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Appalachian Center for Ethnobotanical Studies ACES

Tai Sophia Institute

An Appalachian Plant Monograph

Goldenseal

Hydrastis canadensis L.

1. Taxonomy

Hydrastis canadensis L.
Family: Ranunculaceae (buttercup family)
Hydrastis is a monotypic genus, which some authors have placed in a separate family – Hydrastidaceae (Tobe & Keating, 1985) – though more recent genetic studies confirm *H. canadensis* as the basal branch of the Ranunculaceae, albeit with close ties to the Berberidaceae family (Ro, Keener, & McPheron, 1997; Chu, Li, & Qi, 2006).

Common names: golden seal, eyebalm, eyeroot, golden root, ground raspberry, Indian dye, Indian turmeric, jaundice root, orange root, yellowroot, yellow pucoon.

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2. Botanical description and distribution

Hydrastis canadensis is an herbaceous perennial growing from a short yellow rhizome. The rhizome has a knotty appearance bearing remnants of stems or stem scars (Tobe & Keating, 1985). During the first year vegetative growth consists of a pair of leaf-like cotyledons on long petioles. In the second year a few inch long 'footstalk' emerges bearing one palmately-lobed or maple-shaped leaf with biserrate margins near the apex. Over the next two years a true stem arises attaining a height of one foot or more, bearing two or three petiolate leaves arranged alternately on the stem. Small, solitary, greenish-white flowers (up to 75 stamens enclose a fewer number of carpels with short styles) appear at the base of the leaves, born on short pedicels. The flowers develop into a compound fruit consisting of an aggregate of drupelets, with each drupelet bearing 1-2 seeds. The fruit is inedible to humans (Krochmal, Walters and Doughty 1969; Upton, 2001). Detailed macroscopic botanical illustrations from the *Botanical Gazette* (Bowers, 1891) are reproduced in Appendix 1.

H. canadensis is an understory plant found growing in patches in rich open woodlands, hill slopes and along stream banks (Bowers, 1891; Tobe & Keating, 1985). The natural distribution range includes Ontario Canada, through New York south to Tennessee and Georgia, and it was historically abundant in Ohio, Kentucky and West Virginia (McGraw, Sanders, & Van der Voort, 2003), however it has been experiencing significant population decline in recent decades (Sanders & McGraw, 2005). Figure 7 illustrates the natural distribution as determined by the United States Department of Agriculture (USDA).

3. Traditional Use

Traditional use in Appalachia

The root of *H. canadensis* is highly prized in Appalachian culture as a strengthening tonic, stomachic and as a source of yellow dye. It is also applied topically for sore eyes and general ulcerations (Millspaugh, 1974; Crellin & Philpott, 1990). The powder preparation is combined with water and gargled as a sore throat remedy. Interestingly, it once had a reputation for preventing "pitting" scars from smallpox when applied topically in a warm bath (Cavender, 2003). A common complaint of Appalachian folk is that of "sour stomach" or dyspepsia, which in many cases is linked to tooth decay, gum disease with diminished chewing capabilities. For this *H. canadensis*, is widely used, either by chewing on or making a decoction of the root to ease the stomach upset (Cavender, 2003). It has also been used to treat arrow wounds! (Jacobs & Burlage, 1958).

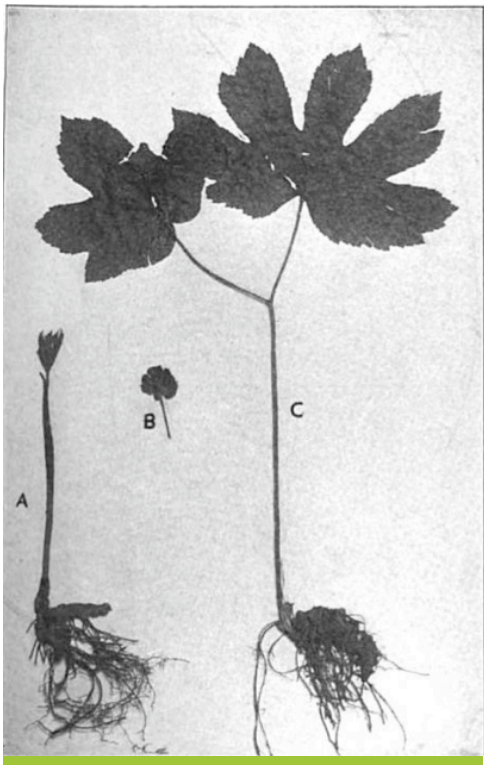


Figure 1. Goldenseal (*Hydrastis canadensis*): A. Young plant with horizontal rhizome and numerous roots. B. Fruit-head of small berries. C, older plant showing the palmately lobed leaves. *Reproduced from Scientific and Applied Pharmacognosy by Henry Kraemer (1915).*

Traditional use outside of Appalachia

Native Americans

H. canadensis was a major medicine for the Cherokee, both as a bitter tonic and as an eyewash for inflamed eyes (Upton, 2001). It was also popular with the Iroquois, whose recorded uses include whooping cough, diarrhea, pneumonia and tuberculosis (Moerman, 1986). More generally, it was widely used for tumors and also as a dermatologic aide topically for ulcers and skin disorders (Duke, 1986). *H. canadensis* was a common remedy for stagnant gastrointestinal function characterized by a "sour" stomach (Moerman, 1998).

Folklore & Home

H. canadensis was used to address dyspepsia and as a tonic for depleted digestive organs. As a wash it was beneficial for sore, inflamed eyes, and as a gargle for sore mouth and throat (Gardner & Aylworth, 1836). The root was generally valued as a household bitter by adding it to wine or brandy. The root was added to wine or brandy and valued as a household bitter (Sumner, 2004).

Physiomedicalism

For Samuel Thomson, regarded as the founding father of Physio-medicalism, *H. canadensis* was a simple bitter, given as a powder in hot water to treat digestive distress and for "correction of the bile" (Thomson, 1835). Howard, an MD and follower of Thomson, recommended *H. canadensis* for debility (weakness), loss of appetite and for recovery from fevers (Howard, 1853). Alvin Curtis, known as a "neo-Thomsonian" and Physiomedicalist, also proclaimed the use as a digestive bitter and as a treatment for bilious colic, as well an ingredient in medicated eye washes (Curtis, 1858). William Cook, (1869) claimed that the root of *H. canadensis* is most beneficial to the mucus membranes, digestive tract and uterine organs. Cook praised it in depleted digestion, "it improves appetite and digestion; and through the stomach proves one of the most acceptable of all general tonics in indigestion, feeble assimilation, biliousness, leucorrhea, prolapses, and all forms of debility" (Cook, 1869, p. 474).

In his classic text *Back to Eden* first published in 1939, Jethro Kloss emphasized the special influence of *H. canadensis* on mucous membranes and skin, and recommended a tea be used as a wash for "open sores, inflammations, eczema, ringworm and erysipelas" (1972, p.244), followed by application of the powdered root and a covering. For ulceration of the mouth, stomach, duodenum, and for tonsillitis he

recommended a tea made with one part goldenseal to one-fourth myrrh - while for pyorrhea and sore gums he brushed the teeth and gums with a toothbrush dipped in the same infusion (Kloss, 1972).

Eclectics

H. canadensis was included in the earliest publications of the Eclectic school, including Wooster Beachs' *American Practice of Medicine* (1833). It was regarded as equally beneficial in catarrhal conditions of the intestines, gall ducts, biliary passages and in jaundice (Felter & Lloyd, 1898). It was considered to act mainly by increasing hepatic secretion in the liver, and was also used for disorders of the mucus membranes. A specific indication was "catarrhal states of the mucous membranes unaccompanied by acute inflammation" Felter (1922). Culbreth (1917) favored its' use as a local treatment for gonorrhea, leucorrhea, otorrhea and numerous other infectious disorders marked by catarrhal discharges.

The homeopath Edwin Hale provided a major contribution on the therapeutic uses and indications for *H. canadensis* (Hale, 1875). With his frequent use of tinctures and herbal teas - often in preference to the potentized homeopathic preparations - Hale was by definition an Eclectic practitioner with his blend of homeopathy and medical botany. His description of the action of *H. canadensis* on mucous surfaces influenced all of the practitioner schools of his day. The mucous secretion for which the herb is indicated is "at first clear, white, transparent and tenacious, it becomes yellow, or thick, green and even bloody, and nearly always tenacious". He goes on to explain "its' secondary effects are exhaustion or destruction of the glandular sources of the mucus - a condition in which the mucus surface is dry, glazed, and its functions destroyed" (Hale, 1875 p. 313).

Regulars

Allopathic physicians employed *H. canadensis* as a stimulating tonic, specific for the digestive tract and liver function. For example, Potter's - the famous British compendium of plant medicines– listed it as an "astringent bitter, promoting appetite and digestion, increasing the secretions of the gastro-intestinal tract, and the flow of bile" (Potter, 1906, p.62). Reported uses included: rhinitis, gastritis, vaginitis, and urethritis, and as a bitter for general debility and digestive impairment. Previously Stille (1874) questioned the reputations of those who asserted *H. canadensis* was effective for such disorders, claiming they lacked an understanding of medicine and disease!

Henry Rutherford, assistant-physician at Chelsea Hospital for Women (U.K.), reported five case studies involving the internal prescription of *H. canadensis* tincture for uterine fibroids in the *British Medical Journal* (Rutherford, 1888). Excessive bleeding associated with the condition was diminished following the treatments in all cases. According to Rutherford the hemostatic properties of the herb were acknowledged in France, Germany, and especially in America where "the drug has had extended trials, and the published results are most satisfactory" (Rutherford, 1888 p. 123).

H. canadensis was included in the *United States Pharmacopoeia* between 1830 and 1926 and the US *National Formulary* from 1926 to1960 (Upton, 2001).

4. Phytochemistry

Alkaloids

The United States Pharmacopoeial Convention has defined goldenseal based on alkaloid concentrations, such that the dried roots and rhizomes comprising no less than 2% hydrastine and 2.5% berberine (USP, 2003). The British Herbal Compendium recommends similar values: 1.5 - 4% hydrastine, 2.5% berberine and 0.5% canadine (Bradley, 1992).

Isoquinoline alkaloids were first found in *H. canadensis* as early as the 1820s. Initially, a yellow-

Table 1. Isoquinoline alkaloids from *H. canadensis*

Major alkaloids	Minor alkaloids
Berberine	Hydrastinine
(—)-(*β*)-hydrastine	Canadaline
(—)-canadine	Isohydrastidine
	1-β-hydrastine
	5-hydroxytetrahydroberberine
	(S)-corypalmine
	(S)-isocorypalmine
	(S)-tetrahydropalmatine
	Berberastine
	8-oxotetrahydrothalifendine
	Canadinic acid

(Galeffi, Cometa, Tomassini & Nicoletti, 1997; Govindan & Govindan, 2000; Upton, 2001; Hwang, Roberts, Chadwick, Wu, & Kinghorn, 2003; Weber et al., 2003; Brown and Roman, 2008)

colored compound named berberine was isolated from the European herb *Berberis vulgaris* L., while in 1828 Rafinesque discovered a yellow-colored compound in *H. canadensis* which he named hydrastine. This name persisted in the US until the 1860s when Mahla established that the *H. canadensis* compound referred to as hydrastine was the salt of an alkaloid, that alkaloid being berberine (Lloyd & Lloyd, 1884). The Eclectics, in particular, were unwilling to forsake the original name introduced by Rafinesque in favour of the European designated name (Lloyd & Lloyd, 1884). Lloyd as pharmacist prepared pure samples of berberine salts which he found crystallized into dark brown-red needles, and submitted them to Professors Power and Coblentz, whose analyses confirmed their identity and established the formula as $C_{20}H_{17}NO_4$ (Lloyd & Lloyd, 1884). Berberine is classed as a quaternary protoberberine alkaloid, occurring mainly as water soluble cations, and with a very wide distribution in the plant kingdom (Grycová, Dostál, & Marek, 2007). Hydrastine is a phthalidisoquinoline alkaloid, possessing a tetracyclic nucleus incorporating a γ-lactone ring. Canadine is a tertiary protoberberine alkaloid, lacking conjugation in ring C (Wagner, Bladt, & Zgainski, 1984; Galle, Müller-Jakic, Proebstle, Jurcic, Bladt, & Wagner 1994; Gocan, Cimpan, & Muresan, 1996).

The colorless phthalidisoquinoline alkaloids hydrastine (not to be confused with Rafinesque's alkaloid mentioned above) and canadine, were first isolated from *H. canadensis* by Perrins in 1862 and Hale in 1873, respectively (Felter & Lloyd, 1898). Lloyd's adaption of Hale's method for extracting (the true) hydrastine 'in a pure state' from *H. canadensis* is described by Professor Power, who contributed a treatise on the compound to the American Pharmaceutical Association confirming the formula as $C_{22}H_{23}NO_6$ (Power, 1884). Despite several attempts, Lloyd was unable to confirm the presence of "Hales's third alkaloid" as he called the substance which later became known as canadine

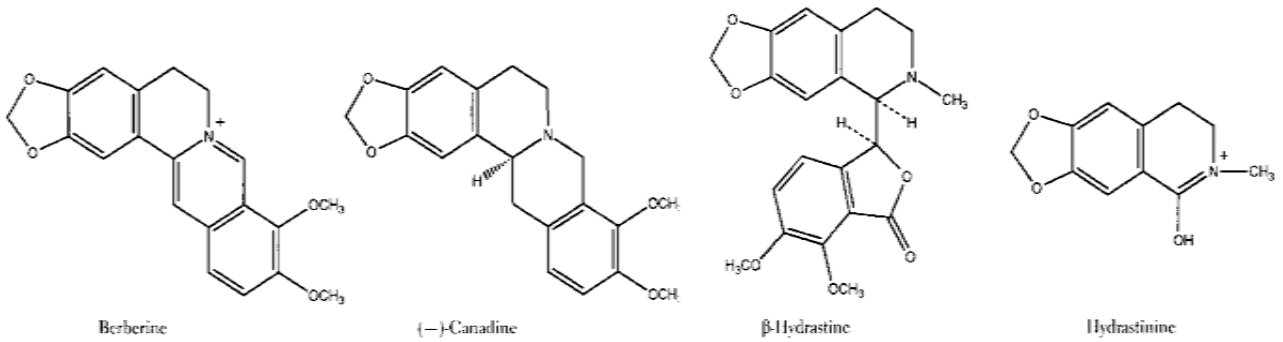


Figure 3. Structures of major alkaloids from *H. canadensis*. Reproduced with permission from *American Herbal Pharmacopoeia* Goldenseal root monograph (Upton, 2001).

(Lloyd & Lloyd, 1884).

By this time the U.S., British and many European pharmacopoeias included *H. canadensis* as an official drug. Quantitative methods such as the Keller-Rusting-Fromme assay were developed, leading to the commercial availability of standardized extracts (Motter & Wilbert, 1912). Given that initially hydrastine was found only in *H. canadensis*, it became the key quality marker for comparing commercial fluid extracts and tinctures, and in one market survey the majority of products tested failed to achieve the minimum levels for hydrastine recommended by the USP (Lancaster & Davidson, 1927). During the twentieth century gravimetric, spectrophotometric, chromatographic, crystallographic and electrophoretic methods were used to determine the presence of hydrastine and berberine in *H. canadensis* (Keenan, 1948; Van Arkel & Meijst, 1952; Zwaving & de Jong-Havenga, 1972; El-Masry, Korany, & Abou-Donia, 1980). Using sensitive fluorometric methods developed in the 1950s, levels of hydrastine could be more accurately determined (Brochmann-Hanssen & Evers, 1951).

The advent of liquid chromatography (LC) methods coupled with a wide array of highly sensitive spectrometric methods has prompted a new generation of phytochemists to investigate the species (Sturm & Stuppner, 1998; Inbaraj, Kukielczak, Bilski, Sandvik, & Chignell, 2001; Chadwick, Wu, & Kinghorn, 2001; Upton, 2001; Weber et al., 2001; Li & Fitzloff, 2002; Edwards & Draper, 2003; Brown, Paley, Roman, & Chan, 2008; Weber, et al., 2003b; Weber & Joseph, 2004; Unger, Laug, & Holzgrabe, 2005; Inbaraj et al., 2006; Gupta, Hubbard, Gurley, & Hendrickson, 2009). Several new alkaloids have been reported including berbastine – a second quaternary protoberberine - however, these are of relatively low concentration. Gentry et al. (1998) isolated a lactam-containing alkaloid 8-oxotetrahydrothalifendine. A list of all currently identified alkaloids in *H. canadensis* is presented in Table 1. Molecular structures of the major alkaloids are shown in Figure 3.

