Department of Pharmacognosy[1], Faculty of Pharmacy, Zagazig University, Zagazig, Egypt and Institut für Pharmazie und Molekulare Biotechnologie[2], Universität Heidelberg, Germany

# Comparative study of the essential oils and extracts of *Achillea fragrantissima* (Forssk.) Sch. Bip. and *Achillea santolina* L. (Asteraceae) from Egypt

A. M. El-Shazly[1,2], S. S. Hafez[1], M. Wink[2]

*Received July 14, 2003, accepted August 20, 2003*

*Prof. Dr. Michael Wink, Institut für Pharmazie und Molekulare Biotechnologie, Abt. Biologie, Universität Heidelberg, Im Neuenheimer Feld 364, 69120 Heidelberg, Germany*
*wink@uni-hd.de*

*Pharmazie 59: 226–230 (2004)*

Essential oils obtained by steam distillation from the aerial parts of *Achillea fragrantissima* (Forssk.) Sch. Bip. and the flower, leaf and stem of *A. santolina* L. as well as their lipophilic constituents obtained by solvent extraction were analysed using GLC and GLC-mass spectrometry. Nineteen constituents in the essential oil of *A. fragrantissima* were identified, in addition to 41 compounds from its *n*-hexane-ether extract. The hydrodistilled oil and the solvent extract contain santolina alcohol, artemisia alcohol, artemisia ketone, *cis*-thujone and *trans*-thujone as major constituents. In *A. santolina* altogether 54 volatile components were detected. The major components were 1,8-cineole, fragranol, fragranyl acetate and terpin-4-ol. Furthermore, the essential oils and the *n*-hexane-ether extracts of the two plants were screened for their antimicrobial activity.

## 1. Introduction

The genus *Achillea* (Fam. Asteraceae) is represented by about 115 species in the temperate regions of the Northern hemisphere, mainly in North Africa, Southeast Europe, and Southwest Asia (Boulos 2002). Among this genus, *A. fragrantissima* (known by its Arabic name Qaysoom) and *A. santolina* (known locally by Beatheran) are represented in Egypt (Täckholm 1974). They are strongly fragrant perennial herbs and have been used by Bedouins as stomachic and anthelmintic (Boulos 1983). *A. fragrantissima* was found to be used as a hypoglycaemic drug (Yaniv et al. 1987). *A. santolina* is used treat toothache (Boulos 1983), chest disorders and as a tonic, carminative (Afsharypuor et al. 1996), insect repellent and in dysentery (Alkofahi et al. 1996). Previous investigations of these plants revealed the isolation and identification of some bioactive compounds (Haddad et al. 1960; Bohlmann and Jastrow, 1962; Shalaby and Steinegger, 1964; Shalaby et al. 1965; Khafagy et al. 1965; Khafagy et al. 1976; Khafagy et al. 1978; Ahmed et al. 1989; Seida et al. 1990; Mustafa et al. 1992; Balboul et al. 1997).

The chemical composition of the essential oil of *A. fragrantissima* has been previously studied and the major constituents were reported to be santolina alcohol, artemisia ketone, *cis*-thujone and *trans*-thujone (Shalaby and Richter, 1964; El-Deeb 1985; Aboutabl et al. 1986a; 1986b; Fleisher and Fleisher 1993; Hifnawy et al. 2001).

The oil of *A. santolina* has not been thoroughly studied with modern analytical methods. Since 1970 only few reports concerning volatile components of this plant growing in Iran (Afsharypuor et al. 1996), Egypt and Turkey have been published (Khafagy and El Fatatry 1970; Brunke et al. 1986) providing the evidence for presence of different chemotypes. In these previous studies, the oils from Egypt and Turkey showed similar components of their monoterpene fractions with 1,8-cineole, camphor, *trans*- and *cis*-sabinene hydrate, linalool, pinocarvone and 4-terpineol as the main constituents. From the Egyptian plant oil, fragranol and its diasteroisomer grandisol were identified. However, the main oil components of *A. santolina* growing in Iran include caryophyllene oxide, *cis*-nerolidol, camphor, olic aldehyde and linalool.

