Journal of Ethnopharmacology 149 (2013) 401–408

Contents lists available at ScienceDirect

Journal of Ethnopharmacology

journal homepage: www.elsevier.com/locate/jep

Review

Review on *Cardamine diphylla* (Michx.) A. wood (*Brassicaceae*): Ethnobotany and glucosinolate chemistry

Sabine Montaut*, René S. Bleeker

Department of Chemistry & Biochemistry, Biomolecular Sciences Programme, Laurentian University, 935 Ramsey Lake Road, Sudbury, ON, Canada P3E 2C6

A R T I C L E I N F O

Article history:
Received 1 February 2013
Received in revised form
11 July 2013
Accepted 11 July 2013
Available online 24 July 2013

Keywords:
Cardamine diphylla
Brassicaceae
Glucosinolates
Alkaloids
Free-radical scavenging activity

A B S T R A C T

Ethnopharmacological relevance: *Cardamine diphylla* (Michx.) A. Wood, commonly called toothwort, is a spring perennial herb belonging to the *Brassicaceae* family. This endemic plant of Eastern North America has been widely used by multiple American First Nations (i.e. indigenous people of North America) for food and medicine for centuries.

Approach and methods: The aim of the review is to describe the botany, ethnopharmacology, phytochemistry, and bioactivity of *Cardamine diphylla*. The review covers literature on *Cardamine diphylla*, and the alternative name *Dentaria diphylla*, from English and French language sources.

Results: Multiple traditional uses of *Cardamine diphylla* by American First Nations are well documented. Initial health studies showed that the tested concentrations of the extract were not toxic against brine shrimp larvae and the same extract had a weak free-radical scavenging activity. However, bioactive compounds in the form of aliphatic and indole glucosinolates and some indole alkaloids have been isolated from this plant. Ecological research regarding *Cardamine diphylla*-insect interactions (such as feeding and oviposition) is also available in the literature.

Conclusions: The wide range of traditional uses by multiple American First Nations suggests that the antibacterial, antiviral, immunostimulant, analgesic, antipyretic, and anti-inflammatory activities of this plant should be explored in in vitro and in vivo tests. Traditional modes of preparation of the plant suggest that some of the medicinal properties could certainly be attributed to glucosinolate degradation products (i.e. isothiocyanates), but a clear assignment of active molecules and mechanisms of action remain to be elucidated. The presence of glucosinolates indicates that the plant could be probed for cancer chemopreventive properties. Overall, the review shows that more investigation is necessary to determine the possible benefits of *Cardamine diphylla* extracts to pharmaceutical companies as a nutraceutic specialty phytotherapeutic agent against respiratory (cold and sore throat) or gastrointestinal problems.

Contents

Abbreviations: $ABTS^{+\bullet}$, 2,2′-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid); CYP, Cytochrome P450; $DPPH^{\bullet}$, Diphenylpicrylhydrazyl radical; GC, Gas chromatography; GL, Glucosinolate; GLs, Glucosinolates; HPLC, High performance liquid chromatography; ITC, Isothiocyanate; ITCs, Isothiocyanates; LC–MS, Liquid chromatography–mass spectrometry; SC_{50}, Half maximal scavenging activity

* Corresponding author. Tel.: +1 705 6751151x2185; fax: +1 705 6754844.
E-mail address: smontaut@laurentian.ca (S. Montaut).

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http://dx.doi.org/10.1016/j.jep.2013.07.020

1. Introduction

Cardamine diphylla (Michx.) A. Wood is a spring perennial herb belonging to the *Brassicaceae* family and an endemic plant to Eastern North America. *Cardamine diphylla* was assigned to the *Cardamine* genus in 1877 by Wood (1877). *Dentaria diphylla* Michx. is a commonly used synonym that was previously assigned by Michaux (1820) in 1820. The word *Cardamine* is derived from the Greek word *kardamon*, a name used by Dioscorides for "cress" (Gray, 1908). *Dentaria* is from Latin, *dens*, meaning "tooth". This refers to the form of the rhizome in certain species (Lamoureux, 1975) and to the bidentate nectary of the corolla (Provancher, 1892). *Diphylla* means "with two leaves" in Latin. The plant was well-known to American First Nations and early European settlers. The common names used for *Cardamine diphylla* in a number of different languages are given in Table 1.

As part of our continued interest in the chemistry and biology of the plants from the *Brassicaceae* family and in particular of *Cardamine diphylla*, we reviewed the literature regarding the occurrence, botanical description, ethnopharmacology, phytochemistry, and biological activity of the plant. The review covers the literature up to 2012. Future research activities and pharmaceutical applications of this under-researched plant emerge from this review.

