Fatty acid composition and preclinical resarches on *Anthemis wiedemanniana* Fisch. & Mey.: Discovery of a new anti-inflammatory agent

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ABSTRACT

Background: *Anthemis* species have been used for the treatment of gastrointestinal disorders, hemorrhoid, stomachache and inflammatory diseases in Turkish folk medicine. *Anthemis wiedemanniana* Fisch. And Mey. is an endemic plant used as painkiller, antispasmodic, sedative and for the treatment of urinary inflammations. **Objective:** The objective of the present study is to evaluate the anti-inflamatory activity of the extracts of *A. wiedemanniana* by using *in vivo* methods. **Materials and Methods:** Carrageenan-, PGE_2- and serotonin-induced hind paw edema, 12-*O*-tetradecanoyl-13-acetate (TPA)-induced mouse ear edema and acetic acid-induced increase in capillary permeability models were used for the anti-inflammatory activity assessment. Moreover, the fatty acid composition of *A. wiedemanniana* was investigated by gas chromatography (GC). **Results:** *n*-Hexane, diethyl ether and total sesquiterpene lactone extracts exhibited significant inhibition in carrageenan-induced hind paw edema and acetic acid-induced increase in capillary permeability model. *n*-Hexane and total sesquiterperne lactone extracts showed anti-inflammatory activity in PGE_2- and serotonin-induced hind paw edema model. However none of the extracts displayed significant activity in TPA-induced ear edema model in mice. $C_{4:0}$ (Buthyric acid), $C_{20:0}$ (Arachidic acid) and $C_{16:1}$ (Palmitoleik acid) were found to be the major fatty acids in these species. Saturated fatty acids (SFA) were found in higher amounts than monounsaturated fatty acids and polyunsaturated fatty acids. SFAs were determined as 63.17%, UFAs as 20.89% and PuFAs as 15.95%. **Conclusion:** This study confirms the traditional usage of *A. wiedemanniana* for inflammatory diseases.

Key words: *Anthemis wiedemanniana, Asteraceae*, Anti-inflammatory, fatty acid

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INTRODUCTION

The genus *Anthemis* L., from the family *Asteraceae*, comprises of approximately 210 species,[1] and is represented with 51 species, 29 of which are endemic to Turkey.[2] *Anthemis* species are used for the treatment of gastrointestinal disorders, hemorrhoid and stomachache in Turkish traditional medicine.[3-5] Extracts, tinctures, salves and tisanes are used as antibacterial, antispasmodic and for the treatmnet of inflammatory diseases in Europe[6] *A. wiedemanniana* Fisch. and Mey. is an endemic species, which is distributed in the southern and eastern regions of Turkey.[2] This plant is called "Papatya" in the western part of Turkey and infusion prepared from *A. wiedemanniana* is used in Turkish traditional medicine against abdominal pain.[4] A decoction prepared from *A. wiedemanniana* is used as painkiller, antispasmodic, sedative and against urinary inflammations in Elazığ region.[7] It is also used for the treatment of cough and cold.[8] The genus *Anthemis* is characterized mainly by the presence of sesquiterpene lactones, belonging to germacranolides, eudesmanolides, guaianolides[9,10] and flavonoids.[11] The literature survey on *A. wiedemanniana* revealed the presence of sesquiterpene lactones of germacrane, eudesmane and guaianolide type.[9-11] According to a previous study, linalool, 1,8-cineole, hexadecanoic acid and chrysanthenone were found to be the major compounds characterized in the essential oil of *A. wiedemanniana*.[6] Fatty acids are known as self defensive agents in organisms and posses various biological activities including anti-inflamation.[12] The plants such as *A. tinctoria* and *A. triumfeti* belonging to the *Asteracea* family have a rich fatty acid composition.[13,14] *Anthemis* species are used in Turkish folk medicine due to their anti-inflamatory activities. However, there have been

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no reports regarding the anti-inflamatory activity and fatty acid composition of *A. wiedemanniana*. Hence, this study was designed to investigate the fatty acid composition and evaluate anti-inflamatory activity of *A. wiedemanniana*.

MATERIALS AND METHODS

Plant material

The aerial parts of *A. wiedemanniana* were collected from Izmir-Bayındır (Allankıyı village), in June 2004 and identified by S.G. Senol from Ege University (Izmir). A voucher specimen (No 1418) is deposited in the Herbarium of the Faculty of Pharmacy, Ege University, Izmir.

