Original article

Salvia macrosiphon seeds and seed oil: pharmacognostic, anti-inflammatory and analgesic properties

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Abstract

Background and objectives: Wild Sage (*Salvia macrosiphon* Boiss.) known as "*Marvak*" in Persian is one of the polymorphic and abundant plants of Lamiaceae. The plants whole seeds usually soaked or boiled in hot water are widely used for inflammatory ailments in folk medicine. Documents have shown that there is scant information on the chemical constituents of this plant seeds. The current study was carried out to assess the phytochemical constituents of *Salvia macrosiphon* seeds as well as anti-inflammatory activities. **Methods**: The seed oil extracted via a Soxhlet extractor was subjected to pharmacognostic assays using High Performance Thin Layer Chromatography (HPTLC), Gas chromatography/mass spectrometry (GC/MS) analysis of fatty acids and sterols as well as evaluation of the possible anti-inflammatory activities in rats. **Results**: Total ash, acid insoluble and water soluble ash values were determined as 51.67±7.53, 10.00±0.02 and 30.01±5.01 mg/g, respectively. HPTLC assessment revealed the presence of different steroids, triterpenes and fatty acids. Amount of sterols in oil was found 2.44, 24.92 and 4.60 mg/g for esterified β-sitosterol, free β-sitosterol and free stigmasterol, respectively. The α-linolenic acid (77.69±6.10%) was the principal fatty acid. Regarding the anti-inflammatory activity, the seed oil showed low activity in the early phase of formalin test; however, could not significantly inhibit the neutrophil-induced damage by reducing MPO activity in the paws of the rat. **Conclusion**: The seed oil did not exhibit satisfactory effects on acute inflammation in this study but considering the rich phytosterols content, the seed and its oil can be introduced as useful dietary supplements.

Keywords: α-linolenic acid, pharmacognostic, *Salvia macrosiphon*, seed oil, β-sitosterol

Introduction

The Lamiaceae family encompasses more than 236 genera and 7000 related species. *Salvia* (commonly known as sage) with about 900 species is the largest genus of Lamiaceae [1,2]. Among all *Salvia* species, 56 are represented in the flora of Iran [3].

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Wild sage (*Salvia macrosiphon* Boiss.) is one of the polymorphic and abundant plants of Lamiaceae and is widely distributed in Iran. This herbaceous and perennial plant appears in pale yellowish green color and has lemon-scented aromatic odor [4]. Documents have shown that there is little information about the chemical constituents of this plant. Studies have demonstrated linalool, hexyl hexanoate, hexyl isovalerate, sclareol, and hexyl octanoate as major essential oil constituents [5]. In other investigations, α-gurjunene, β-cubebene and germacrene-B have also reported as the main ingredients [6,7]. Moreover, some flavonoids such as eupatorin, salvigenin , 13-epi-manoyl oxide and sitosterol have been isolated and identified from the essential oil or the dimethyl ether extract *S. macrosiphon* aerial parts and fresh stem [6,7]; furthermore, compounds such as flavones and flavone glycosides have been recently derived from the ethyl acetate and methanol extracts of *S. macrosiphon* aerial parts [8].

Literature reviews show the lack of pharmacological investigations on *S. macrosiphon*. One report has shown the potent antioxidant activity of the aerial part methanol extract (404.12 ± 27.81 g $FeSO_4$ in 100 g extract) [9]. An experimental study revealed the antitumor activity of the methanol extract obtained from the aerial parts of *S. macrosiphon* (IC_{50} 77 ± 1 μg/mL) [10]. In regard to the antimicrobial activity, *S. macrosiphon* essential oil showed the respective effects on *Streptococcus pneumoniae* [11]. Known as *"Marv"*, *"Tokhm-e-marv"* or *"Marvak"* in Persian traditional medicine, the plants whole seeds usually soaked or boiled in hot water are widely used for inflammatory ailments especially respiratory ailments [12]. In Iranian traditional markets and medicinal plants stores, seeds of this natural medicament are mostly applied for respiratory tract ailments in association with other mucilaginous seeds. The seeds of *S. macrosiphon* involve large amounts of mucilage and thus possess demulcent effects [12]. On the other hand, traditional Persian medical and pharmaceutical manuscripts have indicated that this herb can be used as diuretic, carminative and anti-flatulent. Instillation of the seeds with milk was reported effective in otic inflammations. Seeds have been applied to improve the gastrointestinal weakness and if roasted, they could be effective for diarrhea and intestinal abrasions. Traditional application of *S. macrosiphon* leaves included uterine painful conditions as well as headache and inflammations [13-15].

