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Phenolic composition and biological activities of *Salvia halophila* and *Salvia virgata* from Turkey

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Abstract

The aerial parts of *Salvia halophila* and *Salvia virgata* were subjected to Soxhlet extraction with different solvents such as *n*-hexane, ethyl acetate, methanol, and aqueous methanol (50%). Plants were also extracted with water under reflux. The effects of the extracts were studied in *p*-benzoquinone-induced abdominal constriction test for the assessment of antinociceptive activity and carrageenan-induced hind paw edema and 12-*O*-tetradecanoyl-13-acetate (TPA)-induced ear edema models in mice for the anti-inflammatory activity. The extracts were analysed using a HPLC–PDA method. Results showed that methanol extract of *S. virgata* significantly inhibited carrageenan-induced paw edema and *p*-benzoquinone-induced abdominal constriction at 100 mg/kg dose, while it showed no effect in the TPA-induced ear edema. On the other hand, the other extracts did not show any inhibitory antinociceptive and anti-inflammatory activities in these in vivo models. Rosmarinic acid was found as main constituent in the extracts, while caffeic acid and luteolin derivatives were also detected.
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Keywords: *Salvia halophila*; *Salvia virgata*; Lamiaceae; Anti-inflammatory activity; Antinociceptive activity; Carrageenan-induced edema; 12-*O*-Tetradecanoyl-13-acetate induced edema

1. Introduction

Chronic inflammatory diseases remain one of the world's major health problems (Yeşilada et al., 1997). Currently, both steroidal and nonsteroidal anti-inflammatory drugs (NSAIDs) are used in the treatment of inflammatory disorders. Steroids have an obvious role in the treatment of inflammatory diseases, but due to rate limiting toxicities, can only be prescribed over short periods except in very severe cases where the risks are acceptable. Prolonged use of NSAIDs is also associated with severe side effects, notably gastrointestinal haemorrhage (Miller, 1983; Robert, Hanchar, Lancaster, & Nezamis, 1979). The recently developed cyclooxygenase-2 (COX-2) selective drugs introduced into therapy; however, do not seem to be free of risk (Wallace et al., 1998). Consequently, there is a need to develop new anti-inflammatory agents with minimum side effects.

Salvia is one of the widest-spread members of the Lamiaceae family (Hedge & Salvia, 1982). It features prominently in the pharmacopoeias of many countries throughout the world and several of the ca. 1000 *Salvia* species have been used in many ways *e.g.* essential oils used in perfumery, flowers used as rouge (Baytop, 1999). *Salvia* species, especially *Salvia officinalis*, are an important source of antioxidants used as preservatives and have wider implications for the dietary intake of natural antioxidants (Kintzios, 2000). Turkey is an important country for export and usage of *Salvia* species in the world. The flora of Turkey includes 88 species of the genus *Salvia*. Sage species are traditionally used as herbal tea in Turkey. Sage species have been used as a medication against perspiration and fever;

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as a carminative; a spasmolytic; an antiseptic/bactericidal; an astringent; as a gargle or mouthwash against the inflammation of the mouth, tongue, and throat; a wound-healing agent; in skin and hair care; and against rheumatism (Kintzios, 2000). *Salvia virgata* is known as "yılancık" in Turkey and used for the treatment of skin diseases and wounds. The decoction from aerial parts of *S. virgata* is used against blood cancer in Western Turkey (Baytop, 1999). *Salvia halophila* is an endemic species for Turkey in traditional use as herbal tea.

In the current study, anti-inflammatory and antinociceptive activities of the extracts of *S. halophila* and *S. virgata* were investigated using carrageenan-induced hind paw edema, TPA-induced ear edema models, and *p*-benzoquinone-induced abdominal constriction test in mice.

2. Materials and methods

2.1. Plant material and reagents

S. virgata Jacq., Lamiaceae was collected from Sivrihisar–Eskisehir on 19/6/2004 (ESSE 14417) and *S. halophila* Hedge (endemic) was collected from Konya: Karakulluk-Eskil on 19/6/2004 (ESSE 14418). Chromatographic standards were purchased from Sigma Chemical Company (St. Louis, MO, USA). Ultra-pure water was used throughout and was prepared using a Millipore Milli-RO 12 plus system (Millipore Corp., Bedford, MA, USA). All remaining reagents were of the highest purity available and obtained from the Sigma Chemical Company.

