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Fatty Acid Compositions and Anti-inflammatory Activities of *Tripleurospermum parviflorum* (Willd.) Pobed. and *Tripleurospermum tenuifolium* (Kit.)

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Abstract: In Turkish traditional medicine, *Tripleurospermum* species have been used for the treatment of inflammatory diseases. The present study was designed to investigate the anti-inflammatory potential and fatty acid composition of the extracts prepared from *Tripleurospermum parviflorum* (Willd.) Pobed. and *T. tenuifolium* (Kit.). Anti-inflammatory activity was assessed by using carrageenan-, and serotonin- induced hind paw edema and acetic acid-induced increase in capillary permeability models. The fatty acid compositions of the plants were investigated by gas chromatography (GC). EtOAc extracts of *T. tenuifolium* and *T. parviflorum* exerted notable inhibitory effect in the all *in vivo* anti-inflammatory activity models tested. Generally, C 16:0 palmitic acid and C 18:2 linoleic acid were found to be the major fatty acids in two species. Saturated fatty acids (SFAs) were found in higher amounts than monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) in two species. SFAs were determinated at 63.15% and 58.68% in *T. tenuifolium* and T. *parviflorum*, respectively. The high content of linoleic acid and palmitic acid may be primarily responsible for significant anti-inflammatory activity. The present study confirms the anti-inflammatory activity of *T. parviflorum* and *T. tenuifolium*. Further phytochemical and biological activity studies are needed for the determination of the active principle/s and anti-inflammatory activity mechanism.

Keywords: Anti-inflammatory; Asteraceae; *Tripleurospermum parviflorum; Tripleurospermum tenuifolium*; Fatty acid.

1. Introduction

The genus *Tripleurospermum* Sch. Bip. belongs to the tribe Anthemideae of the family Asteraceae (Compositae) and is composed of about 38 species distributed mainly in Europe and temperate Asia, with a few species also in North Africa [1]. The genus is represented by 26 taxa at the level of species and variety in the flora of Turkey [2]. Most of these are distributed in the North and East Anatolia. *Tripleurospermum* species known locally papatya are extensively used in Turkey as a foodstuff. The decoction and infusion prepared from *T. parviflorum* (Willd.) Pobed. and *T. monticolum* (Boiss. & Huet) Bornm are used against cough and stomachache and as antipyretic. *T. parviflorum* was also reported to have usage against throat diseases and vaginitis [3]. *T. sevanense* (Manden.) Pobed. is used externally for hair care in Turkey [4]. Some of the species of *Tripleurospermum* have been used in Iranian herbal medicine for soothing, calming, relaxation, as

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sedative and against tenseness, exhaustion and stress [5]. The flowers have also been used as a carminative, stimulant and febrifuge [6].

Tripleurospermum species contain a variety of chemical compounds such as terpenes, hydrocarbons, steroids, oxygenated compounds, alcohols, acids and aromatic compounds [7] and have wide range of biological activities such as antioxidant [8], anti-inflammatory, analgesic [5, 9] and antifungal [10]. The anti-inflammatory and analgesic effects of *T. disciforme* were reported in a previous study [5]. However, there have been no reports regarding the anti-inflammatory activity and fatty acid compositions of *T. parviflorum* and *T. tenuifolium.* Hence, this study was designed to evaluate the fatty acid compositions and *in vivo* anti-inflammatory activity of *T. parviflorum* and *T. tenuifolium* comparatively. This is the first report on the fatty acid compositions of the genus *Tripleurospermum*, with two species.

2. Materials and Methods

2.1. Plant materials

Tripleurospermum parviflorum Willd. Pobed. and *T. tenuifolium* Kit. (*Chrysantemum tenuifolium*) were collected from İzmir-Bozdağ in May 2007. They were identified by B. Kıvçak from Ege University, İzmir, Turkey. The voucher specimens (herbarium numbers: 1366 for *T. parviflorum* and 1368 for *T. tenuifolium*) are deposited in the herbarium of the Faculty of Pharmacy, Ege University, İzmir, Turkey.

