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Biological Activity and Composition of the Essential Oils of *Achillea schischkinii* Sosn. and *Achillea aleppica* DC. subsp. *aleppica*

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The essential oils obtained by water distillation from aerial parts of *Achillea schischkinii* Sosn. and *Achillea aleppica* DC. subsp. *aleppica* were analyzed by gas chromatography and gas chromatography/mass spectrometry. 1,8-Cineole (32.5 and 26.1%, respectively) was the main component in both oils. The oil of *A. aleppica* subsp. *aleppica* was also found to be rich in bisabolol and its derivates. When tested for their antimicrobial, antiinflammatory, and antinociceptive activities, the oil of *A. aleppica* subsp. *aleppica* showed significant antiinflammatory, antinociceptive, and moderate antimicrobial activities.

KEYWORDS: Essential oil; GC; GC/MS; antimicrobial activity; antiinflammatory activity; antinociceptive activity

INTRODUCTION

The essential oils of *Achillea* (Compositae) species have been the subject several investigations. Especially, *Achillea millefolium* L. (Yarrow) has been studied for its therapeutic, cosmetic, and fragrant properties. In any, it has been used as an antihelmintic, antiinflammatory, astringent, choleretic, antispasmodic, antiviral, diuretic, analgesic, and antihaemorrhagic (*1*−*3*).

The genus *Achillea* is represented in Turkey by 42 species (*4*), and most of them are used in Turkish folk medicine (*5*). They are generally used as herbal teas, but *Achillea aleppica* has reportedly been used in suppositories for hemorrhoids (*6*). Our research group has previously reported the composition and antimicrobial activity of the essential oils of some *Achillea* species growing in Turkey (*1*, *7*−*12*).

The objectives of this study were to determine the essential oil composition of *Achillea aleppica* DC. subsp. *aleppica* and *Achillea schischkinii* Sosn. (an endemic species in Turkey) and their antimicrobial, antiinflammatory and antinociceptive activities.

MATERIALS AND METHODS

Plant Materials. *A. aleppica* subsp. *aleppica* (ESSE: 14321) was collected while flowering from Sof Mountain, Işıklar Village, 1000 m, Gaziantep (Turkey) in June 2002. *A. schisckinii* (ESSE 14419) was collected from Spikör Mountain, 1800 m, Erzincan (Turkey) in June 2002. Voucher specimens were kept at the herbarium of the Faculty of Pharmacy (ESSE), Anadolu University (Eskişehir, Turkey). The essential oils were obtained by hydrodistillation using a Clevenger type apparatus for 3 h, from air-dried aerial parts of the *A. aleppica* subsp. *aleppica* and *A. schisckinii*. The oil yields were calculated on dry weight bases as 0.1 and 0.3%, respectively.

Gas Chromatography (GC) and Gas Chromatography/Mass Spectrometry (GC/MS). Essential oils were analyzed by GC and GC/MS. GC analysis was carried out using a Hewlett-Packard HP 6890 GC. An Innowax (Carbowax 20M) FSC column (60 m × 0.25 mm Ø, with 0.25 μm film thickness) was used with nitrogen as the carrier gas. The oven temperature was kept at 60 °C for 10 min and increased up to 220 °C at a rate of 4 °C/min and then kept constant at 220 °C for 10 min and increased up to 240 °C at a rate of 1 °C/min. The split ratio was adjusted to 50:1. The injector and flame ionization detection (FID) detector temperatures were 250 °C.

GC/MS analysis was conducted using a Hewlett-Packard GC-MSD system. The same column and operational conditions as in GC were applied. The carrier gas was helium. MS were taken at 70 eV. The mass range was between *m*/*z* 35 and 425. A library search was carried out using Wiley GC/MS Library, Mass Finder Library, Adams Library, as well as in-house BASER Library of Essential Oil Constituents.

Relative percentage amounts of the separated compounds were calculated from FID chromatograms. Alkanes were used as reference points in the calculation of relative retention indices (RI).