In this paper, we present a detailed comparative study between the hydrodistilled oils as well as the *n*-hexane-ether extracts of *A. fragrantissima* and *A. santolina*. Moreover, their antimicrobial activities were assayed.

## 2. Investigations, results and discussion

The hydrodistillation of the fresh aerial parts of *A. fragrantissima* gave pale yellow oil with an aromatic fragrant odour. While the oils obtained from *A. santolina* are yellowish-green with an aromatic, fragrant and pleasant odour. The odour and flavour of the *n*-hexane-ether extracts were believed to be closer to that of those plant oils. Using GLC and GLC-MS, the compounds identified and their proportions are listed according to their retention indices on a OV-1 fused silica capillary column (Table 1).

In *A. fragrantissima,* a total of 49 components were identified. The major compounds in the hydrodistilled oil were found to be *cis*-thujone and santolina alcohol with 29.48 and 18.29%, respectively. In the *n*-hexane-ether extract the percentages were 17.28 and 36.69, respectively. The oil was found to be rich in artemisia ketone (15.24%), *trans*-thujone (10.38%), *trans*-pinocarveol (6.83%) and yomogi alcohol (4.35%). These findings are in agreement with the previous reports of *A. fragrantissima* studies (Shalaby and

**Table 1: Percentage composition of essential oils and n-hexane-ether extracts of *Achillea fragrantissima* (Forssk.) Sch. Bip. and *A. santolina* L. and their retention indices**