Extraction of the plant material

Diethyl ether, *n*-hexane, ethyl acetate, water and methanol extracts were separetely prepared from 40 g batches of the air-dried and powdered plant material by extracting with 400 ml solvent at room temperature for 24 h. Then the solvents were evaporated to dryness in vacuo. The yields of *n*-hexane, diethyl ether, ethyl acetate, methanol and aqueous extracts of A. wiedemanniana were 0.55%, 1.025%, 0.98%, 1.33% and 2.5%, respectively.

Total sesquiterpene lactone extract: Air-dried plant material (40 g) was extracted at room temperature with petroleum ether-Et_2O (peroxides free)-MeOH (1:1:1 *v/v*) and concentrated under vacuum to yield a crude extract. This extract was dissolved with a mixture of cyclohexane-Et_2O (peroxides free)-MeOH (1:1:1 *v/v*). Brine was added in the extract and the aqueous phase was extracted with EtOAc. The organic phase was concentrated under vacuum to dryness and yielded a lipophilic residue (0.14%).[9]

Fatty acid analysis

Oil extraction

The oil extraction of the air dried and powdered aerial parts (10 g) of *A. wiedemanniana* was carried out at 60°C for 6 h by Soxhlet extractor, using petroleum ether as a solvent. The solvent was evaporated by a rotary evaporator.

Fatty acid metyl esters preparation

The fatty acids were esterified in to methyl esters by saponificition with 0.5 N methanolic NaOH and transesterified with %14 BF_3 (v/v) in methanol.[15]

GC conditions

Fatty acid methyl esters (FAMEs) were analyzed on a HP (Hewlett Packard) Agilent 6890 N model GC, equipped with a flame ionization detector (FID) and fitted to a SPTM-2560 Fased Silica capillary column (100 m, 0.25 mm i.d. and 0.20 μm). The injector and detector temperatures were 250 and 260°C respectively. The oven was programmed at an initial temperature of 140°C and initial time of 5 min. Thereafter, the temperature was increased up to 240°C at a rate of 4°C/min^{-1}. The total run time was 40 min. Helium was used as a carrier gas (1 ml min^{-1}). Identification of the fatty acids was carried out by comparing FAME peak relative retention times. The results were expressed as FID response area in relative percentages. Results were given as the average value of three analyses and presented as means ± SD [Table 1].

Pharmacological procedures

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

Table 1: Fatty acid composition of *A. wiedemanniana*

Compound name	Retention time	Area	Area %
Butyric acid methyl ester	3.88	2141198	36.13
Caproic acid methyl ester	4.08	71649	1.21
Caprylic acid methyl ester	4.28	21588	0.36
Capric acid methyl ester	4.91	15568	0.26
Undecanic acid methyl ester	6.16	31393	0.53
Tridecanoic acid methyl ester	8.36	17560	0.30
Myristoleic acid methyl ester	11.43	95113	1.60
Pentadecanoic acid methyl ester	12.78	10269	0.17
cis Pentadecanoic acid methyl ester	13.15	23028	0.39
Palmitic acid methyl ester	14.48	27576	0.47
Palmitoleic acid methyl ester	14.90	918344	15.49
Heptadecanoic acid methyl ester	15.98	14560	0.25
Cis Heptadecanoic acid methyl ester	16.62	20841	0.35
Stearic acid methyl ester	17.67	78048	1.32
Elaidic acid methyl ester	18.32	168566	2.84
Linolelaidic acid methyl ester	19.20	400055	6.75
Arachidic acid methyl ester	20.62	1114121	18.80
Gamma Linoleic acid methyl ester	21.52	92505	1.56
Linoleic acid methyl ester	22.22	91054	1.84
Heneicosanoic acid methyl ester	22.34	10308	0.17
Cis-11;14-Eicosanoic acid methyl ester	23.04	11016	0.19
Behenic acid methyl ester	24.02	133159	2.25
Cis-8'11'14-Eicosatrienoic acid methyl ester	24.51	97060	1.64
Tricosanoic acid methyl ester	25.91	16209	0.27
Cis-13'16-Docosadienoic acid methyl ester	26.65	14542	0.25
Lignoseric acid methyl ester	26.87	40478	0.68
cis-5;8;11;14;17-Eicosapentaenoicacid methyl ester	27.27	124303	2.10
Nervonic acid methyl ester	27.98	12753	0.22
Cis-4'7'10'13'16'19-docosahexaenoic acid m. ester	29.85	96068	1.62
Total		5926995	100.00

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 waterwas allowed. A minimum of six animals were 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.

