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In vivo activity assessment of some *Tanacetum* species used as traditional wound healer along with identification of the phytochemical profile by a new validated HPLC method

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ABSTRACT

Objective(s): *Tanacetum* species are traditionally used as insecticide, and externally wound healer as well as for anti-inflammatory and antihistaminic properties. The *in vivo* wound-healing and anti-inflammatory potential of four *Tanacetum* species, *Tanacetum argenteum* (Lam.) Willd. subsp. *argenteum* (TA), *Tanacetum heterotomum* (Bornm.) Grierson (TH), *Tanacetum densum* (Lab.) Schultz Bip. subsp. *sivasicum* (TD), and *Tanacetum vulgare* L. (TV) was investigated.

Materials and Methods: The chloroform ($CHCl_3$) and methanol:water (80:20) extracts were prepared from the aerial parts of each plant. For assessment of the wound-healing activity, linear incision on rats and circular excision on mice wound models were used and histopathological analyses were conducted on the tissues treated with the test materials. For the evaluation of the anti-inflammatory activity, Whittle Method based on the inhibition of the acetic acid-induced increase in capillary permeability was used. In order to elucidate the phytochemical contents of the extracts, HPLC profiles of active fractions were screened and quantitative analysis was conducted within the scope of HPLC analysis.

Results: The $CHCl_3$ extracts of TD, TA and TV were found to have significant wound healing activity (37.1%, 30.8% and 26.1% tensile strength; 88.05%, 72.93% and 44.88% contraction values, respectively) and anti-inflammatory activities (31.5% and 26.6% inhibition values for TD and TA). Parthenolide content of the $CHCl_3$ extracts of TA, TH and TV were found 242.66±1.53, 190.16±5.62 and 177.51±3.73 µg/100 mg plant material, respectively.

Conclusion: According to the results, the other secondary metabolites present in the aerial parts of the *Tanacetum* species possibly exerted synergistic effects on the observed healing of the wounds.

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Introduction

The genus *Tanacetum* L., which belongs to the Asteraceae family and grows in the temperate regions of Europe and West Asia, comprises about 200 species. The species of this genus are traditionally used for anti-inflammatory, antihistaminic, and stomachic properties and as insecticide (1-3). In addition, these species are used against psoriasis, migraine, nausea, vomiting, tinnitus, dizziness, hysteria, neuralgia, asthma, kidney weakness, constipation, gynecological disorders, and emmenagogue as an anthelmintic food additive and are reported to be used externally as a poultice to heal eruptive skin diseases (4-10). The leaves of *Tanacetum vulgare* L. were reported to be utilized for wound-healing (11) and the infusion prepared from the aerial parts of *Tanacetum densum* (Labill.) Sch. Bip. subsp. *densum* is used for the treatment spelling (12, 13). These species contain sterols, essential oil components, sesquiterpene lactones, resins, bitter substances, acetylenes, flavonoids, coumarins and tannic acid (6, 9, 14-17). Several biological activity studies have also been conducted on *Tanacetum* species revealing their analgesic, antipyretic, antitumor, antioxidant, cardiotonic, spasmolytic, hypoglycemic, diuretic, laxative, acaricidal, antileishmanial, antifungal, antibacterial and herbicidal activities (6-10, 18-24). However, there has been no study on the assessment of the wound-healing activity of *Tanacetum* species despite their previously reported traditional utilizations.

The aim of the present study is to evaluate the *in vivo* wound-healing and anti-inflammatory potentials of four *Tanacetum* species namely *Tanacetum argenteum* (Lam.) Willd. subsp. *argenteum*, *Tanacetum heterotomum*

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(Bornm.) Grierson, *Tanacetum densum* subsp. (Lab.) Schultz Bip. *sivasicum* and *T. vulgare*. Among them, *T. densum* subsp. *sivasicum* and *T. argenteum* subsp. *argenteum* are endemic to Turkey. In order to elucidate the phytochemical contents of the extracts, HPLC profiles of these species were screened and quantitative analysis of three flavonoid aglycones; apigenol, quercetin, kaempferol and a sesquiterpene lactone; parthenolide were conducted within the scope of HPLC analysis.

