

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/286560566>

Comparative Essential Oil Analysis of Calendula arvensis L. Extracted by Hydrodistillation and Microwave Distillation and Antimicrobial Activities

Article in Asian Journal of Chemistry · May 2012

CITATIONS

18

READS

911

6 authors, including:



Gonca Çelik

Karadeniz Technical University

41 PUBLICATIONS 400 CITATIONS

SEE PROFILE



Busra Yayli

Karadeniz Technical University

15 PUBLICATIONS 183 CITATIONS

SEE PROFILE



Tayfun Arslan

Giresun University

36 PUBLICATIONS 462 CITATIONS

SEE PROFILE

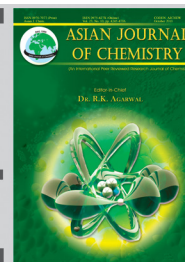


Ahmet Yasar

Karadeniz Technical University

13 PUBLICATIONS 172 CITATIONS

SEE PROFILE



Comparative Essential Oil Analysis of *Calendula arvensis* L. Extracted by Hydrodistillation and Microwave Distillation and Antimicrobial Activities

GONCA TOSUN¹, BÜSRA YAYLI¹, TAYFUN ARSLAN¹, AHMET YASAR¹, SENGÜL ALPAY KARAĞLU² and NURETTİN YAYLI^{1,3,*}

¹Department of Chemistry, Faculty of Science, Karadeniz Technical University, 61080 Trabzon, Turkey

²Department of Biology, Faculty of Arts and Sciences, Rize University, 53100 Rize, Turkey

³Faculty of Pharmacy, Karadeniz Technical University, 61080 Trabzon, Turkey

*Corresponding author: Fax: +90 462 3253196; Tel: +90 462 3772486; E-mail: yayli@ktu.edu.tr

(Received: 24 March 2011;

Accepted: 5 December 2011)

AJC-10802

The present study was to investigate the influence of the extraction methods on yield and chemical composition of the essential oil of *Calendula arvensis* L. The volatiles of *C. arvensis* have been isolated by hydrodistillation (HD) and microwave distillation (MD). The compositions of the essential oils were characterized by GC-FID and GC-MS. A total of 45 and 44 compounds were identified, constituting over 88.3 % and 84.8 % of oil composition of *C. arvensis*, respectively. Sesquiterpenes (HD: 30.5 % and MD: 23.4 %) and monoterpene compounds (HD: 26.3 % and MD: 24.3 %) were shown to be the main group of volatiles. The major terpene constituent of the essential oils of *C. arvensis* was α -selinene (HD, 16.0 % and MD, 0.0 %), α -pinene (HD, 11.9 % and MD, 12.3 %), (Z)- α -santalol (HD, 8.2 % and MD, 7.4 %), δ -amorphene (HD, 0.0 % and MD, 8.0 %), (Z)-sesquilandulol (HD, 4.8 % and MD, 0.0 %), 7-epi-silphiperfol-5-ene (HD, 2.6 % and MD, 3.7 %), viridiflorene (HD, 2.5 % and MD, 1.7 %) and β -pinene (HD, 1.8 % and MD, 2.4 %). Comparative study showed that the amount of total volatiles (88.3 % and 84.8 %) and the identified constituent (45 and 44) were found to be similar in both HD and MD of *C. arvensis*. The terpenoid contents (HD: 73.5 % and MD: 65.3 %) were greater in HD of *C. arvensis*. The antimicrobial activities of the isolated essential oils, hexane, ether and methanolic extracts of the plant were also investigated and only the essential oil (HD) and methanolic extract showed moderate antibacterial activities against *Staphylococcus aureus* and *Bacillus cereus* with in the range of 105-210 μ g, respectively. But all the extract showed good antitubercule activity against *Mycobacterium smegmatis* (13.2-62.5 μ g).

Key Words: *Calendula arvensis* L., Hydrodistillation, Microwave distillation, Essential oil, Antimicrobial activity, GC-MS.

