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Qualitative variation of anti-diabetic compounds in different tarragon (*Artemisia dracunculus* L.) cytotypes

Sasha W. Eisenman^{1,3}, Alexander Poulev¹, Lena Struwe^{1,2}, Ilya Raskin¹, and David M. Ribnicky¹

¹Rutgers University, School of Environmental and Biological Sciences, Department of Plant Biology and Pathology, Cook Campus, 59 Dudley Road, New Brunswick, NJ 08901-8520, USA

²Rutgers University, School of Environmental and Biological Sciences, Department of Ecology, Evolution, and Natural Resources, 14 College Farm Road, New Brunswick, NJ 08901, USA

Abstract

Ethanol extracts of diploid *Artemisia dracunculus* L. (wild tarragon) from populations in the U.S., and polyploid tarragon from a variety of sources, were screened for the anti-diabetic compounds daidigenin; sakuranetin; 2',4'-dihydroxy-4-methoxydihydrochalcone; 4,5-di-O-caffeoylquinic acid; 5-O-caffeoylquinic acid and 6-demethoxycapillarisin using LC-MS. Only decaploid plants contained all six target compounds and were the only plants that contained daidigenin and 2,4-dihydroxy-4-methoxydihydrochalcone. These results exhibit the importance of germplasm selection and provenance when studying plants for medicinal activity. Relying only on the "right species" for consistent medicinal activities may not be sufficient, as intraspecific variation may be highly significant.

Keywords

Artemisia dracunculus; wild tarragon; polyploidy; germplasm; diabetes; sakuranetin

1. Introduction

1.1 *Artemisia dracunculus* L

Artemisia dracunculus L. (tarragon) has a long history of human use and like many other species in the genus *Artemisia*, tarragon produces a wide array of phytochemicals including monoterpenoids, sesquiterpenoids, flavonoids, coumarins, isocoumarins, polyacetylenes, and alkaloids [1-7]. The uniquely fragrant variety, French tarragon (*Artemisia dracunculus* var. *sativa* Besser), is used as a culinary herb and wild or Russian tarragon (*Artemisia dracunculus*, numerous varieties) has been utilized as a medicine throughout its native range (western North America, Asia and Eastern Europe) for the treatment of a wide variety of diseases [8-12]. French tarragon and Russian tarragon differ dramatically in their smell and flavor. French tarragon is favored for its spicy, licorice-like flavor, which has been attributed to high amounts (60-81%) of estragole (methyl chavicol, 1-allyl-4-methoxybenzene) in its

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³corresponding author; current address: Temple University, Department of Landscape Architecture and Horticulture, 201 Dixon Hall, 580 Meetinghouse Road, Ambler, PA 19002 USA. eisenman@temple.edu. Phone: 1-215-268-8168; Fax: 1-267-468-8188.

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essential oil, while in most strains of Russian tarragon, estragole is nearly absent [13-21]. The species is notable for abundant polyploidy with chromosome cytotypes of $2\times$ - $10\times$ being documented [22]. French tarragon is a sterile tetraploid that is reproduced clonally, while Russian tarragon has been found at all ploidy levels.

Extracts of wild tarragon have been shown to be active in a number of different pathways associated with the diabetic condition (see section 1.4), and the bioactive compounds davidigenin (A), 2',4'- dihydroxy-4-methoxydihydrochalcone (B), sakuranetin (C), 6-demethoxycapillarisin (D), 5-O-caffeoylquinic acid (E), and 4,5-di-O-caffeoylquinic acid (F) have been previously isolated via bioassay-guided fractionation (Figure 1) [23-30]. The primary focus of this study was to assess for the presence or absence of these specific anti-diabetic compounds in *A. dracuncululus* extracts prepared from diploid populations found throughout the western United States, as well as clones from these populations and polyploid plants (obtained from a variety of sources) grown in a common garden site to eliminate regional climatic differences. Two congeneric species were analyzed as well.

1.2. Intraspecific variation of phytochemicals

Variation and quality control of active phytochemicals is currently one of the main concerns within the area of medicinal plant use [31]. Both variations in genetic controls of gene expression by differing genotypes, as well as environmental influence on the expression of those genes, can affect the production of various secondary metabolites [32]. For example, flavonoid synthesis in plants can be influenced by a variety of biotic and abiotic factors including UV light radiation, drought, ozone, nutrient availability, diseases, herbivory and developmental stage [33-44]. In addition to variation in chemical production resulting from environmental and temporal conditions, many studies have looked at the variation of chemical groups within genetically related taxa as well. For example, polyacetylenes and related compounds have been shown to segregate various infrageneric groups within *Artemisia* [45,46]. In a chemotaxonomic study of flavonoids found in *Betula* species, Lahtinen et al. [47] found that diploid species did not contain any of the flavanones that were present in the leaves of other polyploid species. Because a number of the biologically active compounds in wild tarragon are flavonoids, and due to the high amount of polyploidy in wild tarragon, a similar finding could have a profound effect on the bioactivity of the extract associated with specific compounds.