Materials and Methods

Plant material

Tanacetum species were collected from different regions of Anatolia (Table 1). Taxonomic identification of the plants was confirmed by Assistant Professor Mehmet Tekin (Department of Pharmaceutical Botany, Faculty of Pharmacy, Trakya University). Voucher specimens were stored in the Herbarium of Cumhuriyet University, Faculty of Science (CUFH).

Dried and powdered aerial parts of each plant (30 g) were extracted with 300 ml $CHCl_3$ during 8 hr for 3 days. Residues were dried and extracted with methanol: water ($MeOH:H_2O$) (80:20) at room temperature during 8 hr for 3 days by continuous stirring. The extracts were filtered and concentrated using evaporator to yield dry crude extracts.

HPLC analysis

Quercetin, kaempferol, apigenin and parthenolide contents of $CHCl_3$ extracts of *Tanacetum* species were investigated using HPLC analysis. HPLC analyses were carried out using Agilent LC 1200 model chromatograph (Agilent Technologies, California, USA). Separation was carried out by using gradient elution on ACE 5 C18 (250 mm× 4.6 mm; 5 µm) column. 0.2% phosphoric acid in water (A) and acetonitrile (B) were used in gradient elution as the mobile phase. The analysis was started with the ratio of A:B 90:10, v/v. Afterwards, the ratio of A:B was linearly changed to 0:100 in 36 min. During the last 4 min of the analysis, the solvent ratio was isocratic at the rate of A:B 0:100, v/v. The analyses were conducted with the flow rate of 1 ml/min and the injection volume was 10 µl. The column temperature was kept at 40 °C during the analyses. Parthenolide and flavonoid aglycones were analyzed at 214 nm and 330 nm, respectively. Peak areas were integrated automatically by computer using Agilent Software.

Preparation of standard solutions and calibration

The stock solution for the reference compound was prepared at the concentration of 0.1 mg/ml. The compound was weighed and dissolved with methanol in volumetric flask and the final volume was adjusted to 10 ml. The stock solution was diluted to obtain the concentration levels between 0.0005 mg/ml and 0.05 mg/ml. Triplicate analyses were carried out for each concentration level and the calibration curve was obtained using peak areas against concentration.

Optimization of the sample extraction procedure and preparation of samples

Aerial parts of the four different *Tanacetum* species were used in this experiment. $CHCl_3$ and $MeOH:H_2O$ (80:20) mixture were used successively for the extraction of plant samples. For HPLC analysis, $CHCl_3$ extracts were prepared from plant material to obtain total extract. Ten mg of $CHCl_3$ extracts of four species were weighed in 10 ml volumetric flask, dissolved in MeOH and adjusted to the final volume separately, and each extract was filtered through 0.45 µm membrane filter after adjusting to a final volume of 10 ml with same solvent. Triplicate 10 µl injections were performed for plant samples.

Validation procedure-Limit of detection and quantification

The injections were repeated 9 times to verify the values of limit of detection (LOD) and limit of quantification (LOQ).

Biological activity tests

Animals

Male Sprague Dawley rats (160-180 g) and Swiss albino mice (20–25 g) were provided from Laboratory of Experimental Animals, Kobay, Turkey. Each group consisted of six animals. Before the experiments, the animals were left 3 days for acclimatization at room temperature, standard humidity and light-controlled (12 hr light/12 hr dark) conditions. The animals were maintained on standard pellet diet and water *ad libitum*. Animals were processed according to the suggested European ethical guidelines for the care of laboratory animals. The study was performed according to the international rules considering the animal experiments and biodiversity rights (Gazi University Ethical Council Project Number: G.U.ET-10.027).

Table 1. Locality of the plant samples of different *Tanacetum* species

Plant species	Locality	Altitude	Date	Herbarium No
T. argenteum subsp. *argenteum* (TA)	Böğrüdelik village, Sivas	1845 m	2012	M.Tekin 1255
T. heterotomum (TH)	Ziyarettepe, Sivas	1402 m	2012	M.Tekin 1315
T. densum subsp. *sivasicum* (TD)	Böğrüdelik village, Sivas	1850 m	2012	M.Tekin 1257
T. vulgare (TV)	Karaçayır, Sivas	1440 m	2012	M.Tekin 1313