Antimicrobial Assay: Microorganisms. The microorganisms were refreshed in Mueller Hinton Broth (Merck) at 35−37 °C and inoculated on Mueller Hinton Agar (Mast Diagnostics, Merseyside, United Kingdom) media for preparation of the inoculum. *Escherichia coli* (NRRL B-3008), methicillin-resistant *Staphylococcus aureus* (MRSA, Clinical isolate, Osmangazi University, Faculty of Medicine, Department of Microbiology), *S. aureus* (ATCC 6538), *Enterobacter aero-*

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Table 1. Composition of the *A. aleppica* Subsp. *aleppica* and *A. schisckinii* Essential Oils[a]

RRI	compounds	A (%)	B (%)	RRI	compounds	A (%)	B (%)
1032	α-pinene	2.1	1.2	1845	*trans*-carveol	0.4	ND
1035	α-thujene	ND	0.3	1857	geraniol	0.2	ND
1076	camphene	0.2	0.7	1864	*p*-cymen-8-ol	0.2	ND
1118	β-pinene	2.2	5.3	1882	*cis*-carveol	0.2	0.4
1132	sabinene	ND	0.3	1889	*cis*-myrtanol	0.4	ND
1195	dehydro-1,8-cineole	0.4	ND	1930	isoamyl benzoate	0.2	ND
1213	1,8-cineole	26.1	32.5	1945	1,5-epoxy-salvial-(4)14-ene	0.3	ND
1280	*p*-cymene	0.7	2.0	1958	(*E*)-β-ionone	0.2	ND
1299	2-methylbutyl isovalerate (2-methylbutyl-3-methyl butyrate)	0.3	ND	2008	caryophyllene oxide	3.4	0.9
1358	artemisia ketone	ND	3.9	2029	perilla alcohol	tr	ND
1403	yomogi alcohol	ND	0.7	2030	methyl eugenol	0.2	ND
1450	*trans*-linalool oxide (furanoid)	0.2	ND	2037	salvial-4(14)-en-1-one	0.6	ND
1474	*trans*-sabinene hydrate	ND	0.6	2056	13-tetradecanolide	0.3	ND
1475	3-methylbutyl-2-methyl-butyrate	0.1	ND	2068	octanoic acid	0.4	ND
1479	linalool-7-oxide-3-one	0.7	ND	2070	humulene epoxide-II	0.2	ND
1497	α-copaene	0.1	ND	2081	cubenol	0.1	ND
1499	α-campholene aldehyde	0.1	ND	2092	β-oplopenone	0.3	0.7
1510	artemisia alcohol	ND	2.7	2131	hexahydrofarnesylacetone	0.2	0.2
1532	camphor	3.1	7.8	2144	spathulenol	2.6	0.7
1553	linalool	1.6	ND	2156	bisabolol oxide	0.6	ND
1556	*cis*-sabinene hydrate	ND	0.6	2163	α-bisabolol oxide B	1.1	ND
1571	*trans*-*p*-menth-2-en-1-ol	ND	0.8	2176	nonanoic acid	0.4	ND
1586	pinocarvone	1.3	0.7	2187	T-cadinol	4.3	1.3
1590	bornyl acetate	0.1	ND	2194	γ-eudesmol	ND	1.4
1611	terpinen-4-ol	0.7	1.6	2205	eremoligenol	ND	0.7
1612	β-caryophyllene	0.1	ND	2209	bisabolone oxide A	0.4	ND
1638	*cis*-*p*-menth-2-en-1-ol	0.1	0.6	2215	valerianol	ND	0.3
1648	myrtenal	0.4	0.5	2222	clovenol	0.2	ND
1661	alloaromadendrene	0.5	ND	2237	α-bisabolol	0.9	ND
1664	*trans*-pinocarveol	0.5	0.7	2247	*trans*-α-bergamotol	0.2	ND
1682	δ-terpineol [= *p*-menth-1(7)-en-8-ol]	0.2	0.5	2255	α-cadinol	2.0	ND
1689	*trans*-piperitol (= *trans*-*p*-menth-1-en-3-ol)	ND	0.3	2257	β-eudesmol	ND	5.7
1690	*trans*-verbenol	0.3	ND	2260	15-hexadecanolide	0.1	ND
1704	myrtenyl acetate	0.2	0.3	2286	decanoic acid	1.6	0.3
1706	α-terpineol	9.3	1.8	2298	oxo-α-ylangene	0.2	ND
1719	borneol	0.5	0.5	2300	tricosane	0.2	ND
1729	*cis*-1,2-epoxy-terpin-4-ol (= *p*-menth-1,2-epoxy-4-ol)	ND	0.3	2316	caryophylla-2(12),6(13)-dien-5β-ol (= caryophylladienol I)	0.5	ND
1742	chrysanthenyl isovalerate I*	ND	0.2	2324	caryophylla-2(12),6(13)-dien-5α-ol (= caryophylladienol II)	1.5	0.5
1748	piperitone	ND	3.8	2332	thujopsenal	0.2	ND
1751	carvone	0.3	ND	2364	caryophylla-2(12),6-dien-5α-ol (= caryophyllenol I)	0.6	ND
1755	bicyclogermacrene	0.5	ND	2385	eudesma-4(15),7-dien-1β-ol	0.4	ND
1758	*cis*-piperitol	ND	0.8	2405	caryophylla-2(12),6-dien-5β-ol (= caryophyllenol II)	0.7	ND
1772	neryl isobutyrate	0.2	ND	2437	6-dodecen-4-olide	1.9	ND
1773	δ-cadinene	0.3	ND	2452	α-bisabolol oxide A	3.6	ND
1776	γ-cadinene	0.8	ND	2499	dodecanoic acid (lauric acid)	0.1	ND
1790	α-campholene alcohol	0.2	ND	2500	pentacosane	0.4	ND
1794	*p*-mentha-1,5-dien-8-ol	0.4	ND	2521	1-octadecyl acetate	tr	ND
1797	nerol	ND	0.3	2533	γ-costol	0.2	ND
1802	chrysanthenyl isovalerate II*	ND	0.9	2607	1-octadecanol	0.4	ND
1807	myrtenol	1.6	0.7	2700	heptacosane	0.4	ND
1820	isogeraniol	0.7	ND	2931	hexadecanoic acid	1.2	0.3