Chemical testing for identity and purity

Adulteration and substitution of *H. canadensis* with

Table 2. Potential adulterants of *H. canadensis* and their alkaloids.

Species	Alkaloids
Berberis vulgaris L. *B. aristata* DC.	Berberine Berbamine Palmatine
Mahonia aquifolium (Pursh). Nutt. *M. nervosa* (Pursh). Nutt.*	Berberine Oxyacanthine Palmatine
Coptis chinensis Franch. *Coptis* spp. Salisb.	Berberine Epiberberine Palmatine Coptisine Jatorrhizine Columbamine
Xanthorhiza simplicissma Marshall	Berberine Jatorrhizine
Rumex crispus L.	None
Chelidonium majus L.	Berberine Chelidonine Cheryletherine Coptisine Protopine Sanguinarine Stypoline

* *Mahonia aquifolium* and *M. nervosa* are synonymous with *Berberis aquifolium* and *B. nervosa* respectively. According to *The Plant List* (2010) classification of these species remains unresolved.

other berberine-containing species or other substances of yellow appearance has been going on since the 1800s (Motter & Wilbert, 1912; Lancaster & Davidson, 1927; Datta, Bose and Ghosh, 1971; Govindan & Govindan, 2000). Chemical methods for validating the identity and purity of *H. canadensis* products are still focused on quantifying berberine and hydrastine levels. The British Pharmacopoeia set levels for the dried rootstock at 3.0% for berberine and 2.5% for hydrastine (British Pharmacopoeia Commission, 2006). The USP further defined goldenseal powdered extracts as containing not less than 5% hydrastine and 10% total alkaloids (USP, 2003).

Tims (2006) has subsequently developed a LC-MS method that was able to resolve ten analytes (berbamine, berberine, canadine, chelerythrine, coptisine, hydrastinine, hydrastine, jatrorrhizine, palmatine and sanguinarine) from six different plant species (*H. canadensis, Coptis japonica, Berberis vulgaris, Chelidonium majus, Mahonia aquifolium* and *Sanguinaria canadensis*), quantitating the presence of adulterants as low as 5%.

Recently an LC method validated in accordance with AOAC single-laboratory guidelines for determining levels of berberine and hydrastine in *H. canadensis* has been reported (Brown et al., 2008; Brown & Roman, 2008). A validated LC/MS method was used to analyse *H. canadensis* root from three suppliers along with the common adulterants (Weber, et al., 2003a; Weber et al., 2003b). Adopting this HPLC method, Kamath, Skeels, & Pai, (2009) identified different alkaloid profiles for *H. canadensis* and two berberine-containing *Coptis* species (see Figure 4).

Other analytical systems have been developed to detect both major and minor *H. canadensis* alkaloids, including capillary electrophoresis-mass spectrometry used for berberastine, berberine, β-hydrastine, canadaline and canadine (Sturm and Stuppner, 1998); a pH-zone refining counter current chromatography used for detecting berberine, canadaline, canadine β-hydrastine and isocorypalmine (Chadwick et al., 2001); and shifted subtracted Raman spectroscopy (SSRS) used for berberine (Bell et al., 2002). An enzyme-linked immunosorbent assay (ELISA) using monoclonal antibodies and linked to a HPLC system has been successfully used for quantitative analysis of

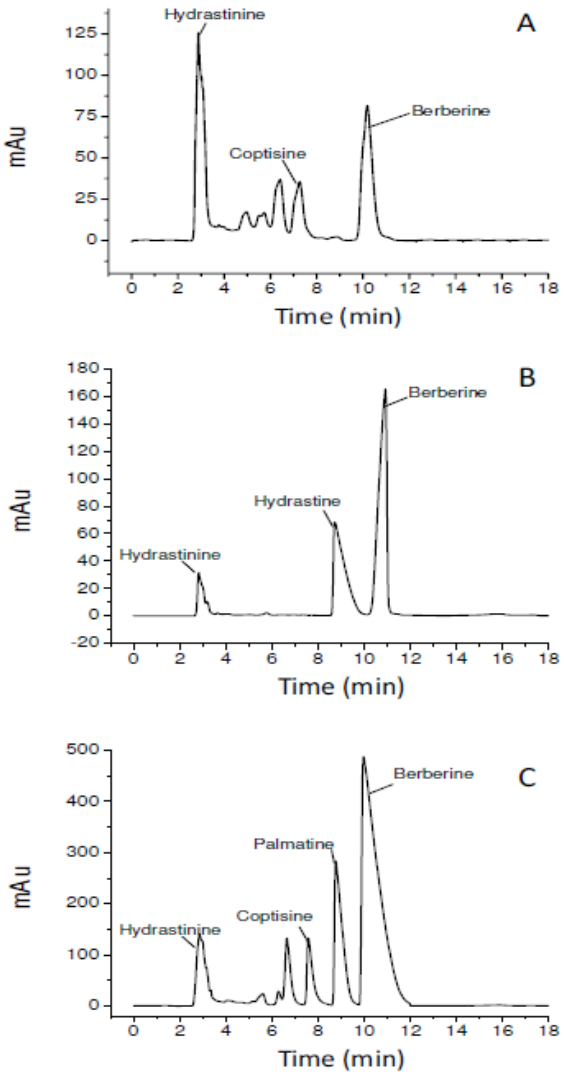


Figure 4. HPLC chromatograms of (A) *Coptis trifolia*, (B) *H. canadensis* and (C) *C. chinensis*. Coptisine and palmatine are absent from *H. canadensis*, while hydrastine is found only in *H. canadensis*. Reproduced from Kamath, Skeels, & Pai (2009). *Chinese Medicine* – Open Access Publication, published by BioMed Central http://www.biomedcentral.com/about/license

berberine in *H. canadensis* and other species (Kim, Tanaka, & Shoyama, 2003).

Despite the increasing sophistication and versatility of LC systems, low cost thin layer chromatography (TLC) methods remain a method of choice within herb industry quality control laboratories. Datta, Bose and Ghosh, (1971) described a TLC and ultra violet (UV) absorption method for authenticating homeopathic tinctures of *H. canadensis.* Two spots present on the chromatograms tested positive for alkaloids, and the application of chemical reagents coupled with UV spectrum analysis indicated the presence of berberine and hydrastine (Datta, Bose, & Ghosh, 1971). Govindan & Govindan, (2000) employed TLC to estimate levels of hydrastine and berberine in ten goldenseal preparations (eight extracted from authenticated raw samples, two from commercial capsules containing powder). Results showed only half of the ten samples tested contained both alkaloids, in varying quantities and ratios. Four of the products tested contained only berberine, while one sample contained neither hydrastine nor berberine, indicating substitution with less expensive alternatives. The findings were verified using HPLC analysis (Govindan & Govindan, 2000).

In their *Goldenseal Root* Monograph, the American Herbal Pharmacopoeia (AHP) assert that mere presence of specific alkaloids is insufficient to rule out the potential of adulteration with other berberine-containing plants, but that screening for alkaloids not found in *H. canadensis* is equally important (Upton, 2001). To this end the AHP adopted a validated high performance TLC (HPTLC) method that analyses palmatine (found in common substitutes), along with berberine, hydrastine and hydrastinine. Hydrastinine is considered a reliable marker for old or poor quality *H. canadensis*, as it is primarily a degradation product of hydrastine (Upton, 2001). This method successfully distinguishes *H. canadensis* from several potential adulterants (see Table 2).

TLC can also be coupled with mass spectrometry (TLC-MS) for improved analyte detection. The technology has been used for fast screening of both high and low molecular weight compounds including alkaloids (Santos, Haddad, Höehr, Pilli, & Eberlin, 2004). Van Berkel and co-workers developed a TLC/desorption electrospray ionization (DESI)-MS method to analyse a selection of commercial goldenseal products and standards (Van Berkel, Tomkins, & Kertesz, 2007). Quantification of berberine and hydrastine agreed closely with the values stated on the labels of two products, however there was marked variation in alkaloid levels of other products, and the authors concluded that one product contained alkaloids from an undeclared herb - thereby implying the presence of an adulterant (Van Berkel et al., 2007).

Distinguishing *H. canadensis* from potential adulterants, proof of principal

Although HPLC is a useful tool for identifying the presence of adulterants, HPTLC, a more practical, affordable and greener technology, is sufficient for identifying medicinal plants such as *H. canadensis* and distinguishing adulterants. Six samples of ethanolic extracts of *H. canadensis radix* from various herb companies were assayed using HPTLC to distinguish the gldenseal alkaloids (Figure 5). In this study other berberine containing plant species - potential adulterants - were also analyzed for comparison to *H. canadensis* (Figure 6).

Figure 5 shows *H. canadensis* extracts from various manufacturers. Lanes 1 and 2 illustrate fresh root extracts, while lanes 4-6 show the dried root extracts. The reference standards berberine, palmatine and β-hydrastine can be seen in lane 7. In spotting the HPTLC plates, the different herb-to-extract ratios were accounted for and normalized. All lanes were loaded with volumes of extract that accounted for approximately the same mass of plant material, based on the herb-to-extract ratio of each extract.

Figures 5A & 5B illustrate the presence of berberine and hydrastine (although with varying band densities) in all extract samples analyzed. Figures 5A and 5B also show the absence of palmatine in all sample lanes. Given the presence of berberine and hydrastine and the absence of palmatine in these samples, these data suggest that none of the *H. canadensis* extracts studied were adulterated with other berberine containing plants.

Figure 5B illustrates the HPTLC plate after derivatization with ninhydrin reagent visualized under 366 nm UV light. Berberine is seen in all samples analyzed. Figure 5B also shows a band above the berberine band (R_f ~0.95) that is of an unidentified phytochemical. The density of this band varies significantly from extract to extract, being absent in the extracts from companies A and F and having the highest density in the extract from company B. However, the significance of this difference in banding densities cannot be interpreted without identification of the corresponding phytochemical. Nonetheless, it does illustrate that there are differences between *H. canadensis* extracts produced by various companies.

Figure 6 illustrates the HPTLC analysis of various medicinal plant species containing berberine: *Mahonia repens* (Lindl.) G. Don (lane 1), *Berberis vulgaris* (lane 2), *Xanthorhiza simplicissima* (lane 3), *H. canadensis* (lane 4) and *Coptis chinensis* (lane 5). Hydrastine at Rf ~0.3 is clearly present in *H. canadensis* (lane 4) but is absent from the other species.

In addition, palmatine (Rf ~0.4) is present in all species except *H. canadensis*. As demonstrated in these relatively quick and inexpensive assays, HPTLC can be sufficient and practical for the identification of *H. canadensis* and its potential adulterants. Moreover, a practical approach to the detection of an adulterant in a *H. canadensis* lot of root material would be the detection of palmatine and hydrastine in the same sample. This would indicate that the *H. canadensis* lot has been adulterated with a berberine containing plant, a common adulterant strategy.

Materials and methods

Chemicals and Standards: All reagents were purchased from VWR (Bridgeport, NJ). Standards were purchased from Chromadex (Santa Anna, CA).

Preparation of material for analysis: Samples of hydroethanolic extracts of *H. canadensis radix* were prepared with a 2:3 (extract:methanol) dilution. To equalize all extracts to the same concentrations various root to menstruum ratios were normalized by adjusting spotting volumes. A confounding issue in the production of fresh root extracts has been the substantial water content of fresh plant. Since fresh root tinctures are generally extracted at 1:2 but this does not include the water content of the plant material, we accounted for the additional water from the root of *H. canadensis* into our sampling. When the water content of the root (≈70%) is considered in these extracts, the actual plant mass (dry equivalent) to menstruum ratio is 1:9. We therefore utilized a 1:9 ratio to calculate a comparative mass of plant material for spotting the fresh plant extracts onto the HPTLC plates.

Chromatographic conditions: HPTLC was performed on CAMAG (Multenz, Switzerland) pre-coated silica gel 60 F254 HPTLC plates (10 x 10 cm, 300 µm layer thickness). Samples were applied to the layers as10 mm-wide bands positioned 10 mm from the bottom and 8 mm from the side of the plate, using a CAMAG Linomat 5 automated TLC applicator with the nitrogen flow providing a delivery speed of 150 nL/second from the syringe. After sample application plates were developed in a CAMAG twin trough glass tank pre-saturated with the mobile phase EtOAc:MeOH:Formic acid:Water (50:10:6:3, v/v). The plates were developed to a distance of 80 mm. The TLC assays were performed under laboratory conditions of 25 ± 5˚C and 27 % relative humidity.

Detection: After drying (RT, 5 min) the chromatographic separation was visualized in a CAMAG UV cabinet (white light, 254 and 366 nm). Post-chromatographic derivatization was performed with ninhydrin reagent (dried 100 ˚C, 2 min). The chromatographic conditions had previously been optimized to achieve the best resolution.

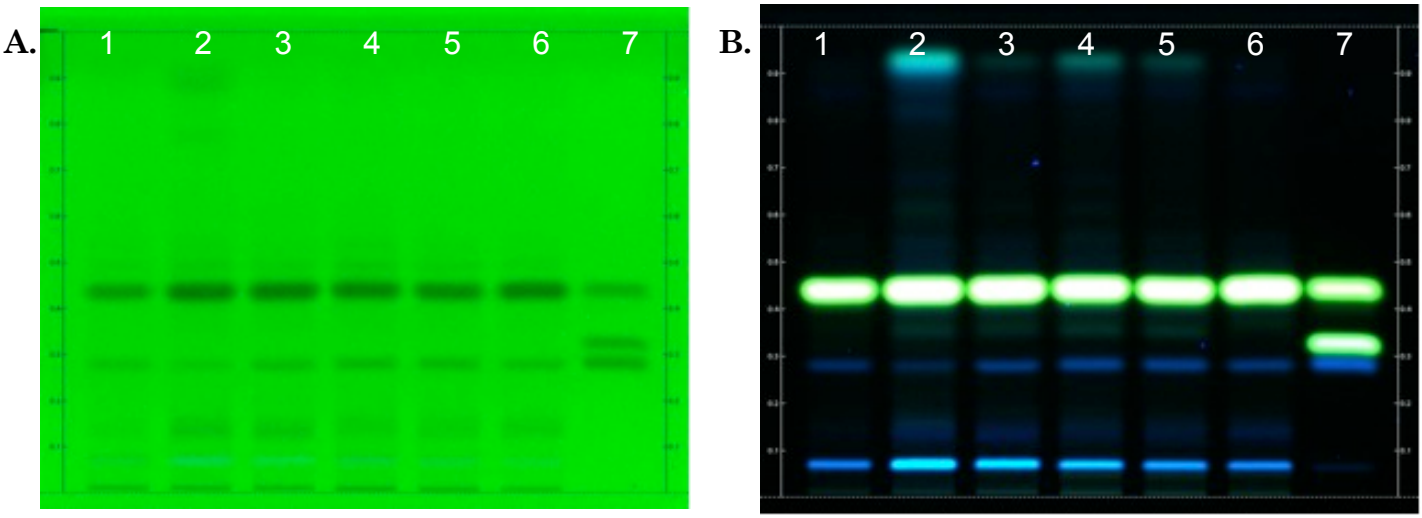


Figure 5. HPTLC Plate of *Hydrastis canadensis radix* ethanol (60%) extracts. From the Herb Pharm Research and Development Laboratory, Analyst Jean Brown. A) wavelength=254 nm B) Ninhydrin derivatization, wavelength=366nm.

Lane 1: Company A, fresh root 1:2; Lane 2: Company B, fresh root 1:2; Lane 3: Company C, dry root 1:3; Lane 4: Company D, dry root 1:4; Lane 5: Company E, dry root 1:5; Lane 6: Company F, dry root 1:5; Lane 7: Reference compounds: berberine (upper), palmitine, hydrastine (lower).

