Volatile Components of Roots, Stems, Leaves, and Flowers of *Echinacea* Species†

G. Mazza* and T. Cottrell

Food Research Program, Agriculture and Agri-Food Canada, Pacific Agri-Food Research Centre, Summerland, British Columbia V0H 1Z0, Canada

The headspace volatile components of roots, stems, leaves, and flowers of *Echinacea angustifolia*, *E. pallida*, and *E. purpurea* were analyzed by capillary gas chromatography/mass spectrometry (GC/MS). Over 70 compounds were identified in the samples. All plant tissues, irrespective of the species, contain acetaldehyde, dimethyl sulfide, camphene, hexanal, β-pinene, and limonene. The main headspace constituents of the aerial parts of the plant are β-myrcene, α-pinene, limonene, camphene, β-pinene, *trans*-ocimene, 3-hexen-1-ol, and 2-methyl-4-pentenal. The major headspace components of root tissue are α-phellandrene (present only in the roots of *E. purpurea* and *E. angustifolia*), dimethyl sulfide, 2-methylbutanal, 3-methylbutanal, 2-methylpropanal, acetaldehyde, camphene, 2-propanal, and limonene. Aldehydes, particularly butanals and propanals, make up 41−57% of the headspace of root tissue, 19−29% of the headspace of the leaf tissue, and only 6−14% of the headspace of flower and stem tissues. Terpenoids including α- and β-pinene, β-myrcene, ocimene, limonene, camphene, and terpinene make up 81−91% of the headspace of flowers and stems, 46−58% of the headspace of the leaf tissue, and only 6−21% of the roots. Of the 70 compounds identified, >50 are reported in *Echinacea* for the first time.

Keywords: *Echinacea species; gas chromatography; mass spectrometry; terpenoids; alcohols; aldehydes; aroma compounds; herbal medicine; medicinal plant; purple coneflower*

INTRODUCTION

Echinacea is a perennial plant of the Compositae family native to the Canadian prairies and the prairie states of the United States (Li and Wang, 1998). It was an important medicinal plant for the native people and early settlers of the North American prairies, but, until recently, it was used only in restricted areas of North America and Germany. Today preparations of *Echinacea* species (*E. angustifolia*, *E. pallida*, and *E. purpurea*) are used as herbal drugs nearly worldwide. In Germany alone, there are currently >300 different *Echinacea* products on sale (Lienert et al., 1998). These preparations contain different mixtures of various forms of *Echinacea*, both alone and in combination with other substances, and are used as self-medication and as prescription drugs for immunostimulation and wound healing (Wagner, 1995; Pamham, 1996). An immunostimulant is defined as a drug capable of stimulating, in a non-antigen-dependent manner, the function and efficiency of a nonspecific immune system to counteract microbial infections or immunosuppresive states (Wagner, 1995).

The mechanism for the immunostimulating effects of *Echinacea* is not well understood, and it is still not known which constituents of *Echinacea* are the bioactive compounds (Melchart et al., 1995).

In recent years, the importance of identifying and characterizing the biologically active constituents of *Echinacea* has been increasingly recognized, and considerable research has been carried out, especially in Germany (Bauer, 1994; Bauer and Wagner, 1991; Bauer et al., 1988a,b, 1989, 1990; Wagner, 1995). Components that have received the most attention include alkamides, polysaccharides, glycoproteins, polycetylenes, and caffeic acid derivatives (Bauer, 1994; Lienert et al., 1998).

The volatile components of *Echinacea*, however, have not been well studied, and no published reports that describe headspace analysis of *Echinacea* species have been identified.

On the basis of the study of Becker (1982), essential oil of *E. purpurea* roots contains caryophyllene, humulene, and caryophyllene epoxide. The study of Bos et al. (1988) showed that the essential oils of the aerial parts of *E. purpurea*, *E. angustifolia*, and *E. pallida* contain borneol, bornyl acetate, pentadeca-8-en-2-one, germacrene D, caryophyllene, caryophyllene epoxide, and palmitic acid. Schulthess et al. (1988) reported the occurrence of the following compounds in the essential oils from the achene of various species: α-pinene, β-farnesene, myrcene, limonene, carvomenthene, caryophyllene, and germacrene D (*E. purpurea*); α-pinene, β-pinene, myrcene, β-farnesene, and epishiobunol (*E. angustifolia*); α-pinene, β-pinene, myrcene, limonene, 1,8-pentadecadiene, and a derivative of germacrene D (*E. pallida*).

