Composition and Antimicrobial Activity of Essential Oil and Hexane–Ether Extract of *Tanacetum santolinoides* (DC.) Feinbr. and Fertig

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Tanacetum santolinoides, Essential Oil Composition, *n*-Hexane–Ether Extract Composition

The essential oil of the aerial parts of *Tanacetum santolinoides* was analyzed by capillary GLC and GLC-MS. Altogether 30 components were identified. The main constituents were thymol (18%), *trans*-thujone (17.5%), *trans*-chrysanthenyl acetate (13.2%), *cis*-chrysanthenyl acetate (9.2%), umbellulone (9.7%) and 1,8-cineole (4.7%). Similar essential oil pattern in addition to palmitic acid methyl ester, palmitic acid, stigmasterol, sitosterol and two flavonoidal aglycons were found in the *n*-hexane-ether extract. The oil showed strong *in vitro* activity against *E. coli, Bacillus subtilis* and *Candida albicans.*

Introduction

Tanacetum santolinoides (DC.) Feinbr. and Fertig (Asteraceae) is the only species among over 50 species of genus *Tanacetum* (*Pyrethrum*) distributed in the extratropical parts of the N. hemisphere which grows in Egypt. The plant is a shrubby, fragrant, perennial herb growing wild in rocky mountains of Southern Sinai with discoid, yellow heads having only tubular florets and bipinnatifid woolly leaves (Taeckholm, 1974). In other population of N. and C. Sinai all plants of the species have radiate heads with short yellow ligules (Feinbrun-Dothan, 1978).

Previous studies on the chemical constituents of the aerial parts of *T. santolinoides* using solvent extraction lead to the isolation of many sesquiterpenes of eudesminolide, germacranolide and secogermacranolide type as well as few sterols, triterpenes and phenolic ether derivatives (El-Sebakhy *et al.*, 1986a, b; Abdel-Mogib *et al.*, 1989; Mahmoud *et al.*, 1994; Hifnawy *et. al.*, 2000a), in addition to various compounds including malabaricane triterpene derivatives from the root (Jakupovic *et al.*, 1987). Two studies have addressed the chemical composition of *T. santolinoides* essential oil. An early study (Ateya, 1992) reported twelve compounds in the essential oil of the flower heads, such as β-eudesmol (13.4%), fenchone (10.8%), thymol (6.9%) and benzyl acetate (6.2%) possibly *cis*-chrysanthenol (27.5%) and *trans*-chrysanthenol (8.9%) as major constituents. In addition, 1,8-cineole, *d*-carvone, pulegone, β-terpineol, *p*-cymene and bisabolene figured as a minor components and. More recently, the oil was analyzed and nineteen compounds were identified (Hifnawy *et al.,* 2000b) including thymol (82%), *p*-cymene (5.9%) and carvacrol (3.8%) as a major components together with α-pinene, β-myrcene, limonene, β-thujene, 1,8-cineole, 2-methyl propanal, 1-methyl-2(1H)pyridinone, 1-methyl-2,3-dihydro-1H-indene, linalool, 6-isopropylidene-bicyclo-[3.1.0]hexane, 2-methyl-4-(1,1-dimethylethyl)phenol, 4-terpineol, α-terpineol, 1,10-decanediol, cumin alcohol and benzyl salicylate as minor constituents. Because of the large variation observed between the two previous reports, a more detailed investigation of the hydrodistilled oil as well as the *n*-hexane-ether extract was the aim of this study. Moreover, the antimicrobial activity of the oil was analysed.

Experimental

Plant material

The flowering plants of *Tanacetum santolinoides* (DC.) Feinbr. and Fertig [synonyms: *Pyrethrum santolinoides* DC., *T. sinaicum* Del. ex DC., *Chrysanthemum sinaicum* (Del.)] were collected from rocky mountains of Wadi Elarbaeen, St. Catherine, Sinai Peninsula, Egypt in April 2000. The

identity of the plant was confirmed by Dr. H. Abdel Baset, Faculty of Science, Zagazig University. A voucher specimens is deposited in the Herbarium of the Department of Pharmacognosy, Zagazig University, Egypt.