Variation in chemical production by con-specific individuals has also been documented in wild tarragon. Both French and wild tarragon have been analyzed to determine if there are differences in their phytochemical compositions. In addition to distinct difference in essential oil profiles [13,15,16,19,48] (see section 1.3), chemical analyses of these tarragon varieties have shown marked qualitative variation. Flavonoids within varieties of *A. dracuncululus* have also been shown to exhibit distinct segregation. Vienne et al. [49] investigated the presence of various flavonols in wild tarragon and French tarragon and found that both types of tarragon contained quercetin glycosides, but only the Russian tarragon contained patuletin glycosides. Chemical variation between cytotypes has also been noted. Using root extracts prepared from different *A. dracuncululus* cytotypes from various geographic sources, Greger [2] conducted an analysis of polyacetylene content and showed that the diploid and decaploid cytotypes had similar qualitative profiles, while hexaploid and octoploids had unique chemical constituents. This within-species variation is particularly important to document because differences in the chemical content of *A. dracuncululus* collections is likely to impact bioactivity.

1.3. Regulatory Considerations and Safety of tarragon

Although originally classified as GRAS (Generally Recognised As Safe), a number of studies have shown that at high doses, estragole is carcinogenic and genotoxic (mostly due to the metabolism into 1'-hydroxyestragole). After reviewing the toxicological literature, the European Commission Scientific Committee on Food could not establish a safe exposure limit and recommended reductions in exposure and restrictions in use [50]. Estragole was also selected for toxicity testing by the National Toxicology Program (an interagency program between the National Institute of Environmental Health Sciences of the National Institutes of Health, the National Institute for Occupational Safety and Health of the Centers for Disease Control and Prevention, and the National Center for Toxicological Research of the Food and Drug Administration). The findings of this three month investigation were released as Toxicity Report Series no. 82 [51]. It stated that examination of reference literature showed no previous documentation of adverse health effects related to human exposure to estragole, but that the carcinogenicity of estragole and its known metabolites have been characterized in rodent bioassays. This report also presented the results of a 3-month study which showed that estragole caused carcinogenic effects in rats of the high dose group. Because rats and mice were exposed for only 3 months, these studies do not assess the full carcinogenic potential of estragole. Additionally, nonneoplastic effects were observed in numerous organs and tissues of study animals.

According to Smith et al. (2002)[52], studies have clearly shown that the conversion of estragole to 1'-hydroxyestragole is dose dependent and that the toxicological risk diminishes markedly at low levels of exposure. They also cite rodent studies that show that the metabolism, metabolic activation, and covalent binding implicated in toxicity and carcinogenicity of estragole are minimal in the dose range of 1–10 mg/kg body weight, which is approximately 100–1000 times the anticipated human exposure to these substances. As such, in their opinion, exposure to methyl eugenol and estragole resulting from food consumption, does not pose a significant cancer risk.

Most populations of Russian tarragon have repeatedly been shown to lack significant amounts of estragole and may or may not contain significant amounts of methyl eugenol (Eisenman et al., unpublished data) [15,16,19,48,49]. Ribnicky et al. (2004) [53] evaluated the toxicological and mutagenic effects of TARRALIN™, an ethanolic extract prepared from Russian tarragon plants. Their results showed that while small quantities of estragole were detected in living Russian tarragon plants, none was detected in either air-dried material, or in the extract prepared from frozen plant material. The loss of estragole was attributed to volatilization during the drying and extraction processes (extraction in ethanol at 80 °C and subsequent rotary evaporation). The extract contained a concentration of methyl eugenol similar to the fresh herb, however, the extract represented a potential 50-fold concentration of some of the herb's components since the yield of extract from fresh herb was 2–3%. Therefore, the overall methyl eugenol content of the extract was greatly decreased from its concentration within the fresh plant. Further analysis revealed that the extract was not active in the Ames test for mutagenicity and there were no histopathological findings that could be attributed to the administration of the extract to the test subjects.

1.4. *Artemisia dracunculus* and diabetes

Type 2 diabetes is a complex metabolic disorder causing elevated blood glucose levels. It is generally the result of a progressive decrease of insulin activity (insulin resistance), and decreased pancreatic insulin secretion, due to failure of insulin producing β -cells [54,55]. According to the World Health Organization more than 220 million people worldwide suffer from diabetes and an estimated 1.1 million people died from it in 2005 [56]. The National Center for Chronic Disease Prevention and Health Promotion has reported that 23.6 million

people or 7.8% of the U.S. population have diabetes, and in adults, type 2 diabetes accounts for about 90 to 95 percent of all diagnosed cases of diabetes [57].

