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Anti-inflammatory Activity of Sesquiterpene Lactones from *Chrysophthalmum montanum* (DC.) Boiss.

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Abstract: The aerial parts of *Chrysophthalmum montanum* (DC.) Boiss. (Asteraceae) is traditionally used for wound healing, as well as for the treatment of common cold, sinusitis, and other inflammatory diseases. The objectives of this study were to identify potential anti-inflammatory effects of the methanol extract, and its different polarity subextracts (*n*-hexane, chloroform, *n*-butanol, and remaining aqueous), and guaianolide-type sesquiterpene lactones [6α-acetoxy-4α-hydroxy-1βH-guaia-9.11(13)-dien-12.8α-olide (**1**), 6α-acetoxy-4α-hydroxy-9β.10β-epoxy-1βH-guaia-11(13)-en-12.8α-olide (**2**), 4α,6α-dihydroxy-1β,5α,7αH-guaia-9(10),11(13)-dien-12,8α-olide (**3**), and (4α,5α,8β,10β)-4,10-dihydroxy-1,11(13)-guaidien-12,8-olide (**4**)] from the aerial parts of *C. montanum*. In order to evaluate the anti-inflammatory activity of *C. montanum*, carrageenan- and PGE$_2$-induced hind paw edema, and acetic acid-induced increase in capillary permeability mice models were used. The methanol extract, the chloroform subextract, and compounds **3**, and **4** were shown to possess anti-inflammatory activity in *in vivo* models at 100 mg/kg dose. The results provide a biological, and phytochemical basis for the traditional use of *C. montanum* aerial parts for inflammatory conditions in Turkish folk medicine.

Keywords: Asteraceae; *Chrysophthalmum montanum;* sesquiterpene lactones; anti-inflammatory; carrageenan; prostaglandins. © 2019 ACG Publications. All rights reserved.

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1. Introduction

Inflammation is a fundamental physiological process of immune system towards a variety of injuries, and tissue damages including bacterial infections, manifested by swelling, pain, heat, redness, and dysfunction. If the inflammation is in acute phase, the physiological response is short, and rapid, normally recovers from harmful stimuli, and pathogens. However, chronic inflammation caused by multiple disorders including asthma, neurodegenerative diseases, arteriosclerosis, and cancer, leads to the adaptive immune response which is controlled by cell-mediated, and humoral mechanisms of acquired immunity. Inflammatory mediators such as nitric oxide (NO) prostaglandin E_2 (PGE_2), and cytokines, as well as chemokines play roles in the initiation, and progression of diverse inflammatory diseases [1].

The genus *Chrysophthalmum* Schultz. Bip, belonging to the family Asteraceae, is represented by four species worldwide. There are three species, namely *Chrysophthalmum montanum* (DC.) Boiss., *Chrysophthalmum dichotomum* Boiss. & Heldr., and *Chrysophthalmum gueneri* Aytac and Anderb., in Flora of Turkey and the East Aegean Islands. *C. montanum* is a common medicinal plant that grows in rock crevices, and limestone cliffs, and mainly distributed in the eastern parts of Turkey, Syria, and into Northern Iraq [2-4]. It is locally named "nezle otu", and "tutça". In Turkish folk medicine, the aerial parts of *C. montanum* have been used against sinusitis, flu, and other inflammatory respiratory diseases as well as for wound healing on the human body, and animals [5,6].

Till today, only a few studies on morphological characteristics of the genus *Chrysophthalmum*, and preliminary evaluation of its biological activities, including antioxidant, and antimicrobial activities, as well as only one phytochemical research on *C. montanum* have been reported [2,7-10]. In our previous study, the bioassay-guided isolation, and identification of the cytotoxic compounds of the aerial parts of *C. montanum* were carried out [11]. In continuation of our study on the genus *Chrysophthalmum*, for the phytochemical, and biological aspects, we screened three *Chrysophthalmum* species from Turkey, *C. montanum*, *C. dichotomum*, and *C. guenerii*, for their toxic effects on brine shrimp, as well as phytotoxic, and insecticidal activities ,and the results showed the *n*-hexane, and the chloroform fractions of the investigated species were determined to be effective sources for further investigation to explore bioactive natural constituents [12-14]. However, anti-inflammatory activity of *Chrysophthalmum* species has never been investigated before by using *in vivo* method. We report here for the first time *in vivo* anti-inflammatory activity of the aerial parts of *C. montanum*.