Apart from the aerial parts of *S. macrosiphon*, investigations on the seeds are very little. Concerning the pharmacognosic and phytochemical analysis of the seeds, only seed gum has been investigated [16]. In this regard, the current study was carried out to assess the pharmacognostic properties of wild sage seeds as well as their anti-inflammatory activities.

Experimental
Materials
N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA), Myeloperoxidase (MPO), fatty acid and phytosterol standards were purchased from Sigma-Aldrich (USA). All other solvents and reagents were obtained from Merck (Germany).

Sample collection
Seeds of *S. macrosiphon* were collected from Banaruiyeh in Fars province ,Iran (May 2015). They were authenticated by the botanist of Department of Pharmacognosy, School of Pharmacy, Shiraz University of Medical Sciences. A voucher specimen (code: PM-58) was preserved for further reference. The plant material was subsequently powdered, sieved and kept in dark closed container.

Physicochemical analysis
Determination of total ash, acid insoluble ash and water soluble ash was employed in line with the standard analysis methods for quality control of herbal medicines described in the World Health Organization (WHO) guidelines [17]. To detect the extractive values and High Performance Thin

Layer Chromatography (HPTLC) fingerprint analysis, 100 g of the powder was extracted with *n*-hexane by a Soxhlet extractor for 6 h. The residue was dried and subsequently macerated in dichloromethane and ethanol (48 h each). Collected hexane, dichloromethane and ethanol fractions were subsequently concentrated using a rotary evaporator. Each concentrated fraction was then dried in a speed vacuum apparatus at 40 ℃. The solvent free *n*-hexane fraction was labeled as the seed fixed oil-containing fraction. Dried fractions were weighed out and kept in Teflon caped tubes at -20 ℃.

Refractive index of the seed oil was measured at 40°C by using an automatic ATAGO Rx7000-α digital refractometer (ATAGO, Japan). Oil thermal behavior was assessed via differential scanning clorimetry (DSC) on Bahr DSC 310 calorimeter (Germany). The temperature was calibrated with indium. At a rate of 5 °C/min, the oil temperature was decreased from room to -20 °C, maintained for 5 min and then increased to 300 °C. An empty DSC pan was used as an inert reference [18].

High Performance Thin Layer Chromatography
To screen for the seed primary and secondary metabolites, 10 µL of dichloromethane and ethanol fractions (5 mg/mL) as well as 2 µL of the oil sample were applied to HPTLC (CAMAG, Switzerland) to the silica gel plate 60F254 (10 - 20 cm, Merck, Germany). The plates were run in non-polar (toluene- acetone, 80:20, MP1), semi-polar (toluene- chloroform-acetone, 40:25:35, MP2) and polar (*n*-butanol-glacial acetic acid- water, 50:10:40, MP3) mobile phases. The TLC profile of the oil was run in chloroform- acetone- water (98:1.99:0.01, MP4). Chromatographic spots were visualized using UV light (254 and 365 nm) and then UV and visible lights following treating with different reagents phosphomolybdic acid (for visible), Dragendorff, 5% potassium hydroxide (for visible and UV$_{365}$ nm), orcinol, NP (ethanolamine diphenylborate)/PEG, UV$_{365}$ nm), Liebermann–Burchard (UV$_{365}$ nm or vis.), 3% FeCl$_3$ (vis.), vanillin-sulfuric acid and anisaldehyde-sulfuric

acid. All chemicals and solvents were of analytical grade purchased from Merck (Germany) or Sigma Aldrich (USA) [19, 20].

