

Chemotypical Variation of Tansy (*Tanacetum vulgare* L.) from 40 Different Locations in Norway

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Between 2001 and 2002, plant collections from wild populations of Norwegian tansy (*Tanacetum vulgare* L.) were studied with a focus on essential oil (EO) yield and composition in order to characterize the chemotypical EO variability. Tansy collections of 40 different locations from North, Mid-, and South Norway were transplanted to the Apelsvoll Research Centre Div. Kise in 2000 and grown for 2 years before the aerial parts (leaves and flower buds) were harvested in June 2002. The EO from individual plants was isolated from dried plant material by hydrodistillation and analyzed by gas chromatography–mass spectrometry (GC-MS) on a DB5 column at the Plant Biocenter. The EO yield ranged between 0.35 and 1.90% (v/w) (average: 0.81%); the most abundant thujone plants were especially rich in EO volatiles (0.95%). On the basis of GC-MS data, seven chemotypes could be identified as follows: A, α -thujone (two individuals); B, β -thujone (22); C, camphor (six); D, chrysanthenyl acetate/chrysanthenol (three); E, chrysanthenone (two); F, artemisia ketone/artemisia alcohol (three); and G, 1,8-cineole (two). The thujone chemotype was dominated by β -thujone (81%) associated with α -thujone, but tansy plants rich in α -thujone were also detected (61%). The chemotypical classification of Norwegian tansy genotypes was underscored by preliminary studies from 2001, indicating the genetic uniformity and biochemical stability of the domesticated plants.

KEYWORDS: Tansy; *Tanacetum vulgare*; chemotypes; essential oil (EO); terpenoids; GC-MS; hydrodistillation

INTRODUCTION

Tansy (*Tanacetum vulgare* L.) is a perennial herb of the Asteraceae family (Compositae), being adapted to the northern climate and growing widely in Europe, Asia, and North America. Belonging to the group of so-called aromatic plants, tansy has traditionally been used as a spicy additive for food, in cosmetics, and as a herbal remedy due to its biologically active compounds. Besides the scientifically important and closely related species feverfew (*T. parthenium*) and Dalmatian insect flower (*T. cinariifolium*), significant applications for tansy have not been found yet. Plant extracts and essential oils (EOs) from tansy are known for their distinct medicinal, antimicrobial, antioxidant, insecticidal, and attractant properties (1). Important secondary metabolites such as sesquiterpene lactones (2, 3), flavonoids (4, 5), and polysaccharides (6, 7) have been isolated and reported in detail. Besides the main active sesquiterpene lactone parthenolide (8, 9), which is commonly found in feverfew, the EO from *Tanacetum vulgare* has especially attracted attention in the past 5 years due to the occurrence of

several interesting irregular monoterpenes and with regard to the plants polymorphic characteristics.

Tansy EO is known for highly infraspecific variability, and many chemotypes from different geographical regions have been investigated (10–22). Chemotypical compounds being reported are monoterpenes and sesquiterpenes, which are widely distributed in the genus *Tanacetum* in general (23–26). The most common tansy chemotypes reported from another Nordic country, Finland, are camphor and thujone, whereas the sabinene and umbellulone types were found less frequently in nature (15, 27). On the basis of the volatile compounds from tansy flower heads, six chemotypes were recently reported by Keskitalo and co-workers: artemisia ketone, camphor, 1,8-cineole, davanone, thujone, and tricyclene + β -myrcene (22).

The aim of the present study was to characterize the specific chemotypical variation of Norwegian tansy by investigating plant collections from 40 different locations in Norway from the South to the North in order to identify unique chemotypes with commercial value for biotechnological, medicinal, and agricultural utilization.

MATERIALS AND METHODS

Plant Material. The geographical locations of the 40 tansy plants collected and used in this study are presented in **Table 1** and **Figure 1**. The plants were transplanted in 2000 to the Apelsvoll Research

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Table 1. Location, Latitude, and Chemotypical Classification of Norwegian Tansy (A = α -Thujone; B = β -Thujone; C = Camphor; D = (*E*-Chrysanthenyl Acetate/Chrysanthenol; E = Chrysanthenone; F = Artemisia Ketone; and G = 1,8-Cineole)