2. Materials and Methods

2.1. General Experimental Procedures

In the experimental study, methanol (MeOH), *n*-hexane, chloroform ($CHCl_3$), and *n*-butanol (*n*-BuOH) were of analytical grade, and purchased from Merck Co. Preperative HPLC was performed on a LC-908W-C60 Recycling Preparative HPLC (Japan Analytical Industry Co., Ltd., Tokyo, Japan) equipped with UV detector 254 nm, and RI detector RI-50. The separation was carried out in a JAIGEL-SIL column (Japan Analytical Industry Co., Ltd., 20 x 250 mm i.d., 10 µm) for normal phase HPLC, and JAIGEL-ODS-M-80 C18 columns (Japan Analytical Industry Co., Ltd., 20 x 250 mm *i.d.*, 10 µm) for reverse phase HPLC. Column chromatography (CC) was performed using Silica gel 60 (70-230 mesh, Merck, Darmstadt, Germany), and Sephadex LH-20 (Sigma-Aldrich, Sweden). Analytical, and preparative TLCs were carried out on precoated silica gel plates (Kieselgel 60 F_{254}, 0.2 or 0.5 mm thickness, Merck). TLC plates were observed under UV light (254, and 366 nm) then visualized by heating after spraying with anisaldehyde reagent [methanol 76 % (Merck), *o*-phosphoric acid 19 % (Riedel-De Haën 85 %), *p*-anisaldehyde 5 % (Merck 98 %)], and 20 % H_2SO_4 (Merck).

2.2 Plant Material

The aerial parts of *C. montanum* were collected in July 2014 from flowering plants grown in the valley of Tohma river, Akçadağ, Malatya, Turkey. Plant material was identified by Prof. Dr. Hayri

Duman from Department of Botany, Faculty of Science, Gazi University. An authenticate voucher specimen (H. Duman 10324) is kept in the Herbarium of GAZI (Ankara, Turkey).

2.3. Plant Extraction, Fractionation, and Purification Procedure

Air-dried powdered aerial parts of *C. montanum* (500 g) were extracted with 80 % MeOH by stirring at room temperature (v/v, 4 x 3 L, 2 days each time). The combined MeOH extract was evaporated under vacuum to dryness to give "MeOH extract" (90.8 g). The MeOH extract was successively partitioned with *n*-hexane (11 x 250 mL), chloroform (8 x 250 mL), and *n*-BuOH (8 x 250 mL) in a separatory funnel, yielding "*n*-hexane subextract" (1.7 g), "CHCl$_3$ subextract" (15.8 g), "*n*-BuOH subextract" (21.4 g), and "remaining aqueous (R-H$_2$O) subextract" (36.4 g). Each of these subextracts, as well as the MeOH extract were tested for their anti-inflammatory activities by *in vivo* assays. Chromatographic separation of the CHCl$_3$ subextract using silica gel, and Sephadex LH 20 column chromatography as well as preparative TLC, and preparative HPLC led us to isolate four compounds identified as known sesquiterpene lactones; i.e. 6α-acetoxy-4α-hydroxy-1βH-guaia-9.11(13)-dien-12.8α-olide (**1**), 6α-acetoxy-4α-hydroxy-9β.10β-epoxy-1βH-guaia-11(13)-en-12.8α-olide (**2**), 4α,6α-dihydroxy-1β,5α,7αH-guaia-9(10),11(13)-dien-12,8α-olide (**3**), and (4α,5α,8β,10β)-4,10-dihydroxy-1,11(13)-guaidien-12,8-olide (**4**).