INTRODUCTION

The genus of *Calendula* belongs to the Asteraceae family¹. *Calendula arvensis* L., the common name of which is field marigold, is an annual herbaceous species (10-100 cm tall) that is widespread in central and southern Europe, Northern Africa and Southwestern Asia¹. Phytochemical studies on the *C. arvensis* have shown various families of phenolic acid, the flavonoid and various natural compounds²⁻¹⁵. Previous studies on the volatiles and the essential oils from *Calendula* included *C. arvensis*^{16,17}, *Calendula officinalis* L.¹⁸⁻²⁵ and *Calendula micrantha*²⁶. The high concentration of sesquiterpenes (δ -cadinene and α -cadinol) were the main components in the essential oil of *C. arvensis*¹⁶ in seasonal variation (winter vs. spring) during the flowering period. *C. officinalis* oils are generally characterized by a high content of sesquiterpenes with cadinane or muurolane skeletons¹⁸⁻²⁵. It should be noted that this species is used as perfumes, pharmaceuticals and in the food industry to add colour or flavour. The main compounds identified in the essential oil from the flower of *C. micrantha*

were (E)- β -caryophyllene, α -gurjunene, α -pinene, aromadendrene, guaiol and benzyl formate²⁶. Antimicrobial activity of the various isolate of *Calendula arvensis* has mentioned²⁷⁻²⁹. However, to our best of knowledge, there are no report dealing with comparative essential oil analysis and antimicrobial activity of the essential oil of *Calendula arvensis*. The first aim of this work is to perform a compositional analysis and compare the essential oil contents of *Calendula arvensis* extracted by hydro and a microwave distillation as well as to evaluate their antimicrobial activities. And the second aim was to characterize the variation in the essential oil composition of *Calendula arvensis* from Turkey and to identify environmental factors associated with differences in essential oil composition.

EXPERIMENTAL

Calendula arvensis was collected in Akçaabat, Trabzon, Turkey (at heights of 300 m) in the northeastern part of Turkey in January 15, 2011. The plant was authenticated by Prof. S. Terzioglu. Voucher specimen was deposited in the Herbarium of the Faculty of Forestry, KATO (KATO: 8783), Karadeniz Technical University, Turkey.

Hydrodistillation apparatus and procedure: The fresh plant material (65 g) were grounded into small pieces and submitted to hydrodistillation (HD) using a Clevenger-type apparatus (4 h) (yield (w/w): 0.16 %). The obtained oil was extracted with HPLC grade *n*-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at -5 °C in a sealed brown vial.

Microwave distillation apparatus and procedure: Microwave distillation (MD) was performed at atmospheric pressure with a Milestone DryDIST microwave apparatus using a fixed power of 750 W for 40 min. Temperature was monitored by an external infrared (IR) sensor. The fresh plant material (65 g) were grounded into small pieces, then placed in a round bottom flask (2l) with 50 mL water and submitted to microwave distillation using a Clevenger-type apparatus (0.5 h) (yield (w/w): 0.86 %). The obtained oil was extracted with HPLC grade *n*-hexane (0.5 mL) and dried over anhydrous sodium sulphate and stored at -5 °C in a sealed brown vial.

Gas chromatography (GC) and Gas chromatography-mass spectrometry (GC-MS) analysis: GC-FID and GC-MS analyses were done as described previously^{30,31}.

Identification of constituents: The constituents of the oils were identified by comparison of their mass spectra with those of a computer library or with authentic compounds (α -pinene, β -pinene, myrcene, γ -terpinene, eicosane, heneicosane, docosane, tricosane, tetracosane and pentacosane) and confirmed by comparison of their retention indices, either with those of authentic compounds or with data published in the literature^{16,30-40}.

Antimicrobial activity: All test microorganisms were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey) and were as follows: *Escherichia coli* ATCC35218, *Yersinia pseudotuberculosis* ATCC911, *Pseudomonas aeruginosa* ATCC43288, *Enterococcus faecalis* ATCC29212, *Staphylococcus aureus* ATCC25923, *Bacillus cereus* 709 Roma, *Mycobacterium smegmatis* ATCC607, *Saccharomyces cerevisiae* RSKK 251 and *Candida albicans* ATCC60193. All extracts were weighed and dissolved in hexane, ether and methanol to prepare extract stock solution of 5.000-10.000 μ g/mL. Essential oils were weighed and dissolved in hexane to prepare extract stock solution of 33.100-186.100 μ g/mL.