Therefore, it was our aim to analyze and identify the constituents of the headspace of *E. angustifolia*, *E. pallida*, and *E. purpurea* roots, stems, leaves, and flowers. The analysis of the volatiles was carried out using a purge and trap headspace gas chromatographic technique in connection with GC/MS.

* Author to whom correspondence should be addressed [telephone (250) 494-6376; fax (250) 494-0755; e-mail mazzag@em.agr.ca].

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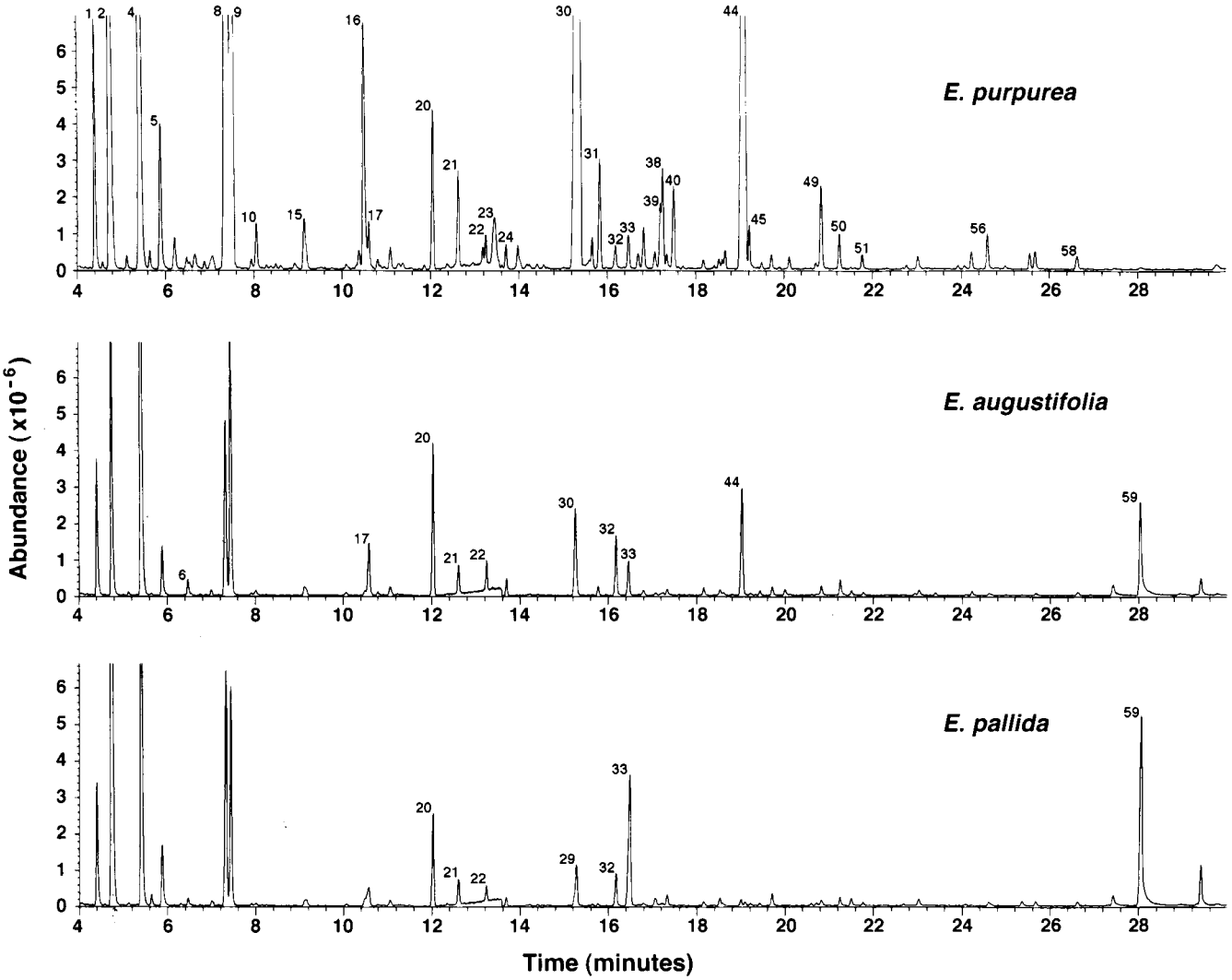

Figure 1. Capillary gas chromatograms of the headspace volatiles from roots of three *Echinacea* species.