Fatty acid analysis of the seed oil
Fatty acid methyl esters were prepared in line with the procedure explained by Official Methods of Analysis of AOAC [21]. Oil or standard fatty acids (0.2 g) were kept in a Teflon caped test tube. Followed by adding 0.1 mL of hexadecanoic acid (2 g/L), as the internal standard a combination of toluene (1 mL) and sulfuric acid in methanol (1%, 2 mL) was added to the samples. The tubes were incubated at 50 °C overnight. Subsequently, NaCl 5% solution was added and the required esters were extracted with *n*-hexane (2×5mL). The extract was washed with sodium bicarbonate 2% solution and dried by anhydrous Na$_2$So$_4$. The tubes were centrifuged at 3000 rpm for 10 min. The upper layer was moved to a test tube and the solvent was removed under a stream of nitrogen and kept at -20 °C. Prior to gas chromatography/mass spectrometry (GC/MS) analysis, 500 µL *n*-hexane was added to dissolve the samples [18].

Isolation and TMS derivatization of sterols
Following addition of 0.2 mg of free cholesterol and cholesteryl heptadecanoate as the internal standards, the oil (300 µL) was applied to a pipette pasture filled and packed with Silica gel and fractionated sequentially with A: 10 mL hexane- diethyl ether (200:1, v/v, fraction 1), B: 10 mL hexane- diethyl ether (96:4, v/v, fraction 2) and C: 10 mL diethyl ether- acetic acid (100:0.2, v/v, fraction 3). Wax esters and sterol esters were eluted in fraction 1, triacylglycerols in fraction 2 and free sterols in fraction 3. Solvent free fractions were obtained after drying under a stream of nitrogen. Sterol esters were saponified in a tube containing 1 mL of KOH (33%) and 4 mL of ethanol (96%). The mixture was refluxed for 1 in 80 ℃ and then cooled to room temperature. The unsaponifiables were extracted with 3 mL *n*-hexane (at least 3 times) after washing the mixture with 2.5 mL distilled water. The pooled *n*-hexane fractions were dried under a

stream of nitrogen. TMS-derivatives of the sterols were prepared by incubating the sterols in 200 of N, O-bis (trimethylsilyl) trifluoroacetamide (BSTFA) at 60 °C for 2 h [18,22].

GC/MS analysis of fatty acids and sterols
Derivatized fatty acid and sterols were analyzed by GC/MS analysis which was carried out using a Hewlett-Packard 6890. The gas chromatograph was equipped with an HP-5MS capillary column (phenylmethylsiloxane, 25 m, 0.25 mm i.d.). The injector temperature was 250 °C and positive ion electron impact mass spectra were recorded at ionization energy of 70 eV. Helium (1 mL/min) was considered as the carrier gas. For fatty acid methyl esters, the oven temperature was programmed from 160 °C (2 min) to 230 °C at a rate of 8 °C/min and held at this temperature for 20 min. In order to analyze TMS derivatives of sterols, the column temperature was held at 230°C for 1 min, increased to 275 °C at 1 °C/min, and finally held at 275 °C for 30 min. The injector temperature was 275 °C [18,22].
Reference compounds as β-sitosterol, stigmasterol, compesterol and standard fatty acids were purchased from Sigma (United States). Fatty acids and sterols were identified by comparing the mass spectra and retention times with those of reference compounds, or with mass spectra in the literature. For those compounds for which neither standard compounds nor reference spectra were available, chemical structures were postulated according to the general patterns of mass spectrometric fragmentation of different sterols. The GC-MS analysis of the seed oil was performed in triplicate. To prepare calibration curves, 6 concentrations of fatty acids and sterols standards were injected to GC-MS apparatus for five consecutive days (three times in a day) to consider inter day and intraday variations.