Swanston-Flatt [58] listed tarragon as a traditionally used plant medicine for the treatment of diabetes in the United Kingdom and early bioactivity research showed that tarragon significantly reduced hyperphagia and polydipsia associated with streptozotocin-induced diabetes in mice [59]. Tarragon also slowed body weight loss but did not significantly alter plasma glucose or insulin concentrations. More recent research has shown that an extract of commercially available wild tarragon reduced hyperglycemia in both streptozotocin-induced and genetically diabetic KK-A^y mice and that the extract significantly reduced the expression of phosphoenolpyruvate carboxykinase (PEPCK) mRNA [25]. This enzyme catalyzes the first step in liver glucose production and is normally over-expressed in insulin-resistant diabetic rats. Using *in vitro* skeletal muscle cell cultures (from both human and obese Zucker rats), an alcoholic extract of *A. dracunculus* was found to improve glucose disposal in these insulin sensitive tissues [27]. A bioassay-guided fractionation experiment using quantitative polymerase chain reaction (qPCR) to measure decreased PEPCK expression led to the isolation of two compounds, 6-demethoxycapillarisin and 2',4'-dihydroxy-4-methoxydihydrochalcone [28]. The experiment showed that 6-demethoxycapillarisin exhibited stronger inhibitory activity on PEPCK expression and when combined with insulin it decreased PEPCK expression twice as much as insulin alone. Like insulin, 6-demethoxycapillarisin was shown to function by activating the PI3K pathway, in contrast to the effect of 2',4'-dihydroxy-4-methoxydihydrochalcone, which was not associated with this pathway.

Additional bioassay guided fractionation experiments revealed that four compounds (4,5-di-O-caffeoylquinic acid, davidigenin [4,2',4'-trihydroxydihydrochalcone], 6-demethoxycapillarisin, and 2',4'-dihydroxy-4-methoxydihydrochalcone) contained in wild tarragon extract, inhibited aldose reductase activity [26]. In cells, excess glucose is converted to sorbitol by the aldose reductase enzyme which then accumulates in the cells causing a number of secondary diseases associated with the diabetic condition. In an *in vitro* analysis assessing the bioactivity of fractions derived from an alcoholic extract of *A. dracunculus*, three compounds showed inhibitory effects on protein tyrosine phosphatase-1B activity. Protein tyrosine phosphatase-1B is a key enzyme in the insulin signaling pathway and acts as a negative regulator and studies have shown that mice lacking the protein tyrosine phosphatase-1B (PTP-1B) have enhanced insulin sensitivity. The compounds with inhibitory effects on PTP-1B activities were identified as sakuranetin (4,2',4'-trihydroxydihydrochalcone), 2',4'-dihydroxy-4-methoxydihydrochalcone and its positional isomer 2',4'-dihydroxy-4'-methoxydihydrochalcone [23,60,61].

2. Materials and methods

2.1. Plant material and extract preparation

In order to collect all plants at the same time of year, to reduce potential variation due to difference in phenological stage, collection expeditions were conducted during the last week of August and the first week of September in 2006 and 2007. Sites were found by using locality records obtained via the Global Biodiversity Information Facility (GBIF) portal and the California Consortium of Herbaria database, as well as by direct examination of specimens from the herbaria BYU, CS, GH, KANU, NY, PH, RENO, RM, RSA, US and UT (abbreviations according to Index Herbariorum). In total, 115 extracts were collected from 26 sites throughout the western United States and an effort was made to sample from populations across an altitudinal gradient (Table 1; Figure 2). Field-collected samples consisted of diploid *Artemisia dracunculus* L. (104 samples), *A. campestris* L. subsp. *caudata* (Michaux) H. M. Hall & Clements (a closely related species in subgenus

Dracunculus; 5 samples), *A. ludoviciana* Nutt. subsp. *incompta* (Nutt.) D. D. Keck (a distantly related species in subgenus *Artemisia*; 2 samples), *A. ludoviciana* Nutt. subsp. *ludoviciana* (3 samples), and *A. ludoviciana* Nutt. subsp. *candicans* (Rydberg) D. D. Keck (1 sample). Each sample was numbered using the site number followed by the unique individual identifier (eg., 26-220 is site 26, individual 220). Because plants can exhibit intraspecific variation of phytochemicals between genotypes, and can be influenced by environmental conditions, clones were made from a subset of the wild plants, and were cultivated in a common garden at Rutgers University (Table 2). For comparison within *A. dracunculus*, tetraploid French tarragon plants were purchased from Pantry Garden Herbs (Cleveland, MO), an octoploid plant was grown from seed collected in Kyrgyzstan, and three decaploid plants grown from purchased seeds (Sheffield's Seeds Co., Locke, NY) were also cultivated at Rutgers. In the results, these individuals are abbreviated FRENCH, KYRGYZ and RUGH, respectively.

To prepare the phytochemical extracts, 1 gram of fresh leaves was collected from the upper portion of each plant. The leaf sample was ground with a mortar and pestle in 10 mL of 95% ethanol and poured into a scintillation vial. An additional 10 mL of ethanol was used to rinse the mortar and added to the vial. The ground material was stored in the dark for 24 hours and then filtered through a folded Kimwipes EX-L (Kimberley-Clark) into a new scintillation vial. Upon returning to Rutgers University, the extracts were kept in the dark at 36° C until the analysis was conducted. Herbarium vouchers, collected from each plant sample, were deposited in the Rutgers University's Chrysler herbarium (CHRB).