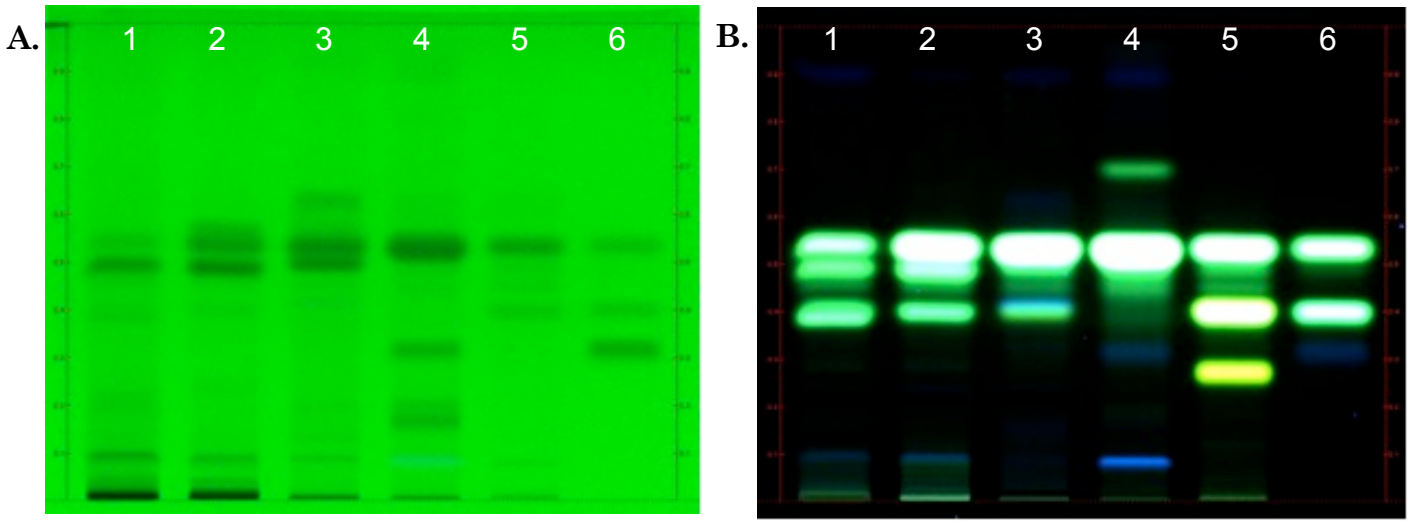


Figure 6. HPTLC Plate of multiple plant species containing berberine. From the Herb Pharm Research and Development Laboratory, Analyst Jean Brown. A) wavelength=254 nm B) Ninhydrin derivatization, wavelength=366nm.
Lane 1: *Mahonia repens*; Lane 2: *Berberis vulgaris*; Lane 3: *Xanthorhiza simplicissima*; Lane 4: *Hydrastis canadensis*; Lane 5: *Coptis chinensis*; Lane 6: Reference compounds: berberine (upper), palmitine, hydrastine (lower).

Biosynthetic studies

For many decades scientists have been investigating possible biosynthetic pathways towards the formation of isoquinoline alkaloids in plants. Zenk and co-workers described the biosynthesis of berberine from the amino acid L-tyrosine via a series of 13 enzymatic reactions, while *(S)*-reticuline was found to be the key branch-point intermediate in the biosynthesis of individual alkaloids (Zenk, Rueffer, Amann, Deus-Neumann, & Nagakura, 1985; Rueffer & Zenk, 1985; Zenk, 1995). This was the first completely described alkaloid biosynthetic pathway, with all 13 enzymes identified and named (Otani et al., 2005). Complementary DNA (cDNA) of several of the enzymes has been identified and cloned, opening up prospects for metabolic engineering of this and other alkaloids (Sato et al., 2001). Berberine bridge enzyme (BBE) catalyzes the initial step in converting *(S)*-reticuline to the quaternary form of berberine (Bird & Facchini, 2001). Using RNA interference (RNAi) technology Fujii and co-workers transformed cultured California poppy (*Eschscholzia californica* Cham.) cells by down-regulating the BBE reaction, which led to accumulation of the key metabolite reticuline without influencing cell growth (Fujii, Inui, Iwasa, Morishige, & Sato, 2006).

Studies on the berberine-containing *Coptis japonica* Thunb. Makino reveal the presence of a cDNA encoding a multidrug-resistance protein (MDR). The transport protein slows down the influx of berberine into the cells of rhizomes via an ATP-dependent process, thereby helping to prevent the accumulation of toxic levels of the alkaloid (Shitan et al., 2003). Otani et al. (2005) identified a second transport mechanism in *C. japonica* rhizomes, an H+/antiporter that effluxes berberine rapidly from the cytosol to the vacuole via a proton gradient. Since *Coptis* is also in the Ranunculaceae it is likely that similar alkaloid transport mechanisms are present in *H. canadensis*.

Phenylpropanoids

Chlorogenic acid, neochlorogenic acid – highest in leaves (McNamara, Perry, Follett, Parmenter, & Douglas, 2004)

Flavonoids

Using nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectroscopy, two new *C*-methyl flavonoids were identified: 6,8-*C*-dimethylluteolin 7–methyl ether, 6-*C*-methylluteolin 7–methyl ether (Hwang et al., 2003). In a bioactivity-guided fractionation study utilizing preparative HPLC, three flavonoids previously reported in *Eucalyptus* spp. were isolated: sideroxylin, 8-desmethyl-sideroxylen, 6-desmethyl-sideroxylin (Junio et al., 2011).

Organic acids

Quinic acid derivatives, hycandinic acid esters (Gentry et al., 1998). A newly discovered compound 5-*O*-(4‘-[β-d-glucopyranosyl]-*trans*-feruloyl) quinic acid could serve as a non-alkaloidal marker for *H. canadensis roots* (McNamara et al., 2004).

Sterols

β- sitosterol 3-*O*- *β*-D-glucoside (Hwang et al., 2003).

Other constituents

Volatile oil, resin, fatty acids (Upton, 2002).

5. Pharmacology and Toxicology

Pharmacokinetics

Analysis of human urine following oral administration indicates the bioavailability of berberine is quite low, however significant levels of berberine conjugates have been detected (Yu et al., 2000; Pan et al., 2002b). A validated LC-MS/MS method for determining levels of *H. canadensis* alkaloids in human serum has been reported. Berberine was shown to be absorbed more rapidly

following oral administration of the *H. canadensis* test supplement compared to administration of isolated berberine (Gupta et. al., 2009).

A Chinese study involving beagle dogs demonstrated low bioavailability for berberine hydrochloride following oral administration (Shen, Sun, & Wang, 1993). This finding was subsequently confirmed in rats, where <5% bioavailability was observed for berberine (Pan, Wang, Liu, Fawcett, & Xie, 2002). Berberine and other *H. canadensis* alkaloids are substrates for the xenobiotic efflux pump p-glycoproteins (P-gp), and observed interactions with P-gp inhibitors suggests P-gp inhibits net absorption of berberine in the gut (Pan et al., 2002a). Phase I metabolites of berberine - known to be biologically active - have been identified using LC/MS, and found to remain in the plasma of rats for sustained periods (Zuo, Nakamura, Akao, & Hattori, 2006). *In vitro* microbial transformation of hydrastine using a fermentation broth model representing human metabolism has been demonstrated (Herath, Ferreira, & Khan, 2003).

H. canadensis alkaloids possess methylenedioxyphenyl (MDP) moieties which interact with cytochrome P450 (CYP) enzymes to form complexes (Chatterjee & Franklin, 2003). Both the root extracts and individual alkaloids inhibited CYP reactions, particularly the CYP3A4 isoform, in which hydrastine was more inhibitory than berberine. CYP inhibitory activity for *H. canadensis* extracts was confirmed in humans for both CYP 3A4 and CYP2D6 isoforms, at doses between 2.7g and 3.9g daily taken in three separate doses (Gurley et al., 2005; Gurley et al., 2008a; Gurley et al., 2008b). *In vitro* studies in human liver microsomes indicate that *H. canadensis* extract, berberine, hydrastine and canadine inhibit the CYP2E1 isoform, with hydrastine the most potent of the alkaloids tested (Raner et al., 2007). Using an 'N-in-one cocktail' method that allows nine major CYP enzymes to be monitored simultaneously with human liver microsomes, methanolic extracts of *H. canadensis* caused significant inhibition of isoenzymes CYP2B6, CYP2C19, CYP3A4, CYP2D6 and CYP2E1 (Sevior et al., 2010). The implication of these collective findings is that ingestion of *H. canadensis* could inhibit the metabolism of pharmaceutical drugs if taken concomitantly (Chatterjee & Franklin, 2003; Gurley et al., 2005; Budzinski et al., 2007) (see below). A review of the pharmacokinetics of *H. canadensis* alkaloids, including a proposed metabolic scheme for berberine, was recently published by the National Institute for Health (National Toxicology Program, 2010).

Pharmacodynamics

There is a large body of literature concerning antimicrobial, antiparasitic, antitumor and other activities associated with berberine (Anonymous, 2000; Mills & Bone, 2000; Grycová et al., 2007). Quaternary protoberberine alkaloids such as berberine react with nucleophiles, disrupt cell membranes, bind to microtubules, act as DNA intercalators, inhibit DNA synthesis and uncouple oxidative phosphorylation (Schmeller, Latz-Brüning, & Wink, 1997; Ball et al., 2006; Grycová et al., 2007). The ability of berberine to affect so many molecular targets helps to rationalize the numerous recorded biological activities for *H. canadensis*, however it is evident that other constituents and mechanisms not associated with berberine are also involved.

Antimicrobial Activity

In an antimicrobial screening program *H. canadensis* extract exhibited significant activity against multiple drug resistant strains of *Mycobacterium tuberculosis*, other *Mycobacterium* species as well as other human pathogens. Bioassay-guided fractionation revealed berberine to be the active constituent, however berberine alone was less active than the crude extract (Gentry et al., 1998). Scazzocchio and co-workers found *H. canadensis* extracts were active against both Gram positive and Gram negative strains of pathogenic microorganisms. In this case canadine's activity was similar to berberine, but canadaline provided the strongest inhibitory activity, especially

of the Gram positive organisms tested – *Staphylococcus aureus* (2 strains) and *Streptococcus sanguis* - based on killing times and minimal inhibitory concentrations (MIC values) (Scazzocchio, Cometa, Tomassini, & Palmery, 2001). Once again the whole-plant extract demonstrated equivalent or superior antibacterial activity compared to the individual alkaloids. Similarly *H. canadensis* extract inhibited the growth of resistant strains of *Neisseria gonorrhoeae*, while berberine alone was less effective (Cybulska et al., 2011).

Berberine and two flavonoids from *H. canadensis*, but no other constituents, showed inhibitory activity against oral pathogens *Streptococcus mutans* and *Fusobacterium nucleatum*, the results suggesting one or more of the flavonoids potentiated the effect of berberine (Hwang et al., 2003). *H. canadensis* extracts also inhibited growth of *Helicobacter pylori* in a screening survey of medicinal plants using a micro-dilution assay (Cwikla et al., 2010).

These studies suggest that while berberine is a key constituent, on its own it cannot reproduce the demonstrated antimicrobial properties of *H. canadensis*. In fact berberine is regarded as a rather weak antibacterial at low concentrations (Stermitz, Lorenz, Tawara, Zenewicz, & Lewis, 2000). However mechanisms for infection control and relief of symptoms that do not involve inhibition of bacterial growth have been established for berberine. Sack & Froehlich, (1982) demonstrated antisecretory effects against *Vibrio cholerae* and *E. coli* enterotoxins, providing a rationale for the traditional use of berberine-containing species in treating diarrheal disease. Berberine also reduces adherence of *E. coli* and *Streptococcus pyogenes* to epithelial cells (Sun, Abraham, & Beachey, 1988; Sun, Courtney, & Beachey, 1988). Reduction of the *E. coli* load in the gut by these mechanisms is likely to also reduce the migration of pathogenic bacteria to the urinary tract, hence helping prevent urinary tract infections (Amalaradjou & Ventikanarayanan 2011).

Bacterial resistance

Isoquinoline alkaloid cations are known substrates for bacterial multi-drug resistant (MDR) efflux pumps, which in turn effectively pump constituents such as berberine back out of bacterial cells (Tegos, Stermitz, Lomovskaya, & Lewis, 2002). However Stermitz et al. (2000) demonstrated that in the presence of an MDR inhibiting compound (5'-methoxyhydnocarpin {5'-MHC}), found in certain North American species of *Berberis*, the antimicrobial potency of berberine was potentiated due to increased accumulation in the bacterial cell. Synergistic combinations of berberine with MDR inhibitors have been used as models for new antimicrobial drugs that avoid bacterial resistance (Ball et al., 2006). With respect to *H. canadensis*, extracts of aerial portions (but not the roots) were shown to contain efflux pump inhibitors, however this effect was not associated with berberine, hydrastine or canadine, which are more concentrated in the roots (Ettefagh, Burns, Junio, Kaatz, & Cech, 2010). Synergism between the berberine and the *H. canadensis* aerial parts extract led to enhanced antibacterial action. Subsequently synergy-directed fractionation of *H. canadensis* leaf extracts tested against *Staphylococcus aureus* led to the identification of three flavonoid MDR pump inhibitors in the leaves (Junio et al., 2011). The authors conclude that a combination of berberine containing roots and flavonoid containing leaves prepared from *H. canadensis* could produce an extract with optimal antibacterial activity. Unlike berberine, canadine does not act as a substrate for MDR inhibitors (Abidi, Chen, Kraemer, Li, & Liu, 2006).

Fewer investigations have been conducted into effects on viruses, but in a recent study berberine inhibited two strains of H1N1 influenza virus *in vitro* by several orders of magnitude more than the anti-influenza A drug amantadine. *H. canadensis* extracts were also effective - but only at high concentrations (Cecil, Davis, Cech, & Laster, 2011).

Immunological Activity

Despite the widespread belief that *H. canadensis* is an immunostimulant (Chatterjee & Franklin, 2003), there are few studies to substantiate the claim (Bergner, 1997). Studies on rats exposed to the KLH antigen indicate augmented immunoglobulin (IgM) response for the first two weeks of a six week treatment period, but failed to improve the IgG response (Rehman et al., 1999). Berberine has been shown to inhibit production of TNF-α and the prostaglandin PGE_2 from macrophages induced by influenza A virus, in an *in vitro* study (Cecil et al., 2011).

Anti-cancer effects

Berberine (as berberine sulfate) inhibited the activity of two tumor promoting compounds in cell cultures in dose-dependent manner, and produced significant reduction of the tumor yield and percentage of tumor-bearing mice (Nishino, Kitagawa, Fujiki, & Iwashima, 1986). Mechanisms for the purported anticancer activity of berberine include complexation with DNA and RNA, inhibition of *N*-acetyltransferase (NAT) in tumor cells, inhibition of cycloxygenase II (Cox-2), inhibition of teleromerase and topoisomerase, antiproliferent effects on tumor cells, activation of multiple signaling pathways, antioxidant activity, inhibition of nuclear factor-κB activation, inhibition of metastases and improvement of multi-drug resistance (Sun, Xun, Wang, & Chen, 2009).

Little work has been conducted on *H. canadensis* itself. In the form of a potentized homeopathic drug (200C), *H. canadensis* decreased chemically-induced tumors in rats compared to controls, while decreasing levels of the elevated marker enzymes ALP, GPT, and GOT (Kumar et al., 2007). In a separate study the homeopathic 'mother tincture' (equivalent to a 1:10 herbal tincture) was highly cytotoxic in a variety of cancer cell lines, while in Ehrlich carcinoma cells the 200C potency showed higher cytotoxicity. The mother tincture and the 30C potency, but not the 200C potency, induced apoptosis in DLA cells (Sunila, Kuttan, Preethi, & Kuttan, 2009).

Cardiovascular effects

Investigations involving berberine indicate numerous beneficial effects on the cardiovascular system (Lau, Yao, Chen, Ko, & Huang, 2001). These include positive inotropic, antiarrhythmic, vasodilatory and antihypertensive effects (Lau et al., 2001). *H. canadensis* extract markedly upregulated low density lipoprotein receptor (LDLR) expression in HepG2 (hepatic) cells, thereby decreasing cholesterol and lipid accumulations in plasma and the liver *in vivo* (Abidi et al., 2006). In this study the alkaloid canadine demonstrated greater activity on LDLR expression than did berberine.

Influence on glucose metabolism

Berberine has long been investigated for purported hypoglycemic activity, and two pilot studies in humans indicate that it may be as effective in Type-2 diabetes as the prescribed drug metformin (Yin, Xing, & Ye, 2008). Various mechanisms of action have been proposed. Investigations using the Caco-2 cell line indicate berberine decreases glucose absorption from the intestine by α–glucosidase inhibition (Pan et al., 2003). Berberine may also improve insulin action by activating 5' adenosine monophosphate-activated protein kinase (AMPK) – a key enzyme in cellular energy homeostasis (Steriti, 2010). When *H. canadensis* extract was tested on streptozotocin-induced diabetic mice there was no influence on blood sugar or insulin levels, however hyperphagia and polydipsia – symptoms associated with diabetes – were significantly reduced (Swanston-Flatt, Day, Bailey, & Flatt, 1989).