Table 2. Effects of the test materials on linear incision wound model

Material	Extract type	Tensile strength ± SEM	(%Tensile strength)
Vehicle		15.42 ± 2.18	7.2
Negative Control		14.39± 2.21	-
T. argenteum subsp. *argenteum*	$CHCl_3$	20.17 ± 1.65	30.8**
	MeOH:H_2O	18.41 ± 1.79	19.4
T. heterotomum	$CHCl_3$	18.26 ± 1.94	18.4
	MeOH:H_2O	17.46 ± 1.78	13.2
T. densum subsp. *sivasicum*	$CHCl_3$	21.14 ± 1.89	37.1**
	MeOH:H_2O	15.32 ± 2.25	-
T. vulgare	$CHCl_3$	19.44 ± 1.86	26.1*
	MeOH:H_2O	15.95 ± 2.52	3.3
Madecassol®		23.16 ± 1.50	50.2***

*: P<0.05; **: P<0.01; ***: P<0.001; SEM.: Standard error of the mean, percentage of the tensile strength values: The vehicle group was compared to the negative control group; The test materials and the reference material were compared to vehicle group

Preparation of test samples for bioassay

For the assessment of wound-healing activity, the test samples were prepared in an ointment base (glycol stearate, 1,2 propylene glycol, liquid paraffin, 3:6:1) in 1% concentration and applied 0.5 g onto the wounded sites. Madecassol® (Bayer) was used as the reference ointment. For the evaluation of anti-inflammatory activity, extract suspensions were prepared as described previously (25).

For the anti-inflammatory activity evaluation, test samples were administered orally to the test animals after suspending in a mixture of distilled H_2O and 0.5% sodium carboxy methyl cellulose (CMC). The control group animals received vehicle only (26). Indomethacin (10 mg/kg) in 0.5% CMC was used as a reference drug (27).

Wound-healing activity

Linear incision wound model

Linear incision wound model was performed on the rats according to the model, which was previously described by Lodhi *et al.* 2006 (28), and Suguna *et al.* 2002 (29) with some modifications (25). Two linear incisions were made on the dorsal part of the rat. Tensile strength of the treated skin was measured with a tensiometer (Zwick/Roell Z 0.5, Germany)

Circular excision wound model

A circular wound was created on the dorsal region of each mouse according to the method described by Süntar *et al.* 2013 (25). After treatment of the wounds, wound contraction was calculated as percentage of the reduction. A specimen sample of tissue was taken in order to be analyzed histopathologically (30).

Histopathology

The tissues were stained and histopathologically examined (25).

Anti-inflammatory activity

Acetic acid-induced increase in capillary permeability

Inhibitory activity of the test samples on the increased vascular permeability induced by acetic acid in mice was evaluated according to Whittle method (31) with some modifications (32).

Statistical analysis of the data

One-way analysis of variance (ANOVA) and Students-Newman-Keuls *post hoc* tests were used to analyze the data. The values of $P \leq 0.05$ were considered statistically significant. No statistical tests were performed for histopathological data, which were considered to be nonparametric.

Results

In the present study, *in vivo* wound-healing and anti-inflammatory activities of various *Tanacetum* species were investigated. Among these species, *T. densum* subsp. *sivasicum, T. vulgare* and *T. argenteum* subsp. *argenteum* were found to have wound-healing activity potential in both wound models. The highest activity was observed for the $CHCl_3$ extract of TD, with the tensile strength value of 37.1% in linear incision wound model (Table 2) and with the contraction value of 88.05% in circular excision wound model (Table 3). Histopathological findings also supported the biological activity results. Phases in wound-healing processes (inflammation, proliferation, and remodeling) were observed within the experimental groups with different degree (Table 4). Comparing the other experimental groups, best remodeling was observed in the reference group and then in the *T. densum* subsp. *sivasicum* $CHCl_3$ extract group. Delayed wound-healing processes were observed in the negative control and vehicle groups. Histopathological results are shown in Figure 1, which stained with hematoxylin & eosin (HE) and Van Gieson (VG).

The results of the anti-inflammatory activity assessment revealed that $CHCl_3$ extracts of *T. densum* subsp. *sivasicum* and *T. argenteum* subsp. *argenteum* possess anti-inflammatory activity in Whittle Method by displaying 31.5% and 26.6% inhibition values, respectively (Table 5).