Preparation of test samples for bioassay

All of the extracts were administered in 100, 200 and 400 mg/kg[1] doses after suspending in 0.5% sodium carboxymethylcellulose (CMC) suspension in distilled water. The control group animals received 0.5% CMC suspension. Indomethacin (10 mg/kg^{-1} and 0.5 mg/ear) in 0.5% CMC was used as reference drug.

Anti-inflammatory activity models

Carrageenan-induced hind paw edema model

Carrageenan-induced hind paw edema model was used for determination of anti-inflammatory activity[16] 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^{-1}) 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.

Table 2: Effect of the extracts from *Anthemis wiedemanniana* on carrageenan-induced paw edema model in mice

Material	Dose (mg kg^{-1})	Swelling thickness (x 10^{-2} mm)±SEM (Inhibition %)			
		90 min	180 min	270 min	360 min
Control		46.3±3.0	49.1±3.0	53.6±2.5	54.0±3.1
n-Hexane extract	100	38.5±3.0 (16.8)	51.7±3.9 -	56.2±3.2 -	59.1±4.0 -
	200	39.1±3.6 (15.6)	36.6±2.1 (25.5)**	40.7±2.2 (24.1)*	48.2±3.0 (3.5)
	400	48.2±3.7 -	49.8±3.2 -	52.4±3.3 (2.2)	52.1±3.4 (3.5)
Diethyl ether extract	100	40.6±3.2 (12.3)	42.7±2.6 (13.0)	44.5±3.7 (16.9)	56.2±3.8 -
	200	36.4±2.2 (21.4)	37.4±2.8 (23.8)*	43.5±3.2 (18.8)	49.7±3.2 (7.9)
	400	41.7±2.8 (9.9)	47.7±2.9 (2.9)	47.3±3.0 (11.8)	52.3±2.8 (3.1)
Ethyl acetate extract	100	38.1±3.4 (17.7)	41.3±2.5 (15.9)	52.6±3.8 (1.9)	50.2±2.4 (7.0)
	200	42.8±3.6 (7.6)	42.6±2.3 (13.2)	48.2±3. (10.1)	55.6±3.3 -
	400	43.3±3.1 (6.5)	50.3±3.0 -	49.9±2.8 (6.9)	58.5±3.1 -
MeOH extract	100	39.4±2.8 (14.9)	46.2±3.2 (5.9)	50.7±2.9 (5.4)	52.3±3.6 (3.1)
	200	39.3±3.1 (15.1)	50.1±3.7 -	48.1±3.6 (10.3)	48.6±2.9 (10.0)
	400	45.2±2.7 (2.4)	49.5±3.5 -	45.0±3.0 (16.0)	50.1±3. (7.2)
Aqueous extract	100	44.1±3.2 (4.8)	40.0±3.3 (18.5)	52.4±3.8 (2.2)	55.4±2.7 -
	200	38.1±2.6 (17.7)	51.3±2.8 -	49.1±2.6 (8.2)	56.6±3.9 -
	400	40.2±3.5 (13.2)	42.5±3.8 (13.4)	50.2±3.5 (6.3)	49.4±3.2 (8.5)
Total sesquiterpene lactone extract	100	34.0±2.3 (26.6)**	38.1±2.6 (22.4)	47.2±3.6 (11.9)	52.4±2.6 (2.9)
	200	32.1±2.7 (30.7)**	35.7±2.5 (27.3)**	39.3±2.3 (26.7)**	53.2±3.5 (1.5)
	400	47.4±2.9 -	45.1±3.1 (8.1)	48.1±3.5 (10.3)	49.8±2.2 (7.8)
Indomethacin	10	31.2±2.8 (32.6)**	31.3±2.4 (36.3)***	36.7±1.7 (31.5)**	30.2±2.2 (44.1)***

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

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^{-1}) was used as the reference drug.

