Pharmacognosy Magazine
ISSN: 0973-1296

PHCOG MAG.
An official Publication of Phcog.Net

PHCOG MAG.: Research Article
Antiplatelet, antibacterial and antifungal activities of *Achillea falcata* extracts and evaluation of volatile oil composition

Talal Aburjai* and Mohammad Hudaib

Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan, Amman, Jordan
**Address for correspondence: E-mail: aburjai@ju.edu.jo ; Phone: +962-6-5355000, Fax: + 962-6-5339649,*
[1]This work has been conducted during a sabbatical leave given to Dr. Talal Aburjai by University of Jordan.

ABSTRACT - The antiplatelet, antibacterial and antifungal effects of various extracts obtained from Jordanian *Achillea falcata* L. were studied. Chemical composition of the volatile oil hydrodistilled from the plant aerial parts was also evaluated by GC and GC/MS. Among the 41 constituents identified in the oil, camphor (17.0%), 1,8-cineole (15.9%), *p*-cymene (11.3%) and β-thujone (9.8%) were the major components. The volatile oil exhibited potent antibacterial activity against standard and resistant strains of both gram positive and gram negative bacteria. The oil showed also significant inhibition against *Candida albicans* and *Trichosporon cutaneum*. Alcoholic extract revealed good activity against *Escherichia coli*, resistant strain of *Pseudomonas aeurogenosa* and *C. albicans*. It also showed significant inhibitory effect against platelet aggregation induced by collagen and ADP (IC_{50}: 48.1, 112.1 µg/ml respectively). The aqueous extract revealed moderate activity against fungal species, in particular *C. albicans*, while it showed no inhibition against blood aggregation induced either by collagen or ADP.
KEYWORDS- *Achillea falcata L., volatile oil, camphor, 1,8-cineole,* p-*cymene, β-thujone, antiplatelet, antibacterial, antifungal, GC/MS.*

INTRODUCTION

The genus *Achillea* (F. Asteraceae) comprises more than 200 species, most indigenous to Europe and the Middle East (1). Six species are widely distributed in Jordan; *A. aloppica* L., *A. biebersteinii* L., *A. falcate* L., *A. fragrantissima* L., *A. membranacea* L. and *A. santolina* L. Phytochemical studies of different Achillea species revealed the presence of different constituents such as flavonoids (aglycones and glycosides), sesquiterpene lactones and essential oils (2, 3). *A. fragrantissima* is considered the most important species and is thoroughly studied. This plant from the wild plants used in Bedouin folk medicine has the Arabic common name Al-Qisum. It grows in Negev Desert, the Judean Desert and the Lower Jordan Valley. It is characterized by a pleasant smell due to its high content of essential oils. It is used in folk medicine for the treatment of gastro-intestinal disturbances and various infections, among them infection of the eye (4), and as hypoglycemic agent (5). Essential oil from *A. fragrantissima* exerted a bactericidal effect on several gram-positive and gram-negative bacterial strains, as well as on *Candida albicans* (6). Moreover, insecticidal and rodenticidal activities of *A. fragrantissima* oil were also demonstrated by Hifnawy et al. (7). The young flowering branches of *A. santolina*, locally named as Jeaidat Al-sabian or Qort, are used as anti-diabetic while the leaves are used for tooth pain, stomach ailments, dysentery and colic pain (8). *A. wilhelmsii* C. Koch, has been shown to be effective in lowering blood lipids and blood pressure (9).

A. falcata L. is a perennial herb, 20-40cm long with woody base and spreading stems covered by dense hairs. It has the growth-form of dwarf shrub of medium height, with a well-developed primary root system; older plants exhibited a thick primary root, thus indicating that the plant may get very old (10). This species is not well studied from phytochemical and pharmacological point of view. The infusion of the leaves and flowers is used by the laymen to stop internal hemorrhage, for uterus hemorrhoid, stomach ailment, for gastritis and bladder stones (11). Volatile oil hydrodistilled from the aerial parts of plants grown in Lebanon showed inhibitory action against gram-positive bacteria (12). Chemically, composition of the oil has been recently evaluated in plants growing in Turkey and Lebanon (12, 13). In plants growing in Antalya (Turkey), the major oil component were camphor (24.0%), artemisia alcohol (20.1%), and 1,8-

cineole (14.4%) in one location and 1,8-cineole (23.8%), α-pinene (12.3%), and piperitone (10.3%) in another; where as in Lebanon the oil was characterized by abundant levels of grandisol (21.4%) and fragranol (16.8%).