Animals
Sprague-Dawley female rats (180-220 g) were sampled from laboratory animal center of Shiraz University of Medical Sciences, Shiraz, Iran. The animals were acclimated for one week under 12 h light and 12 h dark cycles at room temperature. Chow diet and water were provided a*d libitum.* Animal care and treatment procedures were conformed to the Institutional Guidelines and Animal Ordinance (Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran). Rats with free access to water which had been fasted overnight (18 h) were randomly divided into 6 groups (n=5). Treatments were as follows: (I) control (vehicle-treated rat, DMSO 250 µL/kg, *i.p.*), (II) formalin group (50 µL of 2.5% formalin (v/v in distilled water), (III) positive control group rats received the standard drug paracetamol (APAP, 100 mg/kg, *i.p*) and groups (IV-VIII), *S. macrosiphon* seed oil at doses of 250, 500, 1000, 2000 and 4000 µL/kg, *i.p* respectively.
Anti-nociceptive and anti-inflammatory properties of all groups were assessed according to flowing methods.

Formalin-induced paw licking in rats
Analgesic activity assessment was performed via observing the reaction time in the test groups. Sixty minutes after vehicle, acetaminophen (APAP) and oil treatment, formalin was subcutaneously injected into the plantar surface of the animals' right hind paw. Nociception was rated using the original formalin test protocol [23]. Briefly, the pain scoring measurement was as follows: 0, no response behavior of the injected paw; 1, limping during locomotion or resting the paw lightly on the floor; 2, elevation of the injected paw; 3, licking or biting of the injected paw, or grooming [24].
Behavioral responses were observed and recorded for 45 min after the formalin injection. The first 5 min was considered as the early phase and the period between 15 and 45 min as the second phase. Following sub-plantar injection of formalin, the animals were immediately placed in a chamber with a mirror placed under, with a 45 ° angle underneath the floor to allow an unobstructed view of the formalin injected paw.

All animals were brought to the test chamber 1 h prior to the experiment.

Myeloperoxidase activity

Myeloperoxidase (MPO) is an enzyme found primarily in the azurophilic granules of the neutrophils and has been used extensively as a biochemical marker of granulocyte infiltration into various tissues. The MPO activity assay measurement has been described previously by Bradley *et al.* [25]. Intraperitoneal injection of dimethyl sulfoxide (DMSO, 250 µL/kg, *i.p.* control, n=5) or the oil (250, 500, 1000, 2000 and 4000 µL/kg, *i.p*, n=6) was performed 60 min prior to the intra-plantar injection of formalin. Four hours later, when the inflammation was at a maximum, paw tissues were collected under pentobarbital anesthesia (50 mg/kg, i.p.) and homogenized (IKA Homogenizer, Germany) in a solution containing 0.5% of hexadecyltrimethylammonium bromide (HTAB) dissolved in 50 mM potassium phosphate buffer (pH 6) and then centrifuged at 3000 rpm for 20 min at 4 °C. An aliquot of the supernatant (0.1 mL) or standard (Sigma, Germany) was then allowed to react with a 2.9 mL solution of 50 mM potassium phosphate buffer at pH 6 containing 0.167 mg/mL of O-dianisidine dihydrochloride and 0.0005% H_2O_2. After 5 min, the reaction was stopped with 0.1 mL of 1.2 M hydrochloric acid. The rate of change in absorbance was measured by a spectrophotometer (Cecil 9000, UK) at 400 nm. Myeloperoxidase activity was expressed in milliunits (mU) per 100 mg weight of wet tissue [26].

Statistical analysis

The data were expressed as means ± SEM. SPSS software (Computer Statistical Package, SPSS 15) was used to perform statistical analyses. Analysis of Variance (ANOVA) followed by Tukey's multiple comparisons was used to analyze the data. $p < 0.05$ was considered as statistically significant for all comparisons

Results and Discussion

Total ash which represents physiological ash derived from the seed tissues and the non-physiological ash derived from environmental contamination (soil or sand), was determined as 51.67 ± 7.53 mg/g. Acid insoluble and water soluble ash values were determined as 10.00 ± 0.02 and 30.01 ± 5.01 mg/g, respectively.