PGE_2-induced hind paw edema model

PGE_2-induced hind paw edema model was used for the determination of anti-inflammatory activity.[17] 60 min after the oral administration of a test sample or dosing vehicle, each mouse was injected with freshly prepared suspension of PGE_2 (5 μg 5 μl^{-1}) in Tyrode's solution into subplantar tissue of the right hind paw. As the control, 5 μl Tyrode's solution was injected into that of the left hind paw. Paw edema was measured in every 15 min during a period of 75 min after the 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 mean values of a control group andstatistically analyzed. Indomethacin (10 mg/kg^{-1}) was used as reference drug.

Serotonin- induced hind paw edema model

The method of Kasahara *et al.,* was used.[17] 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^{-1}) 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.

Table 3: Inhibitory effect of the extracts from *Anthemis wiedemanniana* on acetic acid-induced increase in capillary permeability

Material	Dose (mg kg^{-1})	Evans blue concentration (μg/ml)±SEM	Inhibition (%)
Control		10.13±1.23	
n-Hexane extract	100	8.78±0.72	13.3
	200	7.12±0.63	29.7**
	400	9.02±1.14	10.9
Diethyl ether extract	100	8.14±0.88	19.6
	200	7.81±1.02	22.9*
	400	8.43±0.93	16.8
Ethyl acetate extract	100	9.33±0.79	7.9
	200	9.28±0.96	8.4
	400	8.62±0.84	14.9
MeOH extract	100	10.25±0.55	-
	200	9.12±0.69	9.9
	400	9.28±0.95	8.4
Aqueous extract	100	10.44±1.32	-
	200	10.02±1.27	1.1
	400	8.45±0.80	16.6
Total sesquiterpene lactone extract	100	8.06±0.92	20.4
	200	7.61±0.81	24.9*
	400	9.14±0.61	9.8
Indomethacin	10	5.16±0.32	49.1***

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

TPA-induced mouse ear edema

Each mouse received 2.5 μg of TPA (12-*O*-tetradecanoylphorbol 13-acetate) dissolved in 20 μl of EtOH 70%.[18] 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%), simultaneously with TPA. Indomethacin (0.5 mg/ear) was used as reference drug. For the evaluation of the activity, two different measurements were taken as given below.

The thickness of each ear was measured 4 h after induction of inflammation using gauge calipers (Ozaki Co., Tokyo, Japan). The edema was expressed as the difference between the right and left ears due to TPA application and consequently inhibition percentage was expressed as a reduction thickness with respect to the control group.

After 4 h of the administration 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 the ear thickness.

Acetic acid-induced increase in capillary permeability

Effect of the test samples on the increased vascular permeability induced by acetic acid in micewas determined according to Whittle method with some modifications.[19,20] Each test sample was administered orally to a group of 10 mice in 0.2 ml 20 g^{-1} 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 10ml with distilled water, 0.1ml of 0.1N NaOH solution was added to the flask and the absorption of the final solution was measured at 590nm (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.

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.

Gastric-ulcerogenic effect

After the employment of PGE_2-induced hind paw edema model antinociceptive activity experiment, 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.

Statistical analysis of data

Data obtained from animal experiments were expressed as the mean 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 [* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$].

RESULT AND DISCUSSION

Histamine, serotonin, bradykinin, and prostaglandins play important role in carrageenan-induced paw edema model.[21] In the early phase (90-180 min) of the inflammation, histamine and serotonin are released. In the later phase (270-360 min) prostaglandins (PG), proteases and lysosomes are activated.[22] Among the most predominant of these chemicals are prostaglandins. PGE_2 is pain-producing class of prostaglandins. Greater amounts of the PGE_2 increase the propensity towards pain. So, if acute inflammation continues for long periods of time,

Table 4. Effect of the extracts from *Anthemis wiedemanniana* on PGE_2-induced paws edema in mice