Effects on smooth muscle

Many isoquinoline alkaloids have spasmolytic properties analogous to papaverine. At low concentrations berberine induced aortic relaxation in rats by an endothelium dependent mechanism (Wong, 1998). An *H. canadensis* alcoholic extract

induced smooth muscle relaxant effects in rat uterus and guinea-pig trachea. Some but not all of this activity was attributed to interaction of *Hydrastis* alkaloids with β–adrenergic receptors (Cometa, Abdel-Haq, & Palmery, 1998). Other possible mechanisms for relaxant effects (on isolated guinea-pig trachea) include interaction with adenosinic receptors, K^{+}, or Ca^{2+} channels, and the releasing of nitric oxide (Abdel-Haq et al., 2000).

Toxicology

While there is no acute toxicity data available for *H. canadensis*, oral LD50s for berberine in mice (329 mg/kg) and berberine sulphate in rats (>1,000 mg/kg) have been published, indicative of high level of safety when used orally (National Toxicity Program, 2010). An investigation into acute toxicity of hydrastine and hydrastinine determined LD50s for intraperitoneal administration in rats (Poe & Johnson, 1954). Hydrastine showed 'a fairly high toxicity' but the relevance of these findings to oral consumption is unclear.

No evidence of fetus malformation or developmental toxicity was seen in mice or rats in reproductive toxicity studies conducted by the National Toxicity Program (NTP) (National Toxicity Program, 2010). Reproductive screening tests conducted at the University of Sydney provided contrasting results between *in vivo* and *in vitro* studies (Yao, Ritchie, & Brown-Woodman, 2005). There was no increase in pre-or post- implantation losses or any malformations in female rats given acute doses equivalent to 65 times the daily human dose of *H. canadensis*, however cytotoxic effects were observed in cultured rat embryos exposed to the extract. The authors conclude that on the basis of their *in vivo* findings, *H. canadensis* at prescribed human doses is safe for use during pregnancy, and the discrepancy with the *in vitro* findings is most likely due to poor absorption from the small intestine (Yao et al., 2005).

In the most recent NTP investigation (TR 562), male and female rats fed diets containing between 9,000-50,000 ppm *H. canadensis* root powder for 2 years incurred increased incidences of hepatocellular adenomas or carcinomas. There was also some evidence of carcinogenic activity in male but not female mice exposed to the same regime (National Toxicity Program, 2010). While survival rates in treated male rats were reduced, treated female rats had increased survival rates (significantly so at the 9,000 ppm dose p= 0.025) over the 2 years compared to controls. There was no change in survival rates for mice. Although the doses that proved toxic in this study were at the high end of the equivalent human dose range, clearly long term oral consumption of the herb should be avoided.

One case study was cited in which an 11-year-old African American girl with type-1 diabetes who had taken 1,000-1,500mg goldenseal supplement daily for at least 2 weeks, presented with diabetic ketoacidosis (Bhowmick, Hundley, & Rettig, 2007). No further details of the supplement are given. Investigations revealed the patient had severe hypernatremia and hyperosmolality, which the authors attribute to the herbal supplement. There are suggestions that *H. canadensis* acts as an aquaretic, allowing for excretion of water while sodium is retained (Chavis, 2003).

Drug interactions

As noted above, *in vitro* and *in vivo* studies indicate *H. canadensis* inhibits some human cytocrome P-450 isoenzymes, potentially influencing the bioavailability of pharmaceutical drugs taken concomitantly. One such isoenzyme - CYP3A4 – metabolizes the anti-HIV drug indinavir. However in a study of 10 healthy human volunteers *H. canadensis* extract had no influence on the bioavailability of indinavir (Sandhu, Prescilla, Simonelli, & Edwards, 2003). Despite this finding, many apparently authoritative sources advise against the use of *H. canadensis* while taking indinavir or other prescription drugs known to be metabolized by CYP3A4 or CYP2D6 isoenzymes (Cassileth, Yeung & Gubili, 2010). Unfortunately, at this time many drug-herb interactions are based on *in vitro* research

giving rise to weakly supported theoretical concerns (Freeman & Spelman, 2008).

Another mechanism by which *H. canadensis* could potentially influence drug bioavailability is via multi-drug resistance (MDR) regulation by berberine and/or other alkaloids. In one study berberine induced the MDR transporter Pgp-170 expression in a range of cell lines, and reduced response of cancer cells to the cancer drug Paclitaxel (taxol) (Lin, Liu, Wu, & Chi, 1999).

H. canadensis may also inhibit the actions of antihypertensive and anticoagulant drugs, and could potentiate the effects of barbiturates (Cassileth, Yeung & Gubili, 2010).

6. Clinical studies

Berberine has been subjected to a number of clinical investigations, however there are few studies with *H. canadensis* preparations. There have been several published studies on the use of berberine for diarrheal disease coming out of India. These studies have rather mixed results, and the methodologies of some have been criticized (Mahady & Chadwick, 2001). In a study of 127 children with moderate to severe diarrhea, 68.5% responded to berberine treatment (Chauhan, Jain, & Bhandari, 1970). In a randomized controlled trial (RCT) of 165 adults with acute diarrhea linked to *E. coli* enterotoxins or cholera assessed by stool volume data, berberine treated subjects had significantly less diarrhea compare to controls (Rabbani, Butler, Knight, Sanyal, & Alam, 1987). In a previous RCT of 185 adults with cholera set in Burma (Khin-Maung-U, Myo-Khin, Nyunt-Nyunt-Wai, Aye-Kyaw, & Tin-U, 1985) stool volume was also reduced, however berberine-treated subjects suffered more severe symptoms compared with those treated with the antibiotic drug tetracycline.

7. Modern Phytotherapy

Modern therapeutic use of goldenseal reflects pharmacological and clinical research as well as traditional indications. *Naturae Medicina and Naturopathic Dispensatory* (Kuts-Cheraux 1953) notes many indications due to its healing effect on

Table 3: Modern phytotherapeutic uses of *H. canadensis*

Actions	
Bitter tonic	Choleretic
Stomachic	Antiparasitic, antiprotozoac
Antimicrobial	Antihemorrhagic
Antidiarrheal	Oxytocic

Therapeutic indications
Sinusitis, conjunctivitis, tonsillitis, otitis media, respiratory infections with catarrhal discharge
Atonic dyspepsia, gastritis, peptic ulcer, anorexia
Diarrhea, colitis, hepatobiliary disorders
Menorrhagia, dysmenorrhea, salpingitis, leucorrhea
Eczema, acne, boils, abscess, pruritis
Protozoal and tapeworm infestations
Varicose veins, phlebitis
Externally for mouth ulcers, wounds and inflammatory skin disorders, eye baths
In vaginal suppositories for candida infections, trichomonas and gardnerella

(Bradley, 1992; Mills & Bone, 2000; Skenderi, 2003; Trickey, 2003)

damaged mucosa. It is recommended both internally and externally for ophthalmic disorders such as conjunctivitis; naso-pharyngeal disorders where the mucus is thick and membranes swollen; gynecological conditions such as vaginitis; and gastrointestinal disorders such as ulcerative colitis. While acknowledging these uses the British herbalist Frank Roberts adds that *H. canadensis* has a unique action on the venous circulation, namely stimulation of blood returning to the heart (Roberts, 1978). The British Herbal Compendium (Bradley, 1992) repeats traditional indications of "menorrhagia, atonic dyspepsia and gastritis" but also mentions anti-microbial activity of berberine and other alkaloids. Other authors and practitioners extrapolate further from research on the isolated alkaloids and suggest goldenseal to be of benefit in diarrhea, diabetes, hypercholesterolemia and infections in general (Braun & Cohen, 2010). Restoration of mucous membrane integrity is a key effect for modern herbalists (Mills & Bone, 2000). While *H. candensis* has found popularity amongst the American public as a cold and flu preventative or treatment, with rare exceptions (Mitchell, 2003) this use is not supported by modern herbalists and naturopaths. While Tigler (2009) does make reference to the colds and flu indication, she provides in addition this rather more useful symptom picture: "It is used for atonic chronic mucosal problems with pale relaxed tissues as well as sub-acute mucosal mucus membrane problems with red, engorged tissues" (Tigler, 2009, p. 97).

Specific indication

Atonic dyspepsia with hepatic symptoms.

Combinations

With *Commiphora myrrha* (Nees) Engl.and *Echinacea* spp. for boils and abscesses

With *Achillea millefolium* L. and *Geranium maculatum* L. for menorrhagia (Trickey, 2003).

With *Hamamelis virginiana* L. leaf and *Euphrasia* spp. as eye lotion (British Herbal Medicine Association, 1983).

Preparations and dosage

Dried rhizome and root, 0.5-1.2g (Upton, 2001)
Tincture 1:10, 0.3-1mL three times daily (Bradley, 1992)
Tincture 1:5, 8mL daily (American Pharmaceutical Association, 1946)

Side effects and contra-indications

The *Botanical Safety Handbook* classifies *H. canadensis* in Class 2(b): "Not to be used during pregnancy" and noted the fresh plant may cause irritation of the mucosa (McGuffin, Hobbs, Upton, & Goldberg, 1997). According to other sources it should not be used by neonates due to the potential of berberine to displace bilirubin from serum protein (Upton, 2001). Large doses used topically are known to have caused severe skin irritation or ulceration (Upton, 2001). The *British Herbal Pharmacopoeia* (1983) contraindicated *H. canadensis* in hypertension, however that contraindication has been removed in the more recent *British Herbal Compendium* (Bradley, 1992).

Regulatory Status

H. canadensis is regulated in the U.S.A. as a Dietary Supplement.

8. Sustainability and cultivation

Ecological status

Range: *H. canadensis* is native to North America, and can be found growing from Ontario to Arkansas, across the southeastern U.S. to Georgia, and north to Quebec. Cultivation projects in Oregon (Oregon's Wild Harvest, 2012, Baker, 2010), Washington (Frontier Coop, 2012) and British Columbia (Beyfuss, 2012) have been successful in growing the species outside of its native range.

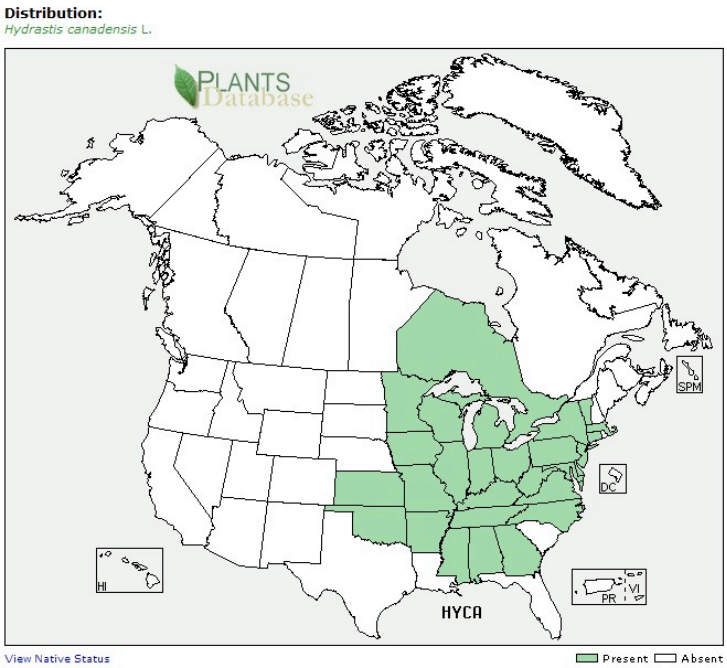


Figure 7. *Hydrastis canadensis* - Natural range and distribution (NRCS, n.d.)

Globally *H. canadensis* is listed as G4: common and apparently secure (NHPMDNR, 2010), though it may be rare in parts of its range, especially at the periphery. According to the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES, 2012), since 1997 *H. canadensis* has been listed in Appendix II which:

> includes species that are not threatened presently with extinction but may become so unless trade is closely controlled. International trade in Appendix-II species requires a permit from the exporting country; no import permit is necessary for these species. Permits are only granted if the exporting country is satisfied that certain conditions are met; above all, that trade will not be detrimental to the species' survival in the wild.

H. canadensis is listed as a threatened plant in Maryland and New York, vulnerable in Pennsylvania, endangered in Connecticut, Georgia, Massachusetts, Minnesota, New Jersey, North Carolina, Vermont and critically endangered in Tennessee (See Table 4). For other states within its' natural range, the lack of a status listing does not imply that the plant is abundant. Some states, such as South Carolina, Louisiana and Florida do not have current estimates of plant populations (NRCS, n.d.).

It should be noted that the status designations are continually evaluated and updated.

Harvesting & Collection regulations

Trade in *H. canadensis* was well-developed by the mid-1800s, and until recently most of this trade was obtained from wild sources. Concern over the possible unsustainable nature of the industry can be found as early as the 1800s (Lloyd & Lloyd, 1884).

Interest in *H. canadensis* as a "crop" can be traced to the early 1900s, as evidenced by a bulletin produced in 1908 by the USDA (Burkhart, 2006).

Global trade and markets for *H. canadensis* are controlled by CITES (US Forest Service, 2010). At the state level, each state varies in its governance and control of *H. canadensis* that is not intended for export. Not all states estimate plant populations or control harvesting/trade (see Table 5). Note: state data frequently changes, please check current state and federal laws before engaging in any harvesting, selling or buying activity with *H. canadensis*.

Market data: Harvesting impact, tonnage surveys

Profits from growing *H. canadensis* vary according to scale of harvest, yield, source, identity cleanliness of product and chemical profile (Burkhart, 2006). Growing *H. canadensis* under artificial shade-cloth may result in greater economic returns due to increases in yield and efficiency, although quality may also be a factor (Burkhart, 2006, 2012).

According to Sharp (2003), *H. canadensis* is one of the top six selling medicinal plants in the United States, and has been listed in the official pharmacopoeias of several European nations, including France, Germany, Italy and Great Britain. Every year ten metric tons of *H. canadensis* roots are exported to the U. K. alone. In 2006, prices per pound for dried roots ranged from $35 wild to $50

Table 4. *H. canadensis* - Status as a rare, threatened endangered plant

Area	Status	Source
Global	G4	Natural Heritage Program Maryland Department of Natural Resources, (2010)
US-federal	none	US Fish and Wildlife Service, (2012)
Connecticut	endangered	Connecticut DEEP, (2010)
Georgia	endangered	Georgia Department of Natural Resources, (2010); US Fish and Wildlife Service, (2012)
Maryland	threatened, S2	Maryland Department of Natural Resources, 2010
Massachusetts	endangered	Natural Heritage and Endangered Species Program, (2008) Massachusetts Division of Fisheries and Wildlife, (1994/2010).
Michigan	threatened, S2	Plants are subject to over-harvesting and habitat destruction. Known in 21 counties, hidden populations are thought to exist in the state (Michigan Natural Features Inventory, 2004)
Minnesota	endangered	At the northern edge of its natural range, *H. canadensis* is known to exist in 5 Minnesota counties. Wild populations continued to be intensively and unsustainably harvested by commercial root diggers. Construction and grazing have also contributed to a loss of local populations and reduced habitat. (Minnesota Department of Natural Resources, 2012)
New Jersey	endangered, S1	NJ Division of Parks and Forestry. (2010)
New York	threatened, S2	New York Department of Environmental Conservation. (2012)
North Carolina		As of December 1, 2010, *Hydrastis canadensis* is no longer listed on the protected plant list for North Carolina. (North Carolina Department of Agriculture and Consumer Services, 2010)
Pennsylvania	S4	The Pennsylvania site linked to NatureServe. January, 2012. (Pennsylvania Natural Heritage Program, nd)
Tennessee	critically endangered, S3	*Hydrastis canadensis* is listed as one of five plants threatened by commercial harvest (Crabtree, 2008)
Vermont	endangered	Note: Vermont offers guidelines for natural plant communities. Vermont Natural Heritage Information Project (2011)

for organic woods-cultivated roots (Burkhart, 2006). From 2006 through 2012 the average price paid for dried goldenseal root has fluctuated between five and nine dollars a pound (Burkhart, 2012).

Table 6 lists the tonnage for wild and cultivated roots over a twelve -year period, while average growers prices over a 42 year period are shown in Figure 8.

Cultivation: Soil Requirements

H. canadensis thrives best in loamy soil with abundant organic matter and a pH between 5.5 and 6.5 (Burkhart, 2006; Eldus, 1996; Cech, 2003; Tilford, 1998). Burkart (2006) notes that *H. canadensis* can be found where there is seasonal flooding as well as in seasonally moist upland areas. Eldus (1996) notes that a 70-80% shaded slope is preferred. Tilford (1998) suggests a dense hardwood canopy on a north-facing hillside. The Natural Heritage Society of Massachusetts (2010) records finding *H. canadensis* in three types of mesic forest: 1. an oak/conifer forest dominated by a black birch canopy, 2. sugar maple forests with *Asarum* species and trout lilies and 3. with *P. quinquefolium* L. under a sugar maple canopy.

