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Essential Oil of *Valeriana officinalis* L. Cultivars and Their Antimicrobial Activity As Influenced by Harvesting Time under Commercial Organic Cultivation

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The essential oil content and the composition of subterranean parts of two valerian (*Valeriana officinalis*, L.) cultivars Select and Anthose, from certified commercial organic fields, were determined by hydrodistillation, followed by gas chromatography (GC) and GC/mass spectrometry analysis. Eight and fourteen month old cv. Select had 0.67 and 0.87% essential oil, while similar aged cv. Anthose contained 0.97 and 1.1% essential oil. Forty-three and fifty-three components from cv. Select and cv. Anthose oils were detected, respectively. The oil composition significantly varied due to the cultivar type, plant age, and/or harvesting time. The major components for cv. Select were valerenal, bornyl acetate, 15-acetoxy valeranone, valerenic acid, and camphene, while cv. Anthose had valerenal, (−)-bornyl acetate, α-humulene, camphene, 15-acetoxy valeranone, and valerenic acid. With further aging of the plants, the valerenal, valerenic acid, and α-humulene contents increased. The oil of cv. Select had a strong antimicrobial effect against *Aspergillus niger*, *Escherichia coli*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae*, while cv. Anthose showed low or no activity against all test microbes, including *Pseudomonas aeruginosa*, suggesting that the inhibitory activity of valerian oil depends on the cultivar and its developmental stage. The oil profile of our cultivars did not match the literature proposed chemotype profiles.

KEYWORDS: ***Valeriana officinalis*; essential oil; valerenal; valerenic acid; bornyl acetate; α-humulene; antimicrobial; cultivars; growth stage; organic cultivation**

INTRODUCTION

The dried underground parts (roots and rhizomes) of valerian (*Valeriana officinalis* L.) from the Valerianaceae family are used to prepare modern phytomedical products, mild traditional sedatives, and antianxiety and digestive formulations (*1*, *2*). Valerian essential oils or extracts are used in the formulations of personal care products, cosmetics, aromatherapy, and veterinary practices. Dried valerian roots alone or combined with other crude herbs are also used as natural repellents of insects, pests, and some rodents, especially to fend off cockroaches from homes and skunks from golf fields and home gardens. The dried roots are sometimes packed in cloth bags and put around stored grain, fruits, berries, or vegetables to fend off insects, rodents, and other damaging animals.

Valerian is the 8th top-selling herbal supplement in North America (*2*), making it very attractive to find and establish the chemical composition of the product in relation to its efficacy. There is a dramatically increasing demand for organically grown herbs (produced in the absence of herbicides, growth hormones, pesticides, and synthetic fertilizers) having a precise botanical identity and a desirable chemical profile. For example, in 1995, the Washington-based American Herb Company sold about 17 000 kg of dry valerian roots for ca. $20/kg to an European company. In 2000, this volume jumped to more than 120 000 kg of dry roots at ca. $19−22/kg (Letchamo, Personal observation, 2000). This sharp increase was the result of an increased application of valerian for human health, expanding demand from the pet products market, and increasing interest for natural crop and lawn protection.

As the active component(s) responsible for the therapeutic properties of valerian are not yet clearly understood, our investigation is directed to evaluate the essential oil content and composition that may contribute to valerian's bioactivity and therapeutic applications. At the present time, most valerian products are made from rather inferior quality raw materials often mixed with adulterants and environmental contaminants from various international sources (*3*−*5*). In most cases, the imported raw material was found to be adulterated with other valerian like species (*6*), thus compromising the product quality and authenticity and exposing the consumers to unpredictable

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Figure 1. Seven month old *V. officinalis* cv. Select (top) and cv. Anthose (bottom). Note their morphological differences between the two cultivars at Heard Farm, Trout Lake, WA.

Figure 2. Roots of cv. Anthose (top left) and cv. Select (top right). Washing and cleaning of *Valerian officinalis* minutes after harvesting at Heard Farm, Trout Lake, WA in 1999.

health risks. To the best of our knowledge, there has been no previously published phytochemical information on the essential oil content of valerian cultivars as influenced by plant age and/or harvesting time under commercial organic cultivation. The objective of this investigation was, therefore, to identify high quality valerian cultivars suitable for commercial organic cultivation and to find optimum harvesting times or plant ages that will provide the maximum root yield and purported active components therein.