2.2. Preparation of the extracts

Air-dried plant material (100 g) was cut to small pieces and sequentially extracted with hexane, ethyl acetate, methanol, and 50% methanol using a Soxhlet apparatus for 8 h each. Thereafter, the extract was filtered and evaporated to dryness in vacuo at 40 °C. Separately, the material was extracted with water under reflux for 3 h. The water phase was filtered and freeze-dried. All extracts were stored at −20 °C. Prior to analysis, 10 mg of each extract was dissolved in 1 mL of 70% methanol and filtered through a 0.45 μm membrane (Whatman, UK) and used in all the experiments.

2.3. Total phenolics, flavonoids and flavonols

Total phenols were estimated as gallic acid equivalents (GAE), expressed as mg gallic acid/g extract (Singleton, Orthofer, & Lamuela-Raventós, 1999). To ca. 6.0 mL H_2O, 100 μL of appropriate concentration of sample were transferred in a 10.0 mL volumetric flask, to which 500 μL undiluted Folin–Ciocalteu reagent was subsequently added. After 1 min, 1.5 mL 20% (w/v) Na_2CO_3 were added and the volume was made up to 10.0 mL with H_2O. After 2 h incubation at 25 °C, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. The data are presented as the average of triplicate analyses.

Total flavonoids were estimated as rutin equivalents (RE), expressed as mg rutin/g extract (Miliauskas & Venskutonis, 2004). One millilitre of plant extract in methanol (10 g/L) was mixed with 1 mL aluminium trichloride in ethanol (20 g/L) and diluted with ethanol to 25 mL. The absorbance of reaction mixture was read at 415 nm after 40 min at 20 °C. Blank samples were prepared from 1 mL plant extract and 1 drop of acetic acid, and diluted to 25 mL. The rutin calibration curve was prepared in ethanolic solutions using the same procedure. All determinations were carried out in quadruplicate and mean values recorded.

Total flavonols were estimated as rutin equivalents (RE), expressed as mg rutin/g extract (Miliauskas & Venskutonis, 2004). The rutin calibration curve was prepared by mixing 2 ml of 0.5–0.015 mg/mL rutin ethanolic solutions with 2 mL (20 g/L) aluminium trichloride and 6 mL (50 g/L) of sodium acetate. The absorbance of reaction mixture was read at 440 nm after 2.5 h at 20 °C. The same procedure was carried out with 2 mL of plant extract (10 g/L) instead of rutin solution. All determinations were carried out in quadruplicate and mean values were calculated.

2.4. Qualitative–quantitative chromatographic analysis

The liquid chromatographic apparatus (Shimadzu LC 10Avp, Kyoto, Japan) consisted of an in-line degasser, pump and controller coupled to a SPD-M10Avp photodiode array detector equipped with an automatic injector interfaced to Class VP chromatography manager software (Shimadzu, Kyoto, Japan). Separations were performed on a 250 × 4.6 mm i.d., 5 μm particle size, reverse-phase Discovery-C18 analytical column (Supelco, Bellefonte, PA, USA) operating at room temperature (22 °C) at a flow rate of 1 mL min^{-1}. Detection was carried out between the wavelengths of 200 and 550 nm. Elution was carried out using a ternary non-linear gradient of the solvent mixture $MeOH/H_2O/CH_3COOH$ (10:88:2, v/v/v) (solvent A), $MeOH/H_2O/CH_3COOH$ (90:8:2, v/v/v) (solvent B) and MeOH (solvent C). The composition of B was increased from 15% to 30% in 15 min, increased to 40% in 3 min and held for 12 min, increased to 100% in 5 min then the composition of C was increased to 15% in 2 min, increased to 30% in 11 min and then returned to the initial conditions in 2 min. Components were identified by comparison of their retention times to those of authentic standards under identical analysis conditions and UV spectra using our in-house PDA-library. A 10 min equilibrium time was allowed between injections. All the standard and sample solutions were injected triplicate.

2.5. Animals

Male Swiss albino mice (20–25 g) were purchased from the animal breeding laboratories of Refik Saydam Central

Institute of Health (Ankara, Turkey). The animals left for 2 days for acclimatization to animal room conditions (22 °C) were maintained on standard pellet diet and water ad libitum. The food was withdrawn on the day before the experiment, but free access of water was allowed. A minimum of six animals was used in each group. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals.