no.	county	location	latitude	chemotype
1-1	Aust-Agder	Risør	58°43'	B
2-1	Rogaland	Vigrestad	58°34'	B
3-1	Telemark	Siljan	59°18'	A
3-2		Siljan	59°18'	B
4-1	Akershus	Asker	59°50'	B
4-2		Asker	59°50'	E
5-1	Buskerud	Lierskogen	59°47'	C
6-1	Sogn og Fjordane	Kaupanger	61°11'	F
6-2		Viksdalen	61°22'	B
6-3		Viksdalen	61°22'	A
6-4		Luster	61°26'	G
7-1	Oppland	Begna	60°34'	C
7-2		Heggenes	61°11'	D
7-3		Bøverdalen	61°44'	C
8-1	Hedmark	Hamar	60°48'	B
8-2		Hamar	60°48'	B
8-3		Brumunddal	60°53'	D
8-4		Alvdal	62° 6'	C
8-5		Alvdal	62° 6'	B
9-1	Møre og Romsdal	Vikebukta	62°37'	B
9-2		Elnesvågen	62°52'	B
10-1	Nord-Trøndelag	Inderøy	63°53'	C
10-2		Nord-Statland	64°30'	B
11-1	Nordland	Brønnøysund	65°28'	B
11-2		Brønnøysund	65°28'	B
11-3		Hattfjelldal	65°36'	B
11-4		Storforshei	66°24'	B
11-5		Røklund	66°49'	E
11-6		Hamarøy	68° 8'	C
11-7		Ankenesstrand	68°25'	G
11-8		Narvik	68°28'	F
12-1	Troms	Borkenes	68°47'	B
12-2		Bardufoss	68°52'	B
12-3		Silsand	69°15'	B
12-4		Lyngseidet	69°34'	D
12-5		Lyngseidet	69°34'	B
12-6		Tromsø	69°40'	B
13-1	Finmark	Svanvik	69°25'	F
13-2		Neiden	69°42'	B
13-3		Gamvik	70°10'	B

Centre, Division Kise, in Hedmark, and grown for 2 years before whole samples of aerial plant parts from individual plants were collected in late June in 2002 and air-dried at 35–40 °C. They were then stored at room temperature in the dark prior to analysis at the Plant Biocenter, Trondheim.

Hydrodistillation. Leaves and flower buds were separated from the stems and submitted to hydrodistillation in a modified Dean and Stark apparatus consisting of a 5 L distillation bottle, a 3 mL graduated receiver, and a jacketed coil condenser. A 100 g amount of dried plant material and 2.5 L of H₂O were used, and the distillation was carried out for 1.5 h after the mixture had reached the boiling point. The gas chromatography (GC) samples (one sample per collection no.) were prepared by diluting 10 μ L of oil in 1 mL of ethanol in brown autosampler flasks and stored at 4 °C prior to analysis.

GC-MS Analysis. A Varian Star 3400 CX gas chromatograph coupled with a Varian Saturn 3 mass spectrometer was used for GC-MS analysis. A J&W DB-5 capillary column was used (30 m \times 0.25 mm i.d.; film thickness, 0.25 μ m) for all analyses in trial year 2002, and the flow of the carrier gas He (12 psi) was held at 50 mL/min (injector) and 30 cm/s (column). The injector temperature was 220 °C (split injection; 1 μ L), and the GC temperature program was 35–220 °C at a rate of 2.5 °C/min and held at 220 °C for 6 min. Preliminary studies on selected plant samples in 2001 (see **Figure 3**) were carried out by using a CP Wax 52CB capillary column (30 m \times 0.32 mm i.d.; film thickness, 0.25 μ m). The injector temperature was 220 °C (splitless injection), and the GC temperature program was 60–220 °C at a rate of 2.0 °C/min and held at 220 °C for 5 min.

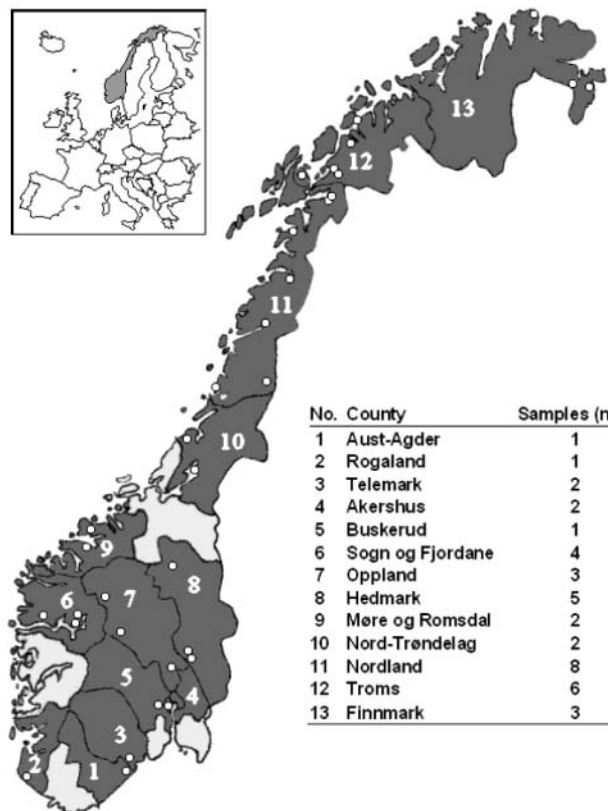


Figure 1. Origin (location) of tansy plant collections from Norway ordered into 13 regions (counties).