2. Occurrence, botanical description and ethnopharmacology

2.1. Occurrence

Cardamine diphylla is an uncommon plant which can be found growing near streams or swampy areas within forested regions (McKay and Catling, 1979). This plant usually grows together in colonies and in shady regions (McKay and Catling, 1979). In Canada, *Cardamine diphylla* is found in the provinces of New Brunswick, Nova Scotia, Quebec and Ontario, while in the United States, it is found from Michigan to Kentucky, South Carolina to Wisconsin, in Tennessee, and Pennsylvania (Erichsen-Brown, 1979; Horsley et al., 2008; Stehn et al., 2011; Weatherbee and Crow, 1992).

The plant is considered to be a good indicator species of sugar maple (*Acer saccharum* Marsh.) health in New Hampshire and Vermont (Horsley et al., 2008).

The compensation point -the amount of sunlight needed for the photosynthetic rate to equal the rate of respiration—is 0.4%. This is notable as being rather low (Greller, 1988). However, this is not surprising due to the forested habitat that the plant prefers. The light saturation point is 3.8% for *Cardamine diphylla* and is unusually low due to its shady habitat. The maximum photosynthetic rate is 36.6 μmol CO_2 g^{-1} s^{-1} (Greller, 1988).

2.2. Botanical description

2.2.1. The rhizome

Cardamine diphylla emerges from underground stems which grow horizontally (Fig. 1), and are referred to as rhizomes (Chambers et al., 1996). The rhizomes are elongated and "toothed" (Torrey and Gray, 1969). They usually grow close to the surface and have a pungent, peppery odour which is a characteristic of the mustard family (McKay and Catling, 1979). The rhizomes are long and continuous, thickened at the nodes, and green or white (Radford et al., 1968). This species tends to spread rapidly from the crisp and edible rootstocks (Smith, 1966). The rhizomes are constricted at intervals representing a single year′s growth, the segments being easily separable (Scoggan, 1978).

2.2.2. The stem

The stem (Fig. 1) is a continuous rootstock and is notched on the way up (Homer, 1918). It grows from 20 to 40 cm in height (Radford et al., 1968). The plant can be easily multiplied by rootstock division when the plant is dormant.

2.2.3. The leaf

The leaves (Fig. 1) appear approximately half way up the stem (Torrey and Gray, 1969). The leaves are described as crenate (meaning having a margin with low, rounded or scalloped projections) or serrated; with the teeth mucronate (meaning ending abruptly in a sharp point). They are rarely lobed, can be ciliate or are otherwise glabrous. The basal leaves are ovate to elliptic and are often evergreen; they measure 10 cm long by up to 6 cm wide (Radford et al., 1968). The leaf has a linear to lanceolate shape. The petioles are 10–15 cm long and the stem has two leaves (Radford et al., 1968). The petioles are sub-opposite, 1–2 cm long (Radford et al., 1968), short-petiolulate and rhombic-ovate or oblong-ovate (Gray, 1908). The lifespan of the leaf is usually greater than ten weeks

Table 1
Vernacular names of *Cardamine diphylla*.

Tribe/language	Name	References
Abnaki	kondouhi′jak meaning "little veins" referring to the winding roots through soil	Lamoureux (1975), Rousseau (1947)
Cherokee	anahlskwalʌ·ski translated to "crowsfoot" or "crowfoot"	Perry (1974)
Delaware	"little burr"	Tantaquidgeon (1942, 1972)
English	Crinkled, crinkleroot, pepper-root, toothache-root, toothwort, twin-leafed toothwort, white flowered toothwort, trickle, trickle-root, two toothed pepper-root	Hamel and Chiltoskey (1975), Yanovsky (1936), Newcomb (1977), Erichsen-Brown (1979), Coffey (1993)
French	Carcajou, corson, dentaire à deux feuilles, snicroût meaning "snake-root" due to its inter-twined roots	Lamoureux (1975), Black (1980)
Iroquois	a-tsa, ιkde′hϵks (Onondaga), o$^{/\varepsilon}$djia$^{\varepsilon}$	Rousseau (1945), Waugh (1916), Herrick (1977)
Malecite	ka/djiwuk "something growing in a hiding place"	Mechling (1959)