Root cuttings from the plants sampled in the wild in 2006 were collected and planted in 7" pots using Fafard Canadian Growing Mix 2 (Agawam, MA), and were cultivated in the Rutgers University research greenhouses in New Brunswick, NJ. In the spring and fall of 2007 all cultivated plants were trimmed back to just above the soil level to produce a new flush of stems. Root cuttings collected in 2007 were planted in the same manner as the 2006 material. During the 2007 collection trip, root cuttings were stored in dry potting mix rather than moistened paper towels. As a result, those collected in 2007 had a much higher survival rate. In April of 2008, all the surviving cultivated plants [53 wild-collected *A. dracunculus* plants, 3 *A. dracunculus* grown from purchased seed, 1 *A. dracunculus* from Kyrgyz seed, and French tarragon plants] were transplanted into 11" pots, trimmed back to just above the level of the soil and placed in an outside growing area. At this time 9 g of Scotts Osmocote Classic 14-14-14 slow release fertilizer (Vero Beach, FL) was also applied to the surface of the soil of each pot. Each plant was subsequently watered twice per week and fertilized monthly with 5.5 g Scotts Peters Professional 20-20-20 fertilizer (Vero Beach, FL). To reduce environmental variability, all cultivated plants were watered thoroughly the day before they were harvested, and all were harvested on the same day in October 2007. The phytochemical extraction process for the cultivated plants was conducted in the same manner as for the wild-collected plant samples.

2.2. Phytochemical analysis

Five mL of each phytochemical extract was dried in a centrifugal evaporator and the dry solids were re-dissolved in a mixture of 2 mL Millipore H₂O and 2 mL ethyl acetate (EA) using a vortex and sonicated at 20° C for 10 minutes. Next the samples were centrifuged for 5 minutes at 3000 rpm. The upper EA fraction was collected and placed in a new tube. The water was partitioned 2 more times with EA to obtain a total of 6 mL. The EA fraction was dried in the centrifugal evaporator and re-dissolved in 500µL of 95% ethanol. Each sample was centrifuged for 5 minutes at 3000 rpm and 20°C and 200 µL of the sample was put into a vial for liquid chromatography/mass spectroscopy (LC-MS) analysis.

The six investigated compounds were identified using liquid chromatography/mass spectroscopy (LC/MS). Each sample (30 μ L injection volume) was analyzed using a LC-MS system consisted of a Waters (Milford, MA) solvent delivery system with a W616 pump and W600S controller, W717plus auto-sampler, and W996 photodiode array detector (PDA). UV data were collected and analyzed with the Waters Millennium[®] v. 3.2 software. After the 996 PDA detector the eluent flow was guided to a Varian 1200L (Varian Inc., Palo Alto, CA) triple quadrupole mass detector with electrospray ionization interface (ESI), operated in negative ionization mode. The electrospray voltage was -4.5 kV, heated capillary temperature was 240 $^{\circ}$ C, sheath gas was air. The mass detector was set to scan from 65 to 1500 atomic mass units. Data from the Varian 1200L mass detector was collected, compiled and analyzed using Varian's MS Workstation, v. 6.41, SP2. Compounds were separated on a Phenomenex[®] Luna C-8 reverse phase column, size 250 $\times$ 4.6 mm, particle size 5 μ m, pore size 100 $\text{\AA}$, equipped with a Phenomenex[®] SecurityGuard[™] pre-column. The mobile phase consisted of 2 components: Solvent A (0.5% ACS grade acetic acid in double distilled de-ionized water, pH 3-3.5), and Solvent B (100% Acetonitrile). The mobile phase flow was adjusted at 0.5 ml/min, and a gradient mode was used for all analyses. The initial conditions of the gradient were 85% A and 15% B; for 30 minutes the proportion reached 5% A and 95% B which was kept for the next 3 minutes; from minute 38 to minute 41 the gradient went back to initial conditions. A 10 minute equilibration interval was included between subsequent injections.

Identification of the compounds was based on deprotonated molecular ions in negative ionization mode and their retention times (Table 2). Due to the diversity of caffeoylquinic acid and chlorogenic acid derivatives in the extracts, and because of the crude nature of the extracts, distinct chromatogram peaks for 4,5-Di-O-caffeoylquinic acid and 5-O-caffeoylquinic acid could not be obtained. As a result, all plants were screened for a mixture of dicaffeoylquinates with a parent ion of m/z 515 and a retention time of 20 minutes, and a mix of chlorogenates with a parent ion of m/z 353 and a retention time of 14 minutes. The liquid chromatography protocol that was used did not sufficiently separate the isomers, 2',4'-dihydroxy-4'-methoxydihydrochalcone and 2',4'-dihydroxy-4-methoxydihydrochalcone. Because of this, plants were screened only for the presence of the molecules and not the specific isomers.