The MS detector was set at 175 °C for all analyses in 2001 and 2002, and a mass range of $m/z = 40$ –250 was recorded. All mass spectra were acquired in EI mode. The EO constituents were identified by the use of a combination of mass spectrum database search (IMS Terpene Library, 1989, and NIST MS Database, 1992), relative retention indices (ESO 2000—Database of Essential Oils, 1999), and comparison of mass spectra from published data. Quantitative analysis (in %) was performed by peak area normalization measurements (TIC = total ion current).

RESULTS AND DISCUSSION

The EO yield from the collected and cultivated tansy plants ranged between 0.35 up to 1.90% (v/w) and showed an average value of 0.81% (see **Table 2**). The most abundant thujone chemotypes (types A and B) were the only samples yielding over 1.00% EO (on average 0.95%) whereas camphor (type C), chrysanthenyl (types D and E), artemisia (type F), and cineole type (type G) oils often showed below average EO contents. In contrast to earlier studies with *Mentha* species (28) and aromatic herbs (29) cultivated at different locations in Norway, no genotypical influence as an effect of the plants' origin (see **Table 1**) on EO yield could be observed. Although genetical variation due to environmental conditions leads to distinct chemotypical expression patterns in tansy, still no correlation between chemotype and leaf morphology and shape could be found. Tansy plants revealing the subvarieties *týpicum*, *tenuisectum*, and *crispum* as described and reviewed by Keskitalo (1) with both lancelike and fine-cut single leaves, rounded edges, and curly shapes, could be observed among the thujone types of the Norwegian collection (see **Figure 2**). Chemotypes rich in thujone and/or camphor showed earlier flowering when cultivated in the greenhouse at The Plant Biocenter, Trondheim, in autumn 2003, which is in accordance to observations by Keskitalo (1) and Németh and co-workers (30).

Table 2. (Continued)^a

KI	compound (area %)	county																		
		Nord-Trøndelag		Nordland						Troms						Finmark				
		10-1	10-2	11-1	11-2	11-3	11-4	11-5	11-6	11-7	11-8	12-1	12-2	12-3	12-4	12-5	12-6	13-1	13-2	13-3
1102	α -thujone		0.4	0.4	0.5	1.2	0.4					0.4	0.4	0.3		0.4	0.7		0.4	0.7
1114	β -thujone		88.8	87.6	82.1	73.7	83.2				74.9	87.4	85.7		75.5	80.6		72.4	88.4	
1128	chrysanthenone	9.1						44.2	4.8	3.7				2.8						
1142	sabinol																			
1146	camphor	29.9						39.2							8.2					
1165	pinocarvone							0.2	0.3					0.8					0.3	
1165	chrysanthenol							0.6	0.3					3.5			2.0	2.3		
1173	artemisyl acetate																3.1			
1169	borneol	10.7		0.7						12.2					2.4					
1175	(Z)-pinocampone																			
1177	4-terpineol					0.3														
1189	α -terpineol																		0.2	
1238	(E)-chrysanthenyl acetate	0.1												4.0	31.9		10.5	5.6		
1289	bornyl acetate	10.7								1.4										
1291	(E)-sabinyl acetate													0.1				0.2	1.1	
1410	α -gurjunene		0.1					0.1											0.1	
1419	β -caryophyllene	0.9	0.2	0.2	0.1	0.4	0.3	0.9	0.2	0.1	0.8	0.4	0.2	0.1	0.9	0.1	0.1	2.0	0.4	0.1
1455	α -caryophyllene	0.1						0.1	tr	0.1	0.1	0.1	1.2		0.1			0.2		
1485	germacrene D	2.7	1.1	0.9	0.9	0.5	1.8	2.3	1.3	1.9	0.8	1.3	0.1	0.5	2.2	0.5	1.0	1.9	1.2	0.7
1500	pentadecane	0.1						0.1										tr		
1523	δ -cadinene	tr						tr						tr				0.1		
1563	(E)-nerolidol	0.2		0.1		0.2	0.2	0.3	0.1		0.2	0.4		0.3		0.1	0.5	0.7	tr	
1578	spathulenol	0.3	0.2	0.2	0.2		0.3	0.3	0.2	0.3	0.2	0.5	0.2		0.5		0.1	0.5	0.7	0.1
1583	caryophyllene oxide	1.0	0.3	0.3	0.3	0.3	0.6	1.9	0.3	0.3	1.2	0.9	0.3	0.1	1.5	tr	0.5	2.6	0.8	0.2
1588	davanone	0.1						0.1		0.1									0.2	
1647	τ -muurolol	0.8					0.9	0.1	0.7		1.8	3.2		0.1	1.9	0.5		3.3		
1650	δ -cadinol	0.1					0.2		0.1		0.3	0.7		0.3	0.5			0.6		
1654	α -cadinol	1.3					1.6		0.9		3.2	5.4		0.1	2.8			5.3		
	total identified (%)	96.4	99.6	99.2	99.7	99.7	98.7	91.4	97.5	99.2	98.2	96.2	99.6	99.8	94.6	99.0	99.9	95.8	98.2	99.6
	EO yield (mL/100 g)	0.80	0.90	1.15	0.95	0.60	0.95	0.55	0.80	0.50	0.75	0.70	0.95	1.05	0.50	0.85	0.67	0.43	0.40	1.08