Essential oil isolation

The air-dried aerial parts (100 g) were hydrodistilled in a clevenger-like apparatus for 5 h. The yield was 0.62% dry weight. Other part was extracted with *n*-hexane–ether mixture (1:1, v/v) and the yield was found to be 1.41% of the dry plant material. The oil was dried with anhydrous sodium sulfate and kept with the residue obtained from the solvent extract at 4 °C in sealed brown vials for analysis. Both the essential oil separated by hydrodistillation and the *n*-hexane-ether extract were analyzed by GLC and GLC-MS.

Analysis

Capillary GLC

A Carlo Erba ICU 600 gas chromatograph equipped with FID, spectra physics integrator and DB1 fused silica capillary column (15 m × 0.317 mm i.d. 0.25 μm film thickness) was employed. GLC condition: carrier gas He (2 ml/min); detector temp. 300 °C; injector temp. 250 °C; oven temp. programme, initial temp. 50 °C 4 min isothermal, 50–90 °C 4 °C/min. 90–300 °C 10 °C/min, then 10 min. isothermal. About 1 mg portion of each sample was dissolved in 1 ml ethylacetate and 1 μl volume was injected. Retention index (RI): Kovats indices (Kovats, 1958) were calculated with respect to a set of co-injected standard hydrocarbons (C9–C24). Percentage of the identified compounds was computed from GLC peak area.

GLC-MS

A Carlo Erba HRGC 4160 gas chromatograph equipped with OV1 capillary column (30 m, 0.25 mm i.d., 0.25 μm film thickness) coupled to a quadrupole mass spectrometer Finnigan MAT 4500 was employed for oil analysis. The ionisation energy for the mass spectrometer was 70 eV. Condition: injector 250 °C; temp. programme 44 °C 4 min isothermal; 44–74 °C at 3 °C/min.; 74–134 °C at 6 °C/min. then to 312 at 12 °C/min; split ratio 1: 20; carrier gas He (1.4 ml/min). The column (DB-1) temperature program adopted for the *n*-hexane-ether extract was raised at a rate of 6 °C/min from 70–300 °C, other conditions were identical to those mentioned above. Identification of the constituents was performed by computer liberary search, retention indices and visual interpretation of mass spectra with those found in the literature (El-Shazly, 1999; Adams, 1995; Asres *et al.*, 1998, Masada, 1967; Ryhage and Sydow, 1963; Sydow, 1963). The identified compounds are recorded in Table I. The compounds are listed in

Table I. Constituents of the steam volatile fraction of *Tanacetum santolinoides* (flowering aerial parts).

Component[a]	RI[b]	Percentage
1 Santolina triene*	903	0.3
2 α-Thujene*	919	0.5
3 α-Pinene	923	1.5
4 Camphene*	934	0.14
5 β-Pinene*	959	1.35
6 1,8-Cineole dehydro*	973	1.16
7 Yomogi alcohol*	991	0.6
8 α-Terpinene*	1004	0.65
9 *p*-Cymene	1007	0.46
10 1,8-Cineole	1014	4.65
11 Artemisia ketone*	1049	3.2
12 *cis*-Sabinene hydrate*	1052	0.21
13 *cis*-Linalool oxide*	1058	0.51
14 *cis*-Thujone*	1081	1.04
15 *trans*-Thujone*	1093	17.51
16 Chrysanthenone*	1094	1.52
17 *trans*-Limonene oxide*	1109	0.41
18 *trans*-Pinocarveol*	1116	0.36
19 Umbellulone*	1146	9.74
20 *cis*-Pinocamphone/ Isopinocampheol•*	1150	tr
21 Terpin-4-ol	1159	1.86
22 α-Terpineol	1170	2.17
23 *trans*-Chrysanthenyl acetate*	1219	13.23
24 *cis*-Chrysanthenyl acetate*	1250	9.23
25 Thymol	1288	17.96
26 *cis*-Carvyl acetate*	1362	0.93
27 γ-Muurolene*	1478	0.54
28 Davanone	1567	2.51
29 β-Eudesmol	1633	2.45
Monoterpene hydrocarbons		4.90
Oxygenated monoterpenes		86.29
Sesquiterpene hydrocarbons		0.54
Oxygenated sesquiterpenes		4.96
Total identified terpenoids		96.69

[a] Compounds listed in order of elution.
[b] RI retention index: measured relative to *n*-alkane on OV1 column under conditions listed in the experimental section.
* New for *Tanacetum santolinoides* essential oil.
• = coeluted; tr = traces < 0.1.

order of elution from DB1 and OV1 capillary columns.