2.6. Preparation of test samples for bioassay

All the extracts were administered 100 mg/kg doses after suspending in 0.5% sodium carboxymethyl cellulose (CMC) suspension in distilled water. The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indomethacin (10 mg/kg and 0.5 mg/ear) or aspirin (acetyl salicylic acid) (100 mg/kg) in 0.5% CMC was used as reference drug.

2.7. Anti-inflammatory activity

2.7.1. Carrageenan-induced hind paw edema model

Carrageenan-induced hind paw edema model was used for determination of anti-inflammatory activity (Yeşilada & Küpeli, 2007). Sixty minutes after the oral administration of test sample or dosing vehicle, each mouse was injected with a freshly prepared (0.5 mg/25 μL) suspension of carrageenan (Sigma, St. Louis, MO, USA) in physiological saline (154 nM NaCl) into subplantar tissue of the right hind paw. As the control, 25 μL saline solutions were injected into the left hind paw. Paw edema was measured every 90 min during 6 h after induction of inflammation. The difference in footpad thickness was measured by a gauge calipers (Ozaki Co.). Mean values of treated groups were compared with mean values of a control group and analysed using statistical methods. Indomethacin (10 mg/kg) was used as the reference drug.

2.7.2. TPA-induced mouse ear edema

Each mouse received 2.5 μg of TPA (12-*O*-tetradecanoylphorbol 13-acetate) dissolved in 20 μL of 70% EtOH (De Young, Kheifets, Ballaron, & Young, 1989). This was applied by an automatic pipette in 20 μL volumes to both anterior and posterior surfaces of the right ear. The left ear (control) received the same volume of solvent (EtOH 70%), Extracts were applied topically (0.5 mg/ear dose) simultaneously with TPA. Indomethacin (0.5 mg/ear) was used as a standard drug. For the evaluation of the activity, two different ways were followed up as given below.

1. The thickness of each ear was measured 4 h after induction of inflammation using a gauge calipers (Ozaki Co., Tokyo, Japan). The edema was expressed as the difference between right and left ears due to TPA application and, consequently, inhibition percentage was expressed as a reduction thickness with respect to the control group.
2. After 4 h, the animals were killed under deep ether anesthesia. Discs of 6 mm diameter were removed from each ear and weighed in balance. The swelling was estimated as the difference in weight between the punches from right and left ears, and expressed as an increase in ear thickness.

2.8. Antinociceptive activity

p-Benzoquinone-induced abdominal constriction test (Okun, Liddon, & Lasagnal, 1963) was performed on mice for the determination of antinociceptive activity. According to the method, 60 min after the oral administration of test samples, the mice were intraperitonally injected with 0.1 mL/10 g body weight of 2.5% (w/v) *p*-benzoquinone (PBQ; Merck, Darmstadt, Germany) solution in distilled H_2O. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the 5th minute after the PBQ injection. The data represent averages of the total number of writhes observed. The antinociceptive activity was expressed as percentage change from abdominal constriction controls. Aspirin at 100 mg/kg doses was used as the reference drug in this test.

2.9. Acute toxicity

Animals employed in the carrageenan-induced paw edema experiment were observed during 48 h and morbidity or mortality was recorded, if any, for each group at the end of the observation period.

2.10. Gastric-ulcerogenic effect

After the antinociceptive activity experiment, mice were killed under deep ether anesthesia and stomachs were removed. Then the abdomen of each mouse was opened through the greater curvature and examined under a dissecting microscope for lesions or bleedings. However, *p*-benzoquinone applied i.p. did not induce any irritation on gastric mucosa, but anti-inflammatory agents of COX-1 inhibitors, i.e., aspirin or indomethacin, orally, cause severe bleedings, without repeated administrations.

2.11. Statistical analysis of data

Data obtained from animal experiments were expressed as mean standard error (±SEM). Statistical differences between the treatments and the control were evaluated by ANOVA and Students–Newman–Keuls post-hoc tests.

$p < 0.05$ was considered to be significant ($^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$).