| Compound | RI* | *A. fragrantissima* | | *A. santolina* | | | |
|---|---|---|---|---|---|---|---|
| | | Essential oil | Hexane extract | Essential oil | | | Hexane extract |
| | | | | Flower | Eaf | Stem | |
| 1 Isopentyl acetate | 876 | – | – | tr | – | – | – |
| 2 Santolina triene | 902 | 1.30 | – | – | – | – | – |
| 3 α-Thujene | 920 | – | – | tr | tr | tr | tr |
| 4 α-Pinene | 924 | 0.10 | – | 0.70 | 0.35 | 0.15 | tr |
| 5 Camphene | 935 | – | – | 0.60 | 0.29 | 0.26 | 0.51 |
| 6 Sabinene | 959 | – | – | tr | tr | – | 1.82 |
| 7 β-Pinene | 962 | – | – | tr | – | – | tr |
| 8 1,8-Dehydrocineole | 974 | – | – | tr | tr | – | – |
| 9 Yomogi alcohol | 991 | 4.35 | – | 0.70 | 0.97 | 0.38 | 1.83 |
| 10 *n*-Decane | 993 | – | – | tr | – | – | – |
| 11 α-Terpinene | 1004 | – | – | 0.50 | 0.47 | tr | – |
| 12 *p*-Cymene | 1008 | – | – | 0.40 | 1.90 | 0.42 | – |
| 13 1,8-Cineole | 1014 | 1.84 | 0.50 | 3.00 | 1.93 | 0.69 | 0.48 |
| 14 Limonene | 1014 | – | – | tr | tr | – | tr |
| 15 Santolina alcohol | 1033 | 18.29 | 36.69 | – | – | – | – |
| 16 Bergamal | 1038 | – | 0.41 | – | – | – | – |
| 17 Artemisia ketone | 1048 | 15.24 | tr | 1.66 | 0.87 | 0.40 | 0.51 |
| 18 γ-Terpinene | 1048 | – | – | tr | 1.22 | 0.98 | – |
| 19 *cis*-Sabinene hydrate | 1053 | – | 0.91 | 1.02 | 1.80 | 1.50 | 3.87 |
| 20 *n*-Octanol | 1055 | – | 1.51 | – | – | – | – |
| 21 Artemisia alcohol | 1074 | 0.27 | 1.21 | 0.9 | 0.52 | 0.39 | – |
| 22 Terpinolene | 1077 | – | – | tr | tr | 0.26 | – |
| 23 *trans*-Sabinene hydrate | 1081 | – | 0.41 | tr | tr | – | – |
| 24 Linalool | 1088 | – | – | 0.52 | tr | – | – |
| 25 *cis*-Thujone | 1090 | 29.48 | 17.28 | – | – | – | – |
| 26 *trans*-Thujone | 1098 | 10.83 | 4.71 | – | – | – | – |
| 27 *iso*-3-Thujanol | 1114 | – | 0.51 | – | – | – | – |
| 28 *trans*-Pinocarveol | 1120 | 6.83 | 0.32 | – | – | – | – |
| 29 Camphor | 1124 | – | – | 3.76 | 3.03 | 3.40 | 6.60 |
| 30 *trans*-dihydro-α-Terpineol | 1143 | – | 0.31 | – | – | – | – |
| 31 *cis*-Chrysanthenol | 1143 | – | 0.11 | – | – | – | – |
| 32 Borneol | 1148 | – | – | 4.50 | 4.80 | 3.56 | 0.34 |
| 33 Lavandulol | 1153 | 2.22 | 0.42 | tr | tr | 0.18 | – |
| 34 Artemisyl acetate | 1153 | 0.72 | 0.63 | – | – | – | – |
| 35 Terpin-4-ol | 1155 | 0.78 | 0.30 | 6.60 | 6.53 | 5.89 | 1.38 |
| 36 α-Terpineol | 1165 | – | 0.91 | 0.34 | 0.30 | 0.12 | 1.24 |
| 37 *cis*-Pinocarveol | 1175 | 0.34 | – | – | – | – | – |
| 38 *cis*-Piperitol | 1178 | – | – | tr | 0.53 | 1.01 | 0.70 |
| 39 Myrtenol | 1178 | – | – | tr | tr | 0.10 | 0.60 |
| 40 Methyl chavicol | 1180 | – | 0.12 | – | – | – | – |
| 41 *trans*-Piperitol | 1188 | – | – | tr | tr | tr | tr |
| 42 Fragranol | 1196 | – | – | 11.84 | 13.22 | 18.69 | 8.78 |
| 43 *cis*-Sabinene hydrate acetate | 1203 | – | – | tr | – | – | – |
| 44 Carvone | 1212 | – | – | tr | – | – | – |
| 45 Carvotanacetone | 1218 | – | 6.67 | – | – | – | – |
| 46 *trans*-Myrtanol | 1246 | – | – | tr | – | – | – |
| 47 *cis*-Chrysanthenyl acetate | 1250 | – | 1.41 | tr | – | – | – |
| 48 *neo*-iso-3-Thujyl acetate | 1251 | – | – | tr | – | – | – |
| 49 Bornyl acetate | 1253 | – | – | 0.30 | 0.17 | 0.15 | 0.53 |
| 50 Hydroxy citronellal | 1263 | – | 0.32 | – | – | – | – |
| 51 Lavandulyl acetate | 1266 | – | – | tr | – | – | – |
| 52 Thymol | 1267 | – | – | 0.90 | tr | 0.31 | 0.99 |
| 53 *trans*-Sabinyl acetate | 1275 | 1.77 | – | – | – | – | – |
| 54 Carvacrol | 1276 | – | – | tr | 0.69 | – | – |
| 55 *iso*-Dihydro carveol acetate | 1302 | – | 0.41 | – | – | – | – |
| 56 Fragranyl acetate | 1335 | – | – | 51.70 | 47.14 | 45.10 | 50.70 |
| 57 α-Terpinyl acetate | 1337 | – | – | 0.40 | 2.30 | 3.88 | 0.67 |
| 58 Eugenol | 1339 | – | 0.91 | – | – | – | – |
| 59 Neryl acetate | 1352 | – | – | tr | tr | tr | – |
| 60 Methyl p-anisate | 1358 | – | – | tr | – | – | – |
| 61 *trans*-Myrtanol acetate | 1368 | – | – | 0.51 | 0.51 | 0.59 | 0.81 |
| 62 (*E*)-α-Damascone | 1377 | – | – | tr | 0.21 | 0.38 | – |
| 63 cis-Jasmone | 1388 | – | tr | – | – | – | – |
| 64 Methyl eugenol | 1367 | – | tr | – | – | – | – |
| 65 *n*-Tetradecane | 1400 | – | 0.10 | | – | – | – |
| 66 (*E*)-Caryophyllene | 1411 | – | – | 0.21 | 0.30 | 0.33 | 0.29 |