Material	Dose (mg kg^{-1})	Swelling thickness (x 10^{-2} mm)± SEM (Inhibition)					
		0 min	15 min	30 min	45 min	60 min	75 min
Control		3.4±1.1	15.4±1.6	22.6±1.5	13.2±1.4	12.1±1.8	7.6±1.0
n-Hexane extract	100	3.1±0.9 (8.8)	13.6±1.8 (11.7)	18.5±1.4 (18.1)	12.6±1.0 (4.5)	11.7±1.2 (3.3)	8.0±1.3 (5.3)
	200	3.6±1.2 -	10.2±1.8 (33.8)**	19.3±1.6 (14.6)	10.4±1.6 (21.2)*	9.3±1.9 (23.1)*	7.1±1.4 (6.6)
	400	2.9±0.5 (14.7)	12.8±1.4 (16.9)	16.1±1.5 (28.8)*	11.0±1.1 (16.7)	11.9±1.9 (1.7)	9.5±1.0 -
Diethyl ether extract	100	4.1±1.6 -	14.5±1.0 (5.8)	24.2±2.1 -	13.5±1.6 -	10.4±2.0 (14.0)	11.1±1.3 -
	200	3.2±0.7 (5.9)	12.6±1.2 (18.2)	19.5±1.9 (13.7)	11.1±1.5 (15.9)	9.6±1.7 (20.7)	6.8±1.1 (10.5)
	400	3.1±0.8 (8.8)	13.2±1.6 (14.3)	20.3±1.9 (10.2)	15.4±1.7 -	13.4±2.0 -	8.4±1.6 -
Ethyl acetate extract	100	2.8±1.6 (17.6)	14.1±1.3 (8.4)	20.4±2.1 (9.7)	14.3±1.9 -	9.7±1.4 (19.8)	6.6±1.6 (13.2)
	200	3.9±1.2 -	14.1±1.5 (8.4)	21.6±1.8 (4.4)	15.1±1.5 -	13.4±2.1 -	7.4±1.8 (2.6)
	400	4.0±1.6 -	16.1±1.4 -	25.3±1.3 -	12.1±1.1 (8.3)	10.3±2.2 (14.9)	7.1±1.2 (6.6)
MeOH extract	100	3.2±1.4 (5.9)	16.3±1.5 -	21.1±1.7 (6.6)	15.1±1.9 -	15.4±2.1 -	8.4±1.5 -
	200	2.8±1.3 (17.6)	12.8±1.3 (16.9)	19.8±1.6 (12.4)	12.5±1.7 (5.3)	16.3±2.4 -	8.2±1.6 -
	400	3.5±0.8 -	14.5±1.2 (5.8)	20.3±1.5 (10.2)	13.7±1.8 -	10.5±1.5 (13.2)	7.9±1.2 -
Aqueous extract	100	3.2±1.2 (5.9)	15.2±0.8 (1.3)	25.3±1.6 -	16.2±2.0 -	12.7±2.1 -	12.0±1.6 -
	200	3.0±0.9 (11.7)	15.9±1.1 -	19.7±1.4 (12.8)	11.1±1.2 (15.9)	13.4±1.4 -	9.3±1.4 -
	400	3.1±0.2 (8.8)	13.4±1.2 (12.9)	18.1±1.9 (19.9)	14.2±1.8 -	11.4±1.8 (5.8)	6.6±1.5 (13.2)
Total sesquiterpene lactone extract	100	3.6±0.7 -	13.3±1.2 (13.6)	18.1±1.3 (19.9)	9.1±1.2 (31.1)**	7.9±1.6 (34.7)**	7.5±1.2 (1.3)
	200	3.3±0.6 (2.9)	9.6±1.3 (37.7)**	17.3±1.5 (23.5)*	10.3±1.6 (21.9)*	8.5±2.5 (29.8)*	7.8±1.5 -
	400	2.9±0.8 (14.7)	12.5±1.7 (18.8)	23.8±1.2 -	15.2±1.8 -	10.2±2.4 (15.7)	8.2±1.9 -
Indomethacin	10	2.7±0.2 (20.6)	12.6±1.4 (18.2)	12.6±1.2 (44.2)***	7.4±1.1 (43.9)***	6.3±1.3 (47.9)***	6.1±0.7 (19.7)

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

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 and so on. In order to test

Table 5. Effect of the extracts from *Anthemis wiedemanniana* on serotonin-induced paw edema in mice