3. Results and discussion

S. virgata and *S. halophila* herbs were sequentially extracted with hexane, ethyl acetate, methanol, and aqueous methanol (50%) using a Soxhlet apparatus. Water extracts were also obtained from the plant materials under reflux. The results of fraction yields, total phenols, total flavonoids, total flavonols and compositional analysis of extracts are presented in Table 1. According to the data presented in Table 1, aqueous methanol, methanol and water extracts of *S. virgata* and aqueous methanol and ethyl acetate extracts of *S. halophila* contained the highest amount of total phenol contents, while the hexane extracts had the lowest in both samples.

The qualitative–quantitative analyses of the extracts, carried out using an HPLC apparatus coupled to a PDA detector, are presented in Table 2, with selected chromatograms presented in Figs. 1a and 1b. Phenolic compounds were identified and quantified at 280, 320, and 360 nm as benzoates, hydroxycinnamates, and flavonoids, respectively. Gallic, *p*-OH-benzoic, caffeic, *o*-coumaric, rosmarinic acids, luteolin-7-*O*-glycoside, and luteolin were identified by comparison with the retention times and UV spectra of authentic standards, while quantitative data were calculated from their calibration curves. For *S. virgata*, the aqueous methanol, methanol and water extracts were found to be the richest in phenolics as measured by both UV spectrophotometry and HPLC. The aqueous methanol extract of *S. halophila* was found to be the richest fraction for total phenolics by UV spectrophotometry, whereas ethyl acetate was in the HPLC measurement. Rosmarinic

Table 1
Total phenol, total flavonoid, total flavonol quantities and yields of the extracts from *S. halophila* and *S. virgata*

	Total phenol[a]		Total flavonoid[b]		Total flavonol[c]		Yield[d]	
	S. halophila	*S. virgata*	*S. halophila*	*S. virgata*	*S. halophila*	*S. virgata*	*S. halophila*	*S. virgata*
(A)[e]	29.2 ± 0.6	28.3 ± 0.6	0.2 ± 0.2	0.8 ± 0.1	tr	tr	12.4	21.5
(B)	98.9 ± 0.8	64.5 ± 1.0	1.3 ± 0.3	0.1 ± 0.1	–	tr	3.6	12.5
(C)	73.2 ± 1.0	133.8 ± 0.8	2.7 ± 0.1	6.5 ± 0.1	–	–	71.2	45.0
(D)	106.7 ± 1.5	212.3 ± 0.4	6.3 ± 0.1	3.6 ± 0.1	–	–	182.0	119.0
(E)	58.5 ± 0.4	116.2 ± 0.8	3.6 ± 0.2	3.9 ± 0.1	tr	tr	151.0	152.0

tr, trace (< 0.05).
[a] $mg_{GAE}/g_{extract}$.
[b] $mg_{RE}/g_{extraxt}$.
[c] $mg_{RE}/g_{extract}$.
[d] $mg_{extract}/g_{drog}$.
[e] (A) hexane; (B) ethyl acetate; (C) methanol; (D) 50% methanol; (E) aqueous extract.

Table 2
The qualitative–quantitative analyses of the extracts carried out using an HPLC from *S. halophila* and *S. virgata*

Compound	Amount of compounds [mg/kg] ± SD				
	Extracts[a]				
	(A)	(B)	(C)	(D)	(E)
S. halophila					
Gallic acid	–	tr	5.3 ± 0.1[b]	2.0 ± 0.1	0.6 ± 0.1
p-OH-Benzoic acid	–	4.8 ± 0.1	13.8 ± 0.1	74.6 ± 3.0	12.7 ± 0.2
Caffeic acid	–	15.6 ± 0.9	2.1 ± 0.1	23.9 ± 0.7	12.1 ± 0.1
o-Coumaric acid	–	7.3 ± 0.4	7.0 ± 0.5	63.7 ± 2.4	12.6 ± 0.1
Rosmarinic acid	–	489.0 ± 21.2	385.9 ± 2.2	271.4 ± 8.9	59.8 ± 0.5
Luteolin-7-*O*-glucoside	–	3.2 ± 0.2	4.5 ± 0.1	23.2 ± 0.9	8.6 ± 0.1
Luteolin	–	4.6 ± 0.2	1.6 ± 0.1	0.8 ± 0.1	0.4 ± 0.0
S. virgata					
Gallic acid	–	tr	4.1 ± 0.3	12.9 ± 0.7	8.3 ± 0.3
p-OH-Benzoic acid	–	0.3 ± 0.1	tr	4.6 ± 0.4	1.1 ± 0.2
Caffeic acid	–	4.8 ± 0.4	5.6 ± 0.2	5.6 ± 0.4	13.5 ± 1.1
o-Coumaric acid	–	8.3 ± 0.4	107.8 ± 2.5	76.3 ± 4.7	13.4 ± 0.2
Rosmarinic acid	–	44.8 ± 1.3	597.5 ± 16.6	484.9 ± 28.4	232.3 ± 4.3
Luteolin-7-*O*-glucoside	–	1.5 ± 0.1	6.9 ± 0.2	4.7 ± 0.3	2.3 ± 0.1
Luteolin	–	0.6 ± 0.1	1.4 ± 0.1	3.6 ± 0.2	5.8 ± 0.2