In the recent years there was emphasis on discovery of medicinal plant extracts and natural products that inhibit the aggregation of platelets. Several alkaloids, naphthalenes, xanthones, coumarins, anthraquinones, flavonoids and stilbenes in free form and in form of their esters and glycosides found to have effect on the platelet aggregation induced by arachidonic acid, collagen, ADP or platelet activating factor (14, 15, 16). In the alcoholic extract of aerial parts of *Rheum palaestinum* it has been found that trans-resveratrol-3-O-ß-D-glucopyranoside and rhaponticin exhibit anti-platelet activity on human blood (17). The former compound with anti-platelet activity was previously isolated from water extract of the seeds of *Erythrophleum lasianthum* (18). From Formosan plants, various xanthones, anthraquinones as well as quercetin among other eleven flavonol-derivatives have been isolated and their anti-platelet activity on washed rabbit platelets has been tested and the role of the sugar moiety on the antiplatelet activity discussed (19, 20). Furthermore for several isolated secondary metabolites the degree of inhibitory effect was shown to be different depending on the type of aggregation inducers (21, 22). Teng et al. (23) classified the antiplatelet agents according to their effects on platelet aggregation, release reaction and signal transductions involved into eight groups. These relatively new group of pharmacological agents derived from medicinal plant resources may be useful as leads to develop effective cardiovascular and antithrombotic drugs and to search for inhibitors of platelet aggregation.

The aim of the present work is to investigate the volatile oil composition of the aerial parts of *A. falcata*. As well, and on the basis of the above data and our ethnopharmacological field observations in Jordan and neighboring countries, we decided to study the antimicrobial and antiplatelet effects of the various extracts, and the volatile oil obtained from Jordanian *A. falcata*.

MATERIALS AND METHODS

Plant material

Samples of native *A. falcata* L. were collected in March 2003 from Madaba, 20km southern of Amman, during the flowering period and the vegetative phase. Taxonomic identity of the plant was confirmed by comparing collected voucher specimen with those of known identity in the herbarium of the Department of Biological Sciences, Faculty of Science, University of Jordan (Amman-Jordan) and with the assistance of Prof. Dr. Barakat Abu-Irmeileh, Faculty of Agriculture, University of Jordan. A voucher specimen (No. AFc. 22-01) has been deposited in the author research laboratory at the Department of Pharmaceutical Sciences, Faculty of Pharmacy, University of Jordan.

Oil distillation

Oils from air-dried, finely ground aerial parts were obtained by hydrodistillation using a Clavenger-type apparatus. Distillation was performed using 100 g of dried plant material in 2.5 L distilled water for 4 hrs. The oil obtained was dried over anhydrous sodium sulfate and stored in a dark glass bottle at 4°C until analysis. The yield of oil (w/w) was 0.6 % of the dried materials.

GC/MS analysis

Varian Chrompack CP-3800 GC/MS/MS-2000 equipped with split-splitless injector and DB-5.625 GC column (30 m × 0.25 mm ID, 0.25 µm film thickness) was used. The injector temperature was set at 220 °C for 5 min with a split ratio of 1:10. A 1 µL volume of 1000 ppm oil solution in a GC-grade n-hexane (Scharlu, Spain) was injected. A linear temperature program was adapted to separate the different oil components as follow: initially the column maintained at 50°C for 2 min, ramped at a rate of 10°C/min to 150°C at which held isothermal for 5 min; a second ramp (20°C/min) was then applied up to 220°C and held isothermal for 10 min. The total run time was 30.5 min. The temperatures of the transfer line and ion source were maintained at 230 °C and 180 °C, respectively. The mass detector was set to scan ions between 40-400 *m/z* using full-scan fixed mode electron impact (EI, 70 eV). Components of the oil were identified by matching their recorded spectra with the data bank mass spectra (Wiley and NIST library databases) provided by the instrument software, and by comparing their retention indices values with those in the literature, measured on columns with identical polarity (24). The databases were compiled using more than 80,000 electron impact (EI) mass spectra. Only matching spectra of large degree of certainty using reverse-fit mode were accepted. Percent contents of the oil components were calculated using their relative peak areas, in the total ion current (TIC)

chromatograms, assuming a unity response by all components.