Screening for antimicrobial activity

Tested microorganisms were *Staphylococcus aureus*, *Bacillus subtilis* (Gram positive bacteria); *Escherichia coli, Pseudomonas aeruginosa* (Gram negative bacteria) and *Candida albicans* (fungus). The microorganisms were obtained from the stock cultures of the Department of Microbiology, Faculty of Pharmacy, Zagazig University. Antimicrobial activity was assayed via the agar diffusion method. Small cups were taken out of the agar which could hold approximately 60 µl of oil solutions. Each cup was filled accurately with 50 µl solution (20 mg oil or *n*-hexane–ether extract were dissolved in 1 ml dimethylformamide, DMF), as well as DMF as a control. The plates were incubated overnight at 37 °C for bacteria and 30 °C for *Candida*. The observed inhibition zones were measured (in mm) and compared against standard antibiotics (ciprofloxacin and nystatin). Results are recorded in Table II.

Results and Discussion

The oil has a pale yellow colour and fragrant pleasant odour. The hexane-ether extract has the same odour but yellowish green colour. Most of their components could be identified unambiguously by direct comparison (mass fragmentation, retention index) with published data as well as computer library search. The unidentified components mainly consisted of a mixture of oxygenated monoterpenes and sesquiterpenes, whose individual abundances were small or in trace amounts. As shown in Table I, ca. 96.69% (30 compounds) of the oil was identified. The oil consisted mainly of oxygenated monoterpene compounds (86.3%) rich in thymol (17.9%), *trans*-thujone (17.5%), *trans*-chrysanthenyl acetate (13.2%), umbellulone (9.7%) and *cis*-chrysanthenyl acetate (9.2%). Other minor constituents were: santolina triene, α-thujene, α-pinene, camphene, β-pinene, 1,8-cineole dehydro, yomogi alcohol, α-terpinene, *p*-cymene, 1,8-cineole, artemisia ketone, *cis*-sabinene hydrate, *cis*-linalool oxide, *cis*-thujone, chrysanthenone, limonene oxide, *trans*-pinocarveol, pinocamphene, pinocamphenol, terpin-4-ol, α-terpineol and *cis*-carvyl acetate. γ-Muurolene, davanone and β-eudesmol represent the sesquiterpenes in our sample. Examination of the *n*-hexane-ether extract of the aerial parts revealed the presence of similar oil constituents in addition to palmitic acid methyl ester and palmitic acid with **RI** 1911 and 1948, respectively (Engel *et al.*, 1998), stigmasterol and β-sitosterol with **RI** 3170 and 3220 respectively (Goad and Akihisa, 1997), 5-hydroxy-6,7,3′,4′-tetramethoxyflavone or its isomer (**RI** 3180), 5-hydroxy-3,6,7,3′,4′-pentamethoxyflavone or its isomer (**RI** 3200) also, two alkanal with C24 and C26 skeleton having **RI** 2615 and 3810, respectively were detected in trace amounts. Thymol was also found to be a major component in the oil of this plant (Hifnawy *et al.,* 2000).

The results obtained differ from all those reported elsewhere in the literature for this species (Ateya, 1992; Hifnawy *et al.*, 2000b). For the first time *trans*-thujone, *trans*-chrysanthenyl acetate, umbellulone, *cis*-chrysanthenyl acetate and artemisia ketone are identified and found in substantial amounts. These dissimilarities may be considered as an indication of several chemical races or chemotypes existing within the species.

Table II. Results of antimicrobial analysis of hydrodistilled oil and hexane–ether extract of *Tanacetum santolinoides.*

Materials	Diameter of inhibition zone [mm]					
	Gram negative bacteria		Gram positive bacteria		Fungi	
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*	*C. albicans*	*A. flavus*
Ciprofloxacin 25 µg/disc	20	10	25	22	–	–
Nystatin 30 µg/disc	–	–	–	–	25	10
Volatile oil*	19	10	15	32	32	8
n-Hexane–ether extract*	19	17	–	18	13	6

* 20 mg of the isolated volatile oil or *n*-hexane-ether extract were dissolved in 1 ml DMF; 50 µl of these solutions were used in these assays.
– = no inhibition.