[a] A, *A. aleppica* subsp. *aleppica* essential oil. B, *A. schischkinii* essential oil. RRI, relative retention indices on a polar column. tr, trace < 0.1%. *, These are cis and trans isomers. The correct isomer was not identified. ND, not detected.

genes (NRRL 3567), *Salmonella typhimurium* (NRRL B-4420), *Bacillus cereus* (NRRL B-3711), *Staphylococcus epidermidis* (ATCC 12228), and *Candida albicans* (Clinical Isolate, Osmangazi University, Faculty of Medicine, Eskişehir, Turkey) were used as pathogen test microorganisms.

Microdilution Broth Method. A microdilution broth susceptibility assay (*13*, *14*) was used for the antimicrobial evaluation of the essential oils. Stock solutions of oils were prepared in DMSO (Carlo-Erba). Dilution series were prepared from 2 to 0.001 mg/mL in sterile distilled water in microtest tubes from where they were transferred to 96 well microtiter plates. Overnight grown bacterial suspensions in double strength Mueller Hinton broth (Merck) were standardized to approximately 10^8 CFU/mL using McFarland no. 0.5 (10^6 CFU/mL for *C. albicans*). One hundred microliters of each bacterial suspension was then added to each well. The last row containing only the serial dilutions of antimicrobial agent without microorganism was used as a negative control. Sterile distilled water and medium served as a positive growth control. After incubation at 37 °C for 24 h, the first well without turbidity was determined as the minimal inhibition concentration (MIC). Chloramphenicol (Sigma) and ketoconazole (Sigma) were used as standard antimicrobial agents.

Pharmacological Test Protocols: Animals. Male Swiss albino mice (20−25 g) were purchased from the animal breeding laboratories of Refik Saydam Central Institute of Health (Ankara, Turkey). The animals were left for 2 days for acclimatization to animal room conditions and were maintained on a standard pellet diet and water *ad libitum*. The

Table 2. Antimicrobial Test Results of the *Achillea* Oils (MIC: μg/mL)[a]

	E. coli	MRSA	*E. aerogenes*	*S. typhimurium*	*B. cereus*	*S. epidermidis*	*C. albicans*	*S. aureus*
A	**62.5**	250	500	250	**62.5**	125	**62.5**	**62.5**
B	250	250	250	125	125	62.5	125	125
S	**3.9**	7.8	**3.9**	**7.8**	**7.8**	**0.9**	**62.5***	**0.9**

[a] A, *A. aleppica* subsp. *aleppica* essential oil. B, *A. schischkinii* essential oil. S, standard antimicrobial agents, chloramphenicol, *ketoconazole. MRSA, methicilin-resistant *S. aureus*.