Preparation of plant extracts and microbial cultures

In addition to the oil, alcoholic and aqueous extracts were also prepared by macerating 100g of dried and finely ground aerial parts in 1 L absolute ethanol and distilled water overnight, respectively. Alcoholic and aqueous dried residues were obtained by Rota-evaporation and freeze-drying, respectively. Alcoholic and water extracts and oils of *A. falcata* were sterilized by filtration and a 20 µg sample of each was loaded on a filter paper disc (Watermann). The impregnated discs were then tested for their antimicrobial activity. Bacterial species including *Pseudomonas aeruginosa* (Schroeter and Migula) ATCC 27853, *Escherichia c*oli (T. Escherich) ATCC 25922, *Staphylococcus aureus* (Rosenbach) ATCC 25923 and *Bacillus subtilis* (Ehrenberg) ATTC 6633, were grown in nutrient broth (Oxoid, UK) overnight at 37°C and maintained on nutrient agar (Oxoid, UK) plates at 4°C. *Candida albicans* (C.P. Robin) ATCC 10231 and *Trichosporon cutaneum* (de Beurmann and Gougerot) were grown in malt broth (Oxoid, UK) at 25°C for 24 hr and maintained on nutrient agar (Oxoid, UK) plates at 4°C. *T. cutaneum* and resistant strains of both *Ps. aeruginosa* and *St. aureus* were clinically isolated from hospitalized patients from the Jordan University Hospital, Amman, Jordan and were confirmed by biochemical tests.

Antimicrobial activity assay

The crude plant extracts and the volatile oil were tested for their antimicrobial activity using the diffusion technique on solid media (25). Sterile 5 mm diameter filter paper discs were impregnated with 20 µg of either alcoholic; water or oil extracts and placed on nutrient agar seeded with the microorganisms (10^6 cfu/ml). The plates were incubated for 24 hrs at 37°C for bacteria and 48 hrs at 25°C for fungi. Control discs were soaked with the same extraction solvents and treated as the sample discs. The experiments were carried out as duplicate three times and corrected for the control discs. Additionally erythromycin, nystatin (The Arab Pharmaceutical Manufacturing Co Ltd., Salt-Jordan), tetracycline (Dar al Dawa, Naur-Jordan) and Chloramphenicol (Pliva, Croatia) were tested as positive standards at a concentration of 20µg/disc. The diameters of the inhibition zones are presented in Table 2.

Platelet aggregation

Samples for aqueous extract were prepared by adding 50µl of 30, 20 and 10 mg/ml of the aqueous extracts and a series of dilutions (30- 1.875 and 20-0.625 mg/ml) to 450µl platelet rich plasma (PRP). Dilution was done using normal saline.

Samples for the alcoholic extract were prepared by adding 50µl of 30 mg/ml of the alcoholic extract and a series of dilutions (30-1.875 and 20-0.625 mg/ml) to 450µl PRP. The stock of 30mg/ml alcoholic extract was obtained from dry alcoholic extract and normal saline. Dilution was done using normal saline.

Samples for pure oil were prepared by adding 50µl of 12 mg/ml of the oil extract to 450µl PRP. The stock of 30mg/ml oil extract was obtained by adding 0.4 % DMSO, 2 % of 96 % ethanol and sufficient quantity of normal saline to the appropriate quantity of the water-distilled oil. Dilution was done using normal saline.

Control for the aqueous and alcoholic extracts was prepared by adding 50µl normal saline to 450µl (PRP). Control for pure oil was prepared by adding 50µl of 0.4 % DMSO and 2 % of 96 % ethanol.

Aggregation was induced using collagen (2µg/ml) and ADP (10µM) for testing the anti-platelet activity of the aqueous, alcoholic extracts and the volatile oil on human PRP.

Human blood taken from the forearm vein of the volunteers was collected by free flow along the side of plastic tube containing 3.8 % sodium citrate (1:9) and centrifuged at room temperature at 1000 rpm for 15min to obtain PRP. Centrifugation of the remaining blood at 3000 rpm for 20 min yielded platelet poor plasma (PPP). Platelets were counted under microscope, and the platelet count was adjusted to 300 000 platelets/µl with PPP. Aggregation was measured by the aggregometric method (14 Aburjai, 2000). Optical aggregometer (Chrono-log 490) connected to dual channel recorder was used for measuring aggregation. The aggregometer was calibrated so that the PRP gave 10% of light transmission while PPP gave 90 % of light transmission (26). The platelet suspension was stirred at 1000 rpm. Platelets were preincubated with the test extracts or compounds or DMSO for 5 min at 37°C before addition of aggregation inducer.