3. Results and discussion

All target compounds were detected in the extracts of the decaploid *Artemisia dracunculus* plants grown from purchased seeds. Example chromatograms for the target compounds are shown in Figure 3. The decaploids were also the only plants in which davidigenin and 2,4-dihydroxy-4-methoxydihydrochalcone were detected. Sakuranetin was detected in 100% of the extracts prepared from diploid *Artemisia dracunculus* plants collected in North America, regardless of whether the extracts were prepared in the field from wild growing plants or from plants grown in the common garden. 6-demethoxycapillarisin, which was detected in 66% of the wild-prepared extracts of *A. dracunculus*, exhibited variation between wild growing plants and their clones cultivated in the common garden samples. In 32 of the North American plants, 6-demethoxycapillarisin was detected in the wild and not detected in the cultivated clones, and in one individual the compound was not detected in the wild yet was detected in the cultivated clone. This variation leads to the assumption that the production of 6-demethoxycapillarisin in these plants is subject to change depending on environmental conditions. Davidigenin, 2,4-dihydroxy-4-methoxydihydrochalcone, sakuranetin and 6-demethoxycapillarisin were not detected in *Artemisia campestris*, *A. ludoviciana*, the tetraploid French tarragon and the octoploid wild tarragon plant from Kyrgyzstan. This had one exception as 6-demethoxycapillarisin was detected in *A. ludoviciana* subsp. *candicans*. Table 3 presents the presence or absence of the target compounds for all the wild and

cultivated samples. All extracts, from both wild-growing and cultivated samples of all species tested, were found to contain complexes of caffeoylquinic acids and dicaffeoylquinic acids. The nomenclature used for caffeoylquinic acids discussed in this paper is the recommended IUPAC numbering system [62,63].

Due to the simple preparation and partitioning of the extracts, and the specific liquid chromatography parameters, 5-O-caffeoylquinic acid (5-CQA) and 4,5-Di-O-caffeoylquinic acid (4,5-diCQA) were co-eluted with their related compounds and therefore did not produce isolated peaks. 3-O-caffeoylquinic acid (3-CQA) and 4-O-caffeoylquinic acid (4-CQA), isomers of 5-O-caffeoylquinic acid, have the same parent ion (m/z 353), as well as similar retention times. In a similar fashion, 3,4-Di-O-caffeoylquinic acid (3,4-diCQA) and 3,5-Di-O-caffeoylquinic acid (3,5-diCQA) share the same parent ion (m/z 515), and can have similar retention times as 4,5-diCQA acid. A revised liquid chromatography protocol with adjusted solvent flow rates would be necessary to adequately separate and quantify the 5-O-caffeoylquinic acid and 4,5-Di-O-caffeoylquinic acid from related compounds. Preliminary results from a revised LC protocol revealed that the wild prepared sample 14-104 contained primarily 4,5-diCQA, with lesser amounts of 3,5-diCQA and 3,4-diCQA, as well as an unknown diCQA in very small concentrations (J. Munafo, per. comm., data not shown). 1,3-diCQA and 1,5-diCQA were found in significant amounts in some Asteraceae such as artichoke (*Cynara*) and one of these could possibly be the unidentified compound [64]. Of the caffeoylquinic acids, 5-CQA was the most abundant, followed by 4-CQA, and very little 3-CQA (J. Munafo, per. comm., data not shown).

This study has shown that the target anti-diabetic compounds are not found ubiquitously in *Artemisia* species. More significantly, there was a distinct qualitative variation in the chemical content of the different *A. dracunculus* cytotypes. This variation is an extremely important factor to consider when selecting material for biological investigations. Although the target compounds davidigenin and 2',4'-dihydroxy-4-methoxydihydrochalcone were not detected in North American populations, sakuranetin was detected in 100% of those samples.

The results suggest that the North American populations lack the capacity to produce some of these medicinal compounds or that they are produced in undetectable quantities, while decaploid plants contain all targeted compounds. These findings are similar to those of Greger [2], who also found that polyacetylene content in wild tarragon was associated with ploidy level, and that the decaploid cytotype had a unique assembly of compounds. It is also interesting that the tetra-, and octoploid plants of *A. dracunculus* lacked nearly all of the target compounds, while many of the diploids and all the decaploids screened contained the sakuranetin and 6-demethoxycapillarisin. This is also similar to Greger's findings in which a hexaploid and octoploid strain differed from the decaploid strain, while chemically, the diploid was more allied to the decaploid.

As documented in many different taxa, polyploidization can cause dramatic change in physiological processes and increase the production of secondary metabolites [65,66]. Based on the results of this study it seems quite possible that the production of 2,4-dihydroxy-4-methoxydihydrochalcone and davidigenin is unique to the decaploid strain. Since the decaploid strain is likely derived from diploids originating in Europe or Asia, it is possible that the diploid progenitors of this decaploid could produce all the target compounds [67]. An allopolyploid hybridization event in the formation of the decaploid *A. dracunculus* could have resulted in the introduction of novel genes and gene products, or the unique chemical profile may be the product of autopolyploidy and result from inter-specific variation. The latter may very well be the case, as it seems that this species has a propensity for genomic

duplication as evidenced by widespread documentation of individuals within the polyploidy series [68].