2.4. In vivo Anti-inflammatory Activity

2.4.1. Animals

Male Swiss albino mice, provided with the Kobay Experimental Animal Research Laboratory, and weighing 20-25 g, were used in experiments. Six mice were used in each experimental group. The mice were held for at least three days in laboratory conditions in order to adapt them to the environment initial the experiment. During this adaptation period, the animals were fed with standard pellet feed, and water, as well as housed at room temperature in a 12 h light/12 h dark cycle. In the experiments, six animals were used for each group. The present study was proceeded according to the international rules governing animal experiments, and biodiversity rights. The experiments were performed, and approved by the ethical committee of Kobay Experimental Animal Research Laboratory Ethical Council (Ethical Number: 227).

2.4.2. Sample Preparation

Test samples were suspended in a mixture of 0.5% sodium carboxymethyl cellulose (CMC), and distilled water. Then, they were applied to test animals with oral. 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. Indomethacin (10 mg/kg) in 0.5 % CMC was used as reference drug.

2.4.3. Carrageenan-induced Hind Paw Edema

The effects of samples on acute inflammation was determined by carrageenan-induced hind paw edema model with slightly modifications [15,16]. To induce inflammation, after 1 h of an administration orally either test sample or dosing vehicle, freshly prepared suspension of carrageenan (0.5 mg/25 µL) (Sigma, St. Louis, Missouri, USA) in physiological saline (154 mM NaCl) was injected into each mouse on subplantar tissue of right hind paw, and the left hind paw was served as the control with 25 µL saline solution. After the inflammation, paw edema, in footpad thickness, was measured in every 90 min during 6 h. The difference between right, and left paw edema was measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo, Japan). The percentage of reduction in paw edema of the treated groups was compared with a control group as mean values, and analyzed using statistical methods.

2.4.4. Acetic Acid-induced Increase in Capillary Permeability

For the determination of effect of the samples on the increased vascular permeability induced by acetic acid in mice was used Whittle method with some modifications [17,18]. Each test sample was applied orally to mice in 0.2 mL/20 g body weight. Thirty minutes later, 0.1 mL of 4 % Evans blue (Sigma, St. Louis, Missouri, USA) in saline solution (i.v.) were injected into tails of each mouse. Then 10 min after the injection of the dye solution, 0.4 mL of 0.5% (v/v) AcOH was injected i.p. on each mouse. After 20 min, the mice were sacrificed by cervical dislocation. The viscera were exposed, and irrigated with distilled water, which was then poured into 10 mL volumetric flasks. Each flask was made up to 10 mL with distilled water, 0.1 mL of 0.1 N NaOH solution was added to the flask. The absorption of the final solution was measured at 590 nm (Beckmann Dual Spectrometer). In control animals, a mixture of distilled water, and 0.5% CMC was given orally, and they were treated in the same manner as described above.

2.4.5. PGE$_2$-induced Hind Paw Edema Model

PGE$_2$-induced hind paw edema model was used for determination of anti-inflammatory activity [15,18]. One hour after the oral application of sample to each mouse received 5 µL of freshly prepared suspension of PGE$_2$ (Fluka Chemie AG, Art. 82475) in Tyrode's solution by injection into the subplantar tissue of the right hind paw except that for control, 5 µL Tyrode's solution was injected into that of the left hind paw. After induction of the inflammation, paw edema was measured in every 15 min during 75 min. The difference between right, and left paw edema was measured, and the mean values were analyzed as earlier described.