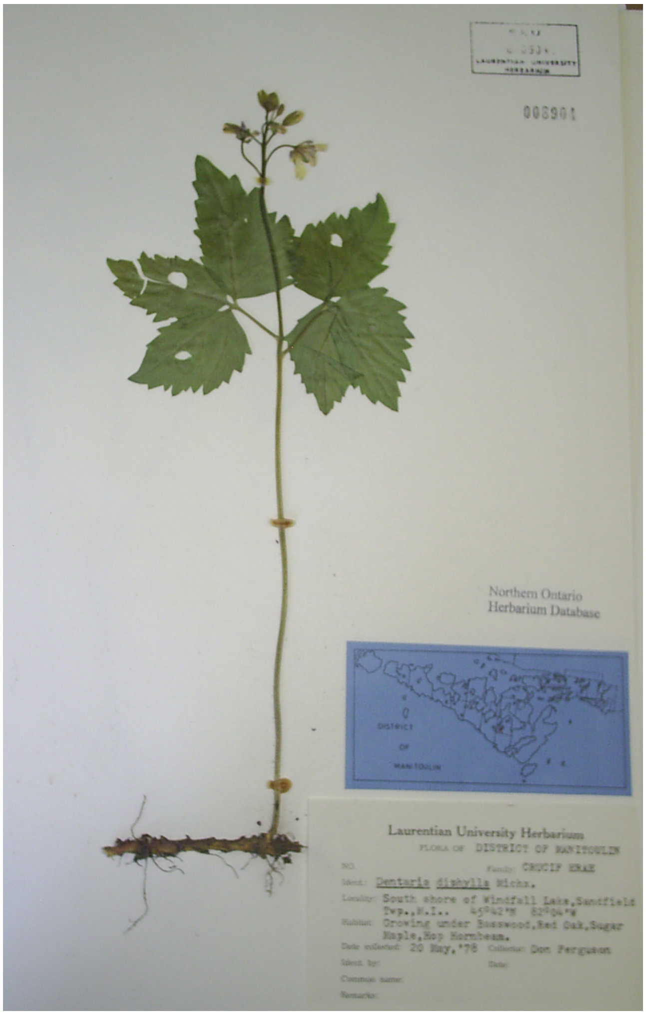

Fig. 1. *Cardamine diphylla* (Michx.) A. Wood.

(Greller, 1988). A quantitative study was undertaken by Turrill et al. to help in the identification of *Cardamine* species, particularly *Cardamine diphylla*, *Cardamine angustata* O.E. Schulz and *Cardamine concatenata* (Michx.) Sw. *Cardamine diphylla's* vegetative characteristics above ground were measured and tabulated (Turrill et al., 1994). The basal leaf length, basal leaf width, inflorescence number, cauline leaf length, cauline leaf width, the node length, the petal length, the sepal length, the serration number and stem length measured 4.31 ± 0.30 cm, 2.91 ± 0.25 cm, 9.30 ± 0.95 cm, 5.91 ± 1.30 cm, 1.97 ± 0.18 cm, 7.77 ± 0.87 cm, 10.82 ± 0.51 mm, 5.17 ± 0.21 cm, 4.17 ± 0.22 cm, and 23.00 ± 1.97 cm, respectively.

Cardamine diphylla was chosen as a test species to determine the role of light and growth regulators in the opening of the *Cardamine* petiolar hook (Yopp, 1973). Large numbers of uniform leaves were easily obtained from a clonal stock of its rhizome. The samples were grown in dark, infra-red, and near-infra-red conditions. It was found that the hook opened even without the leaf in gibberellic acid (0.04% in lanolin) and light. $CoCl_2$ (0.1–1.0 mM) promoted a partial hook opening in the dark, with or without leaf. Also, coumarin (1 mM), indoleacetic acid (1–4% in lanolin), and ethylene ($10\ \mu L\ L^{-1}$) inhibited the opening of hooks with or without lamina. This study showed some of the mechanisms involved in the opening of the petiolar hook and indicated that it is the result of several phytochrome-mediated, intermediate, and morphogenetic events (Yopp, 1973).

Sometimes, the biotrophic oomycete pathogen *Albugo hesleri* Y. J. Choi et Thines infects living leaves of *Cardamine diphylla* (Ploch et al., 2010). It seems possible that *Albugo hesleri* is restricted to this plant and thus to North America and that it is a geographic speciation.

2.2.4. The flower

The flower of *Cardamine diphylla* (Fig. 1) is easy to spot due to its four large white or light purple petals (McKay and Catling, 1979) that range between 1.1 and 1.7 cm long (Erichsen-Brown, 1979). The broad flowers, 14–18 cm in width (Britton and Brown, 1913) are borne in a loose few-flowered raceme (Smith, 1966). The colour variation is due to fading of the petals (Chambers et al., 1996). The sepals are green, linear, pointed, and about half as long as the petals (Smith, 1966). The pistils are long and thin (Smith, 1966).

The flowering season happens from April to early June depending on the location (Chambers et al., 1996; Smith, 1966). The gametophytic development is similar to that of *Cardamine concatenata* (Michx.) O. Schwarz. This includes the formation of a triad of meiotic products (i.e. the antipodal cells), the production of an eight nucleate embryo sac and a three-celled pollen grain. Pollination by honeybees is also possible; however the emerging pollen tubes fail to penetrate the style. The embryo sacs fail to organize and eventually abort (Spooner, 1984).