With its diversity of ploidy levels, *Artemisia dracunculus* provides an interesting look into genomic evolution, and this genetic diversity is reflected in its variable chemistry. The findings of this study show that the proper selection of germplasm material for the development of botanical therapeutics is of the utmost importance, and that the provenance, and more specifically the cytotype and genotype, of the plant material are essential to developing an effective and consistent botanical therapeutic. Although the large series of polyploids known in *A. dracunculus* may be fairly unique, within-species genotypic variation remains an important consideration for other medicinal plants. Reliable germplasm, with plant material that is uniform in chemical composition, is critical for standardization of botanical products.

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Figure 1. Structures of bioactive compounds screened for in *Artemisia dracunculus*: dauidigenin (A), 2',4'-dihydroxy-4-methoxydihydrochalcone (B), sakuranetin (C), 6-demethoxycapillarisin (D), 5-O-caffeoylquinic acid (E), and 4,5-di-O-caffeoylquinic acid (F).

Figure 2.
Geographic distribution of *Artemisia* spp. collection sites across the western United States.

Figure 3.
Representative chromatograms of targeted bioactive compounds in *Artemisia dracunculus*.

Collection localities, coordinates (WGS84) and elevations. *Artemisia dracunculoides* was collected at all sites except site 1 (*A. campestris* subsp. *caudata*), site 4 (*A. ludoviciana* subsp. *incompta* and *A. ludoviciana* subsp. *ludoviciana*), and site 5 (*A. ludoviciana* subsp. *candicans*).

Table 1

collection site	country	state	county	locality	latitude	long	Altitude (m)
site 01	USA	Colorado	Kit Carson	~6.5 km E of Flagler. Growing in the dry bed of Sand Creek. Just S of frontage road bridge crossing creek and 300 m N of Route 70.	39.28	-102.99	1437
site 02	USA	Colorado	Clear Creek	Georgetown. ~0.3 km north of Georgetown lake on NW side of Alvarado Rd. Rocky meadow, opposite a small graveyard.	39.74	-105.69	2566
site 03	USA	Colorado	Saguache	~12.2 km S of Villa Grove. Roadside drainage with <i>Chrysothamnus nauseosus</i> , <i>Bromus</i> sp. Bordering dry, sagebrush dominated rangeland.	38.14	-105.98	2384
site 04	USA	Utah	Emery	Huntington Canyon. ~13 km NW of the town Huntington. N side of Huntington Creek at the turn-off towards Meetinghouse Canyon.	39.39	-111.09	1983
site 05	USA	Utah	San Pete	E side of Skyline Drive, ~5 km south of the intersection with Route 31. Alpine meadow environment.	39.58	-111.31	3054
site 06	USA	Utah	Wayne	~7.0 km SE of Bicknell. N side of highway 24, ~0.55 km W of State Road 117. Sage-brush meadow along edge of pasture.	38.30	-111.48	2113
site 07	USA	Utah	Wayne	W side of the town of Torrey. Roadside N of Route 24, ~0.35 km W of the intersection of Route 24 and River Rd.	38.30	-111.44	2089
site 08	USA	Nevada	Mineral	~2.1 km S of the intersection between U.S. Route 95 and Colorado Drive. Along a dry wash, emptying into SW side of Walker Lake.	38.62	-118.74	1229
site 09	USA	California	Plumas	~3.5 km east of Portola. Immediately N of the junction of Big Grizzly Creek and the middle fork of the Feather River. Meadow area above riparian zone.	39.82	-120.43	1481
site 10	USA	Wyoming	Laramie	~34 km E of Laramie. W side of N Crow rd, 0.8 km N of the intersection between N Crow Rd and Route 210 (Happy Jack Rd). Dry sagebrush grassland.	41.20	-105.27	2337
site 11	USA	Wyoming	Laramie	~34 km E of Laramie. E side of N Crow rd, 5.0 km N of the intersection between N Crow Rd and Happy Jack Rd (Route 210). Edge of riparian area (North Branch Crow Creek) just E of Upper North Crow Reservoir.	41.24	-105.27	2264
site 12	USA	Colorado	Sedgwick	1.7 km SE of Julesburg. E of Rt. 385 and just N of the South Platte River. Sandy area between dried-out branches of the river.	40.97	-102.25	1052
site 14	USA	California	San Bernardino	N of Yucaipa. Among grasses at the base of Crafton Hills. NW of the intersection of Oak Glen Rd and Creekside Drive.	34.04	-117.06	748
site 15	USA	California	San Diego	~20 km N of Escondido. SE side of intersection between Old Highway 395 and Nelson Way. Dry meadow area bordered by large trees.	33.27	-117.15	104
site 16	USA	California	San Diego	~1.25 km NE of Julian. NW of intersection between Rt. 78 and Whispering Pines Dr. Openly wooded area	33.09	-116.59	1244
site 17	USA	California	Inyo	~20.3 km west of the town Independence. Onion Valley campground, growing along the edge of a small stream.	36.77	-118.34	2802
site 18	USA	California	Plumas	~2.6 km south of Crescent Mills, amidst bear paths, just west of Indian Creek and south of Arlington Rd crossing. Just outside riparian zone.	40.08	-120.92	1064

