European elderberry (Sambucus Nigra L.) and American Elderberry (Sambucus Canadensis L.): Botanical, chemical and ….

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European elderberry (*Sambucus nigra* L.) and American Elderberry (*Sambucus canadensis* L.): Botanical, chemical and health properties of flowers, berries and their products

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Abstract

A full evaluation of elderberry botany, production, orchard establishment and technology together with chemical composition of flowers, berries and elder products is presented. The uses of American and European Elderberry are discussed and medical reports on both elder flowers and berries highlighted. The antioxidant, antimicrobial, anti-inflammatory and cancer-chemo preventive properties of elderberry products are reported. Chemical composition of elderberry consists of primary metabolites (organic acids, sugars), secondary metabolites (phenols) and several other constituents (vitamins A and C, cytokines). Elder flowers are distinguished by their intensive, pleasant odor and serve as a basis for industrially produced soft drinks and extracts. They are a rich source of potential bioactive flavonoids and phenolic acids; quercetin-3-rutinoside (rutin), quercetin-3-glucoside (isoquercitrin), kaempferol-3-rutinoside, isorhamnetin-3-rutinoside and isorhamnetin-3-glucoside were identified among flavonoids and among phenolic acids, derivates of quinic acid containing caffeic or *p*-coumaric acid moieties were determined. Flavonol glycosides rutin, kaempferol-3-rutinoside and isorhamnetin-3-rutinoside are the major flavonoids in elder flowers, present in much higher concentrations than in elder fruit. Elder flowers exhibit a much stronger neutralizing activity of free radicals compared to elderberry fruit, which is characterized by high organic acids levels, particularly citric acid, and the most abundant sugars in berries, fructose and glucose. These make the ripe berries ideal for processing to juices, candy and concentrates. Among phenolic compounds in elder berries, anthocyanins, quercetins and other polyphenolic flavonoids are of great interest due to their health-beneficial properties. Cyanidin-3-glucoside and cyanidine-3-sambubioside are the prevalent anthocyanins in elder berries, characterizing their purple color and particularly important for natural pigment industry. Other anthocyanins, such as cyanidin-3-sambubioside-5-glucoside, cyanidin-3,5-diglucoside and cyanidin-3-rutinoside are only presented in minor concentrations. In the group of quercetins, quercetin, rutin and quercetin 3-glucoside were detected; with rutin the predominant one. In elderberry extracts and wine, a high content of both primary and secondary metabolites has also been reported. Anthocyanins, as well as other flavonoids, exhibit antioxidant, immune-stimulating, antibacterial, antialergic and antiviral properties; therefore, their consumption may contribute to prevention of several degenerative diseases such as cardiovascular disease, cancer, inflammatory disease and diabetes.

INTRODUCTION

Berries are rich sources of primary and secondary metabolites, the latter particularly important as they provide a number of beneficial functions for human health such as antioxidant protection and therapeutic benefits including reduced risk of coronary heart disease, reduced risk of stroke, anticarcinogenic activity, improved visual acuity, and improved cognitive behaviour (Prior, 2003; Zafra-Stone et al., 2007; Ozgen et al., 2010). Small fruits containing anthocyanins and other polyphenols, particularly strawberry, raspberry, blueberry, cranberry, and currants, have received much attention; however, some species such as elderberry, are still fairly unknown.

Elderberry flowers and fruit are now predominately used for various processed products and many species and cultivars with high concentrations of organic acids, anthocyanins and other phenolics have been bred for commercial growing. Historically, elderberries have been used medicinally by many indigenous cultures. Native Americans even used the hollow stems and bark of the American elderberry to make toys and musical instruments. The name Elder, is probably derived from the Anglo-Saxon word *aeld*, meaning fire. Old names like Holler, Hylder, Hyllantree, and the German word Holunder all refer to an ancient vegetation goddess and elder-tree was even considered sacred. Therefore, elderberry was often planted around the house and on the farm grounds. Since elders never seemed to get struck by lightning, having it grow near the house was believed to protect the house as well. However, in Christianity, the sacred elder tree became a tree of witches and the old stories were soon distorted. The Church portrayed elder as a tree of sorrow because Judas supposedly hung himself from one after betraying Jesus. Even the cross upon which Jesus was crucified was said to have been made of elder wood. According to Christian mythology this was the reason why elders never since could grow up straight and even to this day barely have the strength to support themselves.