The antimicrobial effects of the substances were tested quantitatively in respective broth media by using double dilution and the minimal inhibition concentration (MIC) values (μ g/mL) were determined⁴¹. The antibacterial and antifungal assays were performed in Mueller-Hinton broth (MH) (Difco, Detroit, MI) at pH 7.3 and buffered Yeast Nitrogen Base (Difco, Detroit, MI) at pH 7.0, respectively. Brain heart infusion broth (BHI) (Difco, Detroit, MI) was used for *M. smegmatis*⁴². The minimal inhibition concentration was defined as the lowest concentration that showed no growth. Ampicillin (10 μ g), streptomisin (10 μ g) and fluconazole (5 μ g) were used as standard antibacterial and antifungal drugs, respectively. Hexane, ether and methanol with dilution of 1:10 were used as solvent controls.

RESULTS AND DISCUSSION

The essential oils obtained after hydrodistillation and microwave distillation of the *C. arvensis* gave an average yields

(w/w) of 0.16 % and 0.86 %, respectively. In total, GC-MS analyzes allowed the identification of 45 and 44 volatile compounds for both hydrodistillation and microwave distillation, accounting for 88.3 % and 84.8 % of the detected GC peak areas, respectively. The list of the identified volatile constituents as well as their grouping into eight classes, namely monoterpene hydrocarbons (26.3 % HD and 24.3 % MD), oxygenated monoterpenes (1.1 % HD and 2.1 % MD), sesquiterpene hydrocarbons (30.5 % HD and 23.4 % MD), oxygenated sesquiterpenes (14.1 % HD and 12.6 % MD), diterpenoids (1.5 % HD and 2.9 % MD), terpene related compounds (1.3 % HD and 1.3 % MD), hydrocarbons (9.0 % HD and 13.4 % MD) and others (4.5 % HD and 4.8 % MD) (Table-1)^{16,30-40}.

TABLE-1
IDENTIFIED COMPONENTS IN THE OILS OF *C. arvensis*^{a,b}
EXTRACTED BY HYDRODISTILLATION (HD) AND
MICROWAVE DISTILLATION (MD)

Compounds	HD Area ^a (%)	MD Area ^a (%)	Exp. RI ^b	Lit. RI
Monoterpene hydrocarbons				
α -Thujene	7.7	4.5	930	930
α -Pinene ^c	11.9	12.3	939	939
Sabinene	0.7	-	971	975
β -Pinene ^c	1.8	2.4	976	979
Myrcene ^c	0.6	0.6	988	991
α -Phellandrene	1.1	0.5	1001	1003
<i>p</i> -Mentha-1(7),8-diene	-	0.6	1004	1004
Limonene ^c	1.2	1.8	1025	1029
(Z)- β -Ocimene	0.3	-	1035	1037
γ -Terpinene ^c	0.4	1.1	1057	1060
<i>p</i> -Cymenene	0.3	0.5	1088	1091
Neo-allo-Ocimene	0.3	-	1128	1132
Oxygenated monoterpenes				
Terpinen-4-ol	0.3	0.7	1170	1172
Anethofuran	-	0.4	1183	1187
α -Terpinen-7-al	0.8	1.0	1280	1285
Sesquiterpene hydrocarbons				
7-epi-Silphiperfol-5-ene	2.6	3.7	1348	1348
Iso-Ledene	0.2	-	1371	1376
α -Copaene	0.1	0.2	1377	1377
β -Bourbonene	0.5	1.4	1382	1388
β -Elemene	0.2	-	1390	1391
α -Gurjunene	0.3	1.0	1405	1410
E-Caryophyllene	1.2	2.6	1415	1419
β -Gurjunene	0.4	-	1430	1434
α -Humulene	0.8	2.0	1450	1455
(E)- β -Farnesene	-	0.3	1457	1457
α -Guaiene	0.3	-	1460	1460
Germacrene D	0.6	1.3	1482	1485
β -Selinene	0.8	0.9	1486	1490
Viridiflorene	2.5	1.7	1495	1497
α -Selinene	16.0	-	1498	1498
α -Cuprenene	-	0.3	1505	1506
δ -Amorphene	-	8.0	1510	1512
2-Norpinene	2.9	-	1608	MS1
Oxygenated sesquiterpenes				
α -Agrofuran	-	1.2	1548	1550
Ledol	-	0.9	1565	1569
Viridiflorol	-	0.7	1590	1593
(Z)-Sesquilavandulol	4.8	-	1605	1607