Table 3. Effects of the *Achillea* Essential Oils against Carrageenan-Induced Paw Edema in Mice[a]

		swelling thickness ($\times 10^{-2}$ mm) $\pm$ SEM (inhibition %)			
test samples	dose (mg/kg)	90 min	180 min	270 min	360 min
control		40.0 $\pm$ 3.52	45.2 $\pm$ 3.67	48.8 $\pm$ 4.19	53.8 $\pm$ 4.45
A	200	33.0 $\pm$ 4.51 (17.5)	34.4 $\pm$ 3.29 (23.9)	37.6 $\pm$ 2.24 (**22.9**)*	38.6 $\pm$ 2.82 (**28.3**)**
B	200	42.5 $\pm$ 4.20	43.2 $\pm$ 5.52 (4.4)	47.0 $\pm$ 4.93 (3.7)	53.0 $\pm$ 4.59 (1.5)
indomethacin	10	27.5 $\pm$ 1.99 (**31.3**)*	29.0 $\pm$ 1.92 (**35.8**)*	29.5 $\pm$ 2.22 (**39.5**)**	30.8 $\pm$ 2.23 (**42.8**)***

[a] *p < 0.05; **p < 0.01; ***p < 0.001. SEM, standard error of the mean. A, *A. aleppica* subsp. *aleppica* essential oil. B, *A. schischkinii* essential oil.

food was withdrawn on the day before the experiment, but they were allowed free access to water. A minimum of six animals was used in each group. Throughout the experiments, animals were processed according to the suggested ethical guidelines for the care of laboratory animals.

Preparation of Test Samples for Bioassay. All of the materials were given orally to test animals in 200 mg/kg doses after suspending in a mixture of distilled H_2O and 0.5% sodium carboxymethyl cellulose (CMC). The control group animals received the same experimental handling as those of the test groups except that the drug treatment was replaced with appropriate volumes of the dosing vehicle. Either indomethacin (10 mg/kg) or acetyl salicylic acid (ASA) (200 mg/kg) in 0.5% CMC was used as reference drug.

Antiinflammatory Activity. The carrageenan-induced hind paw edema model was used with modifications in measuring periods for the determination of antiinflammatory activity (*15*). The difference in footpad thickness between the right and the left foot was measured with a pair of dial thickness gauge calipers (Ozaki Co., Tokyo, Japan). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Sixty minutes after the oral administration of test sample or dosing vehicle, mice were injected with freshly prepared (0.5 mg/25 μL) suspension of carrageenan (Sigma, St. Louis, MO) 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 measured every 90 min during 6 h after induction of inflammation. The difference in footpad thickness was measured by gauge calipers (Ozaki Co.). Mean values of treated groups were compared with mean values of a control group and analyzed using statistical methods. Indomethacin (10 mg/kg) was used as the reference drug.

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

Antinociceptive Activity. A *p*-benzoquinone-induced abdominal constriction test was performed on mice for determination of antinociceptive activity (*16*). According to the method, 60 min after the oral administration of test samples, the mice were intraperitonally injected with 0.1 mL/10 g body weight of 2.5% (w/v) *p*-benzoquinone (PBQ; Merck) solution in distilled H_2O. Control animals received an appropriate volume of dosing vehicle. The mice were then kept individually for observation, and the total number of abdominal contractions (writhing movements) was counted for the next 15 min, starting on the 5th min after the PBQ injection. The data represent the average of the total number of writhes observed. The antinociceptive activity was expressed as percentage change from writhing controls. Aspirin (ASA) at 100 and 200 mg/kg doses was used as the reference drug in this test.

Gastric-Ulcerogenic Effect. After the antinociceptive activity experiment, mice were killed under deep ether anesthesia and the stomachs were removed. Then, the abdomen of each mouse was opened through the greater curvature and examined under a dissecting microscope for lesions or bleedings.

Statistical Analysis of Data. Data obtained from animal experiments were expressed as mean standard error ($\pm$SEM). Statistical differences between the treatments and the control were evaluated by analysis of variance and Students−Newman−Keuls posthoc tests. $p < 0.05$ was considered to be significant (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$).

RESULTS AND DISCUSSION

The essential oils of *A. aleppica* subsp. *aleppica* and *A. schischkinii* were analyzed by both GC and GC/MS to determine their constituents (**Table 1**). Eighty-three and forty-four components were identified representing 91.8 and 87.3% of the total for the oils, respectively. 1,8-Cineole (32.5 and 26.1%, respectively) was the main component in both oils. The oil of *A. aleppica* subsp. *aleppica* was found to contain bisabolol and its derivatives (6.6%).

The antibacterial and anticandidal activities of the oils are presented in **Table 2**. The results of the antimicrobial assays indicated that *E. coli*, *B. cereus*, and *S. aureus* were inhibited by the oil of *A. aleppica* subsp. *aleppica* moderately with a MIC value of 62.5 μg/mL, which was lower than that of the standard antimicrobial agent. The oil showed a strong inhibitory effect against *C. albicans* with a MIC value (62.5 μg/mL) that was equal to ketoconazole. *A. schischkinii* oil, on the other hand, was less active against the test microorganisms except for *S. epidermidis*. The occurrence of higher content of oxygenated derivatives of mono- and sesquiterpenes in the oil of *A. aleppica* subsp. *aleppica* may be responsible for antimicrobial activity.