There are several cultivation methods currently in use:

Forest Farming

Woods cultivation has been practiced using raised beds within a natural or created forest setting. Raised beds reduce root competition and result in large well-formed roots (Burkhart, 2006). This requires more labor and investment to start but may result in increased yields and better profits. Raised beds may be used to establish plantings for production of seed or transplants for shade or wild-simulated cultivation, however, care must be taken not to erode or otherwise jeopardize the site. To create a 4 foot raised bed, a grower would need to dig an area 6-8 feet wide using the outer soil to create a mound about one foot high. This should be allowed to settle in order to reduce heaving and plant loss during the

Table 5. Examples of regulation of *H. canadensis* by selected states.

State	Regulatory requirements
VT	Fees are to be charged to a person applying to take a threatened or endangered species as follows: (A) To take for scientific purposes, to enhance the propagation or survival of the species, or for educational purposes or special purposes consistent with the federal Endangered Species Act, $50.00 (B) To take for a zoological exhibition or to lessen an economic hardship, $250.00 (State of Vermont, 2010).
MN	Any digging of *H. canadensis* roots in the wild is illegal in Minnestota without a permit from the Minnesota Dept of Natural Resources (MN DNR, 2011).
PA	Buying, trading, or bartering plants listed as vulnerable is prohibited within Pennsylvania without first obtaining a vulnerable plant license. This license is granted on an annual basis to any interested individual provided he/she complies with record-keeping requirements. The DCNR oversees this program and uses information. Collection of *H. canadensis* from state parks in Pennsylvania is not permitted. Similarly, collecting *H. canadensis* from state game lands in the Commonwealth is unlawful (Burkhart, 2006). *H. canadensis* collectors and growers in Pennsylvania are not required to maintain records of their activities if the product is intended to be sold to a buyer within Pennsylvania or the United States. If the grower is gathering or producing *H. canadensis* under contract for an exporter, he only needs to maintain records for the benefit of the exporter.
NC	Requires a permit for cultivation. See: Davis & McCoy (2000)
MI	State legislation allows threatened species to be taken for propagation, scientific study and educational purposes (Michigan State Legislature, 2009).

Table 6. Sources of *Hydrastis canadensis* root and leaf 1999-2010 in pounds (Adapted from AHPA, 2006, 2007, 2012)

		1999	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010
Dried root	wild	91,435	70,396	105,099	41,845	31,802	61,160	47,714	61,170	70,129	71,155	54,460	59,197
	cultivated	47,559	18,963	21,337	15,779	11,070	21,000	33,756	12,406	17,146	16,698	18,354	17,931
	total	138,994	89,359	126,436	57,624	42,872	82,160	81,470	73,576	87,275	87,853	72,814	77, 128
	% cultivated	34%	21%	17%	27%	26%	26%	41%	17%	20%	20%	25%	23%
Fresh root	wild	13,800*	4,000*	5,600*	3,000*	3,000*	1,771	2,303	745	890	0	0	0
	cultivated	7,600*	1,200*	2,000*	8,000*	6,600*	65	217	150	44	135	125	124
	total	21,400	5,200	7,600	11,000	9,600	1,836	2,520	895	934	135	125	124
	% cultivated	36%	22%	26%	73%	69%	6%	21%	17%	5%	100%	100%	100%
Dried leaf	wild	87,524	18,770	47,558	16,612	6,869	5,178	6,939	17,133	9,817	11,332	8,354	10,791
	cultivated	5,487	4,248	4,941	8,926	2,435	2,495	7,092	1,622	1,354	425	1,586	782
	total	93,011	23,018	52,499	25,538	9,204	7,673	14,031	18,755	11,171	11,757	9,940	11,573
	% cultivated	6%	18.5%	9%	35%	26%	33%	51%	9%	12%	4%	16%	7%
Fresh leaf	wild	-	-	-	-	-	0	0	0	0	297	0	0
	cultivated	-	-	-	-	-	120	125	0	0	0	0	0
	total						120	125	0	0	297	0	0
	% cultivated						100%	100%	-	-	0%	-	-

* For 1999-2003, for fresh roots, the harvest numbers were extrapolated from tonnage counts in prior years. For these years, the numbers are rounded, however, the percentage cultivated remains the same.

winter (Burkhart, 2006). Some growers adjust the pH with limestone (Burkhart, 2006) or potash (Eldus, 1996). Fertilizers are not recommended. Some of the disadvantages of this method are a known increase in the incidence of disease and pests as well as an investment in materials, time and labor (Burkhart, 2006).

Wild-simulated

Wild-simulated cultivation involves planting roots, seeds or transplants in a forest environment with minimum intervention. This echnique may take from 2-3 years longer to reach harvest and result in a lower yield when compared to woods cultivation (Burkhart, 2006). Burkhart maintains that the quality of the roots is the same between different cultivation techniques. The most important factors will be quality of plant material and site selection. A site may be thinned to create the optimum shade requirements but should be done without altering the ecology of the site (Burkhart, 2006). Sites that contain plants with shallow roots such as rhododendrons, spruce, hemlock, cedar and other conifers should be avoided because they may

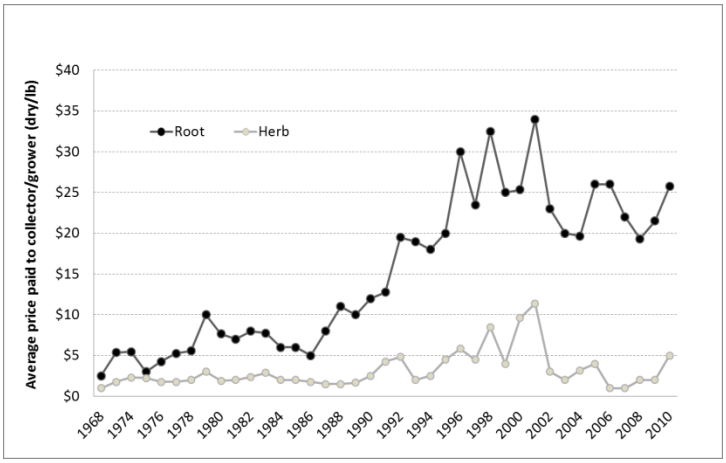


Figure 8. Average price of goldenseal (*Hydrastis canadensis*) root and herb (1968–2010) (Burkhart, 2012).

compete for moisture and nutrients, although *H. canadensis* has been found growing under conifers (Eldus, 1996). In late summer or early autumn, the site is further prepared by brushing the leaf litter aside, sowing the seeds or planting the transplants and then replacing the litter (Burkhart, 2006). Initially the plants/seeds are placed 2-4/square foot and then thinned, if necessary, to one plant per square foot. There may be some natural thinning as a result of animal browsing, insect defoliation or disease, but *H. canadensis* is generally not known to have many pests (Burkhart, 2006; Eldus, 1996). Care should be taken not to trample the area as soil compaction may affect the plants. According to Persons and Davis, (2005), preparing one acre of land for cultivation requires roughly six hundred hours of intense labor.

Eldus (1996) describes four methods for wild-simulated planting of *H. canadensis*. These methods might also be tried for wood or field plantings.

i. Digging a vertical trench from the top of a ridge to the bottom of the creek with plants spaced 6-8 inches.

ii. Placing fallen logs about 5-10 feet apart behind existing trees to create a raised or terraced area for planting.

iii. Companion planting with *Panax quinquefolium*, by alternating with the *H. canadensis* plants.

iv. Companion planting with *P. quinquefolium* forming an inner circle and *H. canadensis* an outer circle.

Field cultivation

Field cultivation relies on shade-cloth, lattice or slat frames to provide the plants with the necessary amount of shade. Plants grown under 70% shade showed almost double the overall weight, increased rootlets and larger leaves compared to plants grown under full sun (Quigley & Mulhall, 2002). Eldus (1996) suggests using raised sloped beds to aid drainage. While shade cultivation may represent an additional cost, it also allows the grower to monitor and protect his crop. During the winter plants should be monitored for exposure from ground heaving which can expose and kill the roots (Burkhart, 2006).

Plant development

H. canadensis has a series of vegetative stages: the cotyledon stage (which may last one or more years), single leaf stage (generally second to third years or more) during which roots develop and the reproductive stage (third-fifth year) when flowering and fruiting occur (Burkhart, 2006). The reproductive stage is characterized by the development of an additional 2-3 leaves. Self-fertile flowers bloom in early spring from March through May, depending on the location, latitude and soil condition. Fruits take from 8-12 weeks to ripen forming a bright red fruit containing one or two small black seeds (Burkhart, 2006, Cech, 2003; Eldus, 1996; Blakley & Sturdivant, 1999).

Starting with seed

At least three months of exposure to cold temperatures (cold stratification) at 35 to 40°F is required before germination will occur, this cold treatment also deters fungal disease (Cech, 2002). Germination may be best when seed and fruit are planted about 1/2 to 1" deep immediately upon harvest (Burkhart, 2006). If the seeds are sown in the fall and mulched, Stoltz (1994) cautions that the

mulch should be removed in spring before the seedlings emerge.

If the seed is to be collected and cleaned, Burkhart (2006) suggests that the fruit be soaked in water 24-48 hours to soften the fruit and facilitate seed removal. Eldus (1996) cautions that care must be taken not to damage the seeds or to allow them to dry out. Once cleaned, seeds can be stored beneath 3-4 inches of sand or mulch in an outside are where they are exposed to natural rain (Eldus, 1996) or refrigerated until planting time. Harvesting and planting seeds is considered responsible stewardship as it helps maintain genetic adaption and diversity (Burkhart, 2006).

Seeds should be planted about four inches apart in rows about twelve inches apart (Tilford, 1998). At Tai Sophia Institute, sowing 10 seeds/fruits in August 2010 resulted in 50% germination by April 2011. All seeds were sown within one foot of the original plant. Adam (2004) notes that once a field of *H. canadensis* is established, the existing stock should provide seeds and rootlets for new plants.

Planting roots

H. canadensis forms colonies, where many stems arise from a single interconnected root system (Burkhart, 2006). A marketable crop may be produced more quickly (3+ years) from large root pieces as compared to small root fragments (5+ years). Although small root fragments may eventually develop into *H. canadensis* plants, ideally a grower should divide roots into pieces larger than 1/2 inches with at least one bud. Division is best just before the plants become dormant during late summer or early fall. Roots should be planted 2-3 inches deep with the bud oriented upwards. Small roots may be used to reduce planting costs but these may remain dormant for a year or more after planting and will take longer to reach harvest size, although they may be used to develop plants for transplant resale (Burkhart, 2006).

Pests and disease

Deer have been known to browse *H. canadensis* (Burkhart, 2006; Eldus, 1996). Moles may sometimes be a problem but can be controlled by placing wire mesh eight to twelve inches into the soil (Eldus, 1996).

Under cultivation, several cases of Botrytis (leaf blight) have been documented (Beyfuss, 2011), as well as root knot nematodes (Eldus, 1996), alternaria, rhizoctonia, and fusarium (Davis & McCoy, 2010). According to Cech (1999), the use of multi-cropping (*P. quinquefolium, Asarum canadense* L.) in cultivated settings will help reduce the risks of pests and disease. Eldus (1996), Beyfuss (2011) and Burkhart (2006) noted that diatomaceous earth may discourage slugs from feeding on *H. canadensis* leaves.

Harvesting

When Sanders & McGraw (2005) examined harvesting practices and subsequent re-growth using leaf size as an indicator of plant health, they found that when roots were dug in midsummer before dormancy, the remaining patches took longer to recover than when harvesting occurred after the plants were dormant.

There is some disagreement regarding the potency of goldenseal roots as the plant ages. According to Beyfuss (2011) the value of *H. canadensis* does not increase with age because as roots become overcrowded growth slows. For optimum production, Beyfuss (2011) suggests that plants should be thinned or spaced after 3-4 years to prevent overcrowding. Quigley & Mulhall (2002) suggest that alkaloid concentration may vary according to the age of the root and/or the speed of growth, and call for further studies in this area.

During the autumn months alkaloid concentrations are at their highest, and water content is at its lowest, suggesting that this is the ideal time to harvest (Douglas et al, 2010). Harvesting times are determined by the method of propagation. Plants

propagated by division will typically mature in their third or fourth year, and plants started from seed will mature in their fifth or sixth (Stoltz, 1994).

Under good growing conditions, one can expect to harvest between one and two thousand pounds of *H. canadensis* per acre (Beyfuss, 2011). Roots should be thoroughly cleaned, ensuring all dirt, stones and organic matter are removed and rinsed before drying (Lockard & Swanson, 2004).

Proper drying from 1-2 weeks will help ensure that spoiling, or molding does not occur, and that the roots contain the highest concentration of medicinal constituents possible (Cech, 2002; Eldus, 1996). Roots will lose from 70-75% of their mass during drying (Eldus, 1996; Stoltz, 1994). Cech (2002) suggests drying at 70° F for one day then increasing the temperature to 95 ° F, rotating the roots daily until the roots snap. Tobacco barns may be used for bulk drying while for smaller scale production a small shed or room may be utilized (Davis & McCoy, 2000). Cech notes that the dried roots are hygroscopic, and should be protected from moisture and condensation. According to Davis and McCoy (2000), yields from shade-grown plants averages around eight hundred pounds per acre.

7. Summary and moving forward

In keeping with some other high profile Appalachian herbs, research into *H. canadensis* has largely focused on one or more of its active chemical constituents, in this case the widely distributed alkaloid berberine, and to a less extent the alkaloid hydrastine. The common finding that activities of isolated alkaloids singly or additively are generally weaker by comparison to *H. canadensis* extracts have led some recent investigators to focus more on potential synergistic activity between constituents, and results from these studies are promising (Junio et al., 2011).

Unlike some other 'at risk' or endangered Appalachian herbs *H. canadensis* is well adapted to cultivation, and can be quite profitable for growers. Hence commercial production is possible without resorting to harvesting of wild populations. Nevertheless protection and monitoring of wild *H. canadensis* sites should remain a high conservation priority.

Findings from a recent toxicological study by NIH suggest potential carcinogenicity to rodents when fed high doses of *H. canadensis* over a long period (2 years) (National Toxicity Program, 2010). Further investigations are needed to establish whether these findings can be replicated in a parallel setting or in alternative models of carcinogenicity.

There is a need for further clinical investigations (preferably RCTs), and the herbal community is best served when such studies are designed so to assess traditional uses. In the case of *H. canadensis*, traditional use would emphasize sub-acute infections as referred to in the Modern Phytotherapy section of this monograph.

Coupled with the clinical investigations, research into the molecular effects of H. canadensis would also ideally continue and explore antimicrobial modes of activity, as well as proteomic and genomic research to further the understanding of this medicinal plant's effects on cellular pathways.

Figure 9. *Hydrastis canadensis*: flower and early leaves. Photo by Jesse Sommerlatt.

9. References

Abdel-Haq, H., Cometa, M. F., Palmery, M., Leone, M. G., Silvestrini, B., & Saso, L. (2000). Relaxant effects of Hydrastis canadensis L. and its major alkaloids on Guinea Pig Isolated Trachea. *Pharmacology & Toxicology*, *87*(5), 218-222. doi:10.1034/j.1600-0773.2000.pto870505.x

Abidi, P., Chen, W., Kraemer, F. B., Li, H., & Liu, J. (2006). The medicinal plant goldenseal is a natural LDL-lowering agent with multiple bioactive components and new action mechanisms. *Journal of Lipid Research*, *47*(10), 2134 -2147. doi:10.1194/jlr.M600195-JLR200

Adam, K. (2004) Ginseng, goldenseal and other native roots. National Sustainable Agriculture Information Service. Retrieved from https://attra.ncat.org/attra-pub/PDF/ginsgold.pdf

AHPA (2006) Tonnage survey of select North American wild-harvested plants, 2002-2003. Silver Spring, MD: American Herbal Products Association. Retrieved from http://www.ahpa .org /Portal s/0/members/02-03_TonnageSurvey.pdf

AHPA (2007) Tonnage survey of select North American wild-harvested plants, 2004-2005. Silver Spring, MD: American Herbal Products Association. Retrieved from http://www.ahpa.org/Portals/ 0/members/04-05_AHPATonnageReport.pdf

American Pharmaceutical Association (1946). *The National Formulary*. Washington, DC: The Association.