^a Tentative EO compound identification by mass spectral database search (KI = Kovats indices; tr = trace compound).

Table 3. Composition of the EO from Samples of the Norwegian Tansy Collection at Planteforsk Research Division Kise (2002), Ordered into Chemical Groups (Data Given as Summarized Peak Area in %; tr = Trace Compound)

chemical group	subgroup	chemotype							
		A	B	C	D	E	F	G	
monoterpenes	hydrocarbons	6.0	5.3	13.5	19.5	19.5	17.9	29.6	
	alcohols	0.6	1.7	14.8	35.5	6.9	10.6	4.4	
	ketones	79.8	82.8	36.9	4.3	35.5	42.1	14.8	
	oxides	10.5	4.4	20.4	16.3	19.3	10.9	44.6	
	esters		1.7	8.2	10.9	4.6	4.9		
	total	96.9	95.9	93.8	86.5	85.8	86.4	93.4	
sesquiterpenes	hydrocarbons	1.4	1.4	2.3	3.0	3.4	3.0	2.1	
	alcohols	0.4	1.0	1.1	3.7	0.9	5.6	0.6	
	ketones	tr	tr	0.1	0.1	0.2		0.1	
	oxides	0.2	0.4	0.6	1.1	1.8	1.5	0.7	
	total	2.0	2.8	4.1	7.9	6.3	10.1	3.5	
straight chain hydrocarbons	total		tr	tr	tr	tr			
	sum total	98.9	98.7	97.9	94.4	92.1	96.5	96.9	

On the basis of the GC-MS data, a total of seven chemotypes could be described within the investigated tansy plant collection with a total of 47 identified compounds (see Table 2). Many EO samples showed transitions between two or more chemotypes, i.e., high variability in EO fingerprints due to the species characteristic frequency of cross-pollination. The most abundant thujone type (24 individuals) was dominated by β -thujone (type B) with extremely high concentrations over 90%, e.g., sample nos. 4-1, 8-2, 8-5, and average concentrations at 81%, which is in accordance to earlier investigations (1, 21, 22, 33, 34). High concentrations of α -thujone (type A; two individuals), scientifically reported only for other *Tanacetum* species (25), could also be detected (average 61%), thus indicating the genetic inde-

pendence of the biosynthetic pathways of α - and β -thujone in *Tanacetum vulgare* and underscoring the synthesis model of thujone by dominant alleles as described by Holopainen and coauthors (31). In general, the β -thujone types were accompanied by smaller amounts of α -thujone and vice versa, whereas camphor was rarely detected.

A chemotype also frequently found (type C; six individuals) presented a 33% camphor average concentration (see also results by 19, 21–23, 36), together with terpenes characteristic of other chemotypes. High camphor concentrations were generally followed by high amounts of the oxygenated monoterpene 1,8-cineole, e.g., sample nos. 5-1, 8-4, 11-6, accompanied by the bornyl acetate/borneol complex (e.g., sample nos. 7-1, 10-1, 11-6), which has been reported by Collin and coauthors as the “camphor–cineole–borneol” chemotype (21). Tansy plants rich in the close-related irregular monoterpenes chrysanthenyl acetate/chrysanthenol (type D; three individuals) and chrysanthenone (type E; two individuals) could be observed. As already described from other countries, the content of chrysanthenyl acetate/chrysanthenol showed concentrations between 30 and 48% (16, 19, 22, 34). In contrast to Finnish studies by Keskitalo and co-workers (22), the main occurring diastereomers in chemotype D were the *trans* and not the *cis* forms as reported from Hungary (35). Amounts of up to 44% chrysanthenone could be detected for chemotype E, which has also been described from Canada (21). Both chemotypes D and E were generally associated with high amounts of α -pinene and 1,8-cineole (>10% each), while camphor was not detected.