**Table 1: (continued)**

| Compound | RI* | *A. fragrantissima* | | *A. santolina* | | | |
|---|---|---|---|---|---|---|---|
| | | Essential oil | Hexane extract | Essential oil | | | Hexane extract |
| | | | | Flower | Eaf | Stem | |
| 67 Lavandulyl isobutyrate | 1425 | – | – | tr | tr | tr | 0.50 |
| 68 Massoia lactone | 1442 | – | tr | – | – | – | – |
| 69 Germacrene D | 1472 | – | – | 1.20 | 1.07 | 1.50 | 0.89 |
| 70 Bicyclogermacrene | 1492 | 3.58 | – | 0.60 | 0.48 | 1.01 | – |
| 71 Butylated hydroxytoluene | 1493 | – | 1.51 | – | – | – | – |
| 72 Lavandulyl-2-methylbutyrate | 1506 | – | – | 1.22 | 0.50 | 0.71 | – |
| 73 β-Sesquiphellandrene | 1510 | 0.58 | – | – | – | – | – |
| 74 Dendrolasin | 1558 | – | – | 0.72 | 0.86 | 1.33 | 1.32 |
| 75 Spathulenol | 1561 | 0.20 | 1.05 | – | – | – | tr |
| 76 Caryophyllene oxide | 1576 | – | – | 0.66 | 0.56 | 0.31 | 3.90 |
| 77 10-*epi*-γ-eudesmol | 1619 | – | – | 0.93 | 0.85 | 0.93 | 3.02 |
| 78 α-Muurolol | 1630 | – | – | 1.23 | 1.25 | 1.18 | 4.25 |
| 79 β-Eudesmol | 1633 | 0.14 | – | – | – | – | – |
| 80 Khusinol | 1655 | – | tr | – | – | – | – |
| 81 Oplopanone | 1695 | – | tr | – | – | – | – |
| 82 Palmitic acid methylester | 1911 | – | 0.10 | – | – | – | – |
| 83 Palmitic acid | 1948 | – | 3.96 | – | – | – | – |
| 84 Phytol | 2100 | – | 1.22 | – | – | – | – |
| 85 Linoleic acid | 2115 | – | 4.55 | – | – | – | – |
| 86 α-Santonine | 2117 | – | – | – | – | – | tr |
| 87 Linolenic acid | 2120 | – | 4.55 | – | – | – | – |
| 88 Methyloctacosane | 2831 | – | – | – | – | – | tr |
| 89 Campesterol | 3110 | – | tr | – | – | – | – |
| 90 Stigmasterol | 3170 | – | tr | – | – | – | – |
| 91 β-Sitosterol | 3220 | – | tr | – | – | – | tr |
| 92 Fucosterol | 3280 | – | tr | – | – | – | – |
| Total identified (%) | | 98.86 | 94.02 | 97.62 | 95.62 | 96.09 | 96.53 |

* RI = retention indices relative to $C_9$–$C_{28}$ *n*-alkanes on the OV-1 column, tr = traces (< 0.1), hex.extract = *n*-hexane-ether extract