The extractive values were determined as 3.88%, 0.04% and 0.14% (w/w) for the *n*-hexane, dichloromethane and ethanol fractions of *S. macrosiphon* (different metabolite content of the seeds).

The HPTLC fingerprints of seeds primary and secondary metabolites which were visualized using UV lamps and variety of chemical reagents have been shown in figure 1.
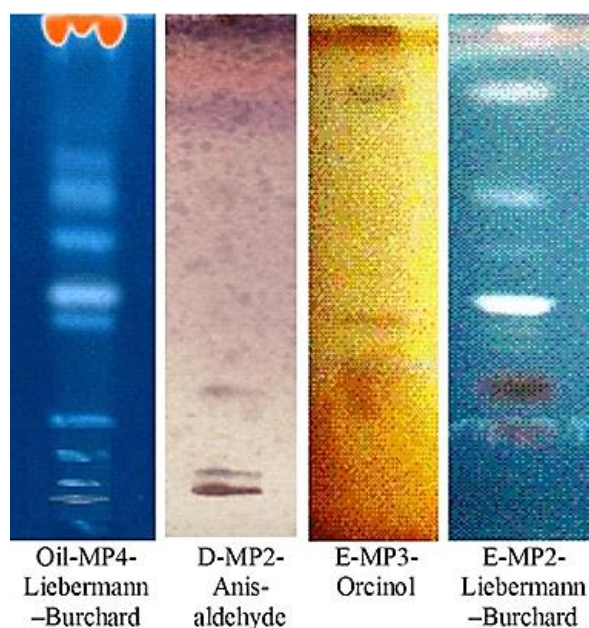

Figure1. HPTLC fingerprints of *S. macrosiphon* seeds fractions run in different mobile phases (MP) and treated with Liebermanne Burchard reagent (under UV_{365} lamp), anisaldehyde-sulfuric acid or orcinol (visible light). Oil: fixed oil, D: dichloromethane fraction, E: ethanol fraction.

The seed oil (*n*- hexane fraction), which was chromatographed in MP4 and visualized with Liebermanne Burchard, revealed the presence of

different steroidal triterpenes and fatty acids. Different polyphenolics, terpenoids and glycosides were detectable in the ethanol and dichloromethane fractions.

Rapid laboratory tests such as ash and extractive values as well as HPTLC finger prints are useful tools for authentication and standardization of herbal medicines in quality control labs. They are also, representative of phytochemical contents of herbs. In the present work, HPTLC finger prints, ash and extractive values of *S. macrosiphon* seeds have been presented for the first time.

According to the results of primary pharmacognostic investigation, the highest extractive value was determined for the seeds *n*-hexane fraction (fixed oil). Furthermore, a higher number of spots for metabolites in HPTLC fingerprints were found in the oil. Thus, the fixed oil was chosen for further investigation on its phytochemical content and possible effects on acute inflammation.

Natural steroids including plant sterols and stanol esters are in a group of secondary metabolites commonly known as phytosterols. They have been detected in variety of plant seeds in different amounts in free form or esterified glycosides or fatty acids [27]. In the present study, esterified β-sitosterol (2.44 mg/g), free β-sitosterol (24.92 mg/g) and free stigmasterol (4.60 mg/g) were detected in the oil. On the contrary, esterified stigmasterol or any other free phytosterols such as campesterol were not detected.

Sesamum indicum seed have been introduced as the richest natural sources of phytosterols such as β-sitosterol (13.9-19.1 mg/g) [28]. According to the findings of the current study, *S. macrosiphon* seed oil can be introduced as one of the richest sources of phytosterols specially β-sitosterol (24.92 mg/g).