MATERIALS AND METHODS

Plant Tissue. *E. angustifolia*, *E. pallida*, and *E. purpurea* flower heads, stems, leaves, and roots from 3-year-old plants grown at the Agriculture and Agri-Food Canada Research Centre, Summerland, BC, were used. Harvesting of the material used for this study was done by hand in the middle of August 1998, when the plants were in full bloom.

The plant roots, leaves, stems, and flowers were washed and patted dry prior to analysis. Amounts of plant material used, their preparation, and purge volumes were as follows: roots, 10 g, chopped and ground, purged for 10 min, 1000 mL; leaves, 10 g, finely chopped, purged for 5 min, 500 mL; flowers, 5 g, finely chopped, purged for 3 min, 300 mL; stems, 15 g, finely chopped, purged for 3 min, 300 mL.

Purge and Trap Conditions. Volatile compounds from the plant parts were trapped using the procedure recently described by Mazza et al. (1998). The sample chamber, which consisted of a 150 mL water-jacketed three-neck Wheaton jar, enabled the sample to be thermostated during the purge cycle. The chamber temperature was held at 50 °C by a circulating water bath. Purge gas connections from the chamber to a Tekmar 2000 sampler used Teflon tubing. Volatiles were trapped on a 100 mg Tenax TA trap (60/80 mesh) packed in a deactivated glass 6 mm o.d. tube. Purge gas flow rate was set at 100 mL/min, and the purge volume was determined by the purge time, as given below. Parameter settings for the Tekmar 2000 were as follows: prepurge time, 0 min; purge time, as given; desorb preheat, 195 °C; desorb 5 min at 200 °C; bake, 30 min at 225 °C; valve temperature, 250 °C; transfer line temperature, 260 °C; and mount temperature, 120 °C. Before the start of the desorb cycle, the GC oven door was opened, and ~10 cm of the transfer line (immediately before the union to the GC column) was immersed in a Dewar flask of liquid nitrogen. At the end of the desorb cycle, the Dewar was removed, the oven door closed, and the GC run initiated.

GC/MS Analysis. All analyses were performed using a Hewlett-Packard 5890A gas chromatograph with a Hewlett-Packard 5970 mass-selective detector. The sample was introduced from the Tekmar 2000 sampler via a heated 0.32 mm deactivated fused silica transfer line, connected via a ZDV union to a 60 m × 0.32 mm DB-Wax column (J&W Scientific) with a 0.25 μm film. Helium (Praxair, prepurified grade) was used as carrier gas for the column and for the Tekmar purge flow, supplied to the column through the Tekmar transfer line at a head pressure of 30 psig. The transfer line from GC to MSD was set at 260 °C, and the oven temperature program was as follows: initial temperature, 35 °C (hold for 5 min); temperature program rate, 5 °C/min; final temperature, 220 °C (hold for 10 min). MSD parameters were scan mode (40−300 amu); threshold, 1500; sample rate, 2.3 scans/s; and EM voltage, 1200 V.

The GC and MSD were controlled by and MS data collected by an HP ChemStation. Mass spectral identification was done using the Wiley MS database.

Reference Compounds. Compounds used for retention time confirmation were obtained from Sigma-Aldrich Canada (Oakville, ON), ICN Biomedicals Canada (Mississauga, ON), and Eastman-Kodak Co. (Rochester, NY).

Table 1. Volatiles in the Headspace of Roots, Stems, Leaves, and Flowers of *E. angustifolia* (*ang*), *E. pallida* (*palli*), and *E. purpurea* (*purp*)[a]