Richter 1964; El-Deeb 1985; Aboutabl et al. 1986a; 1986b; Fleisher and Fleisher 1993; Hifnawy et al. 2001). We report the presence of *trans*-pinocarveol (6.83), artemisyl acetate (0.72%) and β-sesquiphellandrene (0.58%) for the first time in the oil of this plant. Solvent extraction has the advantage of recovering the higher molecular weight natural compounds. Forty-one compounds were identified in this extract. Carvotanacetone (6.67%), *trans*-thujone (4.71%), linolic acid (4.55%) and linolenic acid (4.55%) were major components in the *n*-hexane-ether extract. An interesting finding was the detections of 17 additional volatile components not previously mentioned in the literature e.g. bergamal, *cis*-sabinene hydrate, *n*-octanol, *trans*-sabinene hydrate, *iso*-3-thujanol, *trans*-dihydro-α-terpineol, *cis*-chrysanthenol, carvotan acetone, *cis*-chrysanthenyl acetate, hydroxy citronellal, *iso*-dihydrocarveol acetate, methyl eugenol, *n*-tetradecane, butylated hydroxytoulene, khusinol, oplopanone and phytol. Besides, palmitic acid methyl ester, linolenic acid as well as a traces from campesterol, stigmasterol, β-sitosterol and fucosterol were also detected. Various alkanols such as $C_{24}$, $C_{26}$, $C_{28}$, and $C_{30}$ with retention indices (RI) 2664, 2882, 3043 and 3278, respectively have been also identified. On the other hand, myrecne, nerol, *n*-amyl ketone, β-pinene, limonene and bornyl acetate previously reported as oil components (Shalaby and Richter 1964; El-Deeb 1985) could not be detected. We confirmed the presence of α-pinene, santolina triene, 1,8-cineole, artemisia alcohol, spathulenol and β-eudesmol as minor compounds identified by Hifnawy et al. (2001) as well as the presence of 2-methyl-1-propen-1-one, (2-methylbutyl)-cyclopropane, *p*-cymene, 2-methyl-1-propanol, cyclopropane carboxylic acid methyl ester, propanoic acid 2-methyl-3-methylbutyl ester, butanoic acid 3-methyl-3-methyl butyl ester, 3-hexenyl butanoate (*E*), 3-hexenyl butanoate (*Z*), sabinyl alcohol (sabinol), sabinol isomer and γ-muurolene could not be detected in our sample.

Crushed flowers, leaves and stems of *A. santolina* were separately hydrodistilled. Fifty-four compounds (97.62%) were identified in the flower's oil whereas 43 (95.62%) and 37 (96.09%) compounds were characterized in the oils obtained from the leaves and stems, respectively. Fragranyl acetate, fragranol, lavandulyl isobutyrate, terpin-4-ol and camphor were the main constituents in the investigated parts. The principle components in the *n*-hexane-ether extract of the total aerial parts were fragranyl acetate (50.7%), fragranol (8.78%), camphor (6.6%), *cis*-sabinene hydrate (3.87%). Oxygenated sesquiterpenes present in the plant include α-muurolol (4.25%), caryophyllene oxide (3.9%), spathulenol, dendrolasin and 10-*epi*-γ-eudesmol (3.02%). Another distinguishing feature appeared to be the presence of minor amounts of β-sitosterol, and methyl-octacosane in the extract product, which were apparently absent in flower, leaf and stem distillates. In this context it should also be noted that we found no evidence for the presence of grandisol, *cis*-nerolidol, farnesol, 2-hydroxy-cyclopentadecanone, *cis*-α-santalol acetate and olic aldehyde in the volatile fractions previously reported in this plant (Khafagy and El Fatatry 1970; Brunke et al. 1986; Afsharypuor et al. 1996).

This divergence in the profiles of the volatile constituents of *A. fragrantissima* and *A. santolina* should be possibly attributed either due to geographical and ecological factors or an indication of the existence of different chemotypes of these plants.