Elderberry cultivars are now planted for ornamental purposes, elderflower extracts are used for beverage and food flavouring (Christensen et al., 2007), and elderberry berries are globally utilised as a medicine or a source of dietary supplement (Dawidowicz et al., 2006; Lee and Finn, 2007). The purple-black fruits of elderberries (*Sambucus* spp. L.) are one of the richest sources of anthocyanic pigments and phenolic compounds among small fruits and have strong antioxidant capacity (Koca and Karadeniz, 2009; Lee and Finn, 2007; Veberic et al., 2009). High amounts of anthocyanins, especially cyanidin 3-sambubioside and cyanidin 3-glucoside are important constituents of elderberry fruit and make them ideal for processing to elderberry juices, extracts and alcoholic beverages such as elderberry wine.

SPECIES DISTRIBUTION, HABITAT AND BOTANICAL PROPERTIES

Elderberry (*Sambucus*) genus was previously classified in the honeysuckle family (Caprifoliaceae) but due to new genetic research and reclassification (Donoghue, 2003) it now belongs to moschatel family (Adoxaceae). Plants from this family are mostly woody perennials and include vines, shrubs, and small trees with orange to black berries. Fruit characteristics such as number of berries in umbels, the size and colour of berries as well as branching patterns are found to be particularly important for the classification of individual species (Jordheim et al., 2006).

The *Sambucus* genus consists of 5 to 30 species, native mostly to the Northern hemisphere, although they have become naturalized throughout much of the temperate and subtropical regions. Because their fruit are highly desirable to birds, elderberry rapidly colonizes moist and sunny areas along railways, roadways, forest edges, and fence lines (Lee and Finn, 2007). In most parts of Europe, black or common elder (*Sambucus nigra* L.), also called black elderberry, elder, elderberry common elder, elder bush and European elder, is the most widespread species, whereas in North America American elderberry (*Sambucus canadensis* L.), also called common elder, sweet elder, pie elder, elder-blow and blackberry elder, is the prevailing species. The American and European elderberry are closely related and are combined as two subspecies of *S. nigra* (Finn et al., 2008). Other species and varieties belonging to the *Sambucus* genus are grouped in five complexes: (1) *S. nigra* complex comprising of *S. australis*, *S. canadensis*, *S. cerulea*, *S. javanica*, *S. lanceolata*, *S. nigra*, *S. palmenis*, *S. peruviana*, *S. simpsonii* and *S. valutina;* (2) S. melanocarpa as an intermediate between *S. nigra* and *S. racemosa* group; (3) *S. racemosa* complex comprising of *S. calicarpa*, *S. chinensis*, *S. latipinna*, *S. microbotrys*, *S. pubens*, *S. racemosa* , *S. sieboldiana*, *S. tigranii* and *S. williamsii*, (4) *S.australasica* and *S. gaudichaudiana*; and (5) *S. adnata* and *S. ebulus*. Lesser known species of the *Sambucus* genus native to North and South Americas are *S. melanocarpa*, *S. neomexicana*, *S. Mexicana*, *S. velutina* and *S. coerula* (Kearney and Peebles, 1960). The most widely spread species are *S. nigra* and *S. canadensis*.

Sambucus nigra

Sambucus nigra is a European species with an oceanic to sub oceanic, cool-temperate and west Mediterranean range. The species is common in western and central Europe as well as in North Africa, Scandinavia and Great Britain. Its naturalized distribution area reaches 63^{o} N latitude in western Norway (with scattered naturalized shrubs up to at least 68 ^{o} N) and approximately 55° N in Lithuania (Atkinson and Atkinson 2002). The populations in the Atlas Mountains of Morocco, Algeria and Tunisia have been introduced as well as that on the Azores. *S. nigra* is present in the northern and western part of the Iberian peninsula, in Sicily and mainland Greece but is absent from Crete. It occurs sporadically in western and eastern Turkey, particularly in the northern coastal strip. The eastern limit of its distribution is approximately 55° E. In mountainous regions, *S. nigra* cannot be found in the higher altitudes, above 1500 m in the Alps, 900 m in the Tatra mountains, 2200 m in Morocco and 1200 m in Caucasus. Its precise limits as a native shrub are difficult to establish, because in some places *S. nigra* populates only areas near houses and roads (Tutin et al., 1976).