Previous investigations on the antimicrobial activity of *Achillea* essential oils were consistent with our results. Oxygenated monoterpene-rich *Achillea multifida* oil was shown to possess significant inhibitory effects on *E. coli* and *C. albicans* with MIC values of 125 and 62.5 μg/mL, respectively (*11*). In another study, *Achillea taygetea* and *Achillea frasii* were reported to display moderate inhibitory activities against *E. coli*, *S. aureus*, *S. epidermidis*, and *C. albicans* with MIC values of 1.67−6.87 mg/mL (*17*).

Both *Achillea* oils have been evaluated for their *in vivo* antiinflammatory and antinociceptive activities. Their inhibitory effects on *p*-benzoquinone-induced writhing for the assessment of antinociceptive activity and carrageenan-induced hind paw

Table 4. Effect of the *Achillea* Essential Oils against *p*-Benzoquinone-Induced Writhings in Mice[a]

test samples	dose (mg/kg)	no. of writhings ± SEM	inhibitory ratio (%)	ratio of ulceration
control		47.8 ± 4.27		0/6
A	200	32.6 ± 4.06	31.8**	0/6
B	200	44.8 ± 5.23	6.3	0/6
ASA	200	21.8 ± 1.74	54.4***	5/6

[a] *p < 0.05; **p < 0.01; ***p < 0.001. ASA, acetylsalicylic acid. A, *A. aleppica* subsp. *aleppica* essential oil. B, *A. schischkinii* essential oil.

edema model, a widely used screening protocol for antiinflammatory activity to test the nonsteroidal antiinflammatory drugs, were examined in mice. Results of both assays are given in **Tables 3** and **4**.

While *A. schischkinii* oil did not show any noticeable antiinflammatory activity, *A. aleppica* subsp. *aleppica* oil displayed significant activity (in 200 mg/kg dose) between 22.9 and 23.8% of inhibition in 270−360 min, without inducing any apparent acute toxicity or gastric damage as compared to indomethacine, the reference drug, whose activity was at the range of 31.3−42.8% of inhibition in 90−360 min.

The analgesic activities of the oils were studied by using PBQ-induced writhing model in mice. As shown in **Table 4**, *A. aleppica* subsp. *aleppica* oil inhibited the writhes but was not as potent as acetylsalicylic acid. The antiinflammatory and analgesic activities of *Achillea* species have previously been investigated. The aqueous and methanol extracts of *Achillea ageratum* have been evaluated for analgesic and antiinflammatory properties. The aqueous extract exhibited significant activity in the analgesic and antiinflammatory assays (*18*). Sesquiterpene lactones isolated from *Achillea setacea* (*19*) and a germacrane derivative isolated from *Achillea pannonica* (*20*) showed good antiinflammatory activity. A tincture of *Achillea collina* showed antinociceptive activity in 250 mg/kg (*21*).

In conclusion, *A. aleppica* subsp. *aleppica* essential oil possesses significant antiinflammatory, analgesic, and anticandidal activity but moderate antibacterial activity. Taking into account the mode of action, the species may be suggested as a substitute for yarrow (*A. millefolium*).

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LITERATURE CITED

(1) Kürkçüoğlu, M.; Demirci, B.; Tabanca, B.; Özek, T.; Başer, K. H. C. The essential oil of *Achillea falcata* L. *Flavour Fragrance J.* **2003**, *18*, 192−194.

(2) Montanari, T.; Carvalho, J. H.; Dolder, H. Antispermatogenic effect of *Achillea millefolium* L. in mice. *Contraception* **1998**, *58*, 309−313.

(3) Blumenthal, M. *The Complete German Commission E Monographs: Therapeutic Guide to Herbal Medicines*; Lippincott Williams & Wilkins: United States, 1998.

(4) Davis, P. H. *Flora of Turkey and Aegean Islands*; University Press: Edinburgh, 1982; Vol. 5, p 224.

(5) Baytop, T. *Türkiye'de Bitkiler ile Tedavi Gecmisten Bugüne (Therapy with Medicinal Plants in Turkey-Past and Present)*, 2nd ed.; Nobel Tıp Basımevi: Istanbul, Turkey, 1999.