2.2. Fatty acid analysis

2.2.1. Extraction of oils

The oil extraction of the dried and powdered aerial parts (40 g) have been carried out at 60ºC for 6 h by Soxhlet extractor using petroleum ether as a solvent. The solvent was evaporated by rotary evaporator. The obtained oil was esterified to determine fatty acid composition.

2.2.2. Fatty acid methyl esters (FAMEs) preparation

The fatty acids in the total lipid were esterified into methyl esters by saponification with 0.5N methanolic NaOH and transesterified with 14% BF_3 (v/v) in methanol [11].

2.2.3. GC conditions

Fatty acid methyl esters (FAMEs) were analyzed on a HP (Hewlett Packard) Agilent 6890 N model Gas Chromatography (GC), equipped with a flame ionization detector (FID) and fitted to a Supelco SP-2380 Fused Silica capillary column (60 m, 0.25 mm i.d. and 0.2 μm film thickness). Injector and detector temperatures were set at 250 and 260ºC, respectively. The oven was programmed at an initial temperature of 140ºC and an initial time of 5 min. Thereafter the temperature was increased up to 240°C at a rate of 3°C/min^{-1}. The total run time was 41.33 min. Helium was used as a carrier gas (1 mL min^{-1}). Identification of fatty acids was made by comparing the relative retention times of FAME peaks from samples with standards [12]. The results were expressed as FID response area in the relative percentages. Each reported result is given as the average value of three GC analyses. The results are offered as means ±SD.

2.3. Biological test

2.3.1. Preparation of the plant extracts

n-Hexane, ethyl acetate, methanol and aqueous extracts were separately prepared from 20 g batches of the air-dried and powdered plant materials by extracting with 200 mL solvent at a room temperature at 24 h. Then the solvents were evaporated to dryness in vacuum (60°C). The yields of *n*-hexane, ethyl acetate, methanol and aqueous extracts of *T. tenuifolium* were calculated as 0.39%, 7.35%, 6.43%, and 5.35%, respectively and for the same solvent extracts of *T. parviflorum* the yields were 0.83%, 5.41%, 6.52% and 4.13%, respectively. All the extracts were stored at -20°C.

2.3.2. Pharmacological procedures

2.3.2.1. Animals

Male Swiss albino mice (20-25 g) were purchased from the animal breeding laboratory of Saki Yenilli (Ankara, Turkey). The animals were left for two days for acclimatization to animal room conditions and maintained on standard pellet diet and water ad libitum. The food was withdrawn on the day before the experiment, but free access to water was allowed. A minimum of six animals was used in each group. The study was permitted by the Institutional Animal Ethics Committee and was performed according to the international rules considering the animal experiments and biodiversity right. (Gazi University Ethical Council Project Number: G.U.ET-05.004).

2.3.2.2. Preparation of test samples for bioassay

All of the extracts were administered in 100 and 200 mg/kg doses after suspending in 0.5% sodium carboxymethylcellulose (CMC) suspension in distilled water. The control group animals received the same experimental handling as those of the test groups except the drug treatment was replaced with appropriate volumes of dosing vehicle. Indomethacin (10 mg/kg and 0.5 mg/ear) in 0.5% CMC was used as reference drug [13].

2.3.3. Anti-inflammatory activity

2.3.3.1. Carrageenan-induced hind paw edema model

Carrageenan-induced hind paw edema model was used for determination of anti-inflammatory activity [13]. 60 min after the oral administration of a test sample or dosing vehicle, each mouse was injected with freshly prepared suspension of carrageenan (0.5 mg/25 µL) in physiological saline (154 nM NaCl) into subplantar tissue of the right hind paw. As the control, 25 µL saline solutions were injected into that of the left hind paw. Paw edema was then measured in every 90 min during 6 h after induction of inflammation. The difference in footpad thickness was measured by gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with those of a control group and analyzed by using statistical methods. Indomethacin (10 mg/kg) was used as the reference drug.