According to table 1, α-linolenic acid (C_{18}:3, 77.69 ± 6.10%) was the principal fatty acid of the seed oil. Other major fatty acids were linoleic acid (C_{18}:2), stearic acid (C_{18}:0) and gondoic acid (C_{18}:1). The total unsaturation of the fatty acids for this seed oil was determined as 97.40%, while the highest portion belongs to polyunsaturated fatty acids (PUFAs). Some normal alkanes were also detected in the oil.

Table 1. Fatty acids and alkane profiles of *Salvia macrosiphon* seed oil (The GC-MS analysis of the seed oil was performed in triplicate).

Retention time (min)	Fatty acid	Formula	Percentage (mean ± SD)
3.49	Dodecanoic acid	$C_{14}H_{22}O$	1.78 ± 0.31
5.60	Tetradecanoic acid	C_{14}:0	0.28 ± 0.05
6.44	Octadecane	$C_{18}H_{38}$	0.30 ± 0.05
7.72	9-Hexadecenoic acid	C_{16}:1	0.93 ± 0.09
7.97	Hexadecanoic acid	C_{16}:0	0.19 ± 0.02
8.80	Hexadecnoic acid, 14-methyl	C_{15}:0	0.75 ± 0.01
9.13	Heptadecanoic acid	C_{17}:0	0.76 ± 0.12
9.98	9,12-Octadecadienoic acid	C_{18}:2	15.68 ± 0.34
10.11	9,12,15-Octadecatrienoic acid	C_{18}:3	77.69 ± 14.10
10.29	Octadecanoic acid	C_{18}:1	4.89 ± 3.89
12.43	11-Eicosenoic acid	C_{20}:1	3.09 ± 0.11
12.82	Eicosanoic acid	C_{20}:0	0.87 ± 0.15

Some herbal fixed oils such as Chia or *Salvia hispanica* seed oil (64%) [29], and Kiwi or *Actinidia chinensis* seed oil (62%) [30] were previously reported as rich sources of α-linolenic acid. A study on Mexican Chia Seed (*Salvia hispanica* L.) revealed that α-linolenic, linoleic, oleic, palmitic and stearic acids were the main oil constituents. The seed also contained β-sitosterol (up to 74% of the total unsaponified fraction) [31]. Another investigation reported that the fatty oil content of *Salvia roborowskii* seeds was 43.36%, whereas linoleic acid and oleic acid were the main fatty acids in that species [32]. Also in a study from Iran, seeds of *Salvia leriifolia* were assessed for chemical compositions. The results revealed that oleic and linoleic acids were the major constituents. ; however, the amount of determined α-linolenic acid in that study (1.7 %) was lower than the reports from other species [33].

For the first time, the result of our study demonstrated that *S. macrosiphon* seed oil was even richer in α-linolenic acid (77.69%) and PUFAs (97.40%). Considering that some PUFAs such as α-linolenic acid are essential omega-3 fatty acids needed for normal human growth and

development and cannot be produced within the human body, *S. macrosiphon* seed oil may have the potential to be used as a dietary supplement.

The seed oil appeared in a rich orange-yellow color with the refractive index of 1.4717. Figure 2 has represented the thermal behavior of seed oil with DSC. One major melting peak was exhibited as a single, endothermic peak shown at (-7.920 °C). Since different oils have their own unique features, DSC of the oils is used to detect adulterations. Also oils' DSC can give valuable information on thermal behaviors, fatty acid and triacylglycerol profiles [18]. As seen in Figure 2, *S. macrosiphon* seed oil has an acceptable thermal stability in high heat (up to 300 °C).
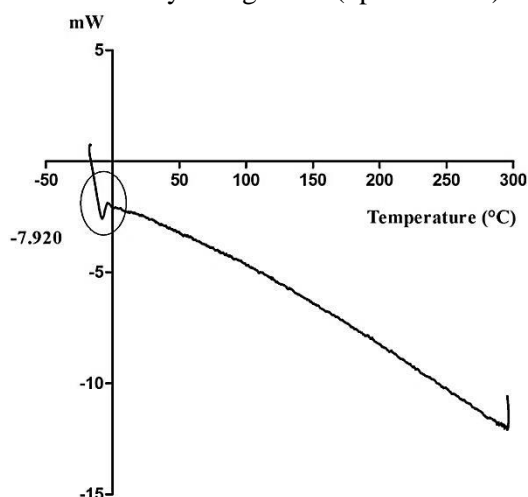