Amalaradjou, M. A. R. & Venkitanarayanan, K. (2011). Natural approaches for controlling urinary tract infections. In Peter Tenke (Ed.), *Urinary tract infections*, ISBN: 978-953-307-757-4, InTech, Available from: http://www.intechopen.com/articles/show/title/natural-approaches-for-controlling-urinary-tract-infections

Anonymous. (2000). Berberine Monograph. *Alternative Medicine Review*, *5*(2), 175-177.

Baker, D. (2010). Goldenseal pays off for La Center's Sego's Herb Farm. *Oregonian,* September 17. Retrieved from http://www.segoherbfarm.com

Ball, A. R., Casadei, G., Samosorn, S., Bremner, J. B., Ausubel, F. M., Moy, T. I., & Lewis, K. (2006). Conjugating berberine to a multidrug resistance pump inhibitor creates an effective antimicrobial. *ACS Chemical Biology*, *1*(9), 594-600. doi:10.1021/cb600238x

Beach, W. (1833). *The American practice of medicine Vols. 1-3.* New York, NY: Betts Anstice.

Bell, S.E., Bourguignon, E.S.O., Dennis, A.C., Fields, J.A. McGarvey, J.J. & Seddon, K.R. (2000). Identification of dyes on ancient Chinese paper damples using the subtracted shifted raman spectroscopy method. *Analytical Chemistry 72*, 234-239.

Bergner, P. 1997. *The healing power of echinacea and goldenseal.* Rocklin, CA: Prima Publishing.

Beyfuss, R. L. (2012). How to grow ginseng and goldenseal in your forest. Cornell Cooperative Extension of Greene County New York State, Department of Environmental Conservation. Retrieved from http://www.dec.ny.gov/animals/7472.html

Bhowmick, S. K., Hundley, O. T., & Rettig, K. R. (2007). Severe hypernatremia and hyperosmolality exacerbated by an herbal preparation in a patient with diabetic ketoacidosis. *Clinical Pediatrics*, *46*(9), 831-834. doi:10.1177/0009922807303042

Bird, D. A., & Facchini, P. J. (2001). Berberine bridge enzyme, a key branch-point enzyme in benzylisoquinoline alkaloid biosynthesis, contains a vacuolar sorting determinant. *Planta*, *213*, 888-897. doi:10.1007/s004250100582

Blakely, T., & Sturdivant, L. (1999). *Bootstrap guide to medicinal herbs in the garden, field, and market place.* San Juan Naturals, Friday Harbor.

Bowers, H. (1891). A contribution to the life history of Hydrastis canadensis. *Botanical Gazette, 16*(3), 73-82.

Brochmann-Hanssen, E., & Evers, J.A. (1951). A fluorometric method for the determination of hydrastine in hydrastis. *Journal American Pharmaceutical Assoc. 40*(12), 620-622.

Bradley, P. (1992). *British herbal compendium vol. 1.* Bournemouth, UK: BHMA

Braun, L. & Cohen, M. (2010). *Herbs and natural supplements. An evidence-based guide*. 3rd ed. Sydney, Australia: Churchill Livingstone Elsevier.

British Pharmacopoeia Commission (2006). *British Pharmacopoeia 2007*. Norwich, U.K. The Stationary Office.

Brochmann-Hanssen, E., & Evers, J. A. (1951). A fluorometric method for the determination of hydrastine in hydrastis. *Journal of the American Pharmaceutical Association. 40*(12), 620-622.

Brown, P. N., & Roman, M. C. (2008). Determination of hydrastine and berberine in goldenseal raw materials, extracts, and dietary supplements by high-performance liquid chromatography with UV: Collaborative study. *Journal of AOAC International*, *91*(4), 694-701.

Brown, P. N., Paley, L. A., Roman, M. C., & Chan, M. (2008). Single-laboratory validation of a method for the detection and/or quantification of select alkaloids in goldenseal supplements and raw materials by reversed-phase high-performance liquid chromatography. *Pharmaceutical Biology*, *46*(1, 2), 135-144.

Budzinski, J. W., Trudeau, V. L., Drouin, C. E., Panahi, M., Arnason, J. T., & Foster, B. C. (2007). Modulation of human cytochrome P450 3A4 (CYP3A4) and P-glycoprotein (P-gp) in Caco-2 cell monolayers by selected commercial-source milk thistle and goldenseal products. *Canadian Journal of Physiology and Pharmacology*, *85*, 966-978. doi:10.1139/Y07-091

Burkhart, E. (2006) Goldenseal. Non-timber forest products (NTFPs) from Pennsylvania 2. State College, PA: Information and Communication Technologies in the College of Agricultural Sciences, Penn State University. Retrieved from http://extension.psu.edu/botanicals

Burkhart, E. (2012). Personal communication. Shaver's Creek Environmental Center. The Pennsylvania State University

Cassilith, B.R., Yeung, K.S., & Gubili, J. (2010) *Herb-drug interactions in oncology, 2nd edition*. Shelton, CT: People's Medical Publishing House.

Cavender, A. (2003). *Folk medicine in southern Appalachia*. Chapel Hill, NC: The University of North Carolina Press.

Cech, Richard A. (1999) Balancing conservation with utilization: Restoring populations of commercially valuable medicinal herbs in forests and agroforests. Herbalgram, 45: 18-22.

Cecil, C. E., Davis, J. M., Cech, N. B., & Laster, S. M. (2011). Inhibition of H1N1 influenza A virus growth and induction of inflammatory mediators by the isoquinoline alkaloid berberine and extracts of goldenseal (Hydrastis canadensis). *International Immunopharmacology 11*, 1706-14. doi:10.1016/j.intimp.2011.06.002

Chadwick, L. R., Wu, C. D., & Kinghorn, A. D. (2001). Isolation of alkaloids from goldenseal (Hydrastis canadensis rhizomes) using pH-zone refining countercurrent chromatography. 2445-2453, *24*(16), 2445-2453.

Chatterjee, P., & Franklin, M. R. (2003). Human cytochrome p450 inhibition and metabolic-intermediate complex formation by goldenseal extract and its methylenedioxyphenyl components. *Drug Metabolism and Disposition*, *31*(11), 1391 -1397. doi:10.1124/dmd.31.11.1391

Chauhan, R. K. S., Jain, A. M., & Bhandari, B. (1970). Berberine in the treatment of childhood diarrhoea. *The Indian Journal of Pediatrics*, *37*(11), 577-579. doi:10.1007/BF02803833

Chavis, P. (2003). Complementary therapy assessment: nutritional supplements: perioperative implications for eye surgery. *American Academy of Ophthalmology Complementary Therapy Task Force 10*, 1-18.

Chu, K. H., Li, C. P., & Qi, J. (2006). Ribosomal RNA as molecular barcodes: a simple correlation analysis without sequence alignment. *Bioinformatics*, *22*(14), 1690 -1701. doi:10.1093/bioinformatics/btl146

CITES (2012) *Hydrastis canadensis*. Species database Retrieved from http://www.cites.org/eng/app/appendices.php

Cometa, M. F, Abdel-Haq, H., & Palmery, M. (1998). Spasmolytic activities of Hydrastis canadensis L. on rat uterus and guinea-pig trachea. *Phytotherapy Research*, *12*(S1), S83-S85. doi:10.1002/(SICI)1099-

1573(1998)12:1+<S83::AID-PTR258>3.0.CO;2-O

Connecticut Department of Energy and Environmental Protection (DEEP). (2010). Endangered, threatened & special concern plants. Hartford, CT: Department of Energy and Environmental Protection (DEEP), State of Connecticut. Retrieved from http://www.ct.gov/dep/cwp/view.asp?a=2702&q=323482&depNav_GID=1628

Compton, J. A., Culham, A., & Jury, S. L. (1998). Reclassification of Actaea to include Cimicifuga and Souliea (Ranunculaceae): Phylogeny inferred from morphology, nrDNA ITS, and cpDNA trnL-F sequence variation. *Taxon*, *47*(3), 593-634. doi:10.2307/1223580

Cook, W. (1869). *The physiomedical dispensatory.* Cincinnati, OH: W.H.Cook.

Crabtree, T. (2008) Rare plant list, Tennessee Natural Heritage Program, Division of Natural Areas, Tennessee Department of Environment and Conservation.

Crellin, J. & Philpott, J. (1990) *A reference guide to medicinal plants*. Durham, NC: Duke University Press

Culbreth, D. (1917). *A manual of material medica and pharmacology.* Portland, OR: Reprinted in 1983 by Eclectic Medical Publications

Curtis, A. (1858). *A synopsis of lectures on medical science* 2nd ed. Cincinnati, OH: Moore, Wilstach, Keys & Co.

Cwikla, C., Schmidt, K., Matthias, A., Bone, K. M., Lehmann, R., & Tiralongo, E. (2010). Investigations into the antibacterial activities of phytotherapeutics against Helicobacter pylori and Campylobacter jejuni. *Phytotherapy Research*, *24*(5), 649-656. doi:10.1002/ptr.2933

Cybulska, P., Thakur, S. D., Foster, B. C., Scott, I. M., Leduc, R. I., Arnason, J. T., & Dillon, J.-A. R. (2011). Extracts of Canadian First Nations medicinal plants, used as natural products, inhibit Neisseria gonorrhoeae isolates with different antibiotic resistance profiles. *Sexually Transmitted Diseases*. doi:10.1097/OLQ.0b013e31820cb166

Datta, D. D., Bose, P. C., & Ghosh, D. (1971). Thin layer chromatography and U. V. spectrphotometry of alcoholic extracts of Hydrastis canadensis. *Planta Medica*, *19*(3), 258-263. doi:10.1055/s-0028-1099639

Davis, J.M., & McCoy, J.A. (2000) Commercial goldenseal cultivation. Horticulture information leaflets. Raleigh, NC: North Carolina Cooperative Extension Service. Retrieved from: http://www.ces.ncsu.edu/depts/hort/hil/hil-131.html

Douglas, J.A., Follett, J.M., Parmenter, G.A., Sansom, C.E., Perry, M.B. & Littler, R.A. (2010). Seasonal variation of biomass and bioactive alkaloid content of goldenseal, Hydrastis canadensis. *Fitoterapia 81*(7), 925-928.

Duke, J.A. (1986). *Handbook of Northeastern Indian medicinal plants.* Lincoln, MA: Quarterman Publications, Inc.

Edwards, D. J., & Draper, E. J. (2003). Variations in Alkaloid Content of Herbal Products Containing Goldenseal. *Journal of the American Pharmacists Association - Article*, *43*(3), 419-423.

Eldus, R. (1996) A Grower's guide to goldenseal: *Hydrastis canadensis*. Marshall, NC: North Carolina Ginseng & Goldenseal Company.

El-Masry, S., Korany, M. A., & Abou-Donia, A. H. A. (1980). Colorimetric and spectrophotometric determinations of hydrastis alkaloids in pharmaceutical preparations. *Journal of Pharmaceutical Sciences*, *69*(5), 597-598. doi:10.1002/jps.2600690534

Ettefagh, K., Burns, J., Junio, H., Kaatz, G., & Cech, N. (2010). Goldenseal (Hydrastis canadensis L.) Extracts synergistically enhance the antibacterial activity of berberine via efflux pump inhibition. *Planta Medica*, *77*, 835-840. doi:10.1055/s-0030-1250606

Felter, H. W., & Lloyd, J. (1898) *Kings American dispensatory*. (18th ed. 3rd rev., Vol. II) Cincinnati, OH: Eclectic Medical Publications.

Felter, H. (1922). *The Eclectic Materia Medica, Pharmacology and Therapeutics.* Portland, OR: Reprinted by Eclectic Medical Publications, 1983.

Freeman, C. & Spelman, K. (2008). A critical evaluation of drug interactions with Echinacea spp. *Molecular Nutrition & Food Research. 52*, 789-98

Frontier Natural Products Co-op (2012) Meet our goldenseal grower, Well Earth Sourcing Program. Retrieved from http://www.frontiercoop.com/wellearth/explorebyproduct/goldenseal.php

Fujii, N., Inui, T., Iwasa, K., Morishige, T., & Sato, F. (2006). Knockdown of berberine bridge enzyme by RNAi accumulates (S)-reticuline and activates a silent pathway in cultured California poppy cells. *Transgenic Research*, *16*, 363-375. doi:10.1007/s11248-006-9040-4

Galeffi, C. Cometa, M.F., Tomassini, L. & Nicoletti, M. (1997). Canadinic acid: an alkaloid from *Hydrastis canadensis. Planta Medica* 63, 194.

Galle, K., Müller-Jacik, B., Proebstle, A., Jurcic, K., Bladt, S., & Wagner, H. (1994). Analytical and pharmacological studies on Mahonia aquifolium. *Phytomedicine 1*, 59-62. http://www.sciencedirect.com/science/article/pii/S0944711311800243 - fn1

Gardner, M. & Aylworth, B. (1836). *The domestic physician and family assistant.* Cooperstown, NY: H. & E. Phinney.

Gao, W. H., Lin, S. Y., Jia, L., Guo, X. K., Chen, X. G., & Hu, Z. D. (2005). Analysis of protoberberine

alkaloids in several herbal drugs and related medicinal preparations by non-aqueous capillary electrophoresis. *Journal of Separation Science, 28*(1), 92-97.

Gentry, E. J., Jampani, H. B., Keshavarz-Shokri, A., Morton, M. D., Vander Velde, D. … Baker, W. (1998). Antitubercular natural products: berberine from the roots of commercial hydrastis canadensis powder. isolation of inactive 8-oxotetrahydrothalifendine, canadine, β-hydrastine, and two new quinic acid esters, hycandinic acid esters-1 and -2. *Journal of Natural. Products*, *61*(10), 1187-1193. doi:10.1021/np9701889

Georgia Department of Natural Resources (GA DNR). (2010) Georgia Rare species and community data, Georgia Department of Natural Resources, Georgia. Retrieved from http://georgiawildlife.com/sites/default/files /uploads /wildlife/nongame/text/html/protected_species/Plants.html

Gocan, S., Cimpan, G., & Muresan, L. (1996) Automated multiple development thin layer chromatography of some plant extracts. *Journal Pharm Biomed Anal 14*, 1221.

Govindan, M., & Govindan, G. (2000). A convenient method for the determination of the quality of goldenseal. *Fitoterapia*, *71*(3), 232-235.

Grycová, L., Dostál, J., & Marek, R. (2007). Quaternary protoberberine alkaloids. *Phytochemistry*, *68*(2), 150-175. doi:10.1016/j.phytochem.2006.10.004

Gupta, P. K., Hubbard, M., Gurley, B., & Hendrickson, H. P. (2009). Validation of a liquid chromatography–tandem mass spectrometric assay for the quantitative determination of hydrastine and berberine in human serum. *Journal of Pharmaceutical and Biomedical Analysis*, *49*(4), 1021-1026. doi:10.1016/j.jpba.2009.01.036

Gurley, B. J., Gardner, S. F., Hubbard, M. A., Williams, D. K., Gentry, W. B. … Shah., A. (2005). In vivo effects of goldenseal, kava kava, black cohosh, and valerian on human cytochrome P450 1A2, 2D6, 2E1, and 3A4 phenotypes. *Clinical pharmacology and therapeutics*, *77*(5), 415-426. doi:10.1016/j.clpt.2005.01.009

Gurley, B J, Swain, A., Hubbard, M. A., Hartsfield, F., Thaden, J. … Tong, Y. (2008a). Supplementation with goldenseal (Hydrastis canadensis), but not kava kava (Piper methysticum), inhibits human CYP3A activity in vivo. *Clinical Pharmacology and Therapeutics*, *83*(1), 61-69. doi:10.1038/sj.clpt.6100222

Gurley, B. J, Swain, A., Hubbard, M. A., Williams, D. K., Barone, G. … Battu, S.K. (2008b). Clinical assessment of CYP2D6-mediated herb–drug interactions in humans: Effects of milk thistle, black cohosh, goldenseal, kava kava, St. John's wort, and Echinacea. *Molecular Nutrition & Food Research*, *52*(7), 755-763. doi:10.1002/mnfr.200600300

Hale, E.M. (1875). *Material medica and special therapeutics of the new remedies. Vol II.* New York, NY: Boericke and Tafel.

Howard, H. (1832). *An improved system of botanical medicine.* Philadelphia, PA.