Compounds	HD Area ^a (%)	MD Area ^a (%)	Exp. RI ^b	Lit. RI
α -Acerenol	-	1.3	1630	1633
Epi- α -Muurolol	1.14	-	1640	1642
α -Muurolol	-	1.1	1644	1646
(Z)- α -Santalol	8.2	7.4	1670	1675
Diterpenoids				
E-Phytol	1.5	2.0	1823	MS2
2E-3,7,11,15-tetramethyl-2-hexadecen-1-ol	-	0.9	2142	MS3
Terpene related compounds				
Z-Jasmone	0.1	-	1392	1393
Hexahydrofarnesylacetone	1.2	1.3	1840	1847
Hydrocarbons				
Eicosane ^c	1.4	1.9	2000	2000
Heneicosane ^c	1.9	0.5	2100	2100
Docosane ^c	1.1	2.9	2201	2200
Tricosane ^c	1.6	1.3	2300	2300
Tetracosane ^c	1.0	1.4	2399	2400
Pentacosane ^c	2.0	5.4	2498	2500
Others				
Benzaldehyde	1.0	-	958	960
Nonanal	1.0	1.7	1101	1101
Methyl salicylate	0.6	0.8	1188	1192
Decanal	0.5	0.7	1200	1202
Ethyl Linoleate	1.4	1.6	1890	1893
Monoterpene hydrocarbons	26.3	24.3		
Oxygenated monoterpenes	1.1	2.1		
Sesquiterpene hydrocarbons	30.5	23.4		
Oxygenated sesquiterpenes	14.1	12.6		
Diterpenoids	1.5	2.9		
Terpene related compounds	1.3	1.3		
Hydrocarbons	9.0	13.4		
Others	4.5	4.8		
Total isolate	88.3	84.8		
MS ¹ : 133(20), 119(100), 105(35), 93(95), 77(40), 69(75). MS ² : 123(60), 109(00), 95(90), 82(80), 68(100), 57(70). MS ³ : 137(10), 123(70), 109(35), 95(90), 82(95), 68(100), 57(87). ^a Percentages obtained by FID peak-area normalization. ^b RI calculated from retention times relative to that of <i>n</i> -alkanes (C ₆ -C ₃₂) on the non-polar HP-5 column. ^c Identified by authentic samples.				

Sesquiterpenes (HD: 30.5 % and MD: 23.4 %) and monoterpene compounds (HD: 26.3 % and MD: 24.3 %) were shown to be the main group of volatiles, respectively. The major terpene constituent of the essential oils of *C. arvensis* was α -selinene (HD, 16.0 % and MD, 0.0 %), α -pinene (HD, 11.9 % and MD, 12.3 %), (Z)- α -santalol (HD, 8.2 % and MD, 7.4 %), δ -amorphene (HD, 0.0 % and MD, 8.0 %), (Z)-sesquilandulol (HD, 4.8 % and MD, 0.0 %), 7-epi-silphiperfol-5-ene (HD, 2.6 % and MD, 3.7 %), viridiflorene (HD, 2.5 % and MD, 1.7 %) and β -pinene (HD, 1.8 % and MD, 2.4 %). Comparative study showed that the amount of total volatiles (88.3 % and 84.8 %) and the identified constituent were found to be similar in both hydrodistillation and microwave distillation of *C. arvensis*. But, the yield of the essential oil is better in microwave distillation (w/w, MD: 0.86 % and HD: 0.16 %). But, the terpenoid contents (HD: 73.5 % and MD: 65.3 %) of the essential oils were greater in hydrodistillation of *C. arvensis*.