MATERIALS AND METHODS

Growing Conditions and the Plant Materials. The cultivation experiment was carried out in certified organic fields that were inspected by the Washington State Department of Agriculture. The farm is located at 46 N, 121 W, altitude 560 m, at Trout Lake, WA. The soil was Mount Adam Series type (brown-colored rocky soil) of volcanic origin, consisting of mostly fine sandy loam soil that easily gets muddy with a little rain and dries fast when it gets warm, windy, and sunny. This soil contains relatively very low organic substances (0.2−2.2%, as compared to up to 9% in black soils of Canada, Russia, and some parts of Ukraine) or nutrients, which is specific to this region, with a pH value of 6.2. The mean temperature of the vegetation period was 27 °C.

The farming practices are founded upon the use of consecutive cover crops right from the beginning of soil preparation to build soil fertility, minimizing the need for the additional use of chemical fertilizers during the plant development. It was assumed that this practice would improve soil health (increased useful microbiological activities) and nutrient balance of the soil prior to planting multiyear root crops such as valerian. Two mixed cover crops, Vienna peas (legume) and barley, were grown for 40 days and then plowed under the soil before planting valerian. The plants were grown with all of the necessary agronomic practices. We used a four-row standard for seeding, transplanting, and cultivation. This provided maximum flexibility and efficiency for everything from special project plots to full-scale field production. The irrigation water was carefully managed to eliminate nitrate leaching and to ensure optimum plant health, i.e., minimize water logging, enable plant root development, and eliminate root rot development.

This experiment was carried out using seed populations of *V. officinalis* cultivars with distinct morphological traits, Select and Anthose (**Figure 1**), obtained from North American commercial seed suppliers. The seeds were first germinated and then propagated under glasshouse conditions for 45 days. The seedlings were transplanted to the fields of Heard Farm at Trout Lake Farm, WA, in September, 1999.

The first root harvest was carried out randomly from each field with three replications on April 25, 2000, about 8 months after transplanting (**Figure 1**), while the second harvesting took place on November 25, 2000, 14 months after transplanting. The plants came to flower; however, before the roots were harvested, the tops were mowed away. After the plants were harvested, the roots were immediately washed with a commercial root washer (**Figure 2**) and subsequently chopped into pieces to ease the further cleaning and processing of the roots. The roots were then dried for 72 h at 38 °C to 11% moisture content, using a forced air commercial propane gas heater. Voucher specimens of the samples are deposited at Heard Farm, Trout Lake, WA.

Essential Oil Isolation. Samples of the dried root were divided into four groups with three replications of 29−30 g. Each sample was subjected to 3 h of hydrodistillation, using a Clevenger type distillation apparatus based upon Deutsche Apotheker Buch (*7*). The resulting oils were dried over anhydrous sodium sulfate for 10 min and immediately placed into a sealed glass tube. The samples were stored in the dark at 4 °C until analyzed.

Physical Tests and Sensory Evaluation of the Oils. The physical and organoleptic traits of the oils were determined after drying with anhydrous sodium sulfate. The viscosity was measured by using a Norcross Viscosity Controller (Norcross Corporation, Newton, MA), where the piston principle of viscosity measurement is applied. In this technique, a piston is periodically raised by an air-lifting mechanism,

Table 1. Physical and Organoleptic Traits of the Essential Oil of *V. officinalis* Cultivars Developed under Certified Organic Cultivation

	plant harvesting time			
oil traits	8 months (S)[a]	14 months (S)[a]	8 months (A)[b]	14 months (A)[b]
yield (mL)	1.3	1.8	1.3	1.2
content (%)	0.67	0.87	0.97	1.1
color	greenish yellow	greenish yellow	light yellow	light yellow
taste	bitter	bitter	bitter	bitter
odor	typical valerian	typical valerian	light valerian	light valerian
viscosity	light fluid	light fluid	light fluid	light fluid
solubility in 80% ethanol	soluble	soluble	soluble	soluble
d^{21} (g/cm^3)	0.9611	0.9621	0.9622	0.9623
root-to-rhizome ratio	92:1	95:1	93:1	96:1
dry root yield (kg/m^2)	15	20	14	18