3. Results and Discussion

The present study was designed to investigate the anti-inflammatory activity of *C. montanum* in mice in order to validate its traditional uses in Turkish folk medicine. For the evaluation of anti-inflammatory activity of *C. montanum*, methanol extract of the aerial parts of the plant was tested on carrageenan-induced hind paw edema model in mice which is a common *in vivo* anti-inflammatory activity model. As shown in Table 1, the MeOH extract displayed a significant anti-inflammatory activity. According to the extraction, and fractionation procedures, the MeOH extract was successively partitioned in increasing polarity by various solvent, and obtained *n*-hexane, CHCl$_3$, *n*-BuOH, and R-H$_2$O subextracts. Each subextracts was tested to experimental animals for all *in vivo* bioassays.

Among the subextracts of *C. montanum*, the anti-inflammatory effect of CHCl$_3$ subextract was prominent as compared to the reference drug indomethacin in carrageenan-induced hind paw edema model. The *n*-hexane, and R-H$_2$O subextracts were found to be totally inactive, while *n*-BuOH subextract displayed no sustained, and considerable effect (Table 1). Results of the carrageenan-induced hind paw edema model were verified by another experimental model, proceeding the effects of the samples on capillary permeability increased by i.p. injection of acetic acid. A very similar activity profile was also observed against these *in vivo* paw edema models (Tables 1, and 2). Moreover, it was also observed that the MeOH extract, and the CHCl$_3$ subextract had prominent effect as compared to the reference drug indomethacin in PGE$_2$-induced hind paw edema model (Table 3). Our phytochemical studies on the CHCl$_3$ subextract resulted in isolation of four guaianolide-type sesquiterpene lactones (guaianolides) **1-4** as the major principles of the plant (Figure 1). Structures of the isolated compounds have determined using spectral techniques, such as ^{1}H and ^{13}C NMR, DEPT, HSQC, HMBC, and mass spectrometry, as well as their detailed spectral data were firstly reported in our previous study [11]. The isolated compounds were characterized to possess a 8,12-guaianolide skeleton, three of which belong to "*trans*", and one of them shows "*cis*" configuration by means of spectroscopic techniques. Compound **1** was isolated as major component of the CHCl$_3$ subextract of *C. montanum*, and was identified as "6α-acetoxy-4α-hydroxy-1βH-guaia-9.11(13)-dien-12.8α-olide" by comparison with those reported ^{1}H NMR [19], and ^{13}C NMR [20] data. In addition, compound **1** was also named as "6α-acetoxy-isoinuviscolide" in the literature [20]. Compound **2** was concluded to be "6α-acetoxy-4α-hydroxy-9β.10β-epoxy-1βH-guaia-11(13)-en-12.8α-olide" by comparing of [1]H

NMR data reported in the literature before [19]. In accordance with the spectral data [21], compound **3** was identified to be "4α,6α-dihydroxy-1β,5α,7αH-guaia-9(10),11(13)-dien-12,8-olide". Compound **4**, with "*cis*-8,12-guaianolide" skeleton, was identified as "(4α,5α,8β,10β)-4,10-dihydroxy-1,11(13)-guaiadien-12,8-olide" by comparing its ¹H NMR spectral data published in the literature [22]. Among them, compounds **1**, and **3** have been isolated from *C. montanum* previously [7]. It should also be noted that the presence of compounds **2**, and **4** was reported for the first time in *C. montanum* by our group [11].
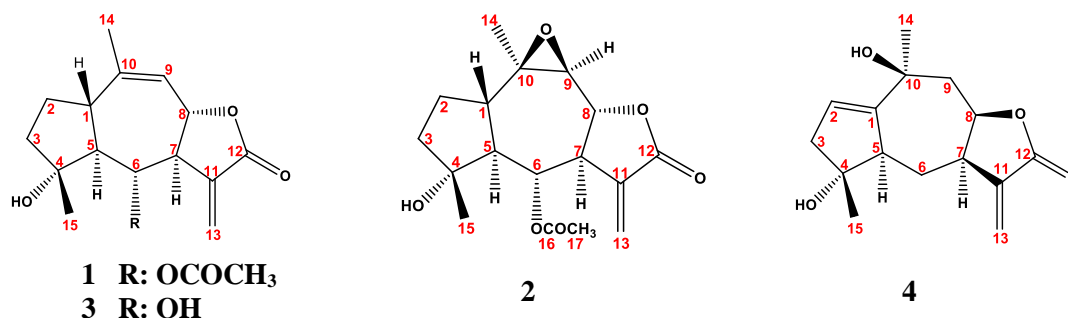