In the literature, the major compounds in the aerial parts of *C. arvensis*¹⁶ were δ -cadinene (15.1 %) and α -cadinol (12.4 %). The main constituents in the essential oil of *C. micrantha*²⁶ were (E)- β -caryophyllene, α -gurjunene, α -pinene, aromadendrene, guaiol and benzyl formate. Cadinane or muurolane skeletons were the major components in the floral volatiles of *C. officinalis*¹⁸⁻²⁵. Generally, the comparison of our data with the literature showed that the main constituents of chemical composition of the investigated *C. arvensis* oils were sesquiterpenes (HD: 30.5 % and MD: 23.4 %) and monoterpene compounds (HD: 26.3 % and MD: 24.3 %) and markedly different from known *Calendula* species¹⁶⁻²⁶. Comparison of the essential oil contents of our *C. arvensis* with the literature¹⁶, (Z)- β -ocimene, γ -terpinene, neo-allo-ocimene, 7-epi-silphiperfol-5-ene, β -elemene, E-caryophyllene, α -guaiene, β -selinene, viridiflorene, α -selinene, α -cuprenene, δ -amorphene, 2-norpinene, viridiflorol, (Z)-sesquilandulol, α -acerenol, epi- α -muurolol, α -muurolol, 2E-3,7,11,15-tetramethyl-2-hexadecen-1-ol and hexahydrofarnesyl acetone were present in our case. The difference of the composition of the oils could be attributed to the geographical source, time of collection of the plant and the specific climate there.

The analysis of variance showed that the distillation method had significant effect on the yield (MD: 0.86 % and HD: 0.16 %) of oil content and the components of *C. arvensis*. Sabinene, (Z)- β -ocimene, neo-allo-ocimene, iso-ledene, β -elemene, α -guaiene, α -selinene, 2-norpinene, (Z)-sesquilandulol, epi- α -muurolol and Z-jasmone were present only in the hydrodistillation oil of *C. arvensis*. *p*-Mentha-1(7),8-diene, anethofuran, (E)- β -farnesene, α -cuprenene, δ -amorphene, α -agorofuran, ledol, viridiflorol, α -acerenol, α -muurolol and 2E-3,7,11,15-tetramethyl-2-hexadecen-1-ol were exist only in the oil of microwave distillation of *C. arvensis*. Therefore, the result clearly showed that extraction methods (HD vs. MD) influenced the contents of essential oils of *C. arvensis*. The highest oil yield was obtained by microwave distillation method. This may be due to the use of high power (750 W) of heat of microwave distillation. But, comparative study showed that the amount and the number of different total volatiles and the major constituent were found to be different in hydrodistillation and microwave distillation of *C. arvensis*. Microwave distillation could be recommended for the essential oil extraction of *C. arvensis* due to the high yield. The results of this study have reinforced the fact that there are quantitative and qualitative differences in the essential components of hydro and microwave distillation for the plant material.

The antimicrobial activity of the isolated essential oils, hexane and ether extracts of the plant were tested quantitatively in respective broth media by using double dilution and the minimal inhibition concentration values ($\mu\text{g/mL}$)^{41,42} with nine microorganisms (*E. coli*, *Y. pseudotuberculosis*, *P. aeruginosa*, *E. faecalis*, *S. aureus*, *B. cereus*, *M. smegmatis*, *C. albicans* and *S. cerevisiae*). Only the essential oil (HD) and methanolic extract of *C. arvensis* showed moderate antibacterial activities against *Staphylococcus aureus* and *Bacillus cereus* with in the range of 105-210 μg , respectively. But all the extract showed good antituberculosic activity against *Mycobacterium smegmatis* (13.2-62.5 μg) (Table-2).