S. nigra is associated with moderately to highly eutrophic and disturbed soils, for example in floodplains, coastal scrub or along forest margins and in forest gaps, or anthropogenically in hedgerows, abandoned gardens, around farm houses and on post-industrial wasteland. *Sambucus nigra* is less frequently found within forests, but it cannot survive under deep shade (Atkinson and Atkinson 2002). In central Europe *S. nigra* is not a typical forest plant, because the most important habitat factors are high light availability and nutrient-rich, neutral to basic soils. It very easily colonizes both natural and man-made forests or shrublands, and in many locations the species has an anthropogenic origin (Kollmann and Reiner 1995). In its introduced range, *S. nigra* occurs mainly in anthropogenic habitats, *e.g.* parks and gardens, near enclosures and countryside houses. In cities it is often found in abandoned places, unmanaged parks, for example in old, disused dumping-grounds, deserted allotments, forest margins, near streets and roads.

Sambucus nigra is a shrub or small tree up to 10 m high, with brownish-grey bark and white pith. Strong erect shoots grow from the base and branches are often arching. Leaves are 20 cm in length, with 5-7 leaflets. Leaflets are 3-9 cm long, ovate, pointy, serrate, and rarely pubescent beneath. Typically they are dark green , although ornamental varieties and selections have been identified that are variegated, lime green and purple and are popular plants for landscaping (Lee and Finn, 2007). Stipules are absent or very small. Inflorescence is flat topped, 10-20 cm in diameter, with 5 primary rays. Corolla is approximately 5 mm in diameter; flowers are hermaphrodite and cream white with cream anthers. Individual fruit is a 3-8 mm black drupe and contains 3-5 seeds (Tutin *et al.* 1976). Collectively hundreds of fruits produce very large clusters. Rubbing the leaf produces a strong odor. *S. nigra* reproduces by seeds and also vegetative. Most shrubs produce copious amounts of fruit and viable seed every year. *S. nigra* usually flowers in its third or fourth year, rarely in its second (Atkinson and Atkinson 2002). Flowering is generally in June and July and flowers have a strong odor, which may deter some visitors, but attracts others such as beetles, particularly longhorn beetles and flies, which pollinate the flowers. Fruits ripen in mid to late summer (August or September) and birds are considered the main dispersal agents of *S. nigra* seeds, which either regurgitate or defecate the seeds after ingesting the fruit (Atkinson and Atkinson 2002).

Sambucus canadensis

Most of the botany, plant morphology and fruit development are very similar between *S. nigra* and *S. canadensis* species, however American elderberry tends to spread more aggressively by underground rhizomes and is usually multi-trunked. European elderberry, on the other hand, is a single-trunked or few-trunked large shrub or small tree.

American elderberries (*S. canadensis*) are erect, stoloniferous, long-lived, perennial shrubs native to eastern regions of North America. *S. canadensis* is spread east of the Rocky Mountains in the United States and Canada. It ranges from Nova Scotia south to Florida and west to Manitoba and Texas (Foote and Jones 1989). It is commonly found throughout the mountain, piedmont, and coastal plain regions of the Southeast as it tolerates a wide variety of climatic conditions, withstanding winter temperatures of -40 degrees C in the northernmost part of its range to summer temperatures of more than 38 degrees C in the southern portion. The species occurs in a variety of habitats from low bottomlands to high mountain elevations (DeGraaf and Witman 1979). American elder is most often found on open or semi-open sites with fertile, moist soils, such as stream edges, fencerows, old fields, pastures, disturbed sites, swamps, bogs, and roadsides; however, it also occurs in alluvial forests and upland woods (Foote and Jones 1989). It is a common species of southern bottomland hardwood forests, where it grows in seasonally to intermittently flooded forests and in transition zones grading from wetland to upland sites. *S. canadensis* thrives in fertile soils but will grow in sandy and bottomland soils, heavy clays, peats, and muck. It is adapted to a wide variety of soil-moisture combinations but generally prefers moist, well-drained sites (Vines 1960). American elder prefers full sun but may occur on sites with partial shade during the day.