peak no.	compd	ID[b]	retention time (min)	root tissue			flower tissue			leaf tissue			stem tissue		
				ang	*palli*	*purp*	*ang*	*palli*	*purp*	*ang*	*palli*	*purp*	*ang*	*palli*	*purp*
1	acetaldehyde	a	4.40	92	87	185	24	34	22	65	27	83	19	25	7
2	dimethyl sulfide	b	4.73	229	606	1054	145	247	206	14	17	18	103	63	20
3	propanal	a	5.11							14	11	20		4	
4	2-methylpropanal + acetone	a	5.42	384	357	822	163	170	121				34	18	56
5	2-propenal	b	5.89	40	49	129									
6	butanal	a	6.47	14											
7	2-butanone	a	6.98				132	101	41						
8	2-methylbutanal	b	7.32	144	194	924	60	59	39			10	23	13	19
9	3-methylbutanal	b	7.43	223	164	834	31	40	20			15	24	15	13
10	ethanol	a	7.99			39		19		18	9		18	17	
11	3-buten-2-one	b	8.12							20	10			9	
12	unknown	b	8.36						128						
13	2-ethylfuran	b	8.40							43	32	42	9		
14	pentanal	a	9.14							95	113	110			
15	1-methylpropyl acetate	b	9.15			60									
16	trichloroacetic acid	b	10.51			242									
17	α-pinene	a	10.81	49		40	3030	848	4779	728	1153	2313	1908	1513	7274
18	α-thujene	b	10.88				193	99		98	79	15	214	100	
19	geranyl acetate	b	11.95					92				26			88
20	camphene	a	12.10	114	69	122	1563	518	299	917	766	49	1870	1831	316
21	hexanal	a	12.63	23	19	81	151	218	180	377	296	559	106	251	44
22	β-pinene	a	13.35	23	11	16	1199	1160	1570	827	1932	355	769	1937	951
23	2-methyl-1-propanol	a	13.46			92									
24	sabinene/β-thujene	b	13.77	13		19	1342	266	541	819	527	238	1025	450	456
25	2-pentenal	b	14.23							42	40	88			
26	unknown	b	14.48				163		228				266		214
27	3-hexenal	b	14.52							156	162			44	
28	2-methyl-4-pentenal	b	14.75				89	32	42	1274	1526	2255	157	402	51
29	β-myrcene	a	15.84		43		7363	10164	9014	7812	6765	5235	9434	9743	9753
30	α-phellandrene	a	15.33	86		1197									
31	α-terpinene	a	16.16	8		94	330	75	302	116	62	35	268	74	76
32	heptanal	a	16.19	50	28	22									
33	limonene	a	16.79	30	134	29	2156	363	837	2030	878	607	2380	566	980
34	2-hexenal (*cis*)	a	16.86								243	432			
35	sabinene/β-thujene	b	17.10				172	38	272	48	179	41	145	102	58
36	1,8-cineole	a	17.22				232	11							
37	2-methyl-1-butanol	a	17.21			45									
38	3-methyl-1-butanol	a	17.26			78									
39	2-hexenal (*trans*)	a	17.44				166	451	614	707	790	1353	141	477	155
40	unknown	b	17.50												
41	ocimene	a	18.04			70	142	29	175	23	36	66	63	41	294
42	γ-terpinene	a	18.39				479	112	146	166	85	38	331	95	130
43	*trans*-ocimene	b	18.62				1868	463	59	458	289	83	362	292	20
44	*p*-cymene	a	19.12	95		790			127			25			
45	hexyl acetate	a	19.31			33		28		41	83	74		16	
46	α-terpinolene	a	19.62				169	46	55	63	38	17	123	43	51
47	unknown	b	20.59						151						
48	3-hexen-1-ol acetate	b	20.74					179		1060	2971	2456	40	428	14
49	unknown	b	20.85			71									
50	6-methyl-5-hepten-2-one	b	21.26	15		28									
51	1-hexanol	a	21.79			13	148	230	358	160	95	74	44	90	16
52	3-hexen-1-ol (*trans*)	a	22.07							46	33	45			
53	*allo*-ocimene	a	22.40				269	60	161	50	55	68	81	61	264
54	3-hexen-1-ol (*cis*)	a	22.75				508	503	368	1957	1780	1767	499	720	110
55	2-hexen-1-ol (*trans*)	a	23.31					176	91					82	15
56	1-octen-3-ol	b	24.60			29									
57	α-cubebene/α-copaene	b	26.09				28	38	60						20
58	benzaldehyde	a	26.64			13									
59	7-dodecenol	b	28.06	110	208										
60	α-ylangene	b	28.35								62	64			21
61	endobornyl acetate	b	28.43				84						405	227	
62	clovene/calarene	b	28.80							29					
63	γ-cadinene	b	28.82								50	46			
64	*trans*-caryophyllene	b	28.97				76	33	97		50	35	62	58	67
65	calarene, α-copaene	b	31.24								19	26			
66	germacrene D/α-cubebene	b	31.81							40	58	60		19	21
67	5-ethyl-2(5*H*)-furanone	b	32.73							206	153	250	35	55	
68	δ-cadinene	b	32.88							22	29	37	18	14	20
69	βα-cubebene/γ-cadinene	b	33.00							22	30	34	17	12	18
70	2,2,3,3-tetramethyl hexane	b	37.24							72	36	43	9	13	