2.3.3.2. Serotonin- induced hind paw edema model

The method of Kasahara et al. was used [14]. Sixty minutes after the oral administration of test sample or dosing vehicle each mouse was injected with serotonin (serotonin creatinin sulfate, Merck, Art. 7768) in Tyrode's solution (0.5 µg/5 µL) into subplantar tissue of the right hind paw and 5 µL of Tyrode's solution into that of the left as secondary control. Measurements were done and evaluated as described above in every 6 min during 30 min.

2.3.4. Acetic acid-induced increase in capillary permeability

Effect of the test samples on the increased vascular permeability induced by acetic acid in mice was determined according to Whittle method with some modifications [15]. Each test sample was administered orally to a group of 10 mice in 0.2 ml/20 g body weight. Thirty minutes after the administration, tail of each animal was injected with 0.1 ml of 4% Evans blue in saline solution (*i.v.*) and waited for 10 min. Then, 0.4 mL of 0.5% (v/v) AcOH was injected *i.p*. After 20 min. incubation, the mice were killed by dislocation of the neck, and the viscera were exposed and irrigated with distilled water, which was then poured into 10 mL volumetric flasks through glass wool. Each flask was made up to 10 mL with distilled water, 0.1 mL of 0.1N NaOH solution was added to the flask, and the absorption of the final solution was measured at 590 nm (Beckmann Dual Spectrometer; Beckman, Fullerton, CA, USA). A mixture of distilled water and 0.5% CMC was given orally to control animals, and they were treated in the same manner as described above.

2.3.5. Acute toxicity

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

2.3.6. Gastric-ulcerogenic effect

After the employment of serotonin-induced hind paw edema model, mice were killed under deep ether anesthesia and the stomachs of each mouse were removed. Then the abdomen of each mouse was opened through the greater curvature and examined under dissecting microscope for lesions or bleedings.

2.3.7. Statistical analysis of data

Data obtained from animal experiments were expressed as the mean (of six data) standard error (±SEM). Statistical differences between the treated and the control groups 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

3.1. Fatty acid composition

Eight fatty acids were identified in oil of *T. tenuifolium* and ten fatty acids were identified in oil of *T. parviflorum*. The results are given in Table 1.

Table 1. Fatty acid composition of *Tripleurospermum tenuifolium*[a] and *T. parviflorum*[a]

Fatty acids	***T. tenuifolium***	***T. parviflorum***
C 10:0 (Capric acid)	-	0.77 ± 0.01
C 14:0 (Myristic acid)	-	4.93 ± 0.02
C 16:0 (Palmitic acid)	47.57[b] ± 0.01	38.61 ± 0.01
C 18:0 (Stearic acid)	6.13 ± 0.02	6.03 ± 0.01
C 20:0 (Arachidic acid)	4.68 ± 0.01	4.08 ± 0.02
C 22: 0 (Behenic acid)	4.77 ± 0.02	4.26 ± 0.02
∑SFA[c]	63.15 ± 0.02	58.68 ± 0.02
C 18:1 (Oleic acid)	8.45 ± 0.02	9.57 ± 0.01
∑MUFA[c]	8.45 ± 0.02	9.57 ± 0.01
C 18:2 (Linoleic acid)	18.42 ± 0.03	20.56 ± 0.03
C 20:1 (Eicosenoic acid)	3.68 ± 0.02	3.71 ± 0.01
C 20:5n3 (Eicosapentanoic acid)	6.79 ± 0.01	7.47 ± 0.02
∑PUFA[c]	28.89 ± 0.02	31.74 ± 0.02

[a] Average of three lots analysed.
[b] Values reported are means ±SD.
[c]SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids.