(6) Başer, K. H. C.; Honda, G.; Miki, W. *Herb Drugs and Herbalists in Turkey*; Institute for the Study of Languages and Cultures of Asia and Africa, Tokyo University of Foreign Studies: Tokyo, 1986; p 181.

(7) Küsmenoğlu, Ş; Başer, K. H. C.; Özek, T. Harmandar, M.; Gökalp, Z. Constituents of the essential oil of *Achillea bieberstenii* Afan. *J. Essent. Oil Res.* **1995**, *7*, 527−528.

(8) Başer, K. H. C.; Demirci, B.; Kaiser, R.; Duman, H. Composition of the essential oil of *Achillea phrygia* Boiss. et Ball. *J. Essent. Oil Res.* **2000**, *12*, 327−329.

(9) Başer, K. H. C.; Demirci, B.; Duman, H.; Aytaç, Z.; Adıgüzel, N. Composition of the essential oil of *Achillea goniocephala* Boiss. et Bal. from Turkey. *J. Essent. Oil Res.* **2001**, *13*, 219−220.

(10) Duman, H.; Başer, K. H. C.; Demirci, B. Composition of the essential oils of two endemic species from Turkey: *Achillea lycaonica* Boiss. et Heldr. and *Achillea ketenoglui*. *Chem. Nat. Compd.* **2001**, *37*, 245−252.

(11) Başer, K. H. C.; Demirci, B.; Demirci, F.; Koçak, S.; Akıncı, Ç.; Malyer, H.; Güleryüz, G. Composition and antimicrobial activity of the essential oil of *Achillea multifida* (DC.) Boiss. *Planta Med.* **2002**, *68*, 939−941.

(12) Tabanca, N.; Ozek, T.; Baser, K. H. C.; Vural, M. Composition of the essential oil of *Achillea sieheana* Stapf and the enantiomeric distribution of camphor. *J. Essent. Oil Res.* **2004**, *16*, 180−181.

(13) Koneman, E. W.; Allen, S. D.; Janda, W. M.; Schreckenberger, P. C.; Winn, W. C. *Color Atlas and Textbook of Diagnostic Microbiology*; Lippincott-Raven Publ.: Philadelphia, 1997.

(14) Amsterdam, D. *Susceptibility Testing of Antimicrobials in Liquid Media, Antibiotics in Laboratory Medicine*, 4th ed.; Lorian, V., Ed.; Williams & Wilkins, Maple Press: Maryland, 1997.

(15) Kasahara, Y.; Hikino, H.; Tsurufiji, S.; Watanabe, M.; Ohuchi, K. Antiinflammatory actions of ephedrines in acute inflammations. *Planta Med.* **1985**, *51*, 325−331.

(16) Okun, R.; Liddon, S. C.; Lasagnal, L. The effect of aggregation, electric shock and adrenergic blocking drugs on a inhibition of the "Writhing Syndrome". *J. Pharmacol. Exp. Ther.* **1963**, *139*, 107−109.

(17) Prokopios, M.; Skaltsounis, A. L.; Chinou, I.; Haroutounian, S. A. Chemical composition and in-vitro antimicrobial activity of the essential oils of three Greek *Achillea* species. *Z. Naturforsch.* **2002**, *57c*, 287−290.

(18) Garcia, M. D.; Puerta, R.; Martinez, S.; Saenz, M. T. Analgesic, antipyretic and antinflammatory effects of *Achillea ageratum*. *Phytother. Res.* **1997**, *11*, 376−379.

(19) Zitterl-Eglseer, K.; Jurenitsch, J.; Korhammer, S.; Haslinger, E.; Sosa, S.; Della-Logia, R.; Kubelka, W.; Franz, C. Sesquiterpenelactones of *Achillea setacea* with antiphlogistic activity. *Planta Med.* **1991**, *57* (5), 444−446.

(20) Sosa, S.; Tubaro, A.; Kastner, U.; Glasl, S.; Jurenitsch, Della-Logia, R. Topical antiinflammatory activity of a new germacrane derivative from *Achillea pannonica*. *Planta Med.* **2001**, *67* (7), 654−658.

(21) Gherase, F.; Pavelescu, M. D.; Stanescu, U.; Grigorescu, E. The experimental evaluation regarding analgesic activity of some extracts isolated from *Achillea collina* J. Becker *ex* Reichenb. *Rev. Med. Chir. Soc. Med. Nat. Iasi*. **2002**, *106* (4), 801−805.

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