collection site	country	state	county	locality	latitude	long	Altitude (m)
site 19	USA	California	Lassen	~13.5 km south of Susanville. Wingfield Rd. Sagebrush dominated edges, outside of fenced pasture.	40.34	-120.57	1318
site 20	USA	California	Inyo	~19 km east of the town Big Pine. Westgard Pass. At the base of dry, rocky slopes.	37.25	-118.16	2054
site 21	USA	California	Inyo	Mojave desert region. ~7.3 km due east of Big Pine. Along Waucoba Road/ Death Valley Rd.	37.17	-118.21	1366
site 22	USA	California	Inyo	~6.5 km W of Highway 395. S side of Nine Mile canyon Rd. Dry, steep sloped ravine.	35.85	-117.94	1222
site 23	USA	California	Tulare	~16.4 km W of Highway 395. S side of Nine Mile canyon Rd. Sage-brush meadow.	35.86	-118.01	1909
site 24	USA	California	Los Angeles	~0.75 km E of Elizabeth Lake, N side of Elizabeth lake Rd. Amid a mixed meadow of shrubby and herbaceous species.	34.66	-118.38	1018
site 25	USA	California	San Bernardino	Mojave Desert at foot of San Bernardino Mountains. SE side of the town Hesperia. In the dry basin of the Mojave River. ~2.6 km mi north of the Mojave Forks Dam.	34.37	-117.24	898
site 26	USA	California	San Bernardino	S side of Rt 18 between Running Springs and Arrowbear Lake, immediately N of Hoffman Elementary School. In open areas of moderately wooded drainage area.	34.20	-117.09	1847

Table 2

Molecular ions of target compounds (in negative ionization mode), and their retention times.

Compound	Mass [M – H] [–] (m/z)	Approximate retention time (minutes)
davidigenin	257	26
6-demethoxycapillarisin	285	25
sakuranetin	285	30
2,4-dihydroxy-4-methoxydihydrochalcone	271	31
caffeoylquinic acids	353	14
di-O-caffeoylquinic acids	515	20

Table 3

Results of the LC-MS analysis assessing for the presence of known anti-diabetic compounds in various *Artemisia* species and *Artemisia dracunculoides* cytotypes.

sample	Species/ploidy ^a	davidigenin	chalcone ^b		6-De-MeO ^c		sakuranetin		chlorogenates		caffeoylquinates	
		W ^d	W	C	W	C	W	C	W	C	W	C
1-1	<i>A. campestris</i>	-	-	-	-	-	-	-	+	-	+	-
1-2	<i>A. campestris</i>	-	-	-	-	-	-	-	+	-	+	-
1-3	<i>A. campestris</i>	-	-	-	-	-	-	-	+	-	+	-
1-4	<i>A. campestris</i>	-	-	-	-	-	-	-	+	-	+	-
1-5	<i>A. campestris</i>	-	-	-	-	-	-	-	+	-	+	-
2-11	<i>A. dracunculoides</i> (2x)	-	-	-	+	-	+	+	+	+	+	+
2-12	<i>A. dracunculoides</i> (2x)	-	-	-	+	+	+	+	+	+	+	+
2-13	<i>A. dracunculoides</i> (2x)	-	-	-	-	-	+	+	+	+	+	+
2-14	<i>A. dracunculoides</i> (2x)	-	-	-	+	+	+	+	+	+	+	+
2-15	<i>A. dracunculoides</i> (2x)	-	-	-	+	+	-	-	+	+	+	+
3-21	<i>A. dracunculoides</i> (2x)	-	-	-	+	+	+	+	+	+	+	+
3-22	<i>A. dracunculoides</i> (2x)	-	-	-	-	-	-	-	+	+	+	+
3-23	<i>A. dracunculoides</i> (2x)	-	-	-	-	-	-	-	+	+	+	+
3-24	<i>A. dracunculoides</i> (2x)	-	-	-	-	-	-	-	+	+	+	+
3-25	<i>A. dracunculoides</i> (2x)	-	-	-	-	-	-	-	+	+	+	+
4-31	<i>A. ludoviciana</i>	-	-	-	-	-	-	-	+	+	+	+
4-32	<i>A. ludoviciana</i>	-	-	-	-	-	-	-	+	+	+	+
4-33	<i>A. ludoviciana</i>	-	-	-	-	-	-	-	+	+	+	+
4-34	<i>A. ludoviciana</i>	-	-	-	-	-	-	-	+	+	+	+
5-36	<i>A. ludoviciana</i>	-	-	-	+	+	-	-	+	+	+	+
6-37	<i>A. dracunculoides</i> (2x)	-	-	-	-	-	+	+	+	+	+	+
6-38	<i>A. dracunculoides</i> (2x)	-	-	-	-	-	+	+	+	+	+	+
7-39	<i>A. dracunculoides</i> (2x)	-	-	-	-	-	+	+	+	+	+	+
7-40	<i>A. dracunculoides</i> (2x)	-	-	-	+	+	+	+	+	+	+	+
7-41	<i>A. dracunculoides</i> (2x)	-	-	-	+	+	+	+	+	+	+	+
7-42	<i>A. dracunculoides</i> (2x)	-	-	-	+	+	+	+	+	+	+	+