American elder is a deciduous flowering shrub that normally grows 2 to 4 m tall, but in favourable conditions it can reach the height of 9 m (Vines 1960). The plant is stoloniferous and thicket-forming, with many tightly clustered stems arising from the base (Vines 1960). The lateral roots form a fibrous, shallow system and upright stems spread vigorously forming an elliptical to round shape. Main stems are thinly woody, with large white pith. Bark is light brown, yellowish brown, or grayish brown and dotted with prominent cork-like lenticels (Foote and Jones 1989). Smaller lateral branches have dark green bark and are nearly herbaceous, usually dying back in the winter (DeGraff and Witman 1979). Buds are medium-sized, conical and somewhat depressed (Harlow 1954). The opposite, pinnately compound leaves have 5 to 11 (usually 7) bright- to medium-green with lanceolate to ovate 10 to 30 cm long leaflets. The margins are finely toothed, and the lower leaflets are occasionally divided into 3 segments (Foote and Jones 1989). Leaflets are rounded to wedge-shaped at the base and tapered to the pointed tips, 6 to 15 cm long and 2.5 to 6 cm wide (Vines 1960). The upper leaf surface is lustrous and smooth; the lower surface is paler and barely pubescent. Petioles are 3 to 10 cm long and may be naked or pubescent. The showy white flowers and dark purple fruits, both borne in umbrella-shaped clusters, are outstanding seasonal features of American elder. The 6 mm, star-shaped flowers are creamy to white and clustered in terminal convex or flattened cymes 5 to 25 cm in diameter (Vines 1960; Foote and Jones 1989). Stalked glands may be present in the forks of the cymes (Radford et al. 1968). The plants bloom in early summer (June to August), bearing hermaphrodite flowers on large cymes produced laterally on perennial canes and terminally on new canes. Individual cymes can have hundreds of mature fruit, botanically described as berries, which ripen from late summer to fall, depending on location. Individual berries are small (8 mm in diameter), with 4 oblong, tan to yellowish seeds.

ELDERBERRY USES AND PRODUCTION

There are several positive socio-economic benefits of growing elderberry, because of its diverse uses. In Europe, it is cultivated for flower and berry production as well as ornamental purposes. The flowers of *S. nigra* are used for the preparation of drinks and medicines; elderberry-flower wine is very popular in England. Elderberry berries are mainly used for food colorants and components in pharmaceuticals, processed to concentrates, syrups, jellies and juices; frequently they are consumed in preserves and alcoholic beverages such as elderberry wine. The flowers, fruits, leaves, bark and roots of *S. nigra* are thus often utilized for medical purposes. For example, dried fruits, flowers and cortex have been used as diaphoretic and diuretic medicines. Tea from *S. nigra* flowers is used against colds, flu, and high temperature. *Sambucus nigra* bark and fruits are potent in the treatment of respiratory problems such as hay fever and asthma. Berries are rich in vitamins and polyphenols and are best used to ward of various winter illnesses. The leaves contain cyanogenic glycosides from which hydrogen cyanide is released by enzyme action. Although European elderberry is not generally considered poisonous, isolated cases of poisoning in animals and man have been reported after eating bark, leaves, berries, roots and stems. Extracts from *S. nigra* are used in horticulture as a repellent against insects as they have an unpleasant odor. *Sambucus nigra* has also been planted for erosion control. It is not used as timber due to its small dimensions and soft wood properties. However, because of its whiteness, close grain, good cutting and polishing properties, the wood is suitable for making pegs and other small wooden items such as musical instruments and toys (Atkinson and Atkinson 2002).

Similarly, *Sambucus canadensis* has been used for a variety of purposes: the dark, juicy berries for making wines, jellies and pies; the flowers for flavouring candies and jellies; the bark for making a black dye; and the leaves, bark and flowers for making a variety of homemade medicinal remedies (Vines 1960). Although the cooked ripe fruit is edible, raw or unripe berries and other plant parts are somewhat toxic with a laxative effect. American elderberry is also a good plant species to use for wildlife habitat improvement and is considered one of the best low, summer-fruiting shrubs for wildlife. Easily established, it can be planted singly or in numbers to form thickets and hedges and can be used in a variety of habitat settings.