2.2.5. The seed

The seeds are contained in lanceolate-shaped siliques which are longer than 2.3 cm (Smith, 1966; Britton and Brown, 1913). The seeds are arranged in a single row and are wingless and flattened. Specimens of the *Cardamine* genus usually have 20–100 seeds per plant. The seeds are $2.8 \times 1.8 \times 1.0$ mm (Montgomery, 1977). Their surface is slightly wrinkled or rugulose, and they are green to brown (Montgomery, 1977). They rarely reach maturity (Chambers et al., 1996). The fact that mature siliques are seldom produced has often been mentioned (Rollins, 1993). In the occasional plant that does produce fruit, these are essentially parthenocarpic (i.e. producing fruit without fertilization). The ovules are aborted during fruit development, and according to Montgomery (Montgomery, 1977), even those seeds which appear to be mature are not viable. Thus, *Cardamine diphylla* is essentially an asexually reproducing species (Rollins, 1993). Finally, *Cardamine diphylla* has the highest known chromosome number within the *Brassicaceae* family with a haploid number of 48 (Radford et al., 1968).

2.3. Ethnopharmacology

Cardamine diphylla has many traditional uses among the American First Nations (i.e. indigenous people of North America), primarily the roots but also the whole plant. The roots of *Cardamine diphylla* can be used for food, and during the spring, the whole plant and the root can be used as medicine (McKay and Catling, 1979). When the plant is administered orally, the rhizome is claimed to have a pleasant, pungent taste (McKay and Catling, 1979) which is similar to that of mustard (Smith, 1966) or horseradish. It can be added to green salads and sandwiches, eaten as a radish-like relish with salt, or grated and prepared like horseradish (Facciola, 1990). Ground *Cardamine diphylla* can be put into vinegar for use as a relish (Black, 1980). *Cardamine diphylla* has been used as a folk remedy for toothaches (Foster and Duke, 1990). The American First Nations chewed the roots for colds or applied poulticed roots for headaches (Foster and Duke, 1990). A tea can be gargled for sore throats, hoarseness, and to clear the throat (Foster and Duke, 1990). Detailed traditional uses of the different parts of the plant are summarized in Table 2.

Table 2
Traditional uses for *Cardamine diphylla*.

Tribe	Location	Method	Application	References
North American First Nations	North America	Chewed parts of the plant	Remedy for toothaches	Moerman (1998)
Abnaki	New England, Quebec, Maritimes	Whole or Ground root	Eaten raw with salt or boiled or ground with meats. Also put in vinegar	Rousseau (1947)
Algonquin	Quebec	Infusion of the plant	Pediatric aid given to children with fevers, for heart disease. Used for tea with root of *Acorus calamus* L	Black (1980), Moerman (1998), Arnason et al. (1981)
Algonquin/Cree	Quebec	Ground roots mixed with salt, sugar, or vinegar	As a condiment or relish	Black (1980)
Cherokee	Eastern and Southern United States of America	Applied as an analgesic poultice	For headaches	Hamel and Chiltoskey (1975), Moerman (1998)
		Chewed root	As a cold remedy	Hamel and Chiltoskey (1975), Moerman (1998)
		Infusion of the root	Gargled for aiding sore throats	Hamel and Chiltoskey (1975), Moerman (1998)
		Leaves used in salad or leaves and stem prepared as boiled greens	As a food source	Perry (1974), Hamel and Chiltoskey (1975)
Delaware	Delaware	Infusion of the root	Gastrointestinal aid and stomach remedy	Moerman (1986, 1998)
			Venereal aid when combined with other plants. For tuberculosis	Tantaquidgeon (1972), Moerman (1977, 1986, 1998)
Delaware, Oklahoma	Oklahoma	Roots	Gastrointestinal aid (stomach remedy), tuberculosis remedy, venereal aid	Moerman (1998)
Iroquois	Southern Ontario, Quebec, Eastern United States of America	Cold infusion of the plant	Used as a febrifuge and taken for fever or "summer complaint"	Moerman (1986, 1998)
		Dried or fresh chewed roots	Throat aid for hoarseness	Moerman (1998)
		Eat whole plant in spring	Stomach tonic.	Rousseau (1945), Arnason et al. (1981)
		Infusion of the plant	Breast treatment to reinforce the lungs. Pulmonary aid for chest pains. Tuberculosis remedy for the first stages of tuberculosis	Moerman (1998)
		Infusion of roots	Psychological aid, used as a love medicine Tonic	Herrick (1977), Moerman (1986, 1998)
		Infusion of the roots with *Cypripedium calceolus* L.	Tuberculosis	Rousseau (1945), Arnason et al. (1981)
		Plant	Thought to cure bad luck in general	Herrick (1977, 1995)
			Without preparation, used as a cold remedy and dietary aid or gastrointestinal aid to stimulate appetite & regulate stomach. Pulmonary aid (for chest pain)	Moerman (1986)
		Poultice of roots	Dermatological aid (applied to swelling)	Moerman (1986, 1998)
		Chewed raw root	For stomach gas	Herrick (1977, 1995), Moerman (1986, 1998)
			Eaten with salt or boiled	Rousseau (1945), Waugh (1916), Sturtevant (1919)
		Roots	Eaten as a vegetable condiment raw or boiled or marinated in vinegar	Rousseau (1945), Waugh (1916)
			Used several ways for heart palpitations or other heart diseases	Moerman (1986)
		Whole plant	"Reinforce the chest"	Rousseau (1945)
Malecite	New Brunswick, Quebec and Maine	Roots	Tonic, sedative and for hoarseness	Mechling (1959), Arnason et al. (1981), Chandler et al. (1979)
Micmac	South Eastern Canada and Maine	Roots	Tonic and sedative To clear the throat and for hoarseness	Moerman (1986, 1998)
Ojibwa	Upper Midwest and Southern Ontario.	Ground roots mixed with salt, sugar, or vinegar	Condiment or relish	Arnason et al. (1981)