[a] Numbers are area counts from total ion chromatogram. [b] Identification by MS and retention time (a) or by MS only (b).

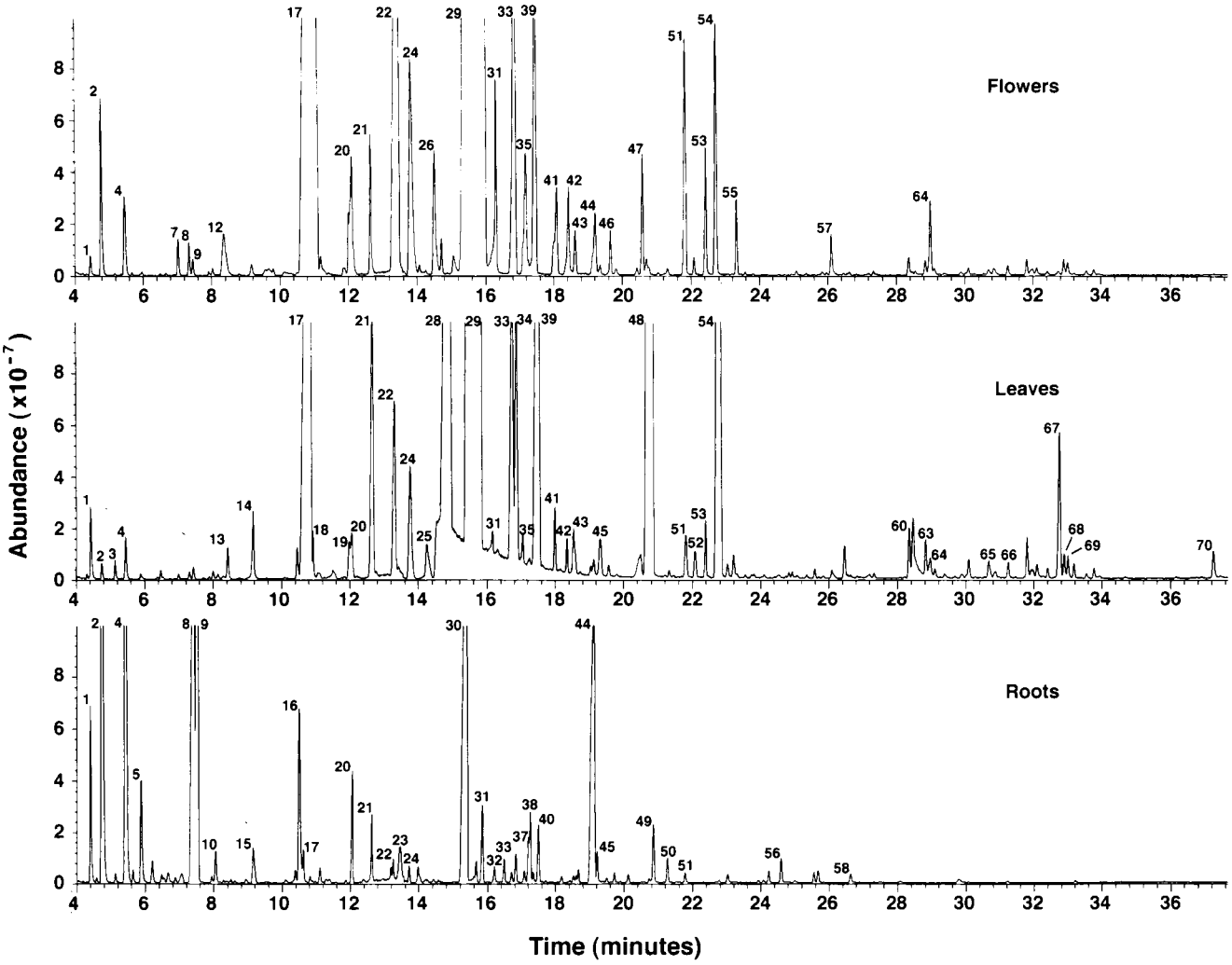

Figure 2. Capillary gas chromatograms of the headspace volatiles of *E. purpurea* flowers, leaves, and roots.