Table 3. Effects of the test materials on circular excision wound model

Material	Extract type	Wound area (mm²) ± SEM (Contraction%)					
		Day 0	Day 2	Day 4	Day 6	Day 8	Day 10
Vehicle		20.25±3.24	18.32±2.45 (2.86)	15.49±2.08 (3.61)	10.24±1.69 (9.70)	6.56±1.89 (7.48)	4.10±0.47 (6.18)
Negative Control		21.16±3.31	18.86±2.78	16.07±2.16	11.34±1.79	7.09±0.91	4.37±0.66
T. argenteum subsp. *argenteum*	$CHCl_3$	22.24±3.83	15.52±1.68 (15.28)	12.29±1.84 (20.66)	7.71±1.92 (24.71)	3.86±0.69 (41.16)*	1.11±0.55 (72.93)***
	MeOH:H_2O	20.75±2.54	16.92±1.95 (7.64)	13.88±1.99 (10.39)	8.91±1.36 (12.98)	5.45±0.99 (16.92)	3.49±0.70 (14.88)
T. heterotomum	$CHCl_3$	21.19±3.04	15.98±1.75 (12.77)	13.13±1.92 (15.24)	8.35±1.86 (18.45)	4.64±0.75 (29.26)	2.81±0.39 (21.71)
	MeOH:H_2O	19.96±3.15	18.42±2.25 -	14.04±2.22 (9.36)	8.91±1.74 (12.99)	5.54±0.91 (15.54)	3.03±0.52 (26.09)
T. densum subsp. *sivasicum*	$CHCl_3$	21.47±2.99	15.56±1.93 (15.07)	12.08±1.79 (22.01)	7.11±1.33 (30.57)*	2.55±0.81 (61.13)**	0.49±0.48 (88.05)***
	MeOH:H_2O	20.68±2.90	17.31±2.10 (5.51)	14.50±2.09 (6.39)	8.99±1.50 (12.21)	5.73±0.91 (12.65)	1.76±0.61 (8.29)
T. vulgare	$CHCl_3$	21.55±3.31	17.91±2.07 (2.23)	14.32±2.11 (7.55)	8.45±2.01 (17.48)	4.02±1.10 (38.71)*	2.26±0.43 (44.88)*
	MeOH:H_2O	20.52±3.41	15.77±2.17 (13.91)	13.38±2.07 (13.62)	8.49±1.70 (17.08)	4.98±0.98 (24.08)	2.66±0.59 (22.92)
Madecassol®		20.44±2.67	15.07±1.91 (17.74)	11.27±1.71 (27.24)	5.29±1.12 (48.34)*	2.01±0.32 (69.36)**	0.00±0.00 (100.00)***

*: $P<0.05$; **: $P<0.01$; ***: $P<0.001$; SEM: Standard error of the mean; percentage of the tensile strength values: The vehicle group was compared to the negative control group; The test materials and the reference material were compared to vehicle group

Table 4. Wound healing processes and healing phases of the experimental groups

Groups	Extract type	Wound Healing Processes								Healing Phases		
		S	U	RE	FP	CD	MNC	PMN	NV	I	P	R
Vehicle		+++	+++	-	+++	+++	+++	+++	+++	+++	+++	-
Negative Control		+++	+++	-	+++	+++	+++	+++	+++	+++	+++	-
T. argenteum subsp. *argenteum*	$CHCl_3$	++/+++	++	-	+++	+++	+++	+++	+++	++/+++	++/+++	-
	MeOH:H_2O	++/+++	++	-	++/+++	++/+++	+++	+++	+++	++/+++	++/+++	-
T. heterotomum	$CHCl_3$	++/+++	++	-	+++	+++	+++	+++	+++	++/+++	++/+++	-
	MeOH:H_2O	+++	+++	-	+++	+++	+++	+++	+++	+++	+++	-
T. densum subsp. *sivasicum*	$CHCl_3$	+	-	++	++	++	++	++	++	++	++	++
	MeOH:H_2O	+++	+++	-	+++	+++	+++	+++	+++	+++	+++	-
T. vulgare	$CHCl_3$	++	++	-	++/+++	++/+++	+++	++/+++	++/+++	++/+++	++/+++	-
	MeOH:H_2O	+++	+++	-	+++	+++	+++	+++	+++	+++	+++	-
Madecassol®		++	-	+/++	++	++	++	++	++	++	++/+++	+/++