RESULTS AND DISCUSSION

Chemical composition of the volatile oil

To our knowledge, this is the first report about composition of the oil obtained from Jordanian *A. falcata.* Water distillation of dried aerial parts of the

Table No.1 - Volatile oil components identified in A. falcata L. oil by GC/MS analysis.

RI*	Compound	% Content
854	(*E*)-2-Hexenal	0.10
910	Santolina triene	0.10
930	α-Thujene	0.70
939	α-Pinene	0.50
954	Camphene	0.30
977	Sabinene	0.60
980	β-Pinene	2.30
992	2,3-Dehydro-1,8-cineole	1.10
1007	α-Phellandrene	0.20
1020	α-Terpinene	2.90
1026	*p*-Cymene	11.32
1033	1,8-Cineole	15.90
1059	γ-Terpinene	1.10
1088	Terpinolene	2.85
1098	Linalool	1.15
1102	β-Thujone	9.84
1143	Camphor	17.03
1166	Borneol	1.02
1173	cis-Pinocamphone	0.50
1177	Terpin-4-ol	5.65
1184	*p*-Cymen-8-ol	tr
1189	α-Terpineol	2.58
1245	Carvone	1.90
1254	Piperitone oxide	Tr
1257	Linalyl acetate	1.28
1288	Isobornyl acetate	0.40
1294	Thymol	0.95
1299	Carvacrol	tr
1373	Methyl-*p*- anisate	0.20
1404	α-Caryophyllene	3.37
1418	β-Caryophyllene	0.72
1439	Aromadendrene	0.63
1481	Germacrene D	1.10
1488	β-Selinene	0.30
1511	β-Bisabolene	0.40
1524	δ-Cadinene	0.50
1577	Spathulenol	1.20
1581	Caryophyllene oxide	2.34
1640	τ-Cadinol	1.10
1685	α-Bisabolol	4.60
1696	α- trans Bergamotol	tr
	Unidentified traces	1.23
	Monoterpene	*22.77*
	Oxygenated monoterpene	*59.50*
	Sesquiterpene hydrocarbons	*7.02*
	Oxygenated sesquiterpenes	*9.24*
	Other below	*0.20*
	Total identified	98.73

** RI: retention index calculated on DB-5 capillary column material. tr: trace (<0.05%).*

plant yielded 0.6% (v/w) of the oil. About 41 constituents (98.7% of the total oil) were identified by means of GC and GC/MS as shown in Table 1. The major compounds identified were camphor (17.0%), 1,8-cineole (15.9%), *p*-cymene (11.3%), β-thujone (9.8%), terpine-4-ol (5.7), bisabolol (4.6%), and α-caryophyllene (3.4%). Compared to *A. falcata* oil obtained from plants growing in other countries mentioned above (12, 13), composition of the Jordanian oil, in particular for camphor and 1,8-cineole, was found comparable with Turkish oil, whereas the oil obtained from plants growing in Lebanon was generally different. Oil composition of *A. falcata* seems to be somehow different from other *Achillea* species growing in Jordan and other countries. Generally, most of the reported *Achillea* spp. oils

Table No.2 - Growth inhibition zones (mm) of water and alcoholic extracts and volatile oils obtained from A. falcata.

	*Microorganism**							
Sample	***E. coli***	***St. aureus***	***St. aureus (R)*** **	***B. subtilis***	***Ps. aeruginosa***	***Ps. aeruginosa (R)*** **	***C. albicans***	***T. cutaneum***
Water extract	+/-	+/-	-	-	-	+/-	8.6±1.8	6.1±.9
Alcoholic extract	13.1±1.5	2.4±1.1	-	+/-	8.1±0.4	11.2±1.1	12.3±0.7	-
Volatile oil	15.6±1.1	14.4±0.7	4.6±0.3	4.5±0.5	4.3±0.4	2.9±0.4	15.6±0.8	14.9±0.8
Erythromycin	28.1±3.1	24.6±2.6	6.7±.09	21.5±2.2	14.2±1.9	11.3±0.9	-	-
Chloramphenicol	-	12.7±1.1	3.3±0.2	9.9±1.8	23.6±1.3	9.1±0.5	-	-
Nystatin	-	-	-	-	-	-	24.3±1.2	26.2±2.4