[a] Sample obtained from cv. Select. [b] Sample obtained from cv. Anthose.

drawing the oil being measured down through the clearance between the piston and the inside of the cylinder into the space that is formed below the piston as it is raised. The assembly is then held up for 20 s and then allowed to fall by gravity, expelling the oil out through the same path as it entered. The time of the fall is a measure of viscosity, with the clearance between the piston and the inside of the cylinder forming the measuring orifice. The viscosity controller measures the time of the fall and displays the resulting viscosity value. A refractive index of the oils was measured using an ATR refractometer (Topac Inc. Instruments, St. Hingham, MA), while the color was determined by simple visual evaluation. Sensory tastes included professionally experienced olfactory testing of the essential oils.

Tests for Antimicrobial Activity and Characterization of Inhibitory Data. The technique to determine the qualitative bactericidal activity was based on the agar overlay technique described earlier (*8*−*12*). Organisms at a concentration of approximately 1.5×10^6 colony forming units/mL were added to each replication. The sterilized membrane disks (reservoirs) of 3 mm diameter impregnated with 5 μL of 1% concentration of the oil to be tested were brought directly into contact with a uniformly inoculated medium in 100 mm × 15 mm Petri dishes. After incubation at 37 °C for 24 (bacteria) and 36 h (fungi), the diameter of the clear zone (inhibition zone) around the disks that were soaked with the oil was measured. However, in a modification of the technique, the reservoir or disks impregnated with the essential oil could also be placed in the lid of the Petri dish, thus avoiding direct contact with the media and excluding the oil transport by diffusion (*9*). The techniques requiring a homogeneous dispersion in water are the so-called "dilution techniques", which may have the difficulty that many essential oils are insoluble in pure water culture medium. This problem could be resolved by solublizing the oil with the addition of Tween or Spans nonionic detergent (*11*).

The effects obtained for the antimicrobial activity of the oil were evaluated by adopting the following: −, halo of inhibition less than 5 mm; +, halo of inhibition between 5 and 9 mm; ++, halo of inhibition between 10 and 14 mm; +++, halo of inhibition between 15 and 19 mm; and ++++, halo of inhibition more than 20 mm. The experiment was replicated three times.

Gas Chromatography (GC) and GC/Mass Spectrometry (MS) Analysis. GC and GC/MS analysis of the oils was performed using an Agilant 6890 apparatus equipped with a network mass selective detector 5973 and an automatic sampler 7683, fitted with a DB-Wax column of 30 m × 0.25 mm i.d., film thickness 0.25 μm column (5% biphenyl, 95% dimethyl−siloxane copolymer) under the following conditions. The carrier gas was He at a rate of 1 mL/min (6 psi) adjusted to a constant flow. The column temperature was programmed from 60 (4 min hold) to 240 °C at 6 °C/min, and finally 250−280 °C at 10 °C/min; injector and detector (MSD) temperatures were set at 280 and 170 °C, respectively. The MSD was operated at 70 eV. The sample injection was 1 mL of a 1% solution in hexane, with a split ratio of 20:1. The mass range was 30−650 Da. The identification of the individual components of the oils was carried out by a combined comparison of retention indices of GC, injection of reference compounds (those commercially available), flame ionization detection area %, comparing the results to the literature values published earlier, and mass spectral data using spectra library Wiley 275 (*13*).