Hematoxylin & eosin (HE) and Van Gieson (VG) stained sections were scored as mild (+), moderate (++) and severe (+++) for epidermal and/or dermal re-modeling. S: Scab, U: Ulcus, RE: Re-epithelization, FP: Fibroblast proliferation, CD: Collagen depositions, MNC: Mononuclear cells, PMN: Polymorphonuclear cells, NV: Neovascularization, I: Inflammation phase, P: Proliferation phase, R: Re-modeling phase

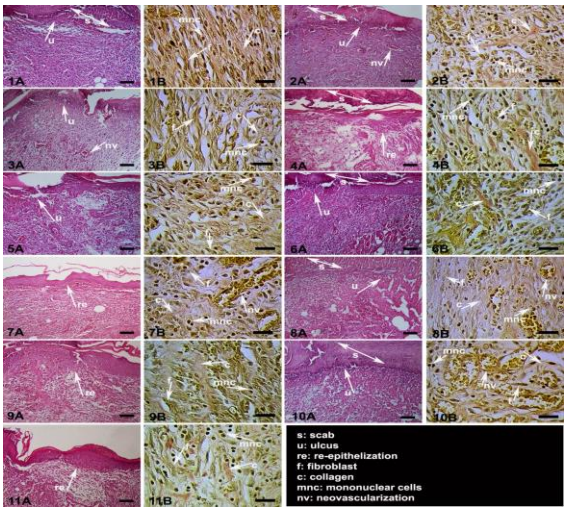

Figure 1. Histopathological view of treated tissues; Skin sections show the hematoxylin & eosin (HE) stained epidermis and dermis in A, and the dermis stained with Van Gieson (VG) in B. The original magnification was x 100 and the scale bars represent 120 µm for figures in A, and the original magnification was x 400 and the scale bars represent 40 µm for B. Data are representative of 6 animal per group. 1) Vehicle group; 2) Negative control group (untreated group); 3) *T. argenteum* subsp. *argenteum* MeOH:H_2O extract group; 4) *T. argenteum* subsp. *argenteum* $CHCl_3$ extract group; 5) *T. heterotomum* MeOH:H_2O extract group; 6) *T. heterotomum* $CHCl_3$ extract group; 7) *T.densum* subsp. *sivasicum* MeOH:H_2O extract group; 8) *T.densum* subsp. *sivasicum* $CHCl_3$ extract group; 9) *T. vulgare* MeOH:H_2O extract group; 10) *T. vulgare* $CHCl_3$ extract group; 11) Reference group (wound tissue treated with Madecassol®); Arrows pointing events during wound healing; s: scab, u: ulcus, re: re-epithelization, f: fibroblast, c: collagen, mnc: mononuclear cells, nv: neovascularization

In the present study, the quantification of three flavonoid aglycones and a sesquiterpene was conducted on the same *Tanacetum* species by using HPLC method. Selected HPLC method was determined by comparing the chromatographic profile and data obtained from the standards and samples, considering the following parameters; retention time, analyzing time for samples, separation, peak shapes and maximum UV absorption of the standards. In order to optimize the suitable chromatographic separation of four compounds in four different *Tanacetum* species, many different isocratic and gradient elution were investigated. Phosphoric acid was also used as modifier. Furthermore, different temperatures (30 ˚C, 35 ˚C and 40 ˚C) and different flow rates (1.2 ml/min, 1 ml/min, 0.8 ml/min) were tested. Finally good separations for the extracts of *Tanacetum* species have been achieved under 40˚C temperature, 1 ml/min flow rate and gradient elution of water (containing 0.2% phosphoric acid) (A), and acetonitrile (B). The chromatograms were obtained at 214 nm and 330 nm for standards and samples. Identification of the peaks was confirmed by comparison of the retention times and UV absorption spectra with acquired standards (Figure 2).