tr, trace (< 0.01).
[a] (A) hexane; (B) ethyl acetate; (C) methanol; (D) 50% methanol; (E) aqueous extract.
[b] Mean ± SD (mg/kg; $n = 3$).

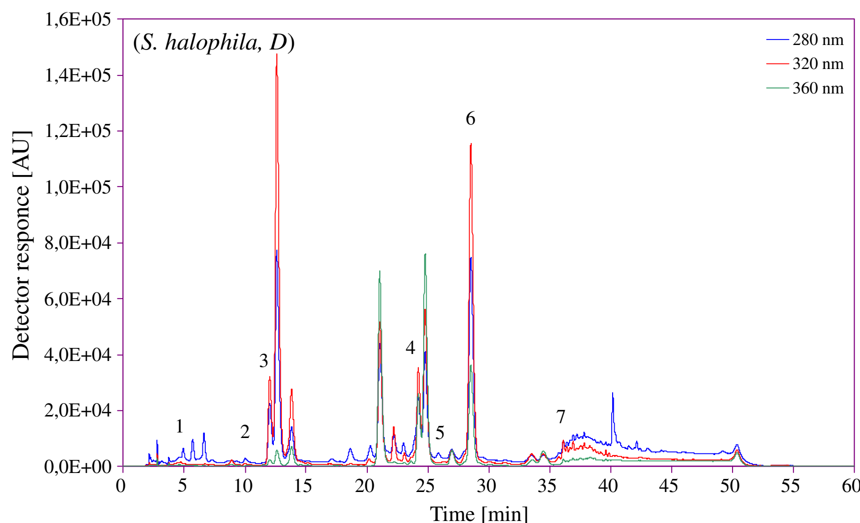

Fig. 1a. HPLC chromatogram of aqueous extract of *S. halophila* (1, gallic acid; 2, *p*-OH-benzoic acid; 3, caffeic acid; 4, luteolin-7-*O*-glycoside; 5, *o*-coumaric acid; 6, rosmarinic acid; 7, luteolin).

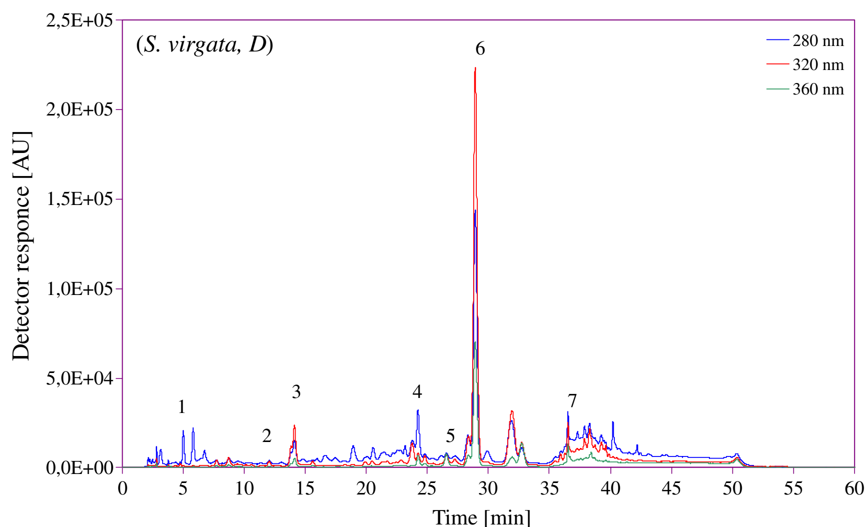

Fig. 1b. HPLC chromatogram of aqueous extract of *S. virgata* (1, gallic acid; 2, *p*-OH-benzoic acid; 3, caffeic acid; 4, luteolin-7-*O*-glycoside; 5, *o*-coumaric acid; 6, rosmarinic acid; 7, luteolin).

acid was the main compound in all the extracts except for the hexane extract. *o*-Coumaric acid was found in the *S. virgata* extracts whereas caffeic acid was found in the *S. halophila* extracts as the second hydroxycinnamic acid. Methanol, aqueous methanol and water extracts had more flavonoids among others. As well known from the literature, rosmarinic acid was found as the principal component.