TABLE-2
SCREENING RESULT FOR ANTIMICROBIAL ACTIVITIES OF *C. arvensis* (µg/00 µL)

Samples	Stok Sol. (µg/mL)	Microorganisms and minimal inhibition concentration								
		<i>Ec</i>	<i>Yp</i>	<i>Pa</i>	<i>Ef</i>	<i>Sa</i>	<i>Bc</i>	<i>Ms</i>	<i>Ca</i>	<i>Sc</i>
Essential oil (HD)	134.600	-	-	-	-	210.3	105.1	13.2	-	-
Essential oil (MD)	136.700	-	-	-	-	-	-	13.4	-	-
Hexane extract	10.000	-	-	-	-	-	-	62.5	-	-
Ether extract	10.000	-	-	-	-	-	-	62.5	-	-
Methanol extract	5.000	-	-	-	-	-	125	31.2	-	-
Ampicillin	10	8	32	>128	2	2	<1			
Streptomisin	10							4		
Fluconazole	5								<8	<8

Ec: *Escherichia coli*, *Yp*: *Yersinia pseudotuberculosis*, *Pa*: *Pseudomonas aeruginosa*, *Ef*: *Enterococcus faecalis*, *Sa*: *Staphylococcus aureus*, *Bc*: *Bacillus cereus* 702 Roma, *Ms*: *Mycobacterium smegmatis*, *Ca*: *Candida albicans*, *Sc*: *S. cerevisiae*. (-) no activity at stock solution concentration; HD = Hydrodistillation; MD = Microwave distillation.

ACKNOWLEDGEMENTS

The authors thank Prof. Dr. Salih Terzioglu for identification of plant material. This work was supported by grants from Karadeniz Technical University Research Fund and State Planning Agency (DPT) of Turkey.