3. Phytochemistry

Only a few phytochemical studies have been performed on *Cardamine diphylla*. The investigations were restricted to the identification of glucosinolates (GLs) and indole alkaloids. No research group has searched rigorously for other phytochemicals in this plant. The number of different natural products identified is 12.

The first investigation studied the seasonal variation in the GL content of *Cardamine diphylla* plants collected in New York State (voucher specimen deposited at the Bailey Hortorium, Cornell University, Ithaca, USA) (Feeny and Rosenberry, 1982). The GLs in the plant extracts were hydrolyzed by the endogenous enzyme myrosinase (β-thioglucoside glucohydrolase; E.C. 3.2.1.147) yielding the corresponding isothiocyanates (ITCs). Each ITC was isolated, analysed by gas chromatography (GC) and identified by comparison of their retention times with commercial standards. The ITCs detected by GC analysis of the material extracted from the leaves of *Cardamine diphylla*, indicated the presence of 1-methylethyl- (**1**), 1-methylpropyl- (**2**), and 2-methylbutyl GL (**3**) (Fig. 2). The major GL was **1** (67.2% in 17 samples until May 16, 76.1% in 8 samples after May 16 and 70.0% in 25 samples for the whole season) followed by **3** (22.2% in 17 samples until May 16, 17% in 8 samples after May16, and 20.6% in 25 samples for the whole season) and **2** (10.6% in 17 samples until May 16, 6.9% in 8 samples after May 16, 9.4% in 25 samples for the whole season). In *Cardamine diphylla* leaves from Connecticut about 95% of the total ITCs was 1-methylethyl ITC and traces of 1-methylpropyl- and 2-methylpropyl ITCs were detected in the volatile hydrolysis products obtained from the leaves (Feeny and Rosenberry, 1982). This indicates that **1**, **2**, and 2-methylpropyl GL (**4**) would be present in this plant organ of *Cardamine diphylla*. Moreover, it was determined that the GLs of *Cardamine diphylla* (plant origin: New York State) have an initial seasonal variation of 0.8–1.8% which declines to virtually 0% fresh weight at the end of the season (Feeny and Rosenberry, 1982). Furthermore, during the same period it was observed that the GL content was higher in shorter and probably younger leaves than in longer and probably older leaves. It was noted that the amount of **1** increased while those of **2** and **3** decreased during the growing season. In fact, **1** reached a maximum of 80% of the total GL content in the leaves, during May. GL levels in leaves of greenhouse-grown *Cardamine diphylla* were lower than those in plants harvested from natural habitats. However, the relative proportions of the compounds **1**–**3** were similar (77.2% of **1**, 3.0% of **2**, and 19.8% of **3**) (Feeny and Rosenberry, 1982). Total GL concentrations in the rhizomes (plant origin: New York State and Connecticut, voucher specimen deposited at the Bailey Hortorium, Cornell University, Ithaca, USA) were similar to those found in young leaves. Relative proportions of **1**–**3** were different (65.9% of **3**, 26.1% of **1** and 8.0% of **2**), however, and seasonal fluctuations of GL contents were less noticeable in rhizomes except that they increased by about 25% during the two weeks immediately before the leaves appeared. Moreover, the relative proportions of **1**–**3** (24.0% of **1**, 10.0% of **2**, and 66.0% of **3**) in one sample of underground shoots were similar to those measured in the rhizomes (Feeny and Rosenberry, 1982).

Our research group has confirmed and added several other GLs and indole alkaloids to the list, from rhizome samples collected in Quebec (voucher specimen number 21024 deposited at the Department of Biology Herbarium at Laurentian University, Sudbury, ON Canada): **1**, **3**, desulfo-2-methylbutyl GL (**5**), 3-methylpentyl- (**6**), 3-indolylmethyl- (**7**), 1-methoxy-3-indolylmethyl- (**8**), 4-methoxy-3-indolylmethyl GL (**9**), 5′-*O*-β-D-glucopyranosyldihydroascorbigen (**10**), 6-hydroxyindole-3-carboxylic acid 6-*O*-β-glucopyranoside (**11**), and tryptophan (**12**) (0.29 μmol per gram of rhizomes dry weight) (Fig. 2). We have isolated these compounds from the methanol extract of *Cardamine diphylla* rhizome using chromatographic techniques and established structures using spectroscopic methods (Bleeker, 2010; Montaut et al., 2010; Montaut and Bleeker, 2010). Compounds **1**, **3**, **6**, **7**, **10**, and **11**