Material	Dose (mg kg^{-1})	Swelling thickness (x 10^{-2}mm)± S.E.M. (Inhibition%)					
		0 min	6 min	12 min	18 min	24 min	30 min
Control		4.5±0.7	9.1±1.6	14.2±1.7	17.8±0.6	20.9±1.4	24.5±1.1
n-Hexane extract	100	4.0±1.7 (11.1)	6.6±1.3 (27.5)**	10.4±1.4 (26.8)*	16.1±1.5 (9.6)	17.3±0.6 (17.2)	20.2±1.2 (17.6)
	200	4.1±0.8 (8.9)	8.2±1.7 (9.9)	10.5±1.9 (26.1)*	13.6±1.2 (23.6)*	16.7±1.6 (20.1)	18.2±1.5 (25.7)*
	400	5.2±0.9 -	10.3±1.8 -	11.6±1.1 (18.3)	19.3±1.2 -	22.6±1.7 -	22.6±1.3 (7.8)
Diethyl ether extract	100	4.6±0.8 -	10.1±1.5 -	12.1±1.4 (14.8)	18.1±1.1 -	17.4±1.3 (16.7)	27.1±1.5 -
	200	4.8±1.0 -	7.2±1.4 (20.9)	13.2±1.5 (7.0)	15.4±1.3 (13.5)	21.7±1.6 -	19.9±1.3 (18.8)
	400	4.0±0.3 (11.1)	9.2±1.3 -	15.8±1.2 -	14.7±1.5 (17.4)	21.5±1.7 -	25.7±1.9 -
Ethyl acetate extract	100	4.7±0.7 -	8.2±0.9 (9.9)	12.4±1.7 (12.7)	19.1±1.8 -	20.1±1.4 (3.8)	23.3±1.4 (4.9)
	200	4.1±0.8 (8.9)	8.3±1.5 (8.8)	13.6±1.8 (4.2)	20.2±1.4 -	22.2±1.3 -	21.6±1.3 (11.8)
	400	3.8±0.9 (15.6)	7.6±1.3 (16.5)	12.3±1.5 (13.4)	15.4±1.9 (13.5)	20.7±1.0 (0.9)	19.9±1.5 (18.8)
MeOH extract	100	3.9±0.5 (13.3)	7.5±1.4 (17.6)	13.1±1.4 (7.7)	15.2±1.3 (12.9)	19.4±1.6 (7.2)	21.3±1.7 (13.1)
	200	4.8±0.4 -	7.3±0.7 (19.8)	13.3±1.6 (6.3)	19.1±1.0 -	18.3±1.4 (12.4)	20.5±2.0 (16.3)
	400	4.6±1.2 -	8.9±1.2 (2.2)	12.4±1.8 (12.7)	18.5±1.2 -	19.2±0.9 (8.1)	23.6±1.6 (3.7)
Aqueous extract	100	4.2±0.8 (6.7)	8.0±1.5 (12.1)	17.3±1.3 -	16.2±1.8 (8.9)	21.6±1.5 -	22.5±1.4 (8.2)
	200	3.7±0.4 (17.8)	7.4±1.8 (18.7)	14.1±1.6 (0.7)	17.6±1.5 (1.1)	19.1±1.2 (8.6)	20.7±1.2 (15.5)
	400	4.2±0.7 (6.7)	7.9±0.9 (13.2)	15.3±1.7 -	17.0±1.6 (4.5)	21.9±1.0 -	23.2±1.8 (5.3)
Total sesquiterpene lactone extract	100	3.9±1.2 (13.3)	6.4±1.1 (29.7)**	13.4±1.2 (5.6)	20.3±1.6 -	17.2±1.5 (17.7)	19.3±1.4 (21.2)
	200	4.2±0.6 (6.7)	6.7±1.6 (26.4)*	10.3±1.4 (27.5)**	13.5±1.7 (24.2)*	23.9±1.8 -	18.7±1.1 (23.7)*
	400	3.8±1.6 (15.6)	8.8±1.2 (3.3)	15.5±1.9 -	18.2±2.0 -	19.1±1.1 (8.6)	24.3±1.8 (0.8)
Indomethacin	10	3.7±0.5 (17.8)	6.2±1.6 (32.2)*	10.8±1.9 (23.9)*	13.1±1.0 (26.4)*	13.5±0.9 (35.4)**	15.1±0.8 (38.4)**

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 *Anthemis wiedemanniana* against TPA-induced ear edema in mice as measurement swelling thickness and weight measurement of edema

Test samples	Dose (mg/ear)	Swelling thickness (mm)±SEM	Inhibition %	Weight edema (mg)±SEM	Inhibition %
Control		328.6±32.6		27.2±3.5	
n-Hexane extract	0.5	294.6±25.4	10.3	21.8±2.8	19.9
Diethyl ether extract	0.5	334.6±25.6	-	28.5±3.5	-
Ethyl acetate extract	0.5	324.9±26.2	1.1	28.3±2.9	-
MeOH extract	0.5	314.7±38.2	4.2	30.2±3.7	-
Aqueous extract	0.5	310.1±30.1	5.6	29.0±2.6	-
Total sesquiterpene lactone extract	0.5	263.7±21.1	19.7	22.4±3.0	17.6
Indomethacin	0.5	95.1±6.3	71.1***	14.2±1.3	47.8***