- *No inhibition,* +/- *slight inhibition (2 mm).*
* *Values for zone of growth inhibition are presented as means (three determinations in duplicate) ± S.E.*
** *Resistant strain*

Table No.3 - Effects of different crude extracts of A. falcata on platelet aggregation induced by collagen and ADP

	IC_{50} *(µg/ml)*[a]				
Inducer	***Aqueous extract***	***Alcoholic extract***	***Volatile oils***	***trans-resveratrol***	***Aspirin***
Collagen	182.0± 2.8x10^3	0.05± 48.1	_	11.4±2.4	_
ADP	37.8± 0.9x10^3	±9.4112.1	_	7.4±1.9	33.4±1.02

[a] *Platelets were preincubated with different concentrations of the various agents or DMSO (0.5%, as control) at 37°C for 3 min, then collagen (2.0 µg/ml) or ADP (10µM) was added. Results are expressed as means ± SD of three replicates experiments.*
- No activity.

contained substantial amounts of 1,8-cineole, camphor, and borneol (27, 28, 29, 30, 31, 32) and some others contained camphene, ascardiol, piperitone, α-thujone, artemisia ketone and santolina alcohol as their major components (2, 33, 34, 35). On the other hand, *A. santolina* grown in Jordan showed mainly 1, 8-cineole, camphor, 4-trpineol and trans-carveol as major constituents, while *A. Biebersteinii* contained cis-ascardiole, *p*-cymene, carvenone oxide and camphor as major constituents (36).

Antimicrobial activity

The *in vitro* antimicrobial tests of the different *A. falcata* extracts and the oil resulted in a range of growth inhibition pattern against the used microorganisms. The results of the antimicrobial activity of the aqueous and alcoholic extracts and the oil of *A. falcata* are given in Table 2. These data revealed that the volatile oil of *A. falcata* exhibits potent antibacterial activity against both gram positive and gram-negative bacteria. However, it exhibited only a weak activity against resistant strains of *Ps. aeruginosa* and *St. aureus*. The oil revealed also significant inhibition against *C. albicans and T. cutaneum.* On the other hand, the alcoholic extract showed good activity against gram-negative bacteria and *C. albicans.* It is noteworthy in particular its effect against resistant *Ps. aeruginosa* which was comparable with erythromycin effect and better than that of chloramphenicol. Finally, water extract showed weak activity against all tested microorganisms except for fungal species where it showed moderate effect in particular against *C. albicans*.

Antiplatelet activity

In this study aspirin and trans-resveratrol were used as positive controls as they are well known to have potential antiplatelet effects (14, 15, 22). Aspirin inhibited platelet aggregation induced by ADP (IC_{50}: 33.4 µg/ml) but not aggregation induced by collagen, while trans-resveratrol inhibited platelet aggregation induced by both ADP (IC_{50}: 7.4 µg/ml) and collagen (IC_{50}: 11.4 µg/ml) as shown in Table 3. The anti-platelet activity studied on the aqueous, alcoholic and oil extracts of *A. falcata* revealed that the oil and up to concentration of 12mg/ml is deprived of any activity when platelet aggregation was induced by either collagen or ADP.

The anti-platelet effect of the aqueous extract showed to be concentration dependent against both collagen and ADP (IC_{50}: $2.8x10^3$ and $0.9x10^3$ µg/ml, respectively). Concentrations of 30 and 20mg/ml gave complete inhibition while concentrations of 2.5, 1.875, 1.25 and 0.625mg/ml showed no anti-platelet activity. The alcoholic extract showed significant inhibitory effect on platelet aggregation induced by both collagen and ADP (48.1, 112.1 µg/ml, respectively). These results might explain the common use of this plant in the folkloric medicine as antiplatelet herb.

CONCLUSION

In conclusion 41 constituents of the volatile oil were identified of which camphor and 1,8-cineole were the principal components. Generally all plant extracts showed good to moderate antimicrobial activity. On the other hand, alcoholic extract exhibited significant inhibitory effect against platelet aggregation induced by either inducers. Future work will be emphasizing upon isolating and identifying the potential principle(s) responsible for the various activities particularly the antiplatelet effect.