Herath, W. H. M. W., Ferreira, D., & Khan, I. A. (2003). Microbial transformation of the phthalideisoquinoline alkaloid, (-)-β-hydrastine. *Natural Product Research*, *17*(4), 269-274. doi:10.1080/1057563021000060158

Hwang, B. Y., Roberts, S. K., Chadwick, L. R., Wu, C. D., & Kinghorn, A. D. (2003). Antimicrobial constituents from goldenseal (the rhizomes of Hydrastis canadensis) against selected oral pathogens. *Planta Medica*, *69*, 623-627. doi:10.1055/s-2003-41115

Inbaraj, J. J., Kukielczak, B. M., Bilski, P., He, Y.-Y., Sik, R. H., & Chignell, C. F. (2006). Photochemistry and photocytotoxicity of alkaloids from Goldenseal (Hydrastis canadensis L.). 2. Palmatine, hydrastine, canadine, and hydrastinine. *Chemical Research in Toxicology*, *19*(6), 739-744. doi:10.1021/tx050356u

Inbaraj, J. J., Kukielczak, B. M., Bilski, P., Sandvik, S. L., & Chignell, C. F. (2001). Photochemistry and photocytotoxicity of alkaloids from Goldenseal (Hydrastis canadensis L.) 1. Berberine. *Chemical Research in Toxicology*, *14*(11), 1529-1534.

Jacobs, M.L. & Burlage, H.M. (1958). *Index of plants of North Carolina with reputed medicinal uses.* Chapel Hill, NC. Henry M. Burlage.

Junio, H. A., Sy-Cordero, A. A., Ettefagh, K. A., Burns, J. T., Micko, K. T.… Cech, N.B. (2011). Synergy-directed fractionation of botanical medicines: a case study with goldenseal (Hydrastis canadensis). *Journal of Natural Products*, *74*(7), 1621-1629. doi:10.1021/np200336g

Kamath, S., Skeels, M., & Pai, A. (2009). Significant differences in alkaloid content of Coptis chinensis (Huanglian), from its related American species. *Chinese Medicine*, *4*(1), 1-4.

Keenan, G. L. (1948). Microscopic identity tests for hydrastis and its principal alkaloids, hydrastine and berberine. *Journal of the American Pharmaceutical Association*, *37*(1), 41. doi:10.1002/jps.3030370114

Khin-Maung-U, Myo-Khin, Nyunt-Nyunt-Wai, Aye-Kyaw, & Tin-U. (1985). Clinical trial of berberine in acute watery diarrhoea. *British Medical Journal (Clinical research ed.)*, *291*(6509), 1601-1605.

Kim, J.-S., Tanaka, H., & Shoyama, Y. (2003). Immunoquantitative analysis for berberine and its related compounds using monoclonal antibodies in herbal medicines. *Analyst*, *129*(1), 87-91. doi:10.1039/B311304C

Kloss, J. (1972). *Back to Eden.* Santa Barbara, CA: Woodbridge Press.

Kraemer, H. (1915). *Scientific and applied pharmacognosy*. Philadelphia, PA: Henry Kraemer.

Krochmal, A., Walters, R. & Doughty, R. (1969). *A guide to medicinal plants from Appalachia*. U.S.D.A. Forest Service Research Paper NE-138. Upper Darby, PA: Northeastern Forest Experiment Station.

Kumar, K.B.H., Sunali, E.S., Kuttan, G., Preethi, K.C. Venugopal, C.N., & Kuttan, R. (2007). Inhibition of chemically induced carcinogenesis by drugs used in homeopathic medicine. *Asian Pacific Journal of Cancer Prevention*, *8*, 98-102

Kuts-Cheraux, A.W. (1953). *Naturae medicina and naturopathic dispensatory*. Chattanooga: TN. American Naturopathic Physicians and Surgeons Association.

Lancaster, H.M. & Davidson, A.L. (1927). Commercial pharmaceutical preparations 4. Hydrastis-golden seal. *Canadian Medical Association Journal, XVII*, 1317-1320.

Lau, C., Yao, X., Chen, Z., Ko, W., & Huang, Y. (2001). Cardiovascular actions of berberine. *Cardiovascular Drug Reviews*, *19*(3), 234-244. doi:10.1111/j.1527-3466.2001.tb00068.x

Li, W., & Fitzloff, J. F. (2002). A validated high performance liquid chromatographic method for the analysis of Goldenseal. *Journal of Pharmacy and Pharmacology*, *54*(3), 435-439. doi:10.1211/0022357021778510

Lin, H.-L., Liu, T.-Y., Wu, C.-W., & Chi, C.-W. (1999). Berberine modulates expression of mdr1 gene product and the responses of digestive track cancer cells to paclitaxel. *British Journal of Cancer*, *81*(3), 416-422. doi:10.1038/sj.bjc.6690710

Lloyd, J.U. & Lloyd, C.G. (1884). *Drugs and medicines of North America Vol. 1. Ranunculaceae.* Reproduced in *Bulletin of the Lloyd Library No. 9*, 1930. Cincinnati, OH.

Lockard, A. & Swanson, A.Q. (2004). *A diggers guide to medicinal plants 2nd ed*. Eolia, MO: American Botanicals.

Mahady, G. B., & Chadwick, L. R. (2001). Goldenseal (Hydrastis canadensis): Is there enough scientific evidence to support safety and efficacy? *Nutrition in Clinical Care*, *4*(5), 243-249.

Maryland Department of Natural Resources (DNR). (2010) Rare, threatened and endangered plants of Maryland. Department of Natural Resources, Maryland. Retrieved from http://www.dnr.state.md.us/wildlife /Plants _Wildlife /rte/rteplants.asp

Massachusetts Division of Fisheries and Wildlife (19942010). Massachusetts list of endangered, threatened, and special concern species. Massachusetts Division of Fisheries and Wildlife, Massachusetts. Retrieved from http://www.mass.gov /dfwele/dfw/nhesp/species_info/mesa_list/mesa_list.htm#PLANTS

McGraw, J.B. Sanders, S.M. & Van der Voort, M. (2003). Distribution and abundance of Hydrastis canadensis L. (Ranunculaceae) and Panax quinquefolius L (Araliaceae) in the Central Appalachian

region. *Journal of the Torrey Botanical Society, 130* (2), 66-69.

McGuffin, M. Hobbs, C. Upton, R. & Goldberg, A. (eds.) (1997). *Botanical safety handbook.* Boca Raton, FL: American Herbal Products Association, CRC Press.

McNamara, C. E., Perry, N. B., Follett, J. M., Parmenter, G. A., & Douglas, J. A. (2004). A new glucosyl feruloyl quinic acid as a potential marker for roots and rhizomes of goldenseal, Hydrastis canadensis. *Journal of. Natural. Products*, *67*(11), 1818-1822. doi:10.1021/np049868j

Michigan State Legislature. (2009). Natural resources and environmental protection act (Excerpt). Act 451 of 1994, 324.36505 Prohibitions; exceptions. Michigan Legislature. Retrieved from http://legislature.mi.gov/doc.aspx?mcl-324-36505

Michigan Natural Features Inventory. (2004). *Hydrastis canadensis* L. - goldenseal. Michigan State University Board of Trustees. Retrieved from http://mnfi.anr.msu.edu/abstracts/botany/hydrastis_canadensis.pdf

Mills, S. & Bone, K. (2000). *Principles and practice of phytotherapy.* Edinburgh, UK: Churchill Livingstone.

Millspaugh, C.F. (1974). *American medicinal plants.* New York, NY: Dover Publications.

Minnesota Department of Natural Resources (MN DNR). (2012) *Hydrastis canadensis*. species profile, rare species guide. Minnesota, DNR. Retrieved from http://www.dnr.state.mn.us/rsg/profile.html?action=elementDetail&selectedElement=PDRAN0F010

Mitchell, W.A. (2003). *Plant medicine in practice.* St. Louis, MO: Churchill Livingstone

Moerman, D.E. (1986). *Medicinal plants of North America Vol. 1*. Ann Arbor, MI: University of Michigan Museum of Anthropology.

Moerman, D. (1998). *Native American ethnobotany.* Portland, OR: Timber Press, Inc.

Motter, M. G., & Wilbert, M. I. (1912). *Digestion of Comments on the Pharmacopoeia of the USA*. Hygenic Laboratory - Bulletin (Vol. 84). U.S. Govt. Print. Off.

National Resources Conservation Service. (NRCS). (n.d.) *Hydrastis canadensis*. Plants Profile. Plants Database, National Resources Conservation Service, USDA. Retrieved from: http://plants.usda.gov/java/profile?symbol=HYCA&mapType=Large&format=Print&photoID=hyca_002_ahp.tif

Natural Heritage and Endangered Species Program (2008). List of rare species in Massachusetts. Code of Massachusetts Regulations, August 8, 2008. Massachusetts Wildlife, Massachusetts Division of Fisheries and Wildlife. Retrieved from: http://www.mass.gov/dfwele/dfw/nhesp/species_info/mesa_list/mesa_list.htm#PLANTS

Natural Heritage Endangered Species Program. (2010). Massachusetts Division of Fisheries and Wildlife. Golden seal. Retrieved from http://www.mass.gov/dfwele/dfw /nhesp/species _info /nhfacts/hydcan.pdf

Natural Heritage Program (NHPMDNR) (2007). Rare, threatened, and endangered plants of Maryland. Natural Heritage Program, wildlife and Heritage Service, Maryland Department of Natural Resources. Retrieved from http://www.dnr.state.md.us/irc/docs/00011184.pdf

National Toxicology Program, (2010). *Toxicology and carcinogenesis studies of goldenseal root powder (Hydrastis canadensis) in F344/N rats and B6C3F1 mice.* Research Triangle Park, NC: National Institute of Health.

New Jersey Division of Parks and Forestry. (2010). List of Endangered plant species and plant species of concern January 2010. Department of Environmental Protection. Trenton, New Jersey. Retrieved from http://www.state.nj.us/dep/parksandforests/natural/heritage/jan2010plantlist.pdf

New York State Department of Environmental Conservation (2012) Regulations and enforcement, Chapter II-Lands and Forests, Part 193: Trees and plants, 193.3 Protected native plants. Retrieved from http://www.dec.ny.gov/regs/15522.html

Nishino, H., Kitagawa, K., Fujiki, H., & Iwashima, A. (1986). Berberine sulfate inhibits tumor-promoting activity of teleocidin in two-stage carcinogenesis on mouse skin. *Oncology*, *43*, 131-134. doi:10.1159/000226349

North Carolina (2010) 02 NCAC 48F .0301 Protected plant species list, Section .0300- Endangered plant species list: threatened plant species list: list of species of special concern. North Carolina Department of Agriculture and Consumer Services. Retrieved from http://www.ncagr.gov/plantindustry/plant/plantconserve/plist.htm

Oregon's Wild Harvest (2012). Oregon's Wild Harvest goldenseal project. Retrieved from http://www.oregonswildharvest.com/archived_newsletter/oregons_wild_harvest_goldenseal_project

Otani, M., Shitan, N., Sakai, K., Martinoia, E., Sato, F., & Yazaki, K. (2005). Characterization of vacuolar transport of the endogenous alkaloid berberine in Coptis japonica. *Plant Physiology*, *138*(4), 1939 - 1946. doi:10.1104/pp.105.064352

Pan, G., Wang, G., Liu, X., Fawcett, J. P., & Xie, Y. (2002a). The involvement of P-glycoprotein in berberine absorption. *Pharmacology & Toxicology*, *91*(4), 193-197. doi:10.1034/j.1600-0773.2002.t01-1-910403.x

Pan, J-F., Yu, C., Zhu, D-Y., Zhang, H., Zeng, J.F.,…Ren, J-Y. (2002b). Identification of three sulfate-conjugated metabolites of berberine chloride in healthy volunteers' urine after oral administration. *Acta Pharmacol Sin., 23*(1), 77-82

Pan, G-Y., Huang, Z- J., Wang, G-J., Fawcett, J. P., Liu, X-D.……Xie, Y-Y. (2003). The

antihyperglycaemic activity of berberine arises from a decrease of glucose absorption. *Planta Medica*, *69*(7), 632-636. doi:10.1055/s-2003-41121

Pennsylvania Natural Heritage Program (nd) Species of concern special lists. Retrieved from http://www.naturalheritage.state.pa.us/Species.aspx

Persons, W. S. & Davis. J. M. (2005). *Growing and marketing ginseng, goldenseal and other woodland medicinals.* Fairview, N.C.: Bright Mountain Books.

Poe, C.F. & Johnson, C.C. (1954). Toxicity of hydrastine, hydrastinine, and sparteine. *Acta Pharmacol. et. Toxicol.* 10, 338-346.

Potter, S. (1902). *A compendium of materia medica, therapeutics, and prescription Writing 6th ed.* Philadelphia, PA: P. Blakiston's Son & Co.

Power, F. B. (1884). Hydrastine. *American Druggist*, *13*, 175-176.

Quigley, M.F. & Mulhall, S. (2002) Effects of variable shading in a greenhouse study on rhizome weight, root length, and bud proliferation in goldenseal. Preliminary and Regional Reports. *Horticultural Technology, 12*(4), 717-720.

Rabbani, G. H., Butler, T., Knight, J., Sanyal, S. C., & Alam, K. (1987). Randomized controlled trial of berberine sulfate therapy for diarrhea due to enterotoxigenic escherichia coli and vibrio cholerae. *Journal of Infectious Diseases*, *155*(5), 979 -984. doi:10.1093/infdis/155.5.979

Raner, G. M., Cornelious, S., Moulick, K., Wang, Y., Mortenson, A., & Cech, N. B. (2007). Effects of herbal products and their constituents on human cytochrome P4502E1 activity. *Food and Chemical Toxicology*, *45*(12), 2359-2365. doi:10.1016/j.fct.2007.06.012

Rehman, J., Dillow, J. M., Carter, S. M., Chou, J., Le, B., & Maisel, A. S. (1999). Increased production of antigen-specific immunoglobulins G and M following in vivo treatment with the medicinal plants Echinacea angustifolia and Hydrastis canadensis. *Immunology Letters*, *68*(2-3), 391-395. doi:10.1016/S0165-2478(99)00085-1

Ro, K.-E., Keener, C. S., & McPheron, B. A. (1997). Molecular phylogenetic study of the Ranunculaceae: Utility of the nuclear 26s ribosomal DNA in inferring intrafamilial relationships. *Molecular Phylogenetics and Evolution*, *8*(2), 117-127. doi:10.1006/mpev.1997.0413

Roberts, F. (1978). *Modern herbalism for digestive disorders.* Wellingborough, UK: Thorsons Publishers Ltd.

Rueffer, M., & Zenk, M. H. (1985). Berberine synthase, the methylenedioxy group forming enzyme inberberine synthesis. *Tetrahedron Letters*, *26*(2), 201-202. doi:10.1016/S0040-4039(00)61879-8

Rutherford, H.T. (1888). The treatment of haemorrhage in fibro-myalgia by Hydrastis Canadensis. *British*

Medical Journal, July. 21.

Sack, R. B., & Froehlich, J. L. (1982). Berberine inhibits intestinal secretory response of Vibrio cholerae and Escherichia coli enterotoxins. *Infection and Immunity*, *35*(2), 471 -475.

Sanders, J.B. & McGraw, J.B. (2005). Hydrastis canadensis L. (Ranunculaceae) distribution does not reflect response to microclimate gradients across a mesophytic forest cove. *Plant Ecology 181*, 279-288.

Sandhu, R. S., Prescilla, R. P., Simonelli, T. M., & Edwards, D. J. (2003). Influence of goldenseal root on the pharmacokinetics of indinavir. *Journal of Clinical Pharmacology*, *43*(11), 1283-1288. doi:10.1177/0091270003258660

Santos, L.S., Haddad, R. Hoehr, N.F., Pilli, R.A., & Eberlin, M.N. (2004). Fast screening of low molecular weight compounds by thin-layer chromatography and "on-spot" MALDI-TOF mass spectrometry. *Analytical Chemistry, 76*(7), 2144-2147

Sato, F., Hashimoto, T., Hachiya, A., Tamura, K.-ichi, Choi, K.-B., Morishige, T., Fujimoto, H., et al. (2001). Metabolic engineering of plant alkaloid biosynthesis. *Proceedings of the National Academy of Sciences*, *98*(1), 367 -372. doi:10.1073/pnas.98.1.367

Saunders S. and McGraw, J. (2004) Harvest recovery of goldenseal, *Hydrastis canadensis* L. *American Midland Naturalist*, *153*, 87-94.