Table 2. Antimicrobial Activities of Essential Oils of *V. officinalis* Developed under Certified Organic Cultivation (Values Are the Means of Three Replications)[a]

	microorganisms				
source of oil	*A. niger*	*E. coli*	*P. aeruginosa*	*S. aureus*	*S. cerevisiae*
8 months old (S)[b]	+++	+++	−	+++	++
14 months old (S)[b]	++++	+++	−	+++	+++
8 months old (A)[c]	++	++	−	++	++
14 months old (A)[c]	++	++	−	++	++
Thymus vulgaris oil[d]	++++	+++	+	+++	+++

[a] −, no growth inhibitory activity; +, very low growth inhibitory activity; ++, slight (weak) growth inhibitory activity; +++, moderate growth inhibitory activity; ++++, strong to complete kill of the activity of microbial growth as seen by the naked eye. [b] Samples obtained from cv. Select. [c] Samples obtained from cv. Anthose. [d] Comparison of the oil of *Thymus vulgaris* L. cv. Laval-1 containing 67% thymol, 1% carvacrol, 11% *p*-cymene, and 4% linalool as reported by Letchamo et al. (*26*).

RESULTS AND DISCUSSION

Oil Content and Physical and Olfactory Traits. It was observed that each cultivar had distinct morphological traits (**Figures 1** and **2**). The root to rhizome proportion was 92:1, 95:1 for cv. Select and 93:1, 96:1 for cv. Anthose harvested after 8 and 14 months of growth, respectively (**Table 1**). All 14 month old plant samples had higher oil contents than the 8 month old ones. We found that the oil content for cv. Select was 0.67, and 0.87%, while cv. Anthose had 0.97 and 1.1% for root samples obtained after 8 and 14 months of growth, respectively (**Table 1**). The oils obtained from cv. Select were of a greenish-yellow color, while cv. Anthose was a light green color. The odor of the roots and essential oils of both valerian had a valerian-like smell, although cv. Select had a heavy typically moldy, dirty socks, valerian smell, while cv. Anthose showed a light aromatic odor. All of the oils were bitter in the taste, light fluid, with a density ranging from 0.9611 to 0.9621 for cv. Select and 0.9622 to 0.9623 for cv. Anthose (**Table 1**).

Antimicrobial Activity. Valerian oil is generally regarded as lacking in antimicrobial activity. However, our studies demonstrated that cv. Select oil shows mild to strong antimicrobial activities against some of the microorganisms of health and environmental importance, such as *Aspergillus niger*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Saccharomyces cerevisiae* (**Table 2**). Cultivar Anthose oil has weak or no antimicrobial activity. However,

Table 3. Essential Oil Composition (%) of *V. officinalis* cv. Select (S) and cv. Anthose (A) at Different Ages and Harvesting Times under Certified Organic Commercial Cultivation at Heard Farm, Trout Lake, WA (Values Are the Means of Three Replications)[a]

