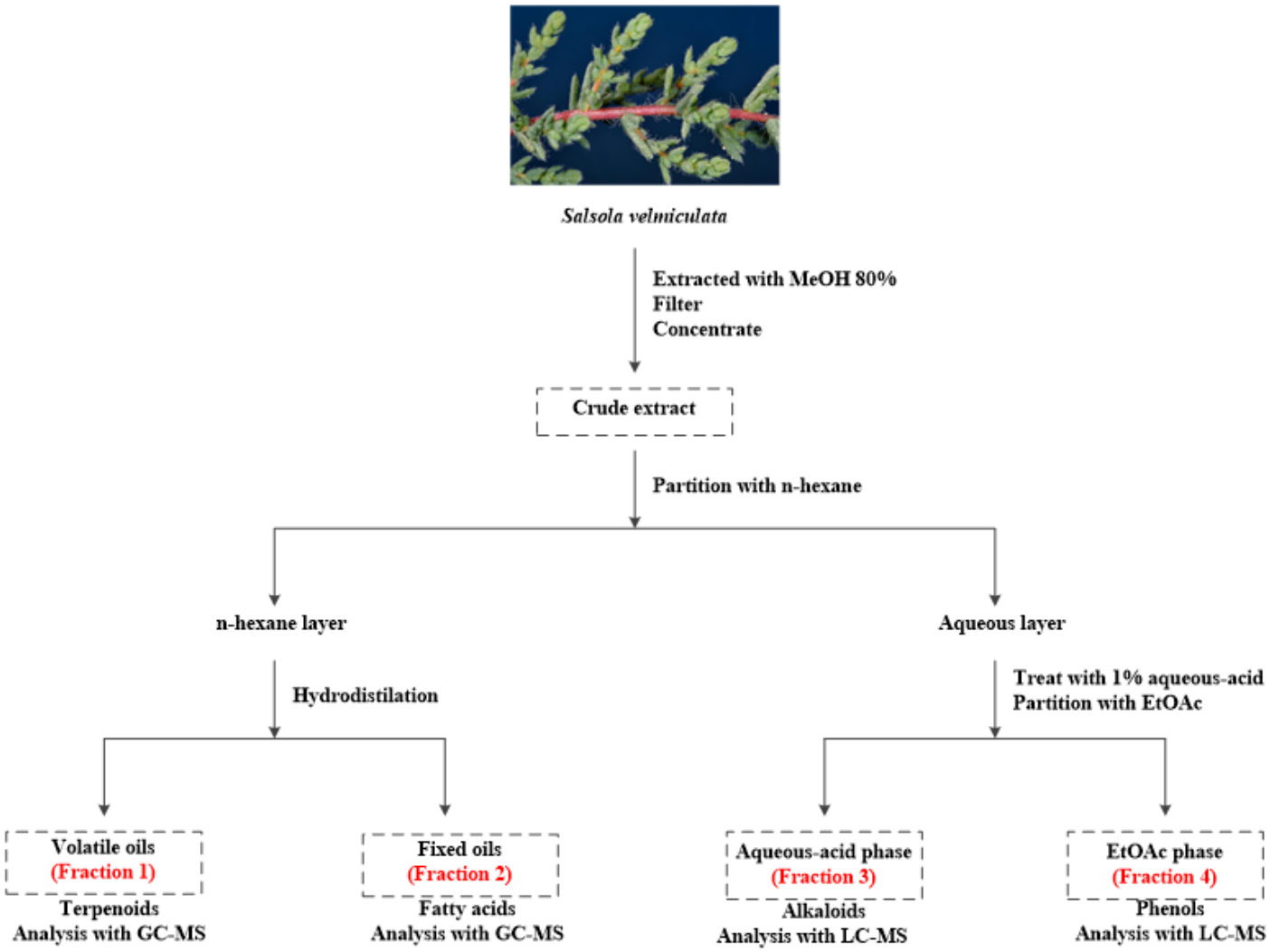

## Figure 2

vitro anti-acetylcholinesterase activity of A) methanolic extracts of different parts of S. vermiculata; B) the different fractions of S. vermiculata roots.




## Figure 3

α-glucosidase inhibitory activity of A) methanolic extracts of different organs of S. vermiculata; B) the different fractions of S. vermiculata leaves




## Figure 4

Schematic diagram of extraction and fractionation of Salsola vermiculata crude extract into different fractions