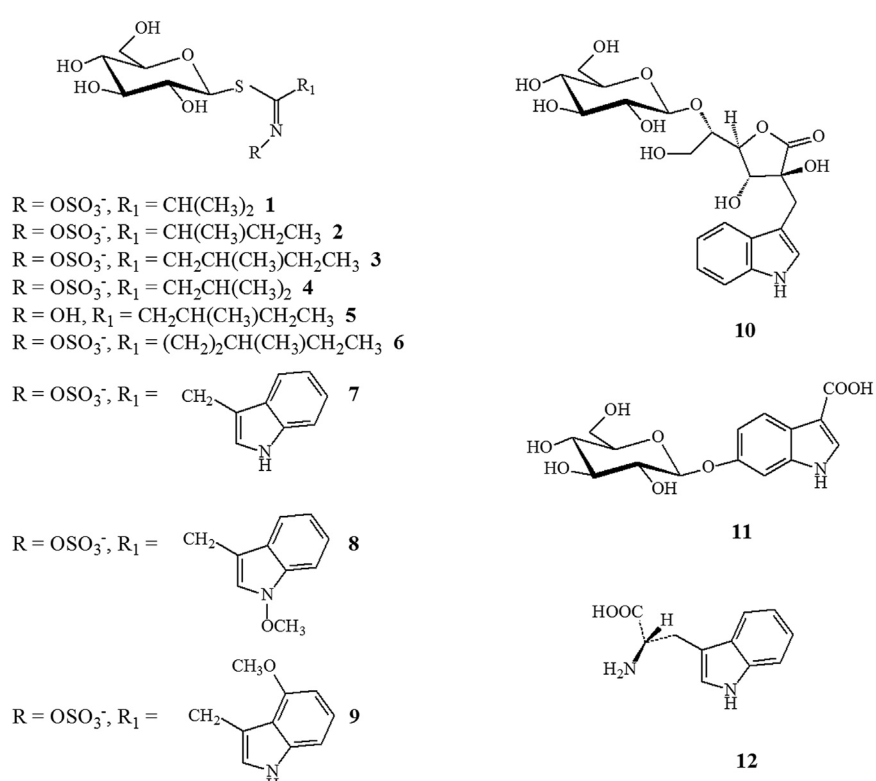

Fig. 2. Phytochemicals identified from *Cardamine diphylla* plant.

were also detected in the aerial parts using HPLC (Bleeker, 2010). Agerbirk et al. (2010) detected **1** as the major GL in the aerial parts of *Cardamine diphylla* (plant origin: Massachusetts) using LC–MS analysis.

Zinc and calcium nutrition have been characterized to some extent (Graves and Monk, 1982). Soils of varying quantities of extractable Zn and Ca did not seem to correlate with quantities of metals found in the plant.

4. Bioactivity

4.1. Non-toxicity and free-radical scavenging activity

Our group began probing the toxicity of the methanol crude extract of *Cardamine diphylla* rhizomes (10, 100 and 1000 ppm concentrations) against *Artemia salina* L. (brine shrimp) larvae. Tested concentrations of the extract were not toxic against the brine shrimp larvae (Bleeker, 2010; Montaut et al., 2010). In addition, we have tested the free-radical scavenging ability of the same extract against the diphenylpicrylhydrazyl radical ($DPPH^{\bullet}$). The half maximal scavenging concentration, SC_{50}, of the methanol crude extract of *Cardamine diphylla* rhizomes, in the $DPPH^{\bullet}$ assay, was 766 μg/mL ($\pm$ 3 μg/mL) (Bleeker, 2010; Montaut et al., 2010). The extract exhibited weak free-radical scavenging capabilities compared to ascorbic acid that showed a half-maximal scavenging concentration, SC_{50}, of 7 μg/mL ($\pm$ 2 μg/mL) (Bleeker, 2010; Montaut et al., 2010). The secondary metabolites responsible for the weak free-radical scavenging activity of the rhizome extract have not been identified. However, GLs **1**–**4**, **6**–**9** and compound **10** are expected to be partly responsible for the activity. In fact, in a previous study, our group has shown that other GLs (6-methylsulfanylhexyl-, 6-methylsulfinylhexyl- and 4-methylsulfanyl-3-butenyl GL) have free-radical scavenging activity towards $DPPH^{\bullet}$ and that these GLs were weaker scavengers than flavonoids (Montaut et al., 2012). In addition, in a recent study, the GL **7** did not display any antioxidant activity towards $DPPH^{\bullet}$, was a weak antioxidant towards $ABTS^{+\bullet}$ (2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)) whereas it was a strong antioxidant in the oxygen radical absorbance capacity assay and superoxide radical scavenging activity assay (Cabello-Hurtado et al., 2012). Moreover, the natural product ascorbigen with a structure related to **10** showed only little scavenging activity toward $DPPH^{\bullet}$ (Wagner et al., 2008). Free radicals provoke inflammatory responses and therefore free radical scavengers can attenuate inflammation. An anti-inflammatory activity would relate to the traditional uses of the plant in reducing swelling and fever. Free radical scavengers would be responsible for the activity.