1   R: OCOCH₃
3   R: OH

2

4

Figure 1. The chemical structures of the isolated compounds **1-4**

Table 1. Effects of the MeOH extract, subextracts, and isolated compounds of *Chrysophtalmum montanum* aerial parts on carrageenan-induced hind paw edema model in mice.

Material	Dose (mg/kg)	Swelling thickness (x 10^{-2}mm)±SEM (Inhibition %)			
		90 min	180 min	270 min	360 min
Control		40.5±5.7	46.3±5.8	51.2±4.2	55.8±3.9
MeOH extract	100	39.2±3.1 (3.2)	42.5±3.4 (8.2)	40.7±3.5 (20.5)	41.6±3.1 **(25.4)***
n-Hexane subextract	100	45.2±3.7	48.9±5.1	54.6±4.9	58.1±4.2
CHCl₃ subextract	100	35.4±3.6 (12.6)	40.1±3.2 (13.4)	39.4±3.3 **(23.0)***	38.5±3.0 **(31.0)****
n-BuOH subextract	100	41.7±3.4	48.5±3.7	47.7±3.5 (6.8)	51.2±3.5 (8.2)
R-H₂O subextract	100	44.6±3.8	47.4±3.1	53.3±3.7	56.9±3.6
Indomethacin	10	32.1±3.0 (20.7)	34.4±2.7 **(32.8)****	30.6±2.9 **(40.2)*****	31.1±2.4 **(44.3)*****
Control		37.1±4.2	39.6±3.7	41.5±4.0	45.2±3.5
1	100	39.4±3.3	40.8±3.4	38.9±3.7 (6.3)	40.1±3.9 (11.3)
2	100	40.2±4.1	42.5±3.9	42.6±3.9	42.0±3.7 (7.1)
3	100	35.3±3.1 (4.9)	37.2±3.2 (6.1)	34.7±3.5 (16.4)	34.1±3.4 **(24.6)***
4	100	37.7±3.8	38.2±3.6 (3.6)	31.5±3.2 **(24.1)***	30.2±3.1 **(33.2)****
Indomethacin	10	33.1±2.9 (10.8)	31.4±2.3 (20.7)	26.9±2.2 **(35.2)****	27.1±2.4 **(40.0)*****

* $p<0.05$. **$p<0.01$. *** $p<0.001$ significant from the control

All isolated compounds were also investigated for their anti-inflammatory activity by *in vivo* bioassays above-mentioned in our study. As shown in Table 1, compounds **3**, and **4** were found to possess a significant anti-inflammatory activity ranging from 4.9 to 24.6 %, and 3.6 to 33.2 %, respectively, in carrageenan-induced hind paw edema model. The anti-inflammatory activity of compound **4** was almost as potent as that of indomethacin (10 mg/ kg) which exerts 10.8-40.0 % anti-

inflammatory activity. Compounds **3**, and **4** displayed prominent inhibition with 26.6, and 28.3 %, respectively, as potent as indomethacin (36.9 %) in capillary permeability induced by acetic acid in mice (Table 2). Moreover, compounds **3**, and **4** exhibited a significant anti-inflammatory activity ranging from 3.4 to 26.2 %, and 2.9 to 30.2 %, respectively, in PGE_2-induced hind paw edema model which was comparable to indomethacin (11.5-43.6 % inhibition) as a reference drug (Table 3). On the other hand, remaining isolated compounds showed no noteworthy effect in all *in vivo* models.

Table 2. Effects of the MeOH extract, subextracts, and isolated compounds of *Chrysophtalmum montanum* aerial parts on increased vascular permeability, induced by acetic acid in mice.