**Table 2: Antimicrobial activity of *Achillea fragrantissima* and *A. santolina* hydrodistilled oils and n-hexane-ether extracts**

| Material* | Diameter of inhibition zone | | | | |
|---|---|---|---|---|---|
| | Gram − ve bacteria | | Gram + ve bacteria | | Fungus |
| | *E. coli* | *P. aeruginosa* | *S. aureus* | *B. subtilis* | *C. albicans* |
| Hydrodistilled oil of *A. fragrantissima*. | 10 | 15 | 15 | 30 | 30 |
| Extract of *A. fragrantissima*** | 17 | 19 | – | 19 | 15 |
| Hydrodistilled oil of *A. santolina* | 8 | 30 | 13 | 21 | 27 |
| Extract of *A. santolina*** | 18 | 17 | – | 17 | 13 |
| Ciprofloxacin (25 μg) | 20 | 10 | 25 | 22 | – |
| Nystatin (25 μg) | – | – | – | – | 25 |

* = All assays consisted of 50 μl of a test solution, consists of 20 mg residue in 1 ml DMF and 25μg/50 μl of standard antimicrobial in DMF.
** = *n*-hexane-ether extract, − = no inhibition.

The susceptibility of various microorganisms to the inhibitory effect of the oils and extracts is represented in Table 2. The results show that: the hydrodistilled oils of *A. fragrantissima* and *A. santolina* exhibited a pronounced antifungal activity however; the *n*-hexane-ether extracts showed moderate effect. Hydrodistilled oil of both plants showed a range of activity against some human pathogens such as *B. subtilis* and *P. aeruginosa.* However, *S. aureus* was completely resistant against the *n*-hexane-ether extracts of *A. fragrantissima* and *A. santolina* which were active against *E. coli, P. aeruginosa* and *B. subtilis.*

## 3. Experimental

### *3.1. Plant material*

Flowering aerial parts of *A. fragrantissima* (Forssk.) Sch. Bip. were collected from wild plants growing in the Sinai desert (vicinity of Sader Hetan) in April 2000, while those of *A. santolina* L. were collected from a wild population growing in the vicinity of Alexandria province, Egypt in April 2000. The identity of the plants have been kindly verified by Prof. Dr. N. El-Hadidi, Faculty of Science, Cairo University. Voucher specimens were deposited in the Herbarium of Pharmacognosy Department, Faculty of Pharmacy, Zagazig University.

### *3.2. Sample preparation*

The fresh aerial parts of *A. fragrantissima* as well as flowers, leaves and stems of *A. santolina* were separately subjected to hydrodistillation. Four-hours produced oils with 1, 0.1, 0.12 and 0.04%, respectively. The aerial parts of the two plants were extracted by percolation with a mixture of *n*-hexane-ether (1 : 1, v/v) and the solvents were removed subsequently under reduced pressure. The yield was 1.3 and 0.9% for *A. fragrantissima* and *A. santolina*, respectively.

### *3.3. Analysis*

The constituents of the volatile oils obtained by steam distillation and of the *n*-hexane-ether extracts were analysed by GLC and GLC-MS as reported (El-Shazly et al. 2002a). Compounds were identified by comparison of their retention indices ($C_9$ to $C_{24}$ *n*-alkane mixture) and mass spectra with those reported in the literature (El-Shazly 1999; El-Shazly et al. 2002a; 2002b; Adams 1995; Asres et al. 1998; Engel et al. 1998; Masada 1976; Ryhage and Sydow 1963; Sydow 1963; Goad and Akihisa 1997).