REFERENCES

1. A.A. Ahmed, A.M. Shalaby, F.R. Melek, T.J. Mabry. Swertisin 2"-arabinoside, a new C-glycosylflavone from *Achillea fragrantissima*. *J. Nat. Prod*. **51**: 971-972 (1988).
2. Z. Fleisher and A. Fleisher. Volatiles of *Achillea fragrantissima* (Forssk.) Sch. Bip. Aromatic plants of the Holy Land and the Sinai. Part XI. *J. Essent. Oil. Res.* **5**: 211-214 (1993).
3. E. Mustafa, S. Abdalla, M. Abu Zarga and S. Sabri. Constituents of *Achillea fragrantissima* and effects of 13-O-desacetyl-1-beta-hydroxyafraglaucolide on rat isolated smooth muscle. *Fitoterapia* **63**: 526-533. (1992).
4. R. Segal, A. Dor, H. Duddeck, G. Snatzke, D. Rosenbaum and M. Kajtar. The sesquiterpene lactones from *Achillea fragrantissima*. I. Achillolide A and B, two novel germacranolides. *Tetrahedron* **43**: 4125-4132 (1987).
5. E.A. Aboutable, F.M. Soliman, S.M. El-Zalani, E.J. Brunke and T.A. El-Kersh. Essential oil of *Achillea fragrantissima* (Forssk.) Sch. Bip. *Egypt J. Pharm Sci***. 27**: 215-19 (1986).
6. S. Asgary, G.H. Naderi, N. Sarrafzadegan, N. Mohammadifard, S. Mostafavi and R. Vakili. Antihypertensive and antihyperlipidemic effects of *Achillea wilhelmsii*. *Drugs Exp. Clin. Res.* **26**: 89-93 (2000).
7. M.S. Hifnawy, O.A. Rashwan and M.A. Rabeh. Comparative chemical and biological investigations of certain essential oils belonging to families Asteraceae, Lamiaceae and Graminae. *Bull. Facul. Pharm.* (Cairo University) **39**: 35-53 (2001).