component	months old				RI[c]
	8 (S)[a]	14 (S)[a]	8 (A)[b]	14 (A)[b]	
oil content (%)	1.3	1.8	1.3	1.2	
α-pinene	1.82	2.12	3.83	2.74	939
camphene	4.96	5.73	7.19	6.99	953
β-pinene	1.01	1.15	1.15	1.02	979
(+)-2-carene	–	0.39	0.69	1.40	1011
α-terpinene	0.83	1.02	0.75	0.73	1017
β-phellandrene	1.01	1.16	1.16	1.1	1030
γ-terpinen	0.15	0.17	–	–	1060
α-terpinolene	0.38	0.34	0.45	0.43	1088
p-cymene	0.24	0.28	0.25	0.23	1091
naphthalene	0.72	0.55	0.76	0.70	1118
α-campholene aldehyde	2.28	0.44	1.57	0.94	1125
borneol	0.57	0.56	0.43	0.41	1165
pulegone	0.24	0.30	–	–	1176
terpinen-4-ol	0.19	0.23	–	–	1177
thymyl methyl ether	0.17	0.19	–	–	1235
(–)-bornyl acetate	10.67	12.68	9.53	9.16	1283
bornyl isvalerate	–	0.36	–	–	1285
carvacrol methyl ether	0.30	025	–	–	1244
trans-caryophyllene	–	–	1.29	1.28	1337
δ-elemene	0.57	0.63	0.51	0.50	1344
cis-caryophyllene	1.36	2.02	0.97	0.90	1362
(+)-cycloisosativene	0.84	0.38	–	–	1368
β-cubebene	–	–	1.17	1.11	1390
β-elemene	0.41	0.51	0.51	0.50	1391
α-elemene	0.77	0.23	–	–	1394
italicene	0.77	–	–	–	1406
α-gurjunene	3.02	3.53	2.59	4.08	1409
β-caryophyllene	1.46	1.87	–	–	1418
β-gurjunene	–	–	4.14	5.15	1432
α-cedrane	3.72	–	–	–	1436
α-humulene	0.58	0.62	7.38	8.46	1455
β-aromadendrene	1.14	–	–	–	1459
alloaromadendrene	1.38	0.69	–	–	1461
6,7-dimethoxy-*m*-cymene	1.73	11	–	–	1467
β-ionene	0.74	1.00	0.74	0.64	1489
β-selinene	0.84	–	1.47	1.82	1490
δ-selinene	1.69	2.27	0.95	0.87	1493
bicyclogermacrene	1.60	1.89	2.19	1.70	1494
α-selinene	–	–	0.95	0.67	1498
isopinocarveol	–	–	0.66	0.64	1499
epizonaren	2.89	–	–	–	1502
germacrene-D	1.43	1.67	–	–	1503
(*E*,*E*)-α-farnesen	–	–	1.51	1.47	1506
γ-cadinene	0.60	–	–	–	1513
γ-salinene	–	1.69	–	1.50	1522
isobornyl 2-methylbutanoate	–	–	0.54	0.39	1524
δ-cadinene	1.51	1.87	–	–	1530
kessyl alcohol	2.13	1.01	0.79	0.70	1539
germacrene-B	2.51	2.79	–	–	1556
longipinanol	–	0.37	–	–	1566
(+)-spathulenol	2.79	3.28	4.63	4.82	1578
(–)-caryophyllene oxide	0.43	0.46	0.97	0.70	1581
benzo-diazepene[d]	0.83	1.08	0.66	0.43	1589
1H-cyclopropanol azulene-4-ol	0.42	0.76	0.69	0.66	1598
15-acetoxyvaleranone	7.11	8.89	5.34	5.67	1628
myrtenalacetate	1.23	1.36	1.38	1.23	1648
α-cadinol	–	3.52	–	–	1653
valerenol	1.82	0.95	2.21	0.98	1655
valerenal	14.02	11.19	12.30	13.30	1668
valeranone	0.94	0.68	–	–	1675
α-bisabolol	0.61	0.91	–	–	1683
valerenic acid	5.02	5.84	2.03	5.92	1686
(*E*,*E*)-farnesol-2	0.52	0.22	2.62	0.70	1697
eremophilene	–	1.12	–	–	1744
isospathulenol	0.66	0.79	0.78	0.79	1771
(*Z*)-valerenyl acetate	2.68	2.55	2.55	0.90	1897
(*E*)-valerenyl isovalerate[d]	1.77	1.85	3.94	4.11	2032

[a] Samples obtained from cv. Select. [b] Samples obtained from cv. Anthose. [c] RI, retention (Kovat's) indices. [d] Tentative identification.

similar to our earlier finding (*14*) on the essential oils of different *Artemisia* species from Siberia, none of the valerian oils under our investigation was able to inhibit *P. aeruginosa* growth (**Table 2**). This is the first report on the antimicrobial activity of the essential oils of valerian roots developed under certified commercial organic cultivation in North America. We also demonstrate for the first time that the antimicrobial activity of valerian oil may depend on its biological origin (cultivar) and the developmental stage of the plant. These results indicate that health care product formulations could be improved based on such differences and selecting the proper chemical profile of the raw material. The differences in the antimicrobial effect could be due to differences in the chemical profile of the oil that included trace amounts of several terpenoids such as thymol in cv. Select. This shows that some valerian oils might be used in formulating personal care products with improved shelf lives of creams and many other products. Further studies are recommended to find valerian cultivars of various origins with suitable chemical profiles for specific areas of applications.