Figure 2. DSC profile of *S. macrosiphon* seed oil

There are numerous studies on the inflammatory activities of aerial parts of different *Salvia* species. Volatile compounds (monoterpenes) of *Salvia officinalis* have shown anti-inflammatory activity in human gingival fibroblasts [34]. Using murine macrophages, these volatile ingredients showed related pharmacological effect via reducing nitric oxide and nuclear kappa B production [35]. The hydroalcoholic extract (polar content) of this species was also effective [36]. However, less investigation has been carried out on the anti-inflammatory activity of *Salvia* seeds. Performing paw edema and human red blood cell membrane stabilization methods as *in vivo* and *in vitro* assessments, *Salvia hispanica*

seeds possessed anti-inflammatory effects dose dependently [37].

The formalin test in mice or rat is a valid and reliable model of nociception (analgesia) and also inflammation and is sensitive for various classes of analgesic and NSAID drugs. In the present study, possible anti-nociceptive properties of *S. macrosiphon* seed oil were investigated using formalin test. Two distinct periods of high licking activity is identified in this test, an early phase lasting the first 5 min and a late phase lasting from 15 to 45 min after the injection of formalin. The effect of seed oil in early (0-5 min) and late (15-45 min) phases of formalin test have been shown in figure 3. As compared to the formalin in early and late phases, the analgesic effect of APAP was significant. The results of this study indicated that the only highest examined dose of the seed oil was somehow effective in early phase of formalin test in rat. In the late phase of formalin test, none of the applied doses of the oil showed significant effect on the nociception compared to formalin group.
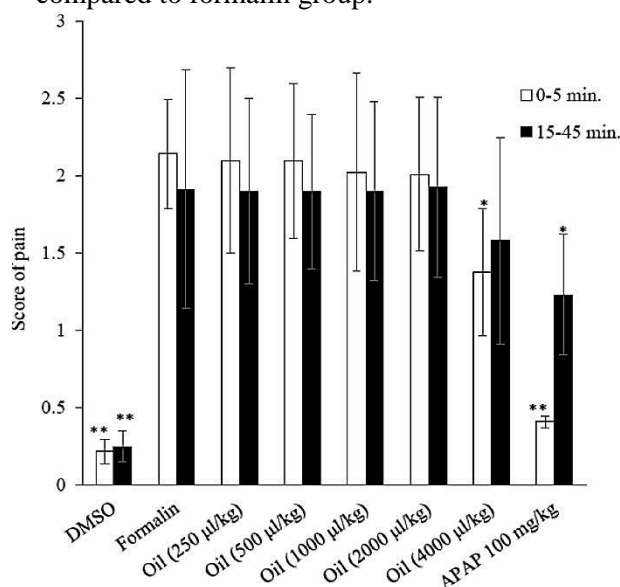

Figure 3. Effects of *Salvia macrosiphon* seed oil on inflammatory pain induced by formalin in rats. The values are expressed as the mean ± SD (n = 6-8). APAP: acetaminophen; *$p<0.05$ indicates statistically significant differences from the formalin group. ** $p<0.001$ indicates statistically significant differences from the formalin group