Another irregular monoterpene, artemisia ketone and its alcohol, could be found in high amounts in three individual tansy plants (type F). This chemotype has been observed in Europe, Asia, and North America (19, 22, 32, 37). 1,8-Cineole was the most abundant (oxygenated) monoterpene being detected in

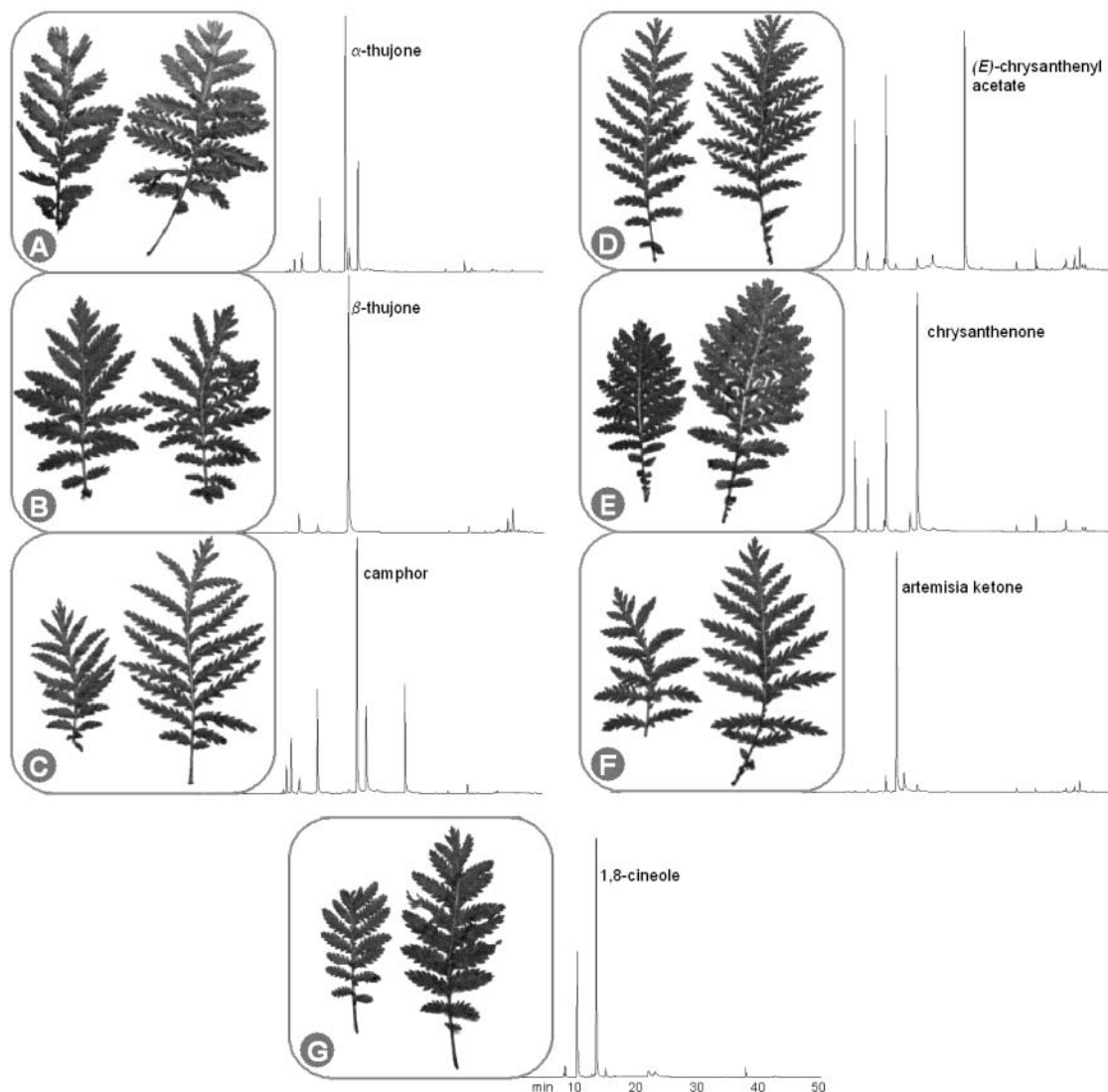


Figure 2. Examples of leaf morphology and GC-MS chromatograms of seven chemotypes of Norwegian tansy.

varying concentrations in all samples. Many samples of chemotypes A–F were associated with cineole contents above 10%, but only two individuals could be characterized as 1,8-cineole chemotype G (sample nos. 11-7 and 12-4) with average concentrations at 44%. Two other monoterpenes, α -pinene and sabinene, were generally present in all samples (except for sample no. 11-4) but never reached levels above 16 and 22%, respectively. These conclusions can also be drawn for the monoterpene alcohol borneol and its acetate, which normally occur pairwise. Although amounts of over 16 and 14% in the EO could be detected, the concentrations were not high and distinct enough to classify them into their own chemotypical groups. In accordance to findings by Hendriks and co-workers (19) and Keskitalo (22), the irregular monoterpene yomogi alcohol (and one unidentified similar structure) could also be detected in one sample (13%) together with relatively high amounts of santolina triene (9%), normally related to other *Tanacetum* species than *T. vulgare* (24–26). The occurrence of davanone (or davanone D), reported as its own chemotype by other research groups (22, 32, 37), was generally limited to concentrations <1%.