Material	Dose (mg/kg)	Evans blue concentration (µg/mL) ± SEM	Inhibition (%)
Control		7.65 ± 0.54	
MeOH extract	100	5.52 ± 0.44	**27.8***
n-Hexane subextract	100	8.13 ± 0.71	-
CHCl₃ subextract	100	5.41 ± 0.38	**29.3***
n-BuOH subextract	100	7.93 ± 0.38	**-**
R-H₂O subextract	100	7.11 ± 0.50	7.1
Indomethacin	10	5.03 ± 0.36	**34.2***
Control		7.02 ± 0.49	
1	100	6.05 ± 038	13.8
2	100	5.82 ± 0.34	17.1
3	100	5.15 ± 0.29	**26.6***
4	100	5.03 ± 0.41	**28.3***
Indomethacin	10	4.43 ± 0.22	**36.9***

* $p<0.05$. ** $p<0.01$. *** $p<0.001$ significant from the control

Table 3. Effects of the MeOH extract, subextracts, and isolated compounds of *Chrysophtalmum montanum* aerial parts on PGE_2-induced hind paw edema in mice.

Material	Dose (mg/kg)	Swelling thickness (x 10^{-2}mm)± SEM (Inhibition)					
		0 min	15 min	30 min	45 min	60 min	75 min
Control		1.6±0.5	12.4±1.3	15.9±1.6	18.8±2.1	13.5±1.9	9.4±1.2
MeOH extract	100	1.6±0.3	12.3±1.1	12.7±1.0 (20.1)	13.4±1.2 (28.7)**	10.1±0.7 (25.2)*	8.8±0.4 (6.4)
n-Hexane subextract	100	1.9±0.4	12.8±1.5	15.8±1.4	19.7±1.7	15.1±1.5	10.8±1.7
CHCl₃ subextract	100	1.7±0.5	11.3±0.9 (8.9)	10.8±1.1 (32.1)*	12.1±0.9 (35.6)***	9.8±0.6 (27.4)**	8.1±0.5 (13.7)
n-BuOH subextract	100	1.7±0.4	11.5±1.1 (7.3)	13.7±1.3 (13.8)	18.9±1.6	14.1±1.5	9.8±1.4
R-H₂O subextract	100	2.0±0.6	13.4±1.7	16.8±1.6	19.2±1.9	14.4±1.9	10.1±1.3
Indomethacin	10	1.7±0.4	10.9±0.7 (12.1)	9.9±0.5 (37.7)**	10.1±0.7 (46.3)***	9.3±0.6 (31.1)**	7.6±0.7 (19.1)
Control		1.7±0.9	13.9±1.5	18.4±1.9	20.2±2.3	15.4±1.7	11.6±1.3
1	100	1.8±0.5	14.1±1.2	18.9±1.5	18.9±1.2 (6.4)	14.3±1.3 (7.1)	12.9±1.7
2	100	1.7±0.6	14.3±1.4	18.1±1.1 (1.6)	19.2±1.4 (4.9)	15.8±1.1	12.6±1.2
3	100	1.7±0.5	13.1±0.9 (5.8)	14.7±1.0 (20.1)	14.9±0.8 (26.2)*	11.7±1.1 (24.0)	11.2±1.1 (3.4)
4	100	1.7±0.4	13.5±1.1 (2.9)	15.9±1.1 (13.6)	14.1±0.6 (30.2)**	10.9±1.0 (29.2)*	10.9±0.7 (6.0)
Indomethacin	10	1.8±0.6	12.3±0.9 (11.5)	13.1±1.0 (28.8)*	11.4±0.7 (43.6)***	10.1±0.6 (34.4)**	10.0±0.5 (13.8)