Commercial elderberry production is scattered across Denmark, Italy, Hungary, and Austria in Europe (*S. nigra* cultivars) and in central Chile (*S. canadensis* cultivars). Historically, the production of American elderberry was concentrated in Oregon in the USA, but production has rapidly decreased in the past few decades. In the last 50 years only, only a few producers in the USA managed to establish small orchards generally for local processing into pies, jams, jellies, and particularly wines. Elderberry wine production in the USA and Canada probably started with the arrival of the first European settlers who brought this tradition from their homeland. Since the early 1990s, elderberry production is slowly increasing in Canada with orchards being established in Ontario, Quebec, Nova Scotia, New Brunswick and Newfoundland (Charlebois, 2007). In some parts of the world (Midwestern USA), wild harvested flowers and fruit is also an important aspect of elderberry processing. The majority of the *S. nigra* cultivars have been developed in Denmark (‘Allesø’, ‘Korsør’, ‘Sambu’, ‘Sampo’) but the origin of the most popular cultivar ‘Haschberg’ can be traced to Klostenburg, Austria. *Sambucus canadensis* cultivars have been developed decades ago at the New York Agricultural Experiment Station and at Agriculture and Agri-Food Canada in Nova Scotia (‘Adams I’, ‘Adams II’, ‘Nova’, ‘York’, ‘Johns’) (Finn et al., 2008).

For commercial harvest, the whole umbel or fruit cluster is picked and the entire crop either processed to flower extract or into juices and purees, when fruit is harvested. Flowers are harvested in early summer (mid June) and berries when fully ripe, reaching a purple black color in late summer and are harvested over a 2 week period. In European countries there is a practice to hand-pick approximately one third of the umbels for flowers so that the rest of the fruit crop increases in size and also attains a better chemical composition. Elderberry fruit is mainly picked by hand, although mechanical harvesting is a possibility. A few umbels are borne in the first year; however, plants reach full production in the third or fourth year after planting. After the fruit is harvested, it must be transported to the processing plant as soon as possible to prevent internal heating in the containers or preferably frozen immediately. Fruit yields from a commercial orchard of 6 to 12 tons per hectare have been reported (Roger, 1981).

PROPAGATION, ORCHARD ESTABLISHEMENT AND CULTIVATION TECHNOLOGY

European and American elderberry can be propagated by seed, seedlings, or cuttings, all of which are commercially available (Vines 1960). Fruits and cuttings can also be easily removed from wild plants without injury to the plant; however, virus free material should be ensured for commercial orchards (DeGraaf and Witman 1979). Stem cuttings from vigorous 1-year-old canes may be taken from spring through fall. They should be approximately 25 to 46 cm long and include 3 sets of opposite buds. Cuttings taken in mid-summer and treated with 0.5% indolebutyric acid powder will root readily, but fall cuttings should be placed in peat moss, kept at 4.4 degrees C through the winter, and transplanted outside in the spring (Bir 1992). Rooted stolons can be severed from the parent plants in early spring or late fall and, if possible, left in place to establish new root systems. When removed, fragile roots of the new plants should be wrapped in plastic or burlap until replanting. Elder is easily grown from seeds, and a young plant will bloom in 3 years. Seeds can be collected by stripping or cutting clusters from the branches as soon as the fruit is ripe. Seeds can be prepared for storage or immediate planted by simply crushing the fruit and drying it or by macerating the seeds with water to remove the pulp before drying. Seeds stored in sealed, airtight containers at cool temperatures will remain viable for several years (Young and Young 1986). Elderberry seeds vary considerably in germination requirements across the plant's range (Bir 1992). The seeds are difficult to germinate because of the hard seed coats. To speed germination, seeds can be scarified with a 10- to 15-minute soak in concentrated sulfuric acid, washed, and chilled at 2.2 to 4.4 degrees C for 2 months before planting. Seeds may also be prepared for planting by placing them in moist sand for 90 days at 20 to 30 degrees C, followed by 90 days of prechilling at 2.2 to 4.4 degrees C (Bir 1992, Young and Young 1992). Treated and untreated seeds should be planted 6 mm deep at a rate of 35 seeds per 30-cm intervals. Seeds may be planted in the spring, or in the late fall if well mulched. Untreated seeds usually will not germinate until the second growing season (Young and Young 1992). Larger seedlings should be transplanted as soon as possible after they are obtained, either in the fall or spring. Late fall or early winter are the best planting times since the plants are dormant, and some root growth may occur during the winter (Foote and Jones 1989). Roots of bare-root seedlings should be soaked in water for a couple of hours prior to planting.