MPO activity was an indication of neutrophil infiltration in the present study. It has already been shown that acetaminophen (paracetamol), a phenol-based drug with analgesic and antipyretic actions, is an efficient inhibitor of myeloperoxidase [38]. Intraperitoneal injection of different doses of *S. macrosiphon* seed oil decreased the MPO activity compared to the formalin group but this decrease in MPO activity was not significant. Figure 4 demonstrated that *S. macrosiphon* seed oil could not significantly inhibit the neutrophil-induced damage by reducing MPO activity in the paws of the rat.
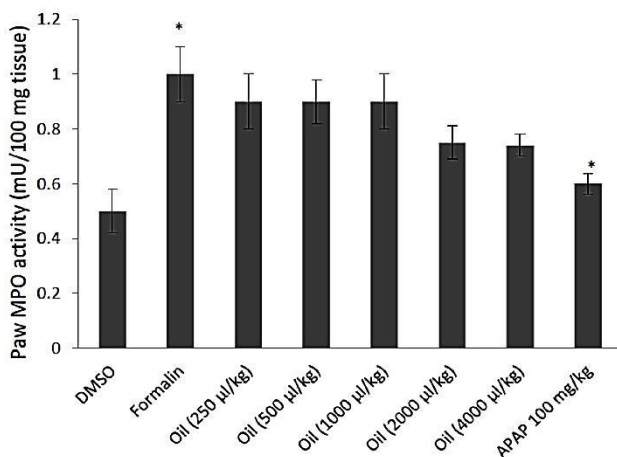

Figure 4. The effect of intraperitoneal injection of *Salvia macrosiphon* seed oil on MPO activity in the paw of rats. The values are expressed as the mean ± S.D (n = 6-8). *$p < 0.05$ indicates statistically significant differences from the formalin group.

Salvia macrosiphon seed oil was consisted of 77.69% α-linolenic acid as a polyunsaturated fatty acid (PUFA). PUFA contribute a considerable amount of energy intake in adults and younger subjects. The major component of PUFA is linoleic acid, while α-linolenic acid (major PUFA in *S. macrosiphon* seed oil) contributes around 10% of PUFA [38]. Studies have demonstrated the anti-inflammatory activity of α-linolenic acid, as an essential omega-3 fatty acid. In an investigation on hypercholesterolemic subjects, dietary α-linolenic acid decreased the vascular inflammation and endothelial

dysfunction via reduction in inflammatory markers such as C - reactive protein (CRP) and vascular cell adhesion molecule 1 (VSAM-1) as compared to the average American diet [39]. Another study demonstrated that increased intakes of dietary α-linolenic acid can exert anti-inflammatory activity via reducing the production of inflammatory cytokines [40]. In addition, it has been proved that α-linolenic acid can downregulate the gene expressions of inflammatory factors such as nitric oxide synthase (iNOS), cyclooxygenase-II (COX-II), and tumor necrosis factor-α (TNF-α). Other PUFAs such as eicosapentaenoic acid (EPA) and docosahexaenoic acid have also exerted anti-inflammatory effects [41].

On the other hand, different phytosterols such as esterified and free β-sitosterol as well as free stigmasterol have been identified in *S. macrosiphon*. Via a bioassay guided fractionation, the mentioned sterols have been isolated from a certain plant and have been evaluated for possible anti-inflammatory effects in murine model of inflammation. Those medicaments could reduce the carrageenan paw and mouse ear oedema in mice (30 and 60 mg/kg; oral). Mechanisms underlying these activities were mentioned as reduction in neutrophil infiltration into inflamed tissues as well as leukocyte granular enzyme release and superoxide generation inhibition [42,43]. Phytosterols extracted from *Eryngium foetidum* L. could reduce both the acute and chronic auricular edema in mouse when applied topically. In that study, MPO activity was significantly decreased in the acute model. It was also claimed that stigmasterol could inhibit several pro-inflammatory mediators and could be used as a beneficial anti-inflammatory agent [44]. Along with those phytosterols, sterol-rich fraction of green microalga has possessed anti-inflammatory activity in LPS-stimulated RAW macrophage. In an investigation, the *n*–hexane fraction of this natural substance which was rich in sterol has exerted highest activity [45].

The current study has demonstrated the high presence of α-linolenic acid as well as β-

sitosterol and stigmasterol in *S. macrosiphon* fixed oil. The seed oil did not exhibit satisfactory effects on acute inflammation in this study but considering the rich content of PUFA and phytosterols in the seed oil, the seed and its oil can be introduced as a useful dietary supplement.

Declaration of interest
The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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