Scazzocchio, F., Cometa, M. F., Tomassini, L., & Palmery, M. (2001). Antibacterial activity of Hydrastis canadensis extract and its major isolated alkaloids. *Planta Medica*, *67*(6), 561-564. doi:10.1055/s-2001-16493

Schmeller, T., Latz-Brüning, B., & Wink, M. (1997). Biochemical activities of berberine, palmatine and sanguinarine mediating chemical defence against microorganisms and herbivores. *Phytochemistry*, *44*(2), 257-266. doi:10.1016/S0031-9422(96)00545-6

Sevior, D. K., Hokkanen, J., Tolonen, A., Abass, K., Tursas, L., Pelkonen, O., & Ahokas, J. T. (2010). Rapid screening of commercially available herbal products for the inhibition of major human hepatic cytochrome P450 enzymes using the N-in-one cocktail. *Xenobiotica*, *40*, 245-254. doi:10.3109/00498251003592683

Sharp, P. (2003) *Hydrastis canadensis* L. Goldenseal. Conservation and research plan for New England. Framingham, MA: New England Wild Flower Society. Retrieved from http://www.newenglandwild.org/docs/pdf/Hydrastiscanadensis.PDF

Shen, M.-P., Sun, Q., & Wang, H. (1993). Studies on the intravenous pharmacokinctics and oral absorbtion of berberine HCI in beagle dogs *Chinese Pharmacological Bulletin*, *9*, 64-67.

Shitan, N., Bazin, I., Dan, K., Obata, K., Kigawa, K., Ueda, K., Sato, F., et al. (2003). Involvement of CjMDR1, a plant multidrug-resistance-type ATP-binding cassette protein, in alkaloid transport in

Coptis japonica. *Proceedings of the National Academy of Sciences*, *100*(2), 751 -756. doi:10.1073/pnas.0134257100

Skenderi, G. (2003). *Herbal vade mecum*. Rutherford, NJ: Herbacy Press.

Stermitz, F. R., Lorenz, P., Tawara, J. N., Zenewicz, L. A., & Lewis, K. (2000). Synergy in a medicinal plant: Antimicrobial action of berberine potentiated by 5′-methoxyhydnocarpin, a multidrug pump inhibitor. *Proceedings of the National Academy of Sciences*, *97*(4), 1433 -1437. doi:10.1073/pnas.030540597

Stille, A. (1874). *Therapeutics and material medica (4th ed.)*. Philadelphia, PA: Henry C. Lea.

Steriti, R. (2010). Berberine for Diabetes Mellitus Type 2. *Natural Medicine Journal 2*(10), 5-6.

Stoltz, L. (1994) Commercial production of ginseng and goldenseal. *New Crops News,* 4, 1. Retrieved from http://www.hort.purdue.edu/newcrop/newcropsnews/94-4-1/ginseng.html

Sturm, S., & Stuppner, H. (1998). Analysis of isoquinoline alkaloids in medicinal plants by capillary electrophoresis—mass spectrometry. *Electrophoresis*, *19*(16-17), 3026-3032. doi:10.1002/elps.1150191639

Sumner, J. (2004) *American household botany. A history of useful plants 1620-1900.* Portland, OR: Timber Press.

Sun, D., Abraham, S. N., & Beachey, E. H. (1988). Influence of berberine sulfate on synthesis and expression of Pap fimbrial adhesin in uropathogenic Escherichia coli. *Antimicrobial Agents and Chemotherapy*, *32*(8), 1274 -1277. doi:10.1128/AAC.32.8.1274

Sun, D., Courtney, H. S., & Beachey, E. H. (1988). Berberine sulfate blocks adherence of Streptococcus pyogenes to epithelial cells, fibronectin, and hexadecane. *Antimicrobial Agents and Chemotherapy*, *32*(9), 1370 -1374. doi:10.1128/AAC.32.9.1370

Sun, Y., Xun, K., Wang, Y., & Chen, X. (2009). A systematic review of the anticancer properties of berberine, a natural product from Chinese herbs. *Anti-Cancer Drugs*, *20*, 757-769. doi:10.1097/CAD.0b013e328330d95b

Sunali, E.S., Kuttan, R., Preethi, K.C. & Kuttan, G. (2009). Dynamized preparations in cell culture. *eCAM, 6*(2), 257-263.

Swanston-Flatt, S. K., Day, C., Bailey, C. J., & Flatt, P. R. (1989). Evaluation of traditional plant treatments for diabetes: Studies in streptozotocin diabetic mice. *Acta Diabetologica Latina*, *26*(1), 51-55. doi:10.1007/BF02581196

Tegos, G., Stermitz, F. R., Lomovskaya, O., & Lewis, K. (2002). Multidrug pump inhibitors uncover remarkable activity of plant antimicrobials. *Antimicrobial Agents and Chemotherapy*, *46*(10), 3133 -

3141. doi:10.1128/AAC.46.10.3133-3141.2002

The Plant List (2010). Version 1. Published on the Internet; http://www.theplantlist.org/

Thomson, S. (1835). *New guide to health.* Boston, MA: Samuel Thomson.

Tigler, S.M. (2009). *Herbal medicine. From the heart of the Earth. 2nd ed*. Pleasant Hill, OR: Wise Acres LLC

Tilford, G. (1998) *From earth to herbalist: An earth-conscious guide to medicinal plants*. Missoula, MT: Mountain Press.

Tims, M.C. (2006). *The chemical ecology of Hydrastis canadensis L. (Ranunculaceae): Effects of root isoquinoline alkaloids on the Hydrastis endophyte, Fusarium oxysporum*. Retrieved from DRUM (Digital Repository at the University of Maryland). http://drum.lib.umd.edu/handle/1903/4052

Tims, M. C., & Batista, C. (2007). Effects of root isoquinoline alkaloids from Hydrastis canadensis on Fusarium oxysporum isolated from Hydrastis root tissue. *Journal of Chemical Ecology*, *33*, 1449-1455. doi:10.1007/s10886-007-9319-9

Tobe, H., & Keating, R. C. (1985). The morphology and anatomy of Hydrastis (Ranunculales): Systematic reevaluation of the genus. *The Botanical Magazine Tokyo*, *98*, 291-316. doi:10.1007/BF02488779

Trickey, R. (2003). *Women, hormones and the menstrual cycle.* Sydney, Australia: Allen & Unwin.

Unger, M., Laug, S., & Holzgrabe, U. (2005). Capillary zone electrophoresis as a tool for the quality control of goldenseal extracts. *Electrophoresis*, *26*(12), 2430-2436. doi:10.1002/elps.200410322

United States Pharmacopeial Convention (2003): *USP 26 – NF21* Supplement 1

Upton, R. (2001). *Goldenseal root. Hydrastis Canadensis.*, Santa Cruz, CA: American Herbal Pharmacopoeia

US Fish and Wildlife Service. (2012). Goldenseal (*Hydrastis canadensis*). Environmental Conservation Online System, Species profile. Retrieved from http://ecos.fws.gov/speciesProfile/profile/speciesProfile.action?spcode=Q0ZO

US Forest Service (2010). Laws and regulations to protect endangered plants. Celebrating Wildflowers. Website last updated October 13, 2010, Retrieved from http://www.fs.fed.us/wildflowers/rareplants/conservation/lawsandregulations.shtml

US Natural Resources Conservation Service (2012) Hydrastis L. Plants profile. Natural Resources Conservation Service, US Department of Agriculture. Retrieved from http://plants.usda.gov/java/profile?symbol=HYDRA2

Van Arkel, C. G., & Meijst, M. (1952). Comparative study on determination of hydrastine in Hydrastic rhizome and liquid Hydrastis extract. *Pharmaceutisch Weekblad*, *87*(49-50), 853-861.

Van Berkel, G.J., Tomkins, B.A., Kertesz, V. (2007) Thin-layer chromatography/desorption electrospray ionization mass spectrometry: investigation of goldenseal alkaloids. *Analytical Chemistry, 79*(7), 2778-2789.

Vermont Natural Heritage Information Project (2011) Threatened and endangered plants of Vermont. Vermont Fish and Wildlife Department. Retrieved from http://www.vtfishandwildlife.com/wildlife_nongame.cfm

Vermont, State of (2010) Protection of endangered and threatened species. Retrieved from http://www.anr.state.vt.us/dec/permit_hb/sheet47_4.pdf

Wagner, H., Bladt, S., & Zgainski, E. (1984). *Plant drug analysis*. New York, NY: Springer-Verlag.

Weber, H. A., & Joseph, M. (2004). Extraction and HPLC analysis of alkaloids in goldenseal. *Sheng Wu Gong Cheng Xue Bao = Chinese Journal of Biotechnology*, *20*(2), 306-308.

Weber, H. A., Zart, M. K., Ferguson, S. L., Greaves, J. G., Clark, A. P. … Smith, C. (2001). Separation and quantitation of isoquinoline alkaloids occurring in goldenseal. *Journal of liquid chromatography & related technologies*, *24*(1), 87-95.

Weber, H. A., Zart, M. K., Hodges, A. E., Molloy, H. M., O'Brien, B. M. … Smith, C.S. (2003). Chemical comparison of goldenseal (Hydrastis canadensis L.) root powder from three commercial suppliers. *J. Agric. Food Chem.*, *51*(25), 7352-7358. doi:10.1021/jf034339r

Weber, H. A., Zart, M. K., Hodges, A. E., White, K. D., Barnes, S. M. … Harris, R.K. (2003b). Method validation for determination of alkaloid content in goldenseal root powder. *Journal of AOAC International*, *86*(3), 476-483.

Wong, K.I. (1998). Mechanism of the aortic relaxation induced by low concentrations of berberine. *Planta Medica 64*, 756-757.

Yao, M., Ritchie, H. E., & Brown-Woodman, P. D. (2005). A reproductive screening test of goldenseal. *Birth Defects Research. Part B, Developmental and Reproductive Toxicology*, *74*(5), 399-404. doi:10.1002/bdrb.20055

Yin, J., Xing, H. & Ye, J. (2008). Efficacy of berberine in patients with type 2 diabetes. *Metabolism,* 57(5), 712-717.

Yu, C., Zhang, H., Ren, J-Y., Pan, J-F., Zhu, D-Y.,…….Xu, X-R. (2000). Determination and preliminary studies of metabolism of berberine in human urine after oral administration. *Chinese Journal of Clinical Pharmacology* 16, 36-39.

Zenk, M. (1995). Chasing the enzymes of alkaloid biosynthesis. *Organic reactivity: physical and biological aspects* (pp. 89-109). UK: Newcastle Upon Tyne: Royal Society of Chemistry.

Zenk, M. H., Rueffer, M., Amann, M., Deus-Neumann, B., & Nagakura, N. (1985). Benzylisoquinoline biosynthesis by cultivated plant cells and isolated enzymes. *Journal of Natural Products*, *48*(5), 725-738. doi:10.1021/np50041a003

Zuo, F., Nakamura, N., Akao, T., & Hattori, M. (2006). Pharmacokinetics of berberine and its main metabolites in conventional and pseudo germ-free rats determined by liquid chromatography/ion trap mass spectrometry. *Drug Metabolism and Disposition*, *34*(12), 2064 -2072. doi:10.1124/dmd.106.011361

Zwaving, J. H., & de Jong-Havenga, E. H. (1972). Determination of hydrastine in hydrastis fluidextract. *Pharmaceutisch Weekblad*, *107*(8), 137-144.

The following WWW sites provide further information related to *Hydrastis canadensis*.

Pennsylvania Department of Conservation and Natural Resources (DCNR): contains information specific to goldenseal in Pennsylvania, including publications, educational opportunities, and dealer contact information. http://www.dcnr.state.pa.us/forestry/wildplant/vulnerable_plants.aspx

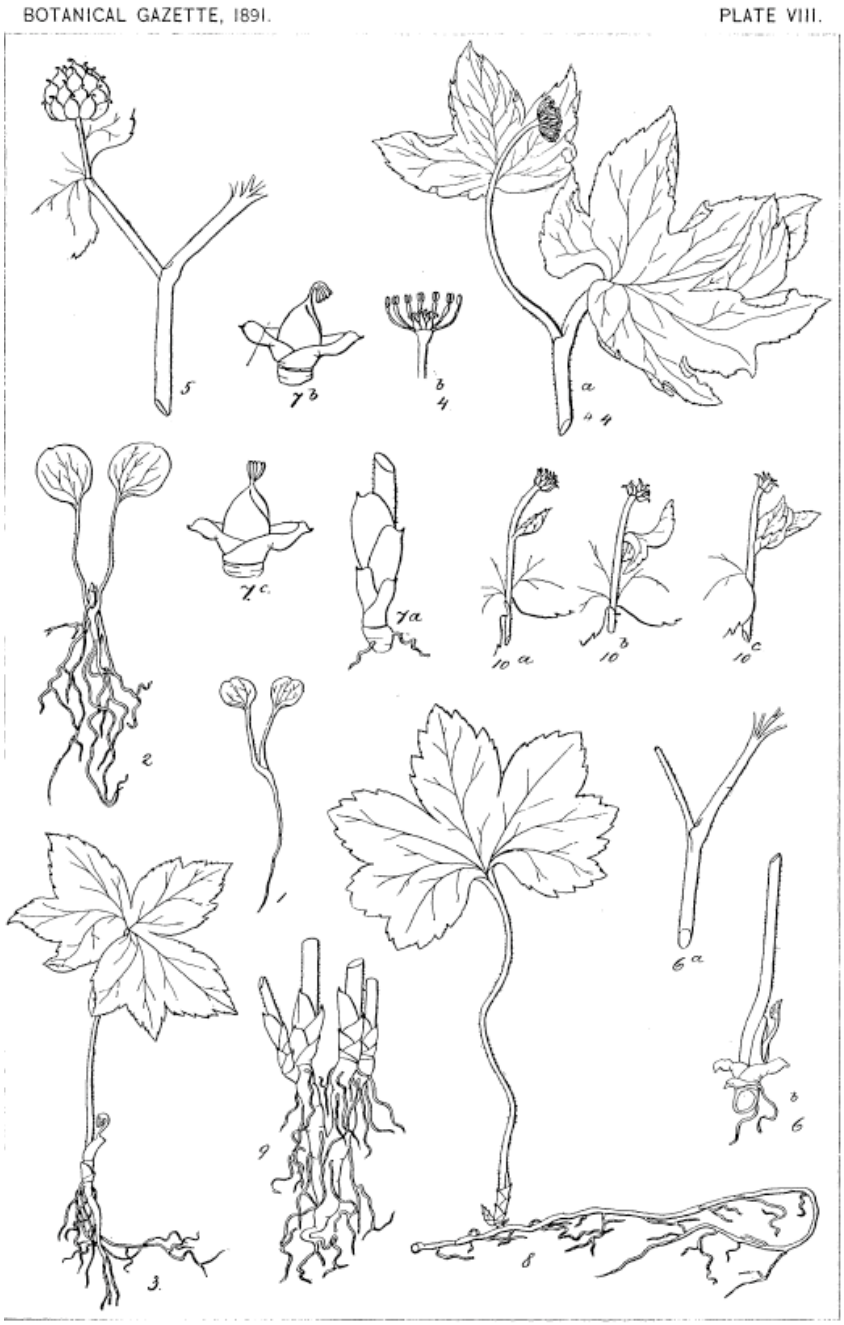
Pennsylvania Flora Project (Morris Arboretum,University of Pennsylvania): contains information about the flora of Pennsylvania, including publications and educational opportunities. http://www.pafl ora.org/

U.S. Fish and Wildlife Service (USFWS): information about CITES and listed species. http://www.cites.org/

U.S. Fish and Wildlife Service (USFWS): contains information about how to obtain a permit to export goldenseal from North America. http://international.fws.gov/permits/plants.html

Appendix 1.

Line drawings of *H. canadensis* botanical features. Reproduced from Bowers, H. in *Botanical Gazette*, 1891



EXPLANATION OF PLATE VIII.—Fig. 1, seedling 2 or 3 days after germination. Fig. 2, seedling at the end of the first season's growth. Fig. 3, second year from the seed. Fig. 4, *a*, top of flowering plant early in May, stipular eminences plainly shown at base of lower leaf; *b*, flower with most of the stamens removed. Fig. 5, part of stem with fruit, late in July. Fig. 6, stipules; *a*, part of fertile stem with amplexicaul petiole of lower leaf, showing tubercular stipules; *b*, caudex with portion of radical leaf petiole, old bud-scales dissected back and showing stipules at base enveloping the rudimentary hibernaculum, depauperate leaf protruding, as observed in June. Fig. 7, stipules as bud-scales; *a*, bud-scales at base of fertile stem, early in May; *b* and *c*, buds formed in the axil of a radical leaf, as they appear in October or November, with outer scales dissected back; *b*, one of the bud-scales surmounted by a depauperate leaf; *c*, same with merely fimbriate attachment; Fig 8, horizontal root fiber, with adventitious bud and radical leaf. Fig. 9, rhizome with most of the fibrous roots removed, showing perpendicular character of axis. Fig. 10, series showing transition from floral bract to leaf, as sometimes observed in exceptional cases where third leaves are produced; *a*, part of upper portion of stem showing attachment of sessile leaf, floral bract, and fruit soon after anthesis; *b* and *c*, same with bracts more leaf-like.