In the present study, we have evaluated the anti-inflammatory and antinociceptive activities of the *n*-hexane, ethyl acetate, methanol and aqueous methanol (50%) extracts from the aerial parts of *S. halophila* and *S. virgata.* The results indicate that methanol extracts of *S. virgata* presents antinociceptive and anti-inflammatory activity. The effect of the extracts was investigated in two models of acute inflammation; the carrageenan-induced induced hind paw edema, and 12-*O*-tetradecanoylphorbol 13-acetate (TPA)-induced ear edema.

The inflammation is a complex process, which is frequently associated with pain and involves several events, such as the increase of vascular permeability, increase of granulocytes and mononuclear cells migration, as well as the granulomatous tissue proliferation.

Anti-inflammatory compounds can act on many steps of pathophysiological process. For example, a compound may block the biosynthesis of proinflammatory mediators by direct interaction with a key enzyme such as inhibition of COX-2 or by decreasing enzyme expression such as steroidal anti-inflammatory compounds or by reducing substrate levels such as decrease of arachidonic acid releasing. In addition, a compound can either act by inhibiting the release of preformed stored mediators such as

histamine release or by blocking mediator receptor interaction on target cells such as histamine receptor antagonists. An anti-inflammatory compound may also act by immunostimulation such as maturation of myeloid cells or stimulation of phagocytosis that in turn promotes an increase removal of the insulting signal molecules, which results in a less aggressive inflammatory response to allergen challenge (Safaihy & Sailer, 1997).

Several experimental protocols of inflammation and pain are used for evaluating the potency of drugs. In the present study, the evaluation of anti-inflammatory and antinociceptive effects was undertaken using different animal models to fully investigate the potential of *S. halophila* and *S. virgata* to be used in the treatment of inflammatory disorders.

Using the carrageenan-induced paw edema, which is the most widely used primary test for the screening of new anti-inflammatory agents (Winter, Risley, & Nuss, 1962), the effective dose was determined as 100 mg/kg. Therefore, this dose was selected to be used in all experiments.

Acute inflammation such as carrageenan-induced edema involves the synthesis or release of mediators at the injured site. These mediators include prostaglandins, especially the E series, histamine, bradykinins, leucotrienes and serotonin, cause fever and pain (Silbernagl & Lang, 2000). Carrageenan-induced hind paw edema is an experimental animal model for acute inflammation and it is believed to be biphasic. The early phase (1–2 h) of this assay is mainly mediated by histamine, serotonin and an increasing synthesis of prostaglandins in the damaged tissue surroundings. The late phase is sustained by prostaglandin release (Brito & Antonio, 1998).

Prostaglandins (PGs) are metabolites of arachidonic acid, which are synthesized and released by most cell types, and cyclooxygenase (COX) enzymes catalyze the first steps in the biosynthesis of PGs (Teather, Packard, & Bazan, 2002). COX-2 is often referred to as the inducible isoform of COX, since levels of COX-2 increase in response to several forms of stimulus, including inflammation process in different types of tissue (Teather et al., 2002). In contrast, COX-1 which is the constitutive form of COX, appears to be involved in housekeeping cellular function (Herschman, 1996). Also, another COX isoform was described (COX-3) in brain cortex and heart tissue (Chandrasekharan et al., 2002).

As shown in Table 3, the obtained results indicated that fraction methanol extracts of *S. virgata* inhibited the formation of the carrageenan-induced hind paw edema significantly, as measured in the 270–360 min of experiment. A 100 mg/kg dose was the most potent and comparable to indomethacin, a well known prostaglandin inhibitor.