The highest content of the parthenolide was detected in *T. argenteum* subsp. *argenteum* $CHCl_3$ extract. The parthenolide contents of the $CHCl_3$ extracts of *T. argenteum* subsp. *argenteum, T. vulgare* and *T. heterotomum* were found as 242.66±1.53 µg/100 mg; 177.51±3.73 µg/100 mg and 190.16±5.62 µg/100 mg, respectively. Parthenolide was not detected in *T. densum* subsp. *sivasicum* $CHCl_3$ extract. Furthermore, quercetin, kaempferol and apigenin have also been investigated in $CHCl_3$ extracts. Quercetin and kaempferol has not been detected even at LOD levels. However, *T. heterotomum* was determined as the only species containing apigenin in quite small amount. According to the HPLC chromatograms and UV absorbances of the peaks, investigated *Tanacetum* species have flavonoids in varying amounts together with other compounds. LOD and LOQ levels of parthenolide were determined as 0.4139 µg/ml and 1.3793 µg/ml, respectively.

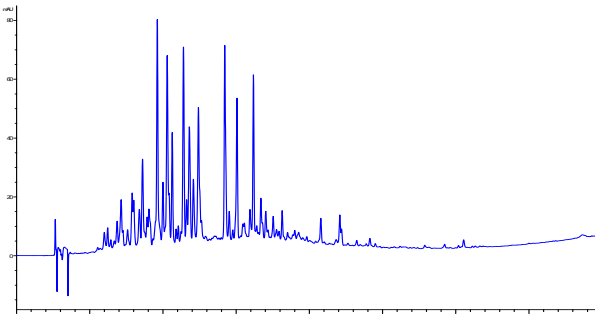

Figure 2. HPLC chromatogram of *T. argenteum* subsp. *Argenteum* (214 nm)

Table 5. Inhibitory effect of the test materials on acetic acid-induced increase in capillary permeability

Material	Extract type	Dose (mg/kg)	Evans blue concentration (µg/ml) ± SEM	Inhibition (%)
Control			12.01 ± 2.18	-
T. argenteum subsp. *argenteum*	$CHCl_3$	100	8.82 ± 0.91	26.6*
	MeOH:H_2O	100	10.91± 1.35	9.2
T. heterotomum	$CHCl_3$	100	11.58 ± 1.23	3.6
	MeOH:H_2O	100	10.94 ± 0.97	8.9
T.densum subsp. *sivasicum*	$CHCl_3$	100	8.23 ± 0.79	31.5**
	MeOH:H_2O	100	9.59 ± 1.44	20.2
T. vulgare	$CHCl_3$	100	10.39 ± 1.46	13.5
	MeOH:H_2O	100	9.65 ± 1.81	19.7
Indomethacin		10	5.97 ± 0.51	50.3***

SEM: Standard error of the mean; *: $P<0.05$. **: $P<0.01$. ***: $P<0.001$ significant from the control

Discussion

Tanacetum species are popular among the people living in rural areas evidenced by the previously published ethnomedicinal data (1-10). Among *Tanacetum* species, *Tanacetum parthenium* (L.) Schultz-Bip. (Feverfew) is popular for its use in migraine prophylaxis (5, 9). The activity potential was confirmed by several clinical trials, which demonstrated its lowering effect on the intensity and frequency of headache, visual disturbance, nausea and vomiting induced by migraine (33-35). The secondary metabolites present in the leaves have been shown to exert the activity synergistically and the whole leaf extract has been suggested to be used for the prevention of migraine (5, 36). *In vivo* and *in vitro* studies revealed that parthenolide-depleted feverfew extract possessed antioxidant activity. According to the *in vitro* studies, the extract decreased cigarette smoke-mediated damage, UV-induced hydrogen peroxide and pro-inflammatory cytokine release (37). Wound-healing and anti-inflammatory activity results exhibited herein could be related to potential antioxidant activity of the tested *Tanacetum* species. The phytochemical studies yielded the chemical constituents of *T. parthenium* as volatile oil components, sesquiterpene lactones, coumarin derivatives and flavonoids (mainly kaempferol, quercetin, apigenin and luteolin derivatives) (9, 14). Besides *T. parthenium,* another species such as *T. vulgare* is also a well-known folk remedy that is externally used as poultice to heal some eruptive skin diseases, sprains, gout, contusions and scabies, or to kill lice, and fleas (4). Previous study by Brown *et al*. (1997) demonstrated that *T. vulgare, Tanacetum ptarmiciflorum* (Webb & Berth.) Schultz. Bip. and *Tanacetum niveum* (Lagasca) Schultz-Bip. contain parthenolide and these species exhibit *in vitro* anti-inflammatory activity. However, lower parthenolide contents of *T. vulgare* and *T. ptarmiciflorum* proved that parthenolide was not the only constituent responsible for such activity (4). *T. vulgare* was further reported to have antibacterial and antihelmintic compounds as well as polysaccharides (6-8, 10). The oil obtained from *T. vulgare* is used by applying on skin as repellent against insects and the common tick *Ixodes ricinus* (1, 15). *T. vulgare* comprises of sesquiterpenes and sesquiterpene lactones, flavonoid derivatives, hydroxycoumarins, sterols, tannic acid, resins and essential oil components (6, 15-17).