In general, the agricultural cultivation (domestication) of tansy plants from wild populations did not affect the chemotype as can be seen from Figure 3. Only weak differences could be found when comparing data of selected plant samples from trial year

2001 with results from trial year 2002 (chemotypes B, C, and F). In contrast to the chemotypically main EO compound, sampling of EO from the same samples resulted in partly higher variability with regard to standard deviation and average values (see chemotype C), which might be explained as an effect of differing climatic factors having a higher impact on EO production and yield than the expression of genetically determined EO patterns. Sesquiterpenes and sesquiterpenols were present in almost all tansy samples from 2002 except for sample nos. 6-3 and 8-3 (see Tables 2 and 3). In general, the thujone types showed lower amounts of sesquiterpene structures and less complex EO matrixes. The most abundant compounds were caryophyllene and its oxygenated analogue caryophyllene oxide, germacrene D, (*E*)-nerolidol, and spathulenol. The chemical group of monoterpenes made up between 86 and 97% of the totally detected compounds, whereas the sesquiterpenes did not reach levels above 10%. The monoterpene subgroups of ketones represented the main chemical structures in the chemotypes A–C, E, F, while alcohols and oxides were dominant in the chemotypes D and G, respectively. In comparison, the chemical group of sesquiterpenes showed relatively high levels of hydrocarbon structures.

In future projects, the applicability of tansy plants will be investigated with regard to agricultural, pharmaceutical, and flavor applications. The main focus will be put on (i) chemo-

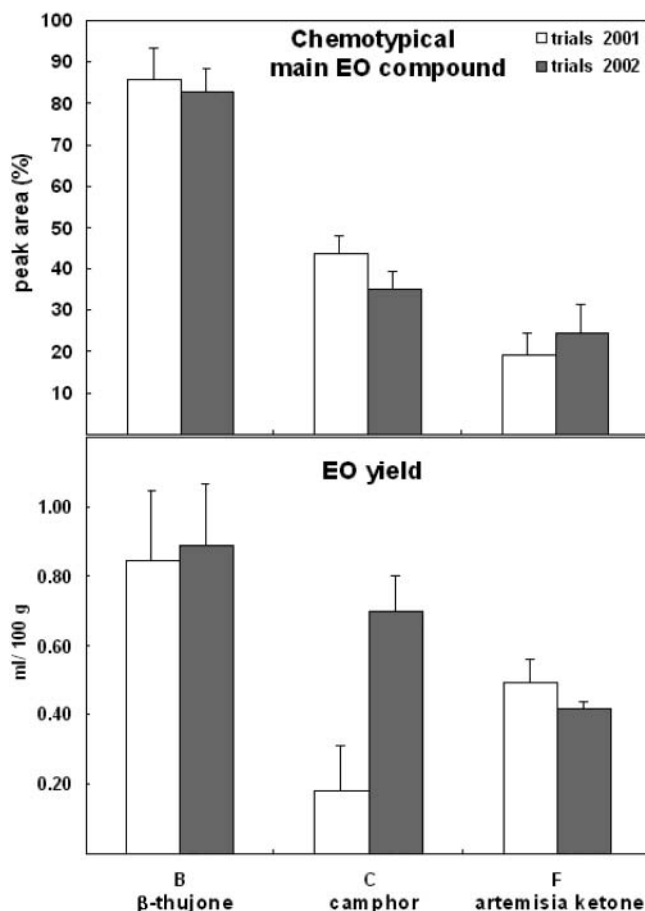


Figure 3. Chemotypical main EO compounds and EO yield of sample plants studied in August 2001 and June 2002: chemotype B ($n = 11$; sample nos. 3-2, 4-1, 8-1, 11-1, 11-2, 11-4, 12-1, 12-2, 12-3, 12-5, and 13-3), chemotype C ($n = 3$; sample nos. 5-1, 7-1, and 10-1), and chemotype F ($n = 2$; sample nos. 6-1 and 13-1).

typical variation, biosynthesis, and within plant distribution of terpenes and (ii) the biological and medicinal activity through antimicrobial testing, determination of antioxidant capacity, flavonoid and phenol content, and antitumor screening in order to identify valuable plant raw material from Norwegian tansy for potential industrial commercialization.