4.2. Plant–insect interactions

Contrary to the investigations of the medicinal properties of *Cardamine diphylla*, there has been more research on *Cardamine diphylla*-insect interactions. Many insects are dependent on *Cardamine diphylla* for food and oviposition. In natural habitats, *Cardamine diphylla* was shown to be exclusively eaten by the flea beetle *Phyllotreta bipustulata* (Fabricius) (Hicks and Tahvanainen, 1974). However, when the plant has been transplanted into open-fields, *Cardamine diphylla* was heavily damaged by the open habitat flea beetles *Phyllotreta bipustulata*, but also by *Phyllotreta cruciferae* Goeze, *Phyllotreta striolata* F., *Phyllotreta zimmermanni* Crotch, *Psylliodes napi* (Fabricius), and *Psylliodes punctulata* Melsheimer (Hicks and Tahvanainen, 1974). This indicated that the plant must be naturally protected against these herbivores by growing in shady woodland habitats. In the laboratory feeding experiments, *Cardamine diphylla* was eaten by *Phyllotreta cruciferae*, *Phyllotreta striolata*, *Phyllotreta bipustulata* and *Psylliodes napi*. The secondary metabolites in the plant could be used to determine the relative amount of feeding on it (Hicks and Tahvanainen, 1974).

Cardamine diphylla is also the primary host plant for *Pieris virginiensis* Edwards, a native woodland butterfly (Benson et al., 2003). Habitat reduction due to crops or pasture land may have reduced the number of *Cardamine diphylla* found in nature, and consequently the number of *Pieris virginiensis* butterflies who use the leaves for oviposition. Another factor contributing to the rarity of *Pieris virginiensis* is the early senescence of *Cardamine diphylla* leaves, therefore stranding the caterpillars, which are unable to find a secondary host. In addition, the granulosis virus, which can reside and build up in soils, vertically transmits to the butterfly, leading to higher mortality (Cappuccino and Kareiva, 1985).

Another study of the *Pieris virginiensis* butterfly showed that oviposition on *Cardamine diphylla* is highly selective (Doak et al., 2006). Interestingly, the butterflies oviposit on only half of the plants they inspect, and fly over most potential host plants. In a study where larvae were placed on *Cardamine diphylla* selected by the butterflies and nearby control *Cardamine diphylla*, it was found that larval survival was greater by a factor of two on the plants chosen by the butterflies. Furthermore, the authors found that eggs laid in the first half of the season have three times greater survival chances due to rapid leaf senescence of *Cardamine diphylla* (Doak et al., 2006).

The pierid butterfly, *Pieris napi oleracea* Harris was observed to utilize *Cardamine diphylla* in wooded areas, which are rarely occupied by *Pieris rapae* L. (Chew, 1981). Furthermore, *Pieris napi oleracea* was observed to change habitats between the first and second generations in Vermont and Massachusetts (USA), with over 95% of eggs from the first generation laid on *Cardamine diphylla*. The number of first generation of *Pieris napi* butterflies that enter diapause increases if eggs are laid on *Cardamine diphylla*. On pre-flower-bud *Cardamine diphylla*, 63% of first instar *Pieris napi* entered diapause in Massachusetts and 86% entered diapause in northern Vermont (Van Driesche et al., 2004).

Finally, the larvae of *Pieris rapae* L. feeding on *Cardamine diphylla* leaves was shown to have an exceptional growth rate, attributed to a higher growth efficiency and nitrogen utilization efficiency (Slansky and Feeny, 1977). It was suggested that *Pieris rapae* larvae have adapted to tolerate the presence of **1**–**3** in *Cardamine diphylla* leaves. The mechanisms by which *Pieris rapae* avoid the toxicity of the GLs were not established. It was hypothesized that the absence of certain GLs (other than aliphatic GLs), that are present in other open-habitat *Brassicaceae* plants and have some inhibitory properties on *Pieris rapae* larval growth, would explain the fast growth of *Pieris rapae* on *Cardamine diphylla* (Feeny and Rosenberry, 1982).

5. Conclusions

This article has reviewed the current knowledge regarding the plant *Cardamine diphylla*. Despite the fact that a wide range of traditional uses of *Cardamine diphylla* by multiple American First Nations are known and that several plant extracts of multiple species of the *Cardamine* genus were shown to possess diverse biological activities (Montaut and Bleeker, 2011), few studies regarding such properties of *Cardamine diphylla* have been reported (Bleeker, 2010; Montaut et al., 2010). Some free-radical scavenging activity of *Cardamine diphylla* has been observed in vitro indicating that an anti-inflammatory activity must be confirmed to correlate with the traditional uses of the plant to reduce fever, headache, pain and swelling. Moreover, the active molecules and the mechanisms of action should be identified. Up to now, no toxicity related to the consumption of *Cardamine diphylla* has been noticed.