Anti-inflammatory activity studies have been conducted on *Tanacetum microphyllum* DC., which is traditionally used for inflammatory conditions and rheumatic diseases. *In vivo* trials showed that flavonoids; 5,7,3'-trihydroxy-3,6,4'-trimethoxyflavone (centaureidin) and 5,3'-dihydroxy-4'-methoxy-7-carbomethoxyflavonol as well as a sesquiterpene lactone, and hydroxyachillin isolated from the aerial parts of this species also exhibited these activities (38, 39). Anti-inflammatory activity of the plant was also confirmed by *in vivo* studies. The flavonoid derivatives; ermanin and 5,3'-dihydroxy-4'-methoxy-7-methoxycarbonylflavonol isolated from *T. microphyllum* were reported to inhibit inducible nitric oxide synthase and cyclooxygenase-2, which were assumed to be the mechanisms of their anti-inflammatory activity (40). *Tanacetum larvatum* (Griseb. ex Pant.) Kanitz is another species that was reported to exhibit anti-inflammatory and antiulcerogenic activity. The mechanism of such activity was supposed to be due to the inhibition of DNA binding of the transcription factor NF-κB (21). In the present study, *T. densum* subsp. *sivasicum* and *T. argenteum* subsp. *argenteum* demonstrated significant anti-inflammatory activity.

The essential oil of *Tanacetum santolinoides* (DC.) Feinbr. and Fertig was found to possess antimicrobial activity on *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis* and *Candida albicans* (19). According to the other records, significant antimicrobial effects of *Tanacetum balsamita* L., *Tanacetum aucherianum* (DC.) Schultz. Bip. and *Tanacetum chiliophyllum* (Fisch. Et Mey.) Schultz. Bip. var. *chiliophyllum* were demonstrated (22, 41). Antimicrobial activity tests were conducted with the aerial part extract of *T. densum* subsp. *sivasicum* and the extract was found to be active against *B. subtilis* and *Klebsiella pneumoniae* (42). Evaluation of antioxidant activities of *T. densum* subsp. *sivasicum, T. densum* subsp. *eginense* and *T. densum* subsp. *amani* revealed that the most active subspecies was *T. densum* subsp. *sivasicum* in compliance with its higher phenolic content (43). The wound-healing activity of *T. densum* subsp. *sivasicum* demonstrated in the present study, could be related to its both antimicrobial and antioxidant effects, which were previously reported.

In previous studies, the secondary metabolites present in the leaves of *T. parthenium* have been shown to exert the analgesic activity synergistically and thus the whole leaf extract has been suggested to be used for the prevention of migraine (5, 36).

Tanacetum species were found to contain sterols, essential oil components, sesquiterpene lactones, resins, bitter substances, acetylenes, flavonoids, coumarins and tannic acid (6,9, 14-17). According to the current results, wound-healing activity does not seem to be in accordance with parthenolide content. In this case, flavonoid aglycones and terpenic compounds that may be found in $CHCl_3$ fraction are supposed to be responsible for such an activity.

In addition, studies on *T. vulgare* and *T. ptarmiciflorum* have shown that parthenolide was not the only constituent responsible for anti-inflammatory activity (4).

Conclusion

Parthenolide appears not to be the only principle compound responsible for the wound-healing activity but other secondary metabolites present in the aerial parts of the *Tanacetum* species studied possibly exerted synergistic effects on the observed healing of the wounds.

Acknowledgment

Throughout the experiments, animals were processed according to the suggested European ethical guidelines for the care of laboratory animals. The present study was performed according to the international rules considering the animal experiments and biodiversity rights (Gazi University Ethical Council Project Number: G.U.ET-10.027).

Conflict of Interest

The authors declare no conflict of interest.

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