Oil Composition. From **Table 3**, it is possible to see that there are significant differences in the composition of the oil with respect to cultivars, plant age, and harvesting times. Forty-three components representing cv. Select and 53 components representing cv. Anthose oil were detected. We found the major components for cv. Select to be valerenal (11.19−14.02%), bornyl acetate (10.67−12.68%), 15-acetoxy valeranone (7.11−8.89%), valerenic acid (5.02−5.84%), and camphene (4.94−5.73%). For cv. Anthose, they were valerenal (12.30−13.30%), (−)-bornyl acetate (9.16−9.53%), camphene (6.99−7.19%), α-humulene (7.38 − 8.46%), 15-acetoxy valeranone (5.34−5.67%), valerenic acid (2.03−5.92%), and gurjunene (4.14−5.15%). α-Humulene, commonly found in hope flowers, was significantly higher in cv. Anthose than in cv. Select and had a tendency to increase with the age of the plants (**Table 3**). Although there was a tendency for many components to increase with aging of the plants or fall harvest time [thus confirming earlier findings by Omidbaigi (*16*) and Bos et al. (*4*)], some components such as valerenal, α-elemene, α-cederene, β-selinene, naphthalene, aromadendrene, epizonaren, γ-cadinene, farnesol-2, and alloaromadendrene showed a decreasing tendency, depending on the cultivar. However, late-harvested roots contained more pyrolol, valerenic acid, (*E*)-valerenyl isovalerate, and α-humulene (**Table 3**).

It is worth mentioning here that the two valerian cultivars under our investigation did not match in the three valerian chemotypes suggested earlier by Titz et al. (*19*) (Type A: elemol, 2.4−4.9%; valeranone, 6.2−8.7%; and valerenal, 13.4−15.9%. Type B: elemol, 9.8−11.7%; valeranal, 10.3−12.0%; and no valeranol. Type C: elemol, 1.9−2.8%; valeranone, 9.3−10.3%; valerenal, 3.3−3.9%). In this report, it is necessary to mention that Hendriks and Bruins (*22*) inferred the presence of α-kessyl acetate supplementing the following quantitative data for type C: elemol, 1.9−10.3%; valeranone, 16.2−18.2%; α-kessyl alcohol, 9.3−10.3%; valeranal, 3.3−3.9%; and α-kessyl acetate, 3.5−4.3%. Unlike the above report, which might have been typical to those sources, none of the valerian root oil samples under our study contained elemol and some other components (**Table 3**). To verify the absence of elemol in our materials, we ran a parallel GC profile using an authentic sample.

The presence of true chemotypes could normally be confirmed with the introduction of germplasm of various origins and conducting genetic and phytochemical studies based on healthy cultivated plants under similar growing conditions (soil,

temperature, humidity, altitude, agronomic practices, multiple growing seasons, harvesting periods, and developmental stages). In the absence of data under such conditions, however, the above findings might suggest the presence of only chemical polymorphism, or ecological differences, rather than the suggested chemotypes. Few published reports on valerian root oils of various geographic origins (*17*−*19*, *22*−*25*) clearly demonstrate remarkable differences in their chemical profiles. Despite the lack of similarity in their chemical profiles, especially in the content of the highly promoted valepotriates with purported responsibility for activity against insomnia and anxiety, valerian roots developed, harvested, and processed in different forms are effectively used in traditional medicine or modern phytomedicine in their countries of origin. This fact suggests that the efficacy of valerian roots may not be due only to one component but that the bioactivity might reside in a complex of another compound(s) that is (are) yet to be discovered.

Our research results, based on commercial organic cultivation, two different cultivars, and plant developmental stages, demonstrate that *V. officinalis* cultivars can be successfully grown with an acceptable root yield, content of essential oils, and desirable chemical profile in the Northwestern United States for various applications. Genetic selection, identification of promising cultivars, breeding, and agronomic and biotechnology programs should now focus not only on valerians' sedative, calming, and antispasmolytic activities but also on its environmental applications, perfumery cosmetic properties, and repellent properties of insects and wild life that may affect crops and the quality of human life. With a view to the increasing limitations of the use of chemical antimicrobial agents and development of drug resistance, it seems necessary to identify, develop, and switch to new natural antimicrobial agents with less side effects from sustainable natural sources.

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