Because elderberries do not bloom until late June, it is not necessary to select a site with excellent air drainage, as is the case with most other fruit crops, such as apple and pear. However, late frosts tend to damage young shoots and locations with heavy winds should also be avoided. Higher altitudes (over 600 m) are also not suitable for elderberry production because of uneven fruit ripening and flower drop due to lower temperatures and high humidity levels. Otherwise, the plants will grow in any good soil, from sandy loamy, with pH levels of about 5.5 to 6.5. Planting should be done in early spring and prior to planting the site should be plowed, well tilled, and soil amended with organic matter similarly to apple orchards (P_2O_5 10-25 mg per 100 g soil, K_2O 10-32 mg per 100 g soil). Fertilizer needs are dependent on the quantity of nutrients previously available in the soil. If the humus level in soil is too low (lower than 2 %), organic matter should be incorporated. The usual planting distance is 4 m apart in the row with rows 5-6 m apart. Preferably two year old plants should be planted in orchards, because pruning is easier and plants can already be commercially harvested in the third or fourth year after planting. After the planting, elderberry branches should be pruned back to 2 buds, and can either be trained as a bush or small tree. The latter training system is prevalent in Europe, in the USA elderberry is mostly pruned back every year as a bush. In the second year after planting, branches are thinned out during the winter; up to 7 are left to form a compact canopy and later 15 to 20 strong branches should be maintained. Healthy, vigorous elderberry plants send up a number of new canes each year and these new shoots attain full height in one season. New canes do not have side shoots (laterals) the first year, but often bear single, large, late-ripening clusters on their terminals. The most fruitful canes are those in their second year, when they produce several lateral branches. Fruit clusters are borne terminally on the wood of the current season's growth. The older trunks of elderberries lose vigor and become weak after two or three years. Little pruning is required. All dead, broken and weak canes should be cutoff before growth starts in the spring. An equal number of 1-, 2- and 3-year-old canes may be left; canes older than 3 years should all be removed to encourage the emergence of new, more fruitful canes. Although, elderberry is winter hardy and rarely dies because of winter injury, small lateral twigs near the tops of canes often freeze back. Bushes will live for 30 years or more but it is not the general practice to keep them that long (Roger, 1981).

Birds can be a serious problem in commercial orchards. In larger plantings, the percentage loss may be less and the destruction less noticeable. Placing nets over the bushes is the only effective control measure for birds. Small plantings should not be located near wooded areas where birds can hide. Other problematic pests include leaf borers, mites, mice, voles and cane borers. These can cause considerable damage to branches. However, the damage is usually not serious enough to justify spraying. When the top of a cane is broken off by wind or during harvest, the adult borer lays its eggs in the exposed pith. The larvae hatch and bore down to the bottom of the cane causing the cane to die. Burning the infested canes can discourage the multiplication of the insect and helps greatly in its control. Mildew on the leaves and berries just before harvest can be a problem, especially if the weather is cool during ripening and if the bushes are planted to closely where air circulation is poor. When mildew is serious, a fungicide spray could control it. Tiny eriophyid mites, visible only under the microscope, sometimes attack the leaves and cause yellow bands which resemble the symptoms of a mottle virus. These can be controlled by dormant sprays but their damage is not usually extensive. Diseases and insects are not generally serious on elderberries and sprays usually are not applied (Roger, 1981).

As elderberry roots are formed very close to the surface caution should be put with mechanical tillage for weed removal. Usually it is best to apply contact herbicides for weed control in the row and mulch the remaining site as often as possible. This often also helps to prevent voles from inhabiting the orchard and supplies elderberry bushes with additional nitrogen. Mineral fertilizers should be used in small doses from March to June, and when flowering is abundant additional nitrogen can be added later in the season to encourage new vigorous growth for the next year.