S.E.M.: Standard error of the mean, *P<0.05. **P<0.01. ***P<0.001 significant from the control, TPA: 12-O-tetradecanoylphorbol 13-acetate

the effects of the extracts on the prostaglandin synthesis and to elucidate the anti-inflammatory mechanism, PGE_2- and serotonin-induced hind paw edema models were used. The mechanism of TPA-induced mouse ear edema is an acute inflammation model, which depends mainly on leukotrienes (LT). LT are synthesized by the lipoxygenases[23] and cause infiltration of macrophages and neutrophil, the induction of TNF-α and IL-1 and the generation of ROS, thus can act as a useful acute model to investigate the anti-arthritic potential of the test materials.[24] In the present study, TPA-induced ear-edema model was employed to assess the activity of the extracts on lipoxygenase metabolites. The extracts prepared from *A. wiedemanniana* were evaluated for the anti-inflammatory effect by carrageenan-, PGE_2- and serotonin- induced hind paw edema, 12-*O*-tetradecanoyl-13-acetate (TPA)-induced ear edema and acetic acid-induced increase in capillary permeability models at doses of 100, 200 and 400 mg kg^{-1} body weight. The experimental results were listed in Tables 1-6. *n*-Hexane, diethyl ether and total sesquiterperne lactone extracts exhibited significant anti-inflammatory activity ranging between 3.5-25.5%, 7.9-23.8% and 1.5-30.7% respectively in carrageenan-induced hind paw edema; and 29.7%, 22.9% and 24.9% inhibition respectively in acetic acid-induced increase in capillary permeability model at dose of 200 mg kg^{-1} [Tables 2 and 3]. Similarly, *n*-hexane and total sesquiterperne lactone extracts provided remarkable anti-inflammatory activity in both anti-inflammatory activity models. 33.8 and 37.7% inhibition values were determined for the *n*-hexane and total sesquiterperne lactone extracts, respectively in PGE_2-induced hind paw edema model [Table 4].The same extracts were shown to have anti-inflammatory effect in serotonin-induced hind paw edema model at 100 and 200 mg/kg^{-1} doses [Table 5]. However none of the extracts displayed anti-inflammatory activity in TPA-induced ear edema model in mice [Table 6].

PUFAs have a role in regulating of inflammatory responses through the production of inflammatory mediators termed eicosanoids.[25,26] PUFA with 20 carbon chains, can play active role in the synthesis of eicosonoids by syclooxygenase and lipoxygenase. Fatty acids constitute an imported component of all cell membranes and influence membrane fluidity and the behavior of membrane-bound enzymes and receptors. They regulate a wide range of functions in the body, including blood pressure, blood clotting and they have several anti-inflammatory effects, but these are dose dependent.[27] The plants belonging to the *Asteracea* family have a rich fatty acid composition such as *A. tinctoria* and *A. triumfeti*.[13,14]

The fermentation of non-starch polysacharides are considered as butyrate sources for intestinal flora. Butyric acid, a short chain fatty acid and butyrate derivatives play an important role in the development of epitelial cells for improved gastrointestinal health.[28,29] In addition to antibacterial properties, butyric acid producing bacteria are promising probiotic tratment for gastrointestinal tract diseases such as inflammatory bowel disese (IBD), Crohn's disese (CD) and ulcerative colitis (UC). In a previous study, butyrate supplementation was reported to prevent and treat diet induced obesity and insulin resistance in mouse models of obesity.[30]

Butyric acid methyl ester is found to be major consitituent of the oil extracted from *A. wiedemanniana*. Therefore, anti-inflammatory activiy of the *n*-hexane extract could be attributed to this compound.

CONCLUSION

There have been no reports on the fatty acid composition and anti-inflamatory activity of *A. wiedemanniana*. In this study, the fatty acid composition of *A. wiedemanniana* was analyzed and anti-inflamatory activity was evaluated. Further anti-inflamatory mechanism studies should be conducted for the discovery of potential agents against inflammatory diseases.

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