Eight fatty acids were identified in oil of *T. tenuifolium* and also ten fatty acids were identified in the oil of *T. parviflorum*. The major fatty acids of *T. tenuifolium* and *T.parviflorum* oils were C 16:0 (palmitic acid) (47.57% and 38.61%), C 18:2 (linoleic acid) (18.42% and 20.56%) and C 18:1 (oleic acid) (8.45% and 9.57%). The principal fatty acid in our two species investigated was palmitic acid (C 16:0). C 18:2 (linoleic acid) was the major PUFAs. PUFAs of *T. tenuifolium* and *T. parviflorum* amounted 28.89% and 31.74% of the total fatty acids, while the MUFAs were 8.45% and 9.57%; SFAs were about 63.15% and 58.68%, respectively. The species have a high content of palmitic acid.

Athough there are many reports dealing with the fatty acid composition of the Asteraceae (Compositae) species there is no study on the fatty acid composition of the *Tripleurospermum.* Generally linoleic acid was determined as the major fatty acid of some Asteraceae species [11, 16-18]. Linoleic and oleic acids are called essential fatty acids, because they can not be synthesized by the human body. Oleic and linoleic acid have the ability of lowering blood cholesterol levels. The intake of these fatty acids have been encouraged by nutritionist and the medical professions [19]. The lack of dietary essential fatty acids such as linoleic acid has been implicated in aetiology of diseases including cardiovascular diseases and its progression [20].

3.2. Anti-inflammatory activity

The other objective of the present study was to investigate the anti-inflammatory activity of *T. parviflorum* and *T. tenuifolium*, which were reported to be used against inflammation in Turkish folk medicine. Therefore, carrageenan- and serotonin- induced hind paw edema and acetic acid-induced increase in capillary permeability models were employed for the evaluation of the anti-inflammatory activity. Carrageenan-induced edema has been commonly used as an experimental animal model for acute inflammation and is a biphasic event. The early phase is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins. The late phase is sustained by prostaglandin release and mediated by bradykinin, leukotrienes, polymorphonuclear cells and prostaglandins produced by tissue macrophages [21, 22]. The results of the present study have shown that EtOAc extracts prepared from *T. parviflorum* and *T. tenuifolium* displayed remarkable anti-inflammatory activity at dose of 200 mg/kg. As shown in Table 2, administration of 200 mg/kg EtOAc extract of *T. parviflorum* displayed 27.4% inhibition in carrageenan-induced hind paw edema model at 270 min. However, no significant effect was detected for any extracts on serotonin-induced paw edema in mice (Table 3). On the other hand, EtOAc extract exhibited notable anti-inflammatory effect on acetic acid-induced increase in capillary permeability model with 29.9% inhibition value at 200 mg/kg dose (Table 4).

Table 2. Effect of the extracts from *Tripleurospermum parviflorum* on carrageenan-induced 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		48.1 ±3.7	52.7±3.5	57.6±4.1	56.2±3.9
n-Hexane	100	41.8±3.0 (13.1)	45.1±3.1 (14.4)	49.1±3.7 (14.8)	52.2±3.0 (7.1)
	200	44.5±3.8 (7.5)	53.6±2.8 -	56.2±3.8 (2.4)	53.6±2.6 (4.6)
EtOAc	100	39.6±3.6 (17.7)	49.6±3.4 (5.9)	48.8±3.6 (15.3)	57.4±3.5 -
	200	39.1±2.3 (18.7)	46.1±3.6 (12.5)	41.8±3.4 **(27.4)****	55.3±3.2 1.6
MeOH	100	38.4±3.2 (20.2)	50.3±2.7 (4.6)	49.7±3.5 (13.7)	57.3±3.2 -
	200	40.8±3.9 (15.2)	49.2±3.5 (6.6)	55.3±4.0 (3.9)	59.1±2.6 -
Aqueous	100	51.1±4.2 -	51.7±3.7 (1.9)	52.2±3.7 (9.4)	50.5±3.4 (10.1)
	200	41.2±3.5 (14.3)	48.8±3.6 (7.4)	56.4±3.2 (2.1)	54.4±2.9 (3.2)
Indomethacin	10	35.2±2.9 **(26.8)***	38.1±2.2 **(27.7)***	38.4±1.5 **(33.3)****	36.2±2.7 **(35.6)*****

S.E.M.: Standard error of the mean; * $p<0.05$. ** $p<0.01$. *** $p<0.001$ significant from the control

Table 3. Effect of the extracts from *Tripleurospermum parviflorum* on serotonin-induced paw edema in mice.