CHEMICAL COMPOSITION OF ELDERBERRY FLOWERS, FRUIT, AND PRODUCTS

Elderberry flowers and their products

Elderberry flowers are distinguished by their intensive, pleasant odor and serve as a basis for industrially produced soft drinks and also as extracts to increase the nutritional value of different foods and diets. Elderflowers are a rich source of potential bioactive flavonoids and phenolic acids; Christensen et al. (2007) reported six flavonol glycosides and eleven phenolic acids in fresh elderflowers and their extracts. Among flavonoids, quercetin-3-rutinoside (rutin), quercetin-3-glucoside (isoquercitrin), kaempferol-3-rutinoside, isorhamnetin-3-rutinoside, isorhamnetin-3-glucoside and quercetin-3-6-acetylglycoside were identified; and among phenolic acids derivates of quinic acid containing caffeic or *p*-coumaric acid moieties were determined. Similar results were also obtained when analyzing elderflower extracts. Flavonol glycosides rutin, kaempferol-3-rutinoside and isorhamnetin-3-rutinoside are the major flavonoids in elderflowers contributing as much as 90% to the total flavonoids content. The major phenolic acids in elderberry flowers are 5-caffeoylquinnic acid and 1,5-di-caffeoylquinnic acid comprising over 70% of the total phenolic acid content. As much as 21.0 mg per g dry weight of rutin is reported in elderflowers (Christensen et al., 2007). Opposed to the chemical composition of elderberry fruit, which is especially rich in anthocyanins, flowers do not contain any pigments from this group. The concentration of flavonoids, however, is greatest in elderberry flowers (Dawidowicz et al., 2006). According to the research of Dawidowicz et al. (2006) elderflowers exhibit a much stronger neutralizing activity of free radicals compared to elderberry fruit and DPPH values between 91.95 and 94.15 are reported.

Products such as cough syrups ad infusions made from elderberry flowers are quite common, and safe taken in moderate doses; however, for bests results, it is recommended that only 30% (m/m) elder flowers are to be used in teas and infusions in mixtures with other plant materials (Cejpek et al., 2009).The consumption of tea infusion is among the most commonly used ways of elderflower antioxidant’s intake; the prevalent phytocemicals being quercetin-rutinoside (rutin) and other flavonols, chlorogenic acid, caffeic acid and flavan-3-ols (catechins). The reported electrochemical activity (EA) of elderflower infusion is equivalent to 21 g ascorbic acid equivalents (AAE) per kg dry flowers and the content of rutin in the infusion 10.9 g per kg of dry flower weight (Cejpek et al., 2009). This compound is mostly responsible for the distinct yellow color of the infusion. Free radical scavenging capacity, using the DPPH assay, revealed a 5.2% antiradical activity of L-ascorbic acid (Cejpek et al., 2009).

Elder flower syrup has a somewhat different composition with compounds from the group of phenolic acids (protocatechulic acid and derivates of caffeic acid) and flavan-3-ols (catechins) are most abundant. When compared to elder flower infusion, the EA was only about 42% (Cejpek et al., 2009). However, EA of elder flower syrups depends greatly on the technology used and the content of elder flower and other ingredients in the maceration process. For example, the addition of citric acid adjusting the pH level to 3.1 very much affects the polyphenoloxidase (PPO) activity and affectively decreases subsequent browning of the syrup. Traditionally prepared syrups from fresh flowers (flower heads soaked in cold water, addition of citric acid – lemons, and sucrose) posses much less EA and are very pale compared to commercially available syrups, where other flower extracts (such as hibiscus and apple concentrate) have been added or prepared from dried elderflowers or extracts.

Elderberry fruit and their products

The fruit of *Sambucus nigra* and *Sambucus canadensis* contains several constituents responsible for its pharmacological activity. Among these are the flavonoids quercetin and rutin, anthocyanins identified as cyanidin-3-*O*-glucoside and cyanidin-3-*O*-sambubioside (Veberic et al., 2009), the hemagglutinin protein Sambucus nigra agglutinin III (SNA-III) (Mach et al., 1991), cyanogenic glycosides including sambunigrin, (Buhrmester et al., 2000) viburnic acid, and vitamins A and C (Duke, 1985).

The juice pressed from elder berries contains many primary metabolites: various sugars and organic acids. High concentrations of organic acids are important in processing, since, unlike sugars, they cannot be added to the final product. Among secondary metabolites, elderberry juice is predominantly characterised by high amounts of anthocyanins. These are a class of flavonoids responsible for the attractive orange to blue colour of flowers, as well as an important fruit quality indicator, since they greatly influence fruit appearance and flavour (Lee and Finn, 2007). They have gained an increasing interest as functional compounds in food colorants and as potent agents against oxidative stress, reducing oxidative damage to the human body. Anthocyanins, as well as other flavonoids – like quercetins, exhibit antioxidant, anticarcinogenic, immune-stimulating, antibacterial, antialergic and antiviral properties; therefore, their consumption may contribute to prevention of several degenerative diseases such as cardiovascular disease, cancer, inflammatory disease and diabetes (Dawidowicz et al., 2006; Thole et al., 2006). These compounds are well known free radical scavengers, reported as potential chemo-preventive agents.