Regarding the phytochemistry, several GLs and some alkaloids have been isolated from this plant. The alkaloid **10** is rare in nature and so far has only been detected in *Cardamine diphylla*. However, the presence of a related structure, glucosylated indole ascorbigen, has been previously reported (Pedras and Zheng, 2010). The occurrence of alkaloid **11** is also rare as it has only been isolated in *Arabidopsis thaliana* (L.) Heynh. (i.e. leaves infected with the virulent or avirulent bacterial pathogen, *Pseudomonas syringae* pathovar tomato strain and in non-infected as well as roots infected by the root-pathogenic oomycete, *Pythium sylvaticum* Campbell & Hendrix) and *Cardamine diphylla* (Bednarek et al., 2005; Hagemeier et al., 2001; Montaut and Bleeker, 2010). This metabolite has a role in disease resistance in *Arabidopsis thaliana* but no particular biological activities or mechanisms have been elucidated. The alkaloids **10**–**11** have never been probed for therapeutic activities. The fact that the alkaloid **11** plays a role in plant resistance to bacterial or fungal attacks and that this metabolite is present in *Cardamine diphylla* indicates that a screening of bacterial and antifungal activities of this plant should be investigated. The presence of the GLs **7**–**9** in *Cardamine diphylla* is worth noting because **7** and the mixture of **7**–**9** can induce an increase in the CYP1A protein (phase I enzyme involved in the detoxification of chemical carcinogens) levels and activities in rats (Bonnesen et al., 1999). The GL degradation products produced during plant extractions at room temperature by the myrosinase enzyme are responsible for several biological activities (Agerbirk et al., 2009; Montaut and Bleeker, 2010). Some ITCs have been shown to possess antibacterial activities against human oral pathogens (Kim et al., 2009) and bacteria from the human gastrointestinal tract (Aires et al., 2009; Fahey et al., 2002). They have also been shown to have some cancer chemoprevention properties (Agerbirk et al., 2009; Navarro et al., 2011; Traka and Mithen, 2009). Traditional modes of preparation of the plant suggest that some of the medicinal properties such as those against colds, sore throat, and gastrointestinal problems could certainly be attributed to the ITCs and GLs. When the plant is used at room temperature raw, chewed, ground, in salt or marinated in vinegar, the GLs will enzymatically degrade quickly into ITCs (and to further degradation products if not consumed rapidly) and ingested by humans. On the contrary, GLs are expected to be ingested when the plant organ is given as an infusion because the deactivation of myrosinase by the heat during the preparation will prevent GL degradation and GLs are water soluble metabolites. However, the length of infusion must be short as GLs can decompose when left too long in boiling water (Bones and Rossiter, 2006). Besides GLs, other families of secondary metabolites should be investigated as such studies could reveal new natural products with pharmacological activity. The plant extracts, using different conditions of extraction, varying the solvent and temperature of extraction, using different plant organs, varying the plant origin and collecting plants in different seasons should be prepared and screened for new or/and more potent bioactive metabolites. In order to evaluate the commercial development of this wild plant, its cultivation on a large scale at different sites should be attempted to check whether the bioactive compounds are still produced in these conditions. A large scale production of *Cardamine diphylla* could also benefit the food industry by using the rhizome in the manner that American First Nations people have been using it. The rhizome of the plant has a pleasant pungent taste due to the release of ITCs from the degradation of GLs. As for horseradish and wasabi rhizomes, *Cardamine diphylla* rhizome could be used and commercialized fresh, as a paste condiment like the one obtained from mustard seeds, as a relish or salted fermented root preparation like kimchi. Similar to rocket leaves, leaves of *Cardamine diphylla* could be cultivated for consumption in salads. A nutraceutic specialty containing *Cardamine diphylla* root powder or GL- or ITC-enriched extract of the rhizome with cancer chemoprevention application could be considered in the future. Overall, this review shows that the antibacterial, antiviral, analgesic, antipyretic, immunostimulant, anti-inflammatory, and cancer chemoprevention activities of this plant should be explored in in vitro and in vivo tests and in clinical trials before an extract of *Cardamine diphylla* could be exploited in the future by pharmaceutical companies as a phytotherapeutic agent.

Acknowledgements

Financial support from the Natural Sciences and Engineering Research Council of Canada (Discovery grant, S.M.) and Laurentian University (teaching assistantship and scholarship to R.B.) is gratefully acknowledged. We also thank Prof. H. Joly for her comments on the manuscript. Information presented herein were part of a M. Sc. thesis (Bleeker, 2010).

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