Material	Dose (mg/kg)	Swelling thickness (x 10^{-2}mm)± S.E.M. (Inhibition%)					
		0 min	6 min	12 min	18 min	24 min	30 min
Control		3.6±0.8	6.2±1.5	12.0±2.4	19.1±0.9	23.2±1.6	24.2±1.3
n-Hexane	100	4.2±1.5 -	8.0±1.2 -	11.5±0.8 (4.2)	16.2±1.9 (15.2)	18.8±1.7 (18.9)	20.4±1.6 (15.7)
	200	4.7±1.2 -	7.1±1.1 -	8.7±1.1	16.1±1.0 (15.7)	26.3± 1.4 -	25.3±1.7 -
EtOAc	100	3.5±1.1 (2.8)	5.9±1.8 (4.8)	10.3±1.2 (14.2)	16.2±1.7 (15.2)	20.7± 1.6 (10.8)	21.5±1.1 (11.2)
	200	3.2±0.5 (11.1)	5.2±1.2 (16.1)	9.7±1.1 (19.2)	15.9±1.4 (16.8)	19.2± 0.8 (17.2)	18.7±1.7 (22.7)
MeOH	100	3.3±0.7 (8.3)	6.9±1.3 -	9.9±1.6 (17.5)	22.1±1.8 -	25.2±1.7 -	26.3±1.6 -
	200	4.6±1.3 -	5.4±0.8 (12.9)	11.0±1.5 (8.3)	18.2±1.6 (4.7)	21.5±1.3 (7.3)	21.3±2.8 (11.9)
Aqueous	100	4.1±0.9 -	6.3±1.0 -	12.2±1.8 -	20.3±1.5 -	24.2± 1.8 -	22.8±1.7 (5.8)
	200	4.0±0.6 -	8.3±1.6 -	13.1±1.5 -	17.1±1.1 (10.5)	21.8±0.9 (6.0)	25.6±1.8 -
Indomethacin	10	3.5±0.6 (2.8)	5.1±0.5 (17.7)	9.1±0.9 **(24.2)***	14.0±0.7 **(26.7)***	16.2±0.8 **(30.2)****	17.2±1.9 **(28.9)***

S.E.M.: Standard error of the mean; * p<0.05. **p<0.01. *** p<0.001 significant from the control

Table 4. Inhibitory effect of *Tripleurospermum parviflorum* on acetic acid-induced increase in capillary permeability.

Material	**Dose (mg/kg)**	**Evans blue concentration (μg/mL) ± S.E.M.**	**Inhibition (%)**
Control		8.75 ± 1.14	
n-Hexane	100	9.10 ± 1.23	-
	200	8.62 ± 1.34	1.5
EtOAc	100	6.55 ± 0.98	**25.1***
	200	6.13 ± 0.61	**29.9****
MeOH	100	8.66 ± 1.02	1.0
	200	7.24 ± 0.87	17.3
Aqueous	100	8.12 ± 1.09	7.2
	200	7.56 ± 0.99	13.6
Indomethacin	10	4.57 ± 0.42	**47.8*****

S.E.M.: Standard error of the mean; * p<0.05. **p<0.01. *** p<0.001 significant from the control

The data obtained from the activity evaluation of the extracts from *T. tenuifolium* was quite identical to the outcome of *T. parviflorum* EtOAc extract. As shown in Table 5, EtOAc extract of *T. tenuifolium* was found to have moderate activity on carrageenan-induced paw edema model in mice**.** At 200 mg/kg dose, the EtOAc extract demonstrated 23.5% inhibitory effect at 12 min on serotonin-induced paw edema model (Table 6). On the acetic acid-induced increase in capillary permeability test EtOAc extract showed 26.3% inhibitory effect at 200 mg/kg dose (Table 7).