### *3.4. Antimicrobial screening*

The hydrodistilled oils and the *n*-hexane-ether extracts of *A. fragrantissima* and *A. santolina* were prepared for antimicrobial screening by dissolving 20 mg (oil or extract) in 1 ml dimethylformamide (DMF) and 50 μl was applied (equivalent to 1 mg). The activity of the solutions was screened by the cup-plate agar diffusion method (Woods and Washington 1995). The available microorganisms (Table 2) were *Staphylococcus aureus*, *Bacillus subtilis* (Gram positive bacteria); *Escherichia coli, Pseudomonas aeruginosa* (Gram negative bacteria) and *Candida albicans* (fungus). The selected microorganisms were obtained from the stock cultures of the Department of Microbiology, Faculty of Pharmacy, Zagazig University. As positive controls, 25 μg of ciprofloxacin in DMF was used as standard antibiotic for bacteria while for fungi nystatin (in DMF) was used at the same concentration. The plates were incubated overnight at 37 °C for bacteria and 30 °C for fungi. The zones of inhibition were observed and recorded (in mm) as shown in Table 2.

Acknowledgements: Thanks are due to Dr. H. Abdel Salam (University of Zagazig) for his help in performing the antimicrobial tests and Prof. Dr. Nabil El-Hadidi for plant identification.

**References**

Aboutabl EA, Soliman FM, El-Zalabani SM, Brunke EJ, El-Kersh TA (1986) Essential oil of *Achillea fragrantissima* (Forssk.) Sch. Bip. Egypt J Pharm Sci 27: 215–219.

Aboutabl EA, Soliman FM, El-Zalabani SM, Brunke EJ, El-Kersh TA (1986) Essential oil of *Achillea fragrantissima* (Forssk.) Sch. Bip. Sci Pharm 54: 37–41.

Adams RP (1995) Identification of essential oil components by gas chromatography/mass spectrometry. Allured Publ. Corp., Carol Stream, Illinois USA.

Afsharypuor S, Asgary S, Lockwood GB (1996) Constituents of the essential oil of *Achillea wilhelmsii* from Iran. Planta Med 62: 77–78.

Ahmed AA., El-Sayed NH, Mabry TJ (1989) Flavonoids of *Achillea santolina* and *A. fragrantissima* (Compositae, Anthemideae). Rev Latinoam Quim 20: 5.

Alkofahi A, Batshoun R, Owais W, Najib N (1996) Biological activity of some Jordanian medicinal plant extracts. Fitoterapia 67: 435–442.

Asres K, Tei A, Moges G, Sporer F, Wink M (1998) Terpenoid composition of the wound-induced bark exudate of *Commiphora tenuis* from Ethiopia. Planta Med 64: 473–475.

Balboul BAAA, Ahmed AA, Otsuka H, Bando M, Kido M, Takedat Y (1997) A guaianolide and a germacranolide from *Achillea santolina*. Phytochemistry 46: 1045–1049.

Bohlmann F, Jastrow H (1962) Polyacetylene compounds. XL. The polynes from the genus *Achillea*. Ber. 95: 1742–1747.

Boulos L (1983) Medicinal Plants of North Africa, Reference Publications, Inc., p. 52.

Boulos L (2002) Flora of Egypt. Al Hadara Publishing, Cairo Egypt, Vol. 3, p. 249.

Brunke EJ, Hammerschmidt FJ, Aboutabl EA (1986) Volatile constituents of *Achillea wilhelmsii* C. Koch (syn. *A. santolina* auct. mult.) from Egypt and the Turkey. Prog. Essent. Oil Res., Proc. Int. Symp. Essent. Oils, 16th. Meeting date 1985. In: Brunke, E. J. (ed.), de Gruyter, Berlin, Fed. Rep. Germany, 85–92.

El-Deeb K (1985) Ph. D. Thesis: Chromatographic and thermal analysis of certain volatile oil containing plants, Faculty of Pharmacy, Cairo University.

El-Shazly AM (1999) Essential oil composition of *Senecio desfontainei* Druce (Compositae). Zagazig J Pharm Sci 8: 1–8.

El-Shazly A, Dorai G, Wink M (2002a) Composition and antimicrobial activity of essential oil and hexane-ether extract of *Tanacetum santolinoides* (DC.) Feinbr. and Fertig. Z Naturforsch 57c: 620–623.