Table 2. Percentage Peak Area Contribution of Compound Classes

	root tissue			flower tissue			leaf tissue			stem tissue		
compd class	*ang*	*palli*	*purp*	*ang*	*palli*	*purp*	*ang*	*palli*	*purp*	*ang*	*palli*	*purp*
alcohols	6	10	4	3	5	4	11	9	10	3	5	1
aldehydes	57	51	41	13	8	9	23	19	29	14	9	6
esters	0	0	2	0	1	0	5	14	13	0	2	0
hydrocarbons	6	2	12	0	0	1	1	1	1	0	0	2
ketones	1	0	0	1	1	0	0	0	0	0	0	0
terpenoids	17	6	21	82	83	83	58	56	46	81	83	91
miscellaneous	13	30	20	1	1	3	1	1	2	2	1	1

RESULTS AND DISCUSSION

Typical gas chromatograms of the headspace of *E. angustifolia*, *E. pallida*, and *E. purpurea* roots are shown in Figure 1. Figure 2 shows chromatograms of the headspace of *E. purpurea* flowers, leaves, stems, and roots, respectively. The volatile compounds were identified by comparison with library mass spectra and capillary gas chromatographic retention times of authentic compounds.

Table 1 gives the list of the chromatographic peaks identified, together with the retention times, a note of how positive is the identification, and the peak area counts for root, flower, leaf, and stem tissues of *E. angustifolia*, *E. pallida*, and *E. purpurea*. Relative percentage abundances of the alcohols, aldehydes, esters, hydrocarbons, ketones, terpenoids, and miscellaneous compounds identified and their contribution to each sample were calculated from the peak area counts of all peaks of the chromatogram and by relating the area of one peak to that of the whole chromatogram as a percentage (Table 2).

The composition of the headspace varied with species and plant tissue. α-Phellandrene, the major constituent in roots of *E. purpurea*, was absent in all tissues of *E. pallida* and in the aerial parts of *E. angustifolia* and *E. purpurea*. β-Myrcene was the major component of flowers, leaves, and stems of all three *Echinacea* species, but was absent in roots of *E. angustifolia* and *E. purpurea*, and was present only in trace amounts in roots of *E. pallida*. All plant tissues, irrespective of the species, contain acetaldehyde, dimethyl sulfide, camphene, hexanal, β-pinene, and limonene. Dimethyl sulfide was a minor component in the leaves, stems, and flowers of all speciess; however, it was the largest

constituent of *E. pallida* roots and the second major component of *E. angustifolia* and *E. purpurea* roots.

Aldehydes, particularly butanals and propanals, make up 41−57% of the headspace of root tissue, 19−29% of the headspace of the leaf tissue, and only 6−14% of the headspace of flower and stem tissues. Terpenoids including α- and β-pinene, β-myrcene, ocimene, limonene, camphene, and terpenene make up 82−91% of the headspace of flowers and stems, 46−58% of the headspace of the leaf tissue, and 6−21% of the roots (Table 2). Also present are 12 alcohols, 7 esters, 14 hydrocarbons, 6 ketones, and 7 miscellaneous compounds.

It is noteworthy that while the terpenoids predominate the aerial parts of *Echinacea* plants, the root tissues are rich in aldehydes, terpenoids, miscellaneous compounds, and alcohols. It is well-known that volatile compounds from plants serve as insect attractants (Metcalf, 1987), and terpenoids have received attention for the anti-inflammatory, analgesic, antimicrobial, and insecticidal properties that some exhibit (Alcaraz and Rios, 1991). Thus, the distribution of the different classes of compounds in the different parts of the *Echinacea* plants may reflect the different biological roles of the compounds identified. Also, it is difficult to assess the relative contributions to flavor and/or aroma of the individual components without their aroma and flavor characteristics and thresholds, but it is likely that most contribute to the overall aroma, flavor, and perhaps the physiological properties of *Echinacea*.

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