* $p<0.05$. ** $p<0.01$. *** $p<0.001$ significant from the control

Prostaglandins, bradykinin, histamine, and serotonin play significant roles in carrageenan-induced hind paw edema model. In the early phase (90-180 min) of the inflammation, mainly serotonin, and histamine which are released from mast cells as well as similar substances are increased. In the later phase (270-360 min) of the inflammation, prostaglandins, lysosomes and proteases are overproduced. Amongst, the most predominant of these substances is prostaglandins. PGE_2 is pain-producing class of prostaglandins. More amounts of the PGE_2 increase the propensity towards pain. Therefore, if acute inflammation continues for long periods of time, the tissues will be affected constantly by the inflammatory mediators which result in chronic inflammation. Chronic inflammation is a major contributor to many diseases such as heart disease, diabetes, obesity causes etc. [23]. In order to test the effects of the extracts on the prostaglandin synthesis, and to elucidate the anti-inflammatory mechanism, PGE_2-induced hind paw edema model was employed in mice.

Sesquiterpene lactones (SLs), the active secondary metabolites, are common in many medicinal plants belonging to Asteraceae family, and have a wide range of biological activities, including antimicrobial, anticancer, antidepressant, antioxidant, anti-migraine, anti-herbivory, insecticidal, anti-inflammatory, and immunomodulatory activities [24]. SLs, obtaining from especially Asteraceae plants, are the bioactive constituents that provide potent anti-inflammatory properties to medicinal plants, such as *Inula viscosa* L. (ilicic acid, and inuviscolide), *Arnica montana* L. (helenalin, 11α, 13-dihydrohelenalin, and their ester derivatives)*, Artemisia annua* L. (artemisinin)*, and *Tanacetum parthenium* (L.) Schultz Bip. (parthenolide) [25,26]. The anti-inflammatory effects of SLs are mediated chemically by *α*, *β*-unsaturated carbonyl functional groups, mainly *α*-methylene-*γ*-lactone. Michael-type addition with nucleophiles, particularly cysteine sulfhydryl groups are reacted by these functional groups. In addition to the primary targets of SLs which were exposed thiol groups in proteins, the activity of SLs may also be influenced by other factors, including molecular geometry, lipophilicity, and the chemical environment of the target sulfhydryl groups. The anti-inflammatory SLs focusing on their effects has been explained by mechanisms of action such as inhibition of the nuclear factor-kB, reactive oxygen, and nitrogen species, cytokines, and lipid mediators, probably by alkylating cysteine-38 in the DNA binding domain of the p65 subunit [27,28]. According to the literature, inuviscolide, the main anti-inflammatory SL possessing 8,12 guaianolide skeleton from *I. viscosa*, was reported having significant topical anti-inflammatory effect against acute inflammation by *in vivo* model. The action mechanism of inuviscolide was found that it can be related to mast cell degranulation, and arachidonic acid pathway [25].

Until now, there has been no data on biological activity of compound **2**, but there has only been one phytochemical report on it [19]. There are few studies of the isolated compounds **1, 3,** and **4** on anti-inflammatory activities [21,29,30]. It was shown that compound **3** is a strong anti-inflammatory agent with IC_{50}=0.13 µM against lipopolysaccharide (LPS) induced nitric oxide (NO) production in RAW264.7 macrophages [21]. In another similar study, compound **1** showed inhibitory effect against LPS-induced NO production in RAW264.7 macrophages, IC_{50}=17.1±1.32 µM [30]. Moreover, it was reported that compound **4** exhibited anti-inflammatory activity on Thromboxane B2, and PGE_2 Production Stimulated by LPS (50 ng/mL) in RAW 264.7 cells with IC_{50}=9.9, and 5.6 µg/mL, respectively [29].

In this study, *in vivo* anti-inflammatory effect of *C. montanum*, and its isolated principal sesquiterpene lactones was investigated for the first time. It was reported that compounds **3,** and **4** possess potent anti-inflammatory activities. These results will form the scientific basis for the future exploration of new anti-inflammatory agents from *C. montanum*.

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