sample	Species/ploidy ^a	davidigenin		chalcone ^b		6-De-MeO ^c		sakuranetin		chlorogenates		caffeoylquinates	
		W ^d	C ^e	W	C	W	C	W	C	W	C	W	C
7-43	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
8-49	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
8-50	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
8-51	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
8-52	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
8-53	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
9-54	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
9-55	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
9-56	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
9-57	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
9-58	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
10-64	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
10-65	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
10-66	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
10-67	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
10-68	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
11-69	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
11-70	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
12-74	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
12-75	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
12-76	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
12-77	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	-	+	+	+	+	+
12-78	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
14-100	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
14-101	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
14-102	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
14-103	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
14-104	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
15-110	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	-	+	+	+

sample	Species/ploidy ^a	davidigenin		chalcone ^b		6-De-MeO ^c		sakuranetin		chlorogenates		caffeoylquinates	
		W ^d	C ^e	W	C	W	C	W	C	W	C	W	C
15-111	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	-	-	-	+	+
15-112	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	-	-	-	+	+
15-113	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	-	-	-	+	+
15-114	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	-	-	-	+	+
16-120	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
16-121	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
16-122	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
16-123	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
16-124	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
17-130	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
17-131	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
17-132	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
17-133	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
17-134	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
18-140	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
18-141	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
18-142	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
18-143	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
18-144	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
19-150	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
19-151	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
19-152	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
19-153	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
19-154	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
20-160	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
20-161	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
20-162	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
20-163	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
20-164	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+

sample	Species/ploidy ^a	davidigenin		chalcone ^b		6-De-MeO ^c		sakuranetin		chlorogenates		caffeoylquinates	
		W ^d	C ^e	W	C	W	C	W	C	W	C	W	C
21-170	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	-	+	-	+	+
21-171	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
21-172	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
21-173	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
21-174	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
22-180	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
22-181	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
22-182	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
22-183	<i>A. dracunculius</i> (2x)	-	-	-	-	+	-	+	+	+	+	+	+
22-184	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
23-190	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
23-191	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
23-192	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
23-193	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
23-194	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
24-200	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
24-201	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
24-202	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
24-203	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
24-204	<i>A. dracunculius</i> (2x)	-	-	-	-	+	+	+	+	+	+	+	+
25-210	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
25-211	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
25-212	<i>A. dracunculius</i> (2x)	-	-	-	-	-	+	+	+	+	+	+	+
25-213	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
25-214	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
26-220	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
26-221	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
26-222	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+
26-223	<i>A. dracunculius</i> (2x)	-	-	-	-	-	-	+	+	+	+	+	+

sample	Species/ploidy ^a	davidigenin		chalcone ^b		6-De-MeO ^c		sakuranetin		chlorogenates		caffeoylquinates	
		W ^d	C ^e	W	C	W	C	W	C	W	C	W	C
26-224	<i>A. dracunculius</i> (2×)	–	–	–	–	–	–	+	+	+	–	+	–
RUGH-1	<i>A. dracunculius</i> (10×)		+		+		+		+		+		+
RUGH-2	<i>A. dracunculius</i> (10×)		+		+		+		+		+		+
RUGH-4	<i>A. dracunculius</i> (10×)		+		+		+		+		+		+
French 1	<i>A. dracunculius</i> (4×)		–		–		–		–		–		–
French 2	<i>A. dracunculius</i> (4×)		–		–		–		–		–		–
French 3	<i>A. dracunculius</i> (4×)		–		–		–		–		–		–
French 4	<i>A. dracunculius</i> (4×)		–		–		–		–		–		–
French 5	<i>A. dracunculius</i> (4×)		–		–		–		–		–		–
French 6	<i>A. dracunculius</i> (4×)		–		–		–		–		–		–
French 7	<i>A. dracunculius</i> (4×)		–		–		–		–		–		–
French 8	<i>A. dracunculius</i> (4×)		–		–		–		–		–		–
French 9	<i>A. dracunculius</i> (4×)		–		–		–		–		–		–
Kyrgyz	<i>A. dracunculius</i> (8×)		–		–		–		–		–		–

^a Ploidy level is based on work by Eisenman and Struwe [55]

^b chalcone = 2,4-dihydroxy-4-methoxydihydrochalcone

^c 6-De-MeO = 6-demethoxycapillarisin

^d W = extracts prepared from plants growing in their wild habitat

^e C = extracts prepared from plants cultivated in a common garden