Although the 12-*O*-tetradecanoyl-13-acetate edema model has limited selectivity in determining the possible mechanism of action, it is appropriate for assessing the anti-inflammatory effects in a first stage trial. The phorbol ester elicited an inflammatory response characterized by a delayed time of onset. It has been established that this agent exerts its inflammatory effect through protein kinase C activation with the subsequent cytosolic phospholipase A2 stimulation, AA mobilization, and biosynthesis of prostaglandins and leukotrienes (Nishizuka, 1988).

Results obtained from TPA-induced ear edema in mice are shown in Table 4. Indomethacin attenuated the inflammatory response in this test. All the extracts were found to be inactive against the TPA-induced ear edema model. In other words, the anti-inflammatory activity of the extracts seems to be unrelated to the inhibition of leukotriene synthesis.

Although the abdominal constriction test has poor specificity (Le Bars, Gozariu, & Cadden, 2001), it is a very sensitive method of screening antinociceptive effects of compounds and with a good correlation between ED_{50} values obtained in animals using this test and analgesic doses in humans (Collier, Dinnin, Johnson, & Schneider, 1968).

Table 3
Effects of the materials against carrageenan-induced paw edema in mice

Samples	Dose (mg/kg)	Swelling thickness ($\times 10^{-2}$ mm) $\pm$ SEM (inhibition% of control)			
		90 min	180 min	270 min	360 min
Control		56.4 ± 4.9	61.8 ± 4.2	67.7 ± 3.9	69.1 ± 4.4
Indomethacin	10	37.2 ± 2.3 **(34.0)****	37.3 ± 2.1 **(39.6)****	40.8 ± 2.4 **(39.7)*****	41.4 ± 1.7 **(40.1)*****
S. halophila					
Hexane	100	59.3 ± 2.5	62.1 ± 3.4	69.0 ± 3.9	69.9 ± 4.2
Ethyl acetate	100	50.4 ± 3.1 (10.6)	54.1 ± 3.6 (12.5)	57.9 ± 3.4 (14.5)	60.1 ± 3.8 (13.0)
Methanol	100	47.4 ± 3.2 (15.9)	51.2 ± 3.9 (17.2)	53.6 ± 3.0 (20.8)	55.3 ± 3.8 (19.9)
Aq. methanol	100	57.8 ± 4.1	64.3 ± 4.6	70.2 ± 4.9	71.5 ± 4.8
Water	100	58.3 ± 3.7	62.9 ± 3.6	64.7 ± 3.3 (4.4)	65.5 ± 3.4 (5.2)
S. virgata					
Hexane	100	57.3 ± 4.6	63.5 ± 5.1	68.0 ± 4.9	67.2 ± 4.1 (2.7)
Ethyl acetate	100	56.9 ± 4.4	62.4 ± 4.1	65.0 ± 3.9 (4.1)	66.2 ± 3.2 (4.3)
Methanol	100	45.1 ± 2.6 (20.0)	49.5 ± 2.4 (19.9)	49.1 ± 3.2 **(27.5)***	52.8 ± 3.4 **(23.6)***
Aq. methanol	100	60.4 ± 4.4	63.6 ± 5.0	69.7 ± 4.2	70.8 ± 4.2
Water	100	57.3 ± 6.1	65.9 ± 5.7	71.3 ± 5.1	74.7 ± 5.4

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control values; SEM: standard error mean.

Table 4
Effect of the extracts and isolated compounds against 12-*O*-tetradecanoylphorbol 13-acetate (TPA) -induced ear edema in mice as measurement swelling thickness and weight measurement of edema

Test samples	Dose (mg/ear)	Swelling thickness (μm) ± SEM	Inhibition %	Weight oedema(mg) ± SEM	Inhibition %
Control		273.4 ± 39.1		37.7 ± 6.2	
Indomethacin	0.5	82.5 ± 21.8	**69.8*****	19.2 ± 5.4	**49.1****
S. halophila					
Hexane	0.5	291.2 ± 43.2	–	44.2 ± 8.6	–
Ethyl acetate	0.5	244.3 ± 29.8	10.6	38.9 ± 7.3	–
Methanol	0.5	230.5 ± 24.0	15.7	33.8 ± 4.9	10.3
Aq. methanol	0.5	265.2 ± 29.8	2.9	35.3 ± 6.8	6.6
Water	0.5	299.2 ± 34.9	–	36.3 ± 6.2	3.7
S. virgata					
Hexane	0.5	286.5 ± 40.8	–	40.2 ± 7.0	–
Ethyl acetate	0.5	235.7 ± 34.7	13.8	35.3 ± 6.1	6.4
Methanol	0.5	240.6 ± 24.5	11.9	31.2 ± 4.0	17.2
Aq. methanol	0.5	283.9 ± 35.2	–	39.7 ± 9.4	–
Water	0.5	304.8 ± 37.5	–	41.5 ± 7.3	–

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control values; SEM: standard error mean.