Table 5. Effect of the extracts from *Tripleurospermum tenuifolium* on carrageenan-induced 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		45.3 ±3.5	50.1±3.2	53.3±3.6	55.1±3.3
n-Hexane	100	43.5±3.3 (3.9)	48.5±2.9 (3.2)	51.2±3.3 (3.9)	50.6±3.3 (8.2)
	200	41.7±3.4 (7.9)	47.6±2.5 (4.9)	50.2±3.7 (5.8)	57.3±2.8 -
EtOAc	100	40.6±3.1 (10.4)	45.9±2.1 (8.4)	47.6±3.5 (10.7)	55.8±3.1 -
	200	39.3±3.0 (13.2)	37.9±2.4 **(24.4)***	42.6±2.3 (20.1)	49.3±2.9 (10.5)
MeOH	100	48.2±3.1 -	49.6±2.8 (0.9)	45.6±3.1 (14.4)	52.7±3.5 (4.4)
	200	39.9±3.3 (11.9)	47.9±3.3 (4.4)	50.5±3.6 (5.3)	56.2±3.8 -
Aqueous	100	50.6±4.0 -	52.5±3.5 -	51.6±3.5 (3.2)	55.7±3.2 -
	200	42.5±3.0 (2.21)	50.0±3.2 (0.2)	52.8±3.0 (0.9)	52.7±3.9 (4.4)
Indomethacin	10	35.3±2.7 **(22.1)***	37.7±2.0 **(24.8)***	37.6±1.8 **(29.5)****	36.6±2.1 **(33.6)*****

S.E.M.: Standard error of the mean; * p<0.05. **p<0.01. *** p<0.001 significant from the control

Table 6. Effect of the extracts from *Tripleurospermum tenuifolium* on serotonin-induced paw edema in mice.

Material	Dose (mg/kg)	Swelling thickness (x 10^{-2}mm)± S.E.M. (Inhibition%)					
		0 min	6 min	12 min	18 min	24 min	30 min
Control	-	4.2±0.9	5.9±1.4	13.2±2.5	21.5±1.1	24.6±1.4	27.5±1.0
n-Hexane	100	4.0±0.7 (4.8)	7.6±1.1 -	11.9±0.9 (9.8)	18.6±1.8 (13.5)	19.9±1.3 (19.1)	23.6±1.2 (14.2)
	200	4.3±1.3 -	6.4±1.5 -	12.6±1.2 (4.5)	19.5±1.4 (9.3)	22.3±1.2 (9.3)	28.2±1.9 -
EtOAc	100	3.9±0.8 (7.1)	5.0±0.9 (15.3)	10.7±1.1 (7.6)	20.4±1.5 (5.1)	19.5±1.3 (20.7)	24.1±1.2 (12.4)
	200	3.7±0.7 (11.9)	4.8±0.7 (18.7)	10.1±0.7 **(23.5)***	19.8±1.2 (7.9)	19.3±1.5 (21.5)	22.1±1.6 (19.6)
MeOH	100	4.0±0.9 (4.8)	5.4±1.2 (8.5)	12.1±1.5 (8.3)	20.6±1.7 (4.2)	20.3±1.1 (17.5)	24.6±1.1 (10.5)
	200	3.9±1.0 (7.1)	5.1±0.9 (13.6)	11.5±1.0 (12.9)	18.7±1.5 (13.0)	25.2± 1.8 -	25.2±1.4 (8.4)
Aqueous	100	4.4±1.3 -	6.7±1.4 -	14.1±1.7 -	21.3±1.3 (0.9)	24.7± 1.7 -	26.9±1.5 (2.2)
	200	4.3±0.9 -	6.2±1.1 -	13.8±1.4 -	21.6±1.2 -	24.9±1.9	26.8±1.5 (2.5)
Indomethacin	10	3.6±0.5 (14.3)	4.5±0.6 **(23.7)***	9.6±0.8 **(27.3)****	15.8±1.2 **(26.5)***	16.3±0.9 **(33.7)****	18.0±0.9 **(34.5)****