REFERENCES

- R.D. Meikle and *Calendula L.*, in ed.: P.H. Davis, Flora of Turkey and the East Aegean Islands, The University Press, Edinburgh, Vol. 5, pp. 170-172 (1975).
- A.A. Ahmed, J. Jakupovic and T.J. Mabry, *J. Nat. Prod.*, **56**, 1821 (1993).
- R. Chemli, A. Babadjamian, R. Faure, K. Boukef, G. Balansard and E. Vidal, *Phytochemistry*, **26**, 1785 (1987).
- N. De Tommasi, C. Pizza, C. Conti, N. Orsi and M.L. Stein, *J. Nat. Prod.*, **53**, 830 (1990).
- H. Kirmizibekmez, C. Bassarello, S. Piacente, C. Pizza and I. Calis, *Z. Nat. Forsch. B*, **61**, 1170 (2006).
- C. Pizza and N. De Tommasi, *J. Nat. Prod.*, **50**, 784 (1987).
- S.A. Radioza, S.V. Ryda and I.Y. Makukh, *Medic. Khim.*, **8**, 72 (2006).
- W. Seebacher, R. Weis, J. Jurenitsch, K. Rauchensteiner and E. Haslinger, *Monatsh. Chem.*, **131**, 985 (2000).
- R. Elias, M. De Meo, E. Vidal-Ollivier, M. Laget, G. Balansard and G. Dumenil, *Mutagenesis*, **5**, 327 (1990).
- R. Chemli, A. Toumi, S. Oueslati, H. Zouaghi, K. Boukef and G. Balansard, *J. Pharm. Belg.*, **45**, 12 (1990).
- E. Vidal-Ollivier, A. Babadjamian, R. Faure, R. Chemli, K. Boukef, G. Balansard and E.J. Vincent, *Spectrosc. Lett.*, **22**, 579 (1989).
- C. Pizza and N. De Tommasi, *Phytochemistry*, **27**, 2205 (1988).
- C. Pizza, Z.L. Zhou and N. De Tommasi, *J. Nat. Prod.*, **50**, 927 (1987).
- A.M. Rizk, H.I. Heiba, H.A. Ma'Ayergi and K.H. Batanouny, *Fitoterapia*, **57**, 3 (1986).
- M. Pinkas, M. Torck and E. Perrin, *Sci. Nat.*, **281**, 447 (1975).
- J. Paolini, T. Barboni, J.M. Desjober, N. Djabou, A. Muselli and J. Costa, *Biochem. Syst. Ecol.*, **38**, 865 (2010).
- S.A. Al-Mazroa, L.H. Al-Wahaibi, A.A. Mousa and H.Z. Alkathlan, *J. Saudi Chem. Soc.*, **9**, 693 (2006).
- J.C. Chalchat, R.P. Garry and A. Michet, *Flav. Fragr. J.*, **6**, 189 (1991).
- N. Crabas, B. Marungiu, A. Piras, T. Pivetta and S. Porcedda, *J. Essent. Oil Res.*, **15**, 350 (2003).
- Z.C. Gazim, G.A. Ferreira, C.M. Rezende, C.V. Nakamura, B.P.D. Filho and D.A.G. Cortez, *Hortic. Bras.*, **25**, 118 (2007).
- Z.C. Gazim, C.M. Rezende, S.R. Fraga, B.P.D. Filho, C.V. Nakamura and D.A.G. Cortez, *J. Pharm. Sci.*, **44**, 391 (2008).
- O.O. Okoh, A.A. Sadimenko and A.J. Afolayan, *J. Appl. Sci.*, **7**, 3806 (2007).
- O.O. Okoh, A.A. Sadimenko, O.T. Asekun and A.J. Afolayan, *J. Biotechnol.*, **7**, 1500 (2008).
- V. Radulescu, C. Doneanu and T. Loloiu, *Rev. Roum. Chim.*, **45**, 271 (2000).
- G. Marczal, Z. Cserjesi, E. Hethelyi and G. Petri, *Herba Hung.*, **26**, 179 (1987).
- K.T. Hussein, *J. Egypt. Soc. Parasitol.*, **35**, 365 (2005).
- S.M. Lavagna, D. Secci, P. Chimenti, L. Bonsignore, A. Ottaviani and B. Bizzarri, *Farmaco*, **56**, 451 (2001).
- N. De Tommasi, C. Conti, M.L. Stein and C. Pizza, *Planta Med.*, **57**, 250 (1991).
- G. Dumenil, R. Chemli, G. Balansard, H. Guiraud and M. Lallemand, *Annales Pharm. Fran.*, **38**, 493 (1980).
- N. Kahrman, G. Tosun, H. Genç and N. Yayli, *Turk. J. Chem.*, **34**, 969 (2010).
- N. Kahrman, G. Tosun, S. Terzioglu, S.A. Karaoglu and N. Yayli, *Rec. Nat. Prod.*, **5**, 82 (2011).
- R.P. Adams, Identification of Essential Oil Components by Gas Chromatography-Mass Spectroscopy, Allured, Carol Stream, IL, USA (2004).
- H.D. Skaltsa, C. Demetzos, D. Lazari and M. Sokovic, *Phytochemistry*, **64**, 743 (2003).
- N. Yayli, A. Yasar, C. Güleç, A. Usta, S. Kolayli, K. Coskunçelebi and S. Karaoglu, *Phytochemistry*, **66**, 1741 (2005).
- N. Yayli, A. Yasar, N.Y. Iskender, N. Yayli, T. Beyza Cansu, K. Coskunçelebi and S. Karaoglu, *Pharm. Biol.*, **48**, 191 (2010).
- O. Üçüncü, N. Kahrman, S. Terzioglu, S.A. Karaoglu and N. Yayli, *Nat. Prod. Comm.*, **5**, 831 (2010).
- N. Kahrman, C.G. Albay, N. Dogan, A. Usta, S.A. Karaoglu and N. Yayli, *Asian J. Chem.*, **22**, 6437 (2010).
- N. Yayli, T.B. Cansu, N. Yilmaz, A. Yasar, M.M. Çetin and N. Yayli, *Asian J. Chem.*, **22**, 3439 (2010).
- T.B. Cansu, M. Yücel, K. Sinek, C. Baltaci, S.A. Karaoglu and N. Yayli, *Asian J. Chem.*, **23**, 1029 (2011).
- N.Y. Iskender, N. Kahrman, M. Yücel, N. Yayli, S.A. Karaoglu, S. Terzioglu and N. Yayli, *Asian J. Chem.*, **23**, 1032 (2011).
- National Committee for Clinical Laboratory Standard. Methods for Determining Bactericidal Activity of Antimicrobial Agents; Approved Guideline. M26-A, 19 (18), NCCLS, Willanova, Pa. 1999.
- G.L. Woods, B.A. Brown-Elliott, E.P. Desmond, G.S. Hall, L. Heifets, G.E. Pfyffer, J.C. Ridderhof, R.J. Jr. Wallace, N.C. Warren and F.G. Witebsky, Approved Standard, NCCLS Document, Vol. 23 (18) (2003).