LITERATURE CITED

- Keskitalo, M. K. Exploring biodiversity to enhance bioactivity in the genus *Tanacetum* through protoplast fusion. Academic dissertation, University of Finland, Department of Plant Production, Section of Crop Husbandry, Publication No. 53, 113 p, 1999.
- Holopainen, M.; Hiltunen, R.; Jarvela, K.; Seppanen, T.; Von Schantz, M. Sesquiterpene lactones of Finnish tansy. *Pharmacol. Weekblad* **1987**, *9*, 236–236.
- Sanz, J. F.; Marco, J. A. NMR-studies of tatrudin A and some related sesquiterpene lactones from *Tanacetum vulgare*. *J. Nat. Prod.* **1991**, *54*, 591–596.
- Williams, C. A.; Harborne, J. B.; Eagles, J. Variations in lipophilic and polar flavonoids in the genus *Tanacetum*. *Phytochemistry* **1999**, *52*, 1301–1306.
- Williams, C. A.; Harborne, J. B.; Geiger, H.; Robin, J.; Hoult, J. R. S. The flavonoids of *Tanacetum parthenium* and *T. vulgare* and their antiinflammatory properties. *Phytochemistry* **1999**, *51*, 417–423.
- Polle, A. Y.; Ovodova, R. G.; Shashkov, A. S.; Ovodov, Y. S. Isolation and general characterization of polysaccharides from tansy *Tanacetum vulgare* L. *Russ. J. Bioorg. Chem.* **2001**, *27*, 45–49.
- Polle, A. Y.; Ovodova, R. G.; Shashkov, A. S.; Ovodov, Y. S. Some structural features of pectic polysaccharide from tansy, *Tanacetum vulgare* L. *Carbohydr. Polym.* **2002**, *49*, 337–344.
- Schinella, G. R.; Giner, R. M.; Recio, M. D.; De Buschiazzo, P. M.; Rios, J. L.; Manez, S. Antiinflammatory effects of south American *Tanacetum vulgare*. *J. Pharm. Pharmacol.* **1998**, *50*, 1069–1074.
- Tournier, H.; Schinella, G.; De Balsa, E. M.; Buschiazzo, H. Effect of the chloroform extract of *Tanacetum vulgare* and one of its active principles, parthenolide, on experimental gastric ulcer in rats. *J. Pharm. Pharmacol.* **1999**, *51*, 215–219.
- Stahl, E.; Schmitt, G. Chemische Rassen bei Arzneipflanzen. *Arch. Pharm.* **1964**, *297*, 385–391.
- Rudloff, E.; Underhill, E. W. Gas-liquid chromatography of terpenes XII. Seasonal variation in the volatile oil from *Tanacetum vulgare* L. *Phytochemistry* **1965**, *4*, 11–17.
- Stahl, E.; Scheu, D. Artemisiaketon als Hauptbestandteil des ätherischen Öles einer neuen Rainfarn-Rasse. *Naturwissenschaften* **1965**, *52*, 394.
- Stahl, E.; Scheu, D. Umbellulon als Hauptbestandteil des ätherischen Öles einer neuen Rainfarnrasse. *Arch. Pharm. (Weinheim)* **1967**, *300*, 456–458.
- Von Schantz, M.; Forsén, K. Begleitstoffe in verschiedenen Chemotypen von *Chrysanthemum vulgare* (L.) Bernh. I. Reine Thujon- und Campher-Typen. *Farm. Aikakauslehti* **1971**, *80*, 122–131.
- Forsén, K. Über die infraspezifische chemische Variation bei *Chrysanthemum vulgare*. *Ann. Acad. Sci. Fenn. Ser. A IV* **1975**, *Biologica* 207, 54 p.
- Nano, G. M.; Bicchì, C.; Frattini, C.; Gallino, M. Wild Piedmontese plants. II. A rare chemotype of *Tanacetum vulgare* L. abundant in Piedmont (Italy). *Planta Med.* **1979**, *35*, 270–274.
- Ekundayo, O. Essential oils. II. Terpene composition of the leaf oil of *Tanacetum vulgare* L. *Pflanzenphysiol.* **1979**, *92*, 215–220.
- Héthelyi, É.; Tétényi, P.; Kettens v.d. Bosch, J. J.; Saleminck, C. A.; Heerma, W.; Versluis, C.; Kloosterman, J.; Sipma, G. Essential oils of five *Tanacetum vulgare* genotypes. *Phytochemistry* **1981**, *20*, 1847–1850.
- Hendriks, H.; Van Der Elst, D. J. D.; Van Putten, F. M. S.; Bos, R. The essential oil of Dutch tansy (*Tanacetum vulgare* L.). Research Report. *J. Essent. Oil Res.* **1990**, *2*, 155–162.
- Héthelyi, É.; Tétényi, P.; Neszmélyi, A. Studies on, and three dimension correlations in some biologically active essential oils of *Tanacetum vulgare* clones. *Acta Hort.* **1992**, *306*, 295–301.
- Collin, G. J.; Deslauriers, H.; Pageau, N.; Gagnon, M. Essential oil of tansy (*Tanacetum vulgare* L.) of Canadian origin. *J. Essent. Oil Res.* **1993**, *5*, 629–638.
- Keskitalo, M.; Pehu, E.; Simon, J. E. Variation in volatile compounds from tansy (*Tanacetum vulgare* L.) related to genetic and morphological differences of genotypes. *Biochem. Syst. Ecol.* **2001**, *29*, 267–285.
- Greche, H.; Hajjaji, N.; Ismaili-Alaoui, M.; Mrabet, N.; Benjilali, B. Chemical composition and antifungal properties of the essential oil of *Tanacetum annuum*. *J. Essent. Oil Res.* **2000**, *12*, 122–124.
- Başer, K. H. C.; Demirci, B.; Tabanca, N.; Ozek, T.; Gören, N. Composition of the essential oils of *Tanacetum armenum* (DC.) Schultz Bip., *T. balsamita* L., *T. chiliophyllum* (Fisch & Mey.) Schultz Bip., var. *chiliophyllum* and *T. haradjani* (Rech. fil.) Grierson and the enantiomeric distribution of camphor and carvone. *Flavour Fragrance J.* **2001**, *16*, 195–200.
- Gören, N.; Demirci, B.; Başer, K. H. C. Composition of the essential oils of *Tanacetum* spp. from Turkey. *Flavour Fragrance J.* **2001**, *16*, 191–194.