Table 5
Effect of the materials against *p*-benzoquinone-induced abdominal constrictions in mice

Test samples	Dose (mg/kg)	Number of abdominal constrictions ± SEM	Inhibition%	Ratio of ulceration
Control		46.9 ± 4.7		0/6
ASA	100	23.4 ± 1.7	**50.1*****	5/6
S. halophila				
Hexane	100	50.4 ± 4.9	–	0/6
Ethyl acetate	100	40.2 ± 2.3	14.3	0/6
Methanol	100	38.2 ± 2.1	18.5	0/6
Aq. methanol	100	41.5 ± 3.5	11.5	0/6
Water	100	53.7 ± 5.7	–	0/6
S. virgata				
Hexane	100	51.2 ± 6.3	–	0/6
Ethyl acetate	100	40.8 ± 3.2	13.1	0/6
Methanol	100	36.3 ± 2.7	**22.6***	0/6
Aq. methanol	100	43.7 ± 2.9	6.8	0/6
Water	100	49.6 ± 5.1	–	0/6

$^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ compared to control values; SEM: standard error mean.

The effect of the extracts on constrition response in mice is shown in Table 5. The methanol extracts of *S. virgata* fraction at 100 mg/kg p.o. caused a significant inhibition on the abdominal constrition response induced by *p*-benzoquinone without inducing any apparent acute toxicity or gastric damage, while the rest of the extracts did not show any activity.

For the acute toxicity evaluation of the extracts, morbidity and mortality was monitored for 48 h period after the administration of the extracts in mice and not a negative symptom that might be attributed to morbidity or death was recorded. As shown in Table 5, the extracts did not induce any apparent gastric lesion in the administered dose.

Hosseinzadeh, Haddadkhodaparast, and Arash (2003) reported that the aqueous extract of seeds of *Salvia leriifolia* showed significant and dose-dependent (1.25–10 g/kg) antinociceptive activity over 7 h, and the activity was inhibited by naloxone pretreatment in hot-plate and tail flick tests. Significant and dose-dependent (2.5–10 g/kg) activity was also observed against acute inflammation induced by acetic acid and in the xylene ear oedema test. In the chronic inflammation test (cotton pellet test) the extract (2.5–5 g/kg) demonstrated considerable significant and dose-dependent anti-inflammatory activity. The aqueous seed extract of *S. leriifolia* which showed substantial effects against acute and chronic inflammation may therefore have supraspinal antinociceptive effects which may be mediated by opioid receptors (Hosseinzadeh et al., 2003).

Moreover, in the course of previous studies for the in vivo anti-inflammatory activity evaluation of *Salvia* species, the methanol extract of *Salvia miltiorrhiza* was found to inhibit production of PGD2 and the ethyl acetate subfraction gave the strongest inhibition. From this ethyl

acetate subfraction, an activity-guided isolation finally gave tanshinone I as an active principle (Kim et al., 2002).

In another study, the topical anti-inflammatory activity of three extracts at increasing polarity (*n*-hexane, chloroform, and methanol) from the leaves of *Rosmarinus officinalis* L. (Lamiaceae) had been tested using the croton oil ear test in mice. Both the *n*-hexane and the chloroform extracts from the leaves showed a dose-dependent activity, the last one possessing an anti-inflammatory potency similar to that of indomethacin, the nonsteroidal anti-inflammatory drug used as a reference drug (ID50) 83 and 93 $\mu g/cm^2$, respectively). The bioassay-oriented fractionation of CE-1 led to the identification of tritepenes, ursolic acid, oleanolic acid, and micromeric acid as the main anti-inflammatory principles (Altınıer et al., 2007).

In conclusion, our results support the traditional use of *Salvia* species in some aching and inflammatory conditions. However, further investigations are needed to elucidate the mechanisms related to the actions of the sage species. As a next step, studies in our laboratory are currently under way to isolate and characterize the active principles of the methanol extracts of *S. virgata*.

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