El-Shazly A, Dorai G, Wink M (2002b) Chemical composition and biological activity of the essential oils of *Senecio aegyptius* var. *discoideus* Boiss. Z Naturforsch 57c: 434–439.

Engel R, Gutmann M, Hartisch C, Kolodziej H, Nahrstedt A (1998) Study of the composition of the volatile fraction of *Hamamelis virginiana*. Planta Med 64: 251–258.

Fleisher Z, Fleisher A (1993) 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.

Goad LJ, Akihisa T (1997) Analysis of sterols. Blackie Academic and Professional, an imprint of Chapman and Hall, London.

Haddad DY, Khafagy SM, El-Fatatry L (1960) *Achillea santolina*. Isolation of two crystalline principles santolin and santolinol. J Pharm Sci (U.A.R.) 1: 75–81.

Hifnawy MS, Rashwan OA, Rabeh MA (2001) Comparative chemical and biological investigations of certain essential oils belonging to familes Asteraceae, Lamiaceae and Graminae. Bull Fac Pharm Cairo Univ 39: 35–53.

Khafagy SM, El-Fatatry LAMB, Amer MS (1965) Preliminary characterization of santolin, bitter principle of *Achillea santolina* growing in Egypt. Acta Pharm Suecica 2: 403–410.

Khafagy SM, El-Fatatry L (1970) Phytochemical study of *Achillea santolina*. III. Examination of the volatile oil. J Pharm Sci (U.A.R.) 11: 239–243.

Khafagy SM, Sabri NN, Soliman FSG, Abou-Donia AH, Mosandl A (1976) Isolation of two flavonoids from *Achillea santolina* L. growing in Egypt. Pharmazie 31: 894–895.

Khafagy SM, Sabri NN, Abou Donia AH (1978) Isolation of four crystalline materials from *Achillea santolina* L. growing in Egypt. Egypt J Pharm Sci 19: 163–168.

Masada Y (1976) Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry. John Wiley and Sons, Inc., New York, London, Sydney, Toronto.

Mustafa EH, Abu Zarga M, Abdalla S (1992) Effects of cirsiliol, a flavone isolated from *Achillea fragrantissima*, on rat isolated ileum. Gen Pharmacol 23: 555–560.

Ryhage R, Sydow EV (1963) Mass Spectrometry of Terpenes. I. Monoterpene hydrocarbons. Acta Chem Scand 17: 2025–2035.

Seida AA, El-Gohary HM, Azzam SM, El-Deeb KS (1990) Flavonoids of *Achillea fragrantissima* (Forssk) Sch. Bip. herb. Bull Fac Pharm Cairo Univ 28: 59–61.

Shalaby AF, Richter G (1964) Chromatographic investigation of the essential oil of *Achillea fragrantissima*. J Pharm Sci 53: 1502–1505.

Shalaby AF, Steinegger E (1964) The phytochemical study of *Achillea fragrantissima* (Forsk.) Sch. Bip. Pharm Acta Helv 39: 756–761.

Shalaby AF, Tsingaridas K, Steinegger E (1965) On the flavonoids from *Achillea fragrantissima* (Forsk.) Sch. Bip. Pharm Acta Helv 40: 19–24.

Sydow EV (1963) Mass Spectrometry of Terpenes II. Monoterpene alcohols. Acta Chem Scand 17: 2504–2512.

Täckholm V (1974) Students' Flora of Egypt. Co-operative Printing Company, 3rd ed., Beirut, p. 577.

Woods GL, Washington JA (1995) Antibacterial susceptibility tests: Dilution and Disk diffusion methods. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH (ed.) Manual of Clinical Microbiology. 6th ed., ASM Press, Washington, D.C. pp. 1327–1341.

Yaniv Z, Dafni A, Friedman J, Palevitch D (1987) Plants used for treatment of diabetes in Israel. J Ethnopharmacol 19: 145–151.