- (26) El-Shazly, A.; Dorai, G.; Wink, M. Composition and antimicrobial activity of essential oil and hexanes–ether extract of *Tanacetum santolinoides* (DC.) Feinbr. and Fertig. *Z. Naturforsch., C* **2002**, *57*, 620–623.
- (27) Sorsa, M.; Schantz, M.; Von Lokki, J.; Forsén, K. Variability of essential oil components in *Chrysanthemum vulgare* in Finland. *Ann. Acad. Sci. Fenn. Ser. A IV* **1968**, *135*, 1–13.
- (28) Rohloff, J.; Skagen, E. B.; Steen, A. H.; Beisvåg, T.; Iversen, T.-H. Essential oil composition of Norwegian peppermint (*Mentha × piperita* L.) and sachalinmint (*Mentha sachalinensis* Briq. (Kudô)). *Acta Agric. Scand.* **2000**, *50*, 161–168.
- (29) Rohloff, J. Essential Oil Drugs—Terpene Composition of Aromatic Herbs. In *Production Practices and Quality Assessment of Food Crops. Vol. 4: Quality Handling and Evaluation*; Dris, R., Jain, S. M., Eds.; Kluwer Academic Publishers: Dordrecht, The Netherlands, 2004; 59 p.
- (30) Németh, É. Z.; Héthelyi, É. Bernáth, J. Comparison studies on *Tanacetum vulgare* L. chemotypes. *J. Herbs, Spices Med. Plants* **1994**, *2*, 85–92.
- (31) Holopainen, M.; Hiltunen, R.; Lokki, J.; Forsén, K.; von Schantz, M. Model for the genetic control of thujone, sabinene and umbellulone in tansy (*Tanacetum vulgare* L.). *Hereditas* **1987**, *106*, 205–208.
- (32) Keskitalo, M. K. Application of protoplast fusion technology to tansy (*Tanacetum vulgare* L.): biodiversity as a source to enhance biological activity of secondary compounds. *J. Herbs, Spices Med. Plants* **2002**, *9*, 197–203.
- (33) Charles, R.; Garg, S. N.; Mehta, V. K.; Kumar, S. (+)-10-hydroxy-3-thujone and other constituents from essential oil of *Tanacetum vulgare* L. from India. *J. Essent. Oil Res.* **1999**, *11*, 406–408.
- (34) Héthelyi, É.; Cseko, I.; Grosz, M.; Mark, G.; Palinkas, J. Chemical variants of wild and cultivated *Tanacetum vulgare*. Chemotaxonomic variety of domestic populations. *Olaj, Szappan, Kozmetika* **1996**, *45*, 109–114.
- (35) Neszmélyi, A.; Milne, G. W. A.; Podanyi, B.; Koczka, I.; Héthelyi, É. Composition of the essential oil of clone 409 of *Tanacetum vulgare* and 2D NMR investigation of trans-chrysanthenyl acetate. *J. Essent. Oil Res.* **1992**, *4*, 243–250.
- (36) Holopainen, M.; Kauppinen, V. Antimicrobial activity of essential oils of different chemotypes of tansy (*Tanacetum vulgare* L.). *Acta Pharmacol. Fenn.* **1989**, *98*, 213–219.
- (37) Héthelyi, E.; Koczka, I.; Bernáth, J. Chemotaxonomical varieties of *Tanacetum vulgare* L. in Hungary and Canada. *Olaj, Szappan, Kozmetika* **2000**, *49*, 143–148.

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