Concerning the antimicrobial activity (Table II), the hydrodistilled oil showed substantial effect on *E.coli*, *P. aeruginosa, B. subtilis* and *C. albicans* as compared with ciprofloxacin and nystatin as antibacterial and antifungal standards, respectively. The activity against *S. aureus* is considered to be moderate. The *n*-hexane-ether extract exhibited a marked inhibition of Gram negative bacteria and moderate activity against *B. subtilis* and *C. albicans*. The antimicrobial effect of the oil had previously been reported (Hifnawy *et al.*, 2000b) against *E. coli* and *B. subtilis*; our results corroborate these findings.

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Abdel-Mogib M., Jakupovic J., Dawidar E. M., Metwelly M. E. and Abou-Elzahab M. (1989), Sesquiterpene lactones from *Pyrethrum santolinoides*. Phytochemistry **28**, 268–271.

Adams R. P. (1995), Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry. Allured Publ. Corp., Carol Stream, Illinois USA.

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

Ateya A. M. (1992), GC-MS analysis of the volatile oil of *Pyrethrum santolinoides*. Az. J. Nat. Prod. **8**, 82–88.

El-Sebakhy N. A. and El-Ghazouly M. G. (1986), Sesquiterpene lactones of *Tanacetum santolinoides* (DC.) Feinbr. and Fertig. Pharmazie **41**, 298.

El-Sebakhy N. A., El-Ghazouly M. G., Seif El-Din A. A. and Zdero C. (1986), A heliangolide from *Tanacetum santolinoides*. Pharmazie **41**, 525–526.

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

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

Feinbrun-Dothan N. (1978), Flora Palaestina, The Israel Academy of Sciences and Humanities. Vol **3,** pp. 350–351.

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

Hifnawy M. S., El-Kashoury E. A., Rashwan O. A. and Rabeh M. A. (2000a), Phytochemical and biological evaluation of *Tanacetum santolinoids* and *Artemisia monosperma* (Family Asteraceae). Alexandria second international conference of pharmaceutical sciences and technology, Oct. 25–27, Abstract book p. 4. Taken from M. Sc. Thesis of Rabeh M. A. Cairo University (2000).

Hifnawy M. S., El-Kashoury E. A., Rashwan O. A. and Rabeh M. A. (2000b), Analysis of certain essential oils belonging to family Asteraceae and their antimicrobial and pesticidal activities. Alexandria second international conference of pharmaceutical sciences and technology, Oct. 25–27, Abstract book p. 2. Taken from M. Sc. Thesis of Rabeh M. A. Cairo University (2000).

Jakupovic J., Eid F., Bohlmann F. and El-Dahmy S. (1987), Malabaricane derivatives from *Pyrethrum santolinoides*. Phytochemistry **26**, 1536–1538.

Kovats E. (1958), Gas-Chromatographische Charakterisierung organischer Verbindungen. Teil I: Retentions-Indices aliphatischer Halogenide, Alkaloide, Aldehyde und Ketone. Helv. Chim. Acta **41**, 1915–1932.

Masada Y. (1967), Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry. John Wiley and Sons, New York, NY.

Mahmoud A. A., Ahmed A. A., Linuma M. and Tanaka T. (1994), 2,3-Secogermacranolides and germacranolides from *Pyrethrum santolinoides*. Phytochemistry **36**, 393–398.

Ryhage R. and Sydow E. V. (1963), Mass spectrometry of terpenes. I. Monoterpene hydrocarbons. Acta Chem. Scand. **17**, 2025–2035.

Sydow E. V., (1963), Mass spectrometry of terpenes. II. Monoterpene alcohol. Acta Chem. Scand. **17**, 2504–2512.

Taeckholm V. (1974), Students' Flora of Egypt. Co-operative Printing Company, Beirute 2nd ed. p. 579.

Yamaguchi K. (1970), Spectral Data of Natural Products, Vol. I. Elsevier Publ., Amsterdam, London & New York.