8. S. Barel and J. Yashphe. Effect of the essential oil from *Achillea fragrantissima* on Escherichia coli cells. *Curr. Microbiol.* **19**: 337-341 (1989).
9. S. Oran and D. Al-Eisawi. Check-list of medicinal plants in Jordan. *Dirasat Med. Biol. Sci.* **25**: 84-112 (1998).
10. Z. Yaniv, A. Dafni, J. Friedman and D. Palevitch. Plants used for the treatment of diabetes in Israel. *J. Ethnopharmacol*. **19**: 145-151 (1987).
11. S. Barel, R. Segal and J. Yashphe. The antimicrobial activity of the essential oil from *Achillea fragrantissima*. *J. Ethnopharmacol*. **33**: 187-191 (1991).
12. F. Senatore, F. Napolitano, N. Apostolides-Arnold, M. Bruno and W. Herz. Composition and antimicrobial activity of the essential oil of *Achillea falcata* L. (Asteraceae). *Flav. Frag. J.* **20**: 291-294 (2005).
13. M. Kurkcuoglu, B. Demirci, N. Tabanca, T. Ozek and K. Baser. The essential oil of *Achillea falcata* L. *Flav. Frag. J.* **18**: 192-194 (2003).
14. T.A. Aburjai. Anti-platelet stilbenes from aerial parts of *Rheum palaestinum*. *Phytochemistry* **55**: 407-410 (2000).
15. J.J. Chen, Y.L. Chang, C.M. Teng, Y.C. Chen and I.S. Chen. A new tetrahydroprotoberberine N-oxide alkaloid and anti-platelet aggregation constituents of *Corydalis tashiroi*. *Planta Med.* **67**: 423-427 (2001).
16. M.I. Chung, J.R. Weng, J.P. Wang, C.M. Teng and C.N. Lin. Antiplatelet and anti-inflammatory constituents and new oxygenated xanthones from *Hypericum geminiflorum*. *Planta Med.* **68**: 25-29 (2002).
17. M.I. Chung, K.H. Gan and C.N. Lin. Antiplatelet effects and vasorelaxing action of some constituents of Formosan plants. *J. Nat. Prod.* **56**: 929-934 (1993).
18. M.I. Chung, S.J. Jou, T.H. Cheng, C.N. Lin, F.N. Ko and C.M. Teng. Antiplatelet constituents of Formosan *Rubia alkane*. *J. Nat. Prod.* **57**: 313-316 (1994).
19. Y.C. Chia, K.S. Chen, Y.L. Chang, C.M. Teng and Y.C. Wu. Antiplatelet actions of aporphinoids from Formosan plants. *Bioorg. Med. Chem. Lett.* **9**: 3295-300 (1999).
20. M.I. Chung, C.M. Teng, K.L. Cheng, F.N. Ko and C. N. Lin. An antiplatelet principle of *Veratrum formosanum*. *Planta Med*. **58**: 274-276 (1992).
21. H. Dong, S.X. Chen, H.X. Xu, S. Kadota, and T. Namba. A New Antiplatelet Diarylheptanoid from *Alpinia blepharocalyx*. *J. Nat. Prod.* **61**: 142-144 (1998).
22. F. Orsini, F. Pelizzoni, L. Verotta, T. Aburjai. Isolation, Synthesis, and Antiplatelet Aggregation Activity of Resveratrol 3-O-beta-D-glucopyranoside and Related Compounds. *J. Nat. Prod.* **60**: 1082-1087 (1997).
23. C.M. Teng and F.N. Ko. Antiplatelet agents isolated from medicinal plants. *Res Commun Mol Pathol. Pharmacol.* **102**: 211-225 (1998).
24. R.P. Adams. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy.* (Allured Publ. Corp.: Carol Stream, IL 1995).
25. A.M. Janssen, J.C. Scheffer and A.B. Svendsen. Antimicrobial activity of essential oils: A 1976-1986 literature review. Aspects of the test methods. *Planta Med.* **53**: 395-398 . (1987).
26. A. Beretz, J.P. Cazenave. In: P.M. Dey and J.B. Harborne, eds. *Methods in Plant Biochemistry*. Vol. 6. 1st ed. New York : Academic Press; 235-260 (1991).
27. J.C. Chalchat, M.S. Gorunovic, and S.D. Petrovic. Aromatic plants of Yugoslavia. I. Chemical composition of oils of *Achillea millefolium* L. ssp. *pannonica* (Scheele) Hayak, A. crithmifolia W. et K., A. serbica Nym. and A. tanacetifolia All. *J. Essent. Oil. Res.* **11**: 306-310 (1999).
28. K.C. Baser, B.Demirci, H.Duman, Z. Aytac and N. Adlguzel. Composition of the essential oil of *Achillea goniocephala* Boiss. et Bal. from Turkey. *J. Essent. Oil. Res.* **13**: 219-220 (2001).
29. A.C. Figueiredo, J.G. Barroso, M. Pais and J.J. Scheffer. Composition of the essential oils from leaves and flowers of *Achillea millefolium* L. ssp. *millefolium*. *Flav. Frag. J.* **7**: 219-222 (1992).
30. M. Unlu, D. Daferera, E. Donmez, M. Polissiou, B. Tepe and A. Sokmen. Compositions and the in vitro antimicrobial activities of the essential oils of *Achillea setacea* and *Achillea teretifolia* (Compositae). *J. Ethnopharmacol.* **83**: 117-121 (2002).
31. N. Simic, R. Palic, V. Vajs, S. Milosavljevic and D. Djokovic. Composition and antibacterial activity of *Achillea chrysocoma* essential oil. *J. Essent. Oil. Res.* **12**: 784-787 (2000).
32. G. Stojanovic, R. Palic, T. Naskovic, D. Dokovic and S. Milosavljevic. Volatile constituents of *Achillea lingulata* W. K. *J. Essent. Oi. Res.* **13**: 378-379 (2001).

33. P. Chatzopoulou, S.T. Katsiotis and A. Baerheium-Svendsen. An ascaridole-containing essential oil of the *Achillea millefolium* L. complex growing wild in northern Greece. *J. Essent. Oil. Res.* **4**: 457-459 (1992).
34. J.C. Chalchat, M.S. Gorunovic, S.D. Petrovic and V.V. Zlatkovic. Aromatic plants of Yugoslavia. II. Chemical composition of essential oils of three wild *Achillea* species: *A. clavenae* L., *A. collina* Becker and *A. lingulata* W. et K. *J. Essent. Oil. Res.* **12**: 7-10 (2000).
35. S. Kuesmenoglu, K.H. Baser, T. Ozek, M. Harmandar and Z. Goekalp. Constituents of the essential oil of *Achillea biebersteinii* Afan. *J. Essent. Oil. Res.* **7**: 527-528 (1995).
36. A. Bader, G. Flamini, P. L. Cioni, I. Morelli. Essential oil composition of *Achillea santolina* L. and *Achillea biebersteinii* Afan. Collected in Jordan. *Flav. Frag. J*. **18**: 36-38 (2003).

****