S.E.M.: Standard error of the mean; * p<0.05. **p<0.01. *** p<0.001 significant from the control

This study highlights fatty acid compositions and anti-inflammatory activities of *T. parviflorum* and *T. tenuifolium*. The content of palmitic acid is much higher in *T. tenuifolium* than in *T.parviflorum*, but the content of linoleic acid is higher in *T. parviflorum* than in *T. tenuifolium*. Fatty acids are known as self-defensive agents in organism and possess various biological activities including anti-inflammation [23-27]. The high content of linoleic acid and palmitic acid may be primarily responsible for significant anti-inflammatory activity. The results of this study could explain the efficiency in medicinal usage of this plant for the anti-inflammation purpose.

Table 7. Inhibitory effect of *Tripleurospermum tenuifolium* on acetic acid-induced increase in capillary permeability.

Material	**Dose (mg/kg)**	**Evans blue concentration (μg/mL) ± S.E.M.**	**Inhibition (%)**
Control		10.43 ± 1.22	
n-Hexane	100	8.44 ± 0.85	19.1
	200	8.92 ± 0.98	14.5
EtOAc	100	8.57 ± 0.72	17.8
	200	7.69 ± 0.74	**26.3***
MeOH	100	9.12 ± 1.10	12.6
	200	8.76 ± 1.11	16.0
Aqueous	100	9.66 ± 1.44	7.4
	200	8.99 ± 1.41	13.8
Indomethacin	10	6.11 ± 0.67	**41.4*****

S.E.M.: Standard error of the mean

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

In the previous biological activity studies, chloroform and hydroalcoholic extracts of *T. disciforme* were shown to have antioxidant and anti-ulcerogenic potential [28, 29]. Moreover, the anti-inflammatory and analgesic effects of *T. disciforme*, a native plant of Iran, were studied using carrageenan induced edema, formalin and tail-flick test. *T. disciforme* extract displayed sigificant anti-inflammatory and antinociceptive acivities in the models tested. The results of the study suggested that the antinociceptive effect of the extract was due to the inhibition of prostaglandin release and other mediators [5]. Primary studies on this plant indicated that the flavonoids and the essential oil components are the main secondary metabolites of the flower parts of this plant [30] and flavonoid type components could be responsible from the anti-inflammatory activity. Since in traditional medicine, plant flavonoids have been used for their antioxidant and anti-inflammatory properties for centuries. Inflammation is the body's natural response to the damage and should be carefully regulated to prevent unwanted immune response. Flavonoids have been shown to prevent excessive inflammation. In the present study the EtOAc extracts of *T. parviflorum* and *T. tenuifolium* displayed significant moderate anti-inflammatory activity in the carrageenan-induced hind paw edema and acetic acid-induced increase in capillary permeability models. In the phytochemical studies on *Tripleurospermum* species, apigenin, apigenin-7-glucoside, apigenin-7-glucuronide, luteolin, luteolin-7-glucoside, luteolin-7-glucuronide, quercetin, quercetin-7-glucoside and chrysoeriol were isolated from this genus [31]. Therefore, it could be suggested that flavonoid type compounds in the EtOAc extracts could probably be responsible from the anti-inflammatory activity [32].

4. Conclusions

This study is the first to demonstrate that the EtOAc extracts of *T. parviflorum* and *T. tenuifolium* possess notable anti-inflammatory activity. Further studies on this plant may yield purification of the active components that might be investigated as leads to develop new medicines. On the other hand, our study has reported for the first time on the fatty acid composition of *Tripleurospermum* species.

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