Elder berry chemical composition has been thoroughly explored and compounds from the groups of primary and secondary metabolites determined (Veberic et al., 2009). The former group consists of various sugars and organic acids. The most abundant sugars in black elderberry fruit are fructose and glucose, sucrose is detected only in small amounts (Veberic et al., 2009). The total sugar content in elderberry fruit averages from 68.53 to 104.16 g per kg fresh weight (FW) and is, like in other fruit, such as sweet cherry (Usenik et al., 2008) and peach (Colaric et al., 2005) strongly dependant on cultivar. Elderberry fruit contains moderate amounts of sugars compared to apple, which contains 115-183 g per kg total sugars (Hofer et al., 2005) and significantly lower amounts of total sugars than sweet cherry, which averagely contains 150-230 g per kg (Usenik et al., 2008). The content level of sugars in elderberry fruit is comparable to sour cherry fruit (*Prunus cerasus* L.), which contain approx. 90 g per kg total sugars and are also mainly used in processing (Bonerz et al., 2006). The amount of sugar in elderberry fruit, however, is not the focal chemical compound for technological processing, as fructose and sucrose can easily be added to the final products (i.e. juices, concentrates, spreads and beverages).

Among organic acids, four have been identified in elder berries: citric acid, malic acid, shikimic acid and fumaric acid (Veberic et al., 2009). Citric acid is the most abundant organic acid, followed by malic acid and minor concentrations of shikimic and fumaric acid. The concentration of citric acid in the fruit of black elderberry is reported in range from 3.11 g per kg to 4.81 g per kg FW (Veberic et al., 2009). Compared to apple, which contains between 0.07-0.52 g per kg FW citric acid (Hofer et al., 2005), sweet cherry, which contains between 0.11-0.54 g per kg FW citric acid (Usenik et al., 2008) and sour cherry, which contains between 0.08-0.14 g per kg FW citric acid (Bonerz et al., 2006), elderberry fruit is exceptionally rich in this organic acid. The content of citric acid in fruit is a particularly important parameter and although elder berries contain lower amounts of total organic acids than apple, which on average contains between 6.00 and 14.00 g per kg FW organic acids

(Hofer et al., 2005) and sweet cherry, which on average contains between 3.50 and 8.20 g per kg FW organic acids (Usenik et al., 2008), it is widely used for processing purposes due to the high levels of citric acid.

Berries are particularly rich in flavonoids compounds, including anthocyanidins and proanthocyanidins as well as flavonols. The most important polyphenols in elderberry fruit are thought anthocyanins, being responsible for the colour (Cejpek et al., 2009). Total monomeric anthocyanin content of different *S. ni*gra cultivars is reported in range from 170 to 343 mg cyanidin-3-*O*-glucoside per 100 g and for *S. canadensis* in range from 106 to 444 mg cyanidin-3-*O*-glucoside per 100 g (Lee and Finn, 2007). Much higher values of total monomeric anthocyanin content were determined in elder berries in a study of Kaack (1997), where values ranged from 518 to 1028 mg cyanidin-3-*O*-glucoside per 100 g.

High performance liquid chromatography analysis of European elder (*Sambucus nigra*) fruit revealed the presence of four main anthocyanins: cyanidin-3-*O*-sambubioside-5-*O*-glucoside, cyanidin-3,5-*O*-diglucoside, cyanidin-3-*O*-sambubioside, cyanidin-3-*O*-glucoside; cyanidin-3-*O*-rutinoside, cyanidin-3-*O*-rhamnoglucoside and cyanidin-3-*O*-xyloglucoside have also been reported (Thole et al., 2006;Veberic et al., 2009). Lee and Finn (2007) reported the presence of non-cyanidin-based pelargonidin-3-*O*-glucoside and delphinidin-3-*O*-rutinoside in *S. nigra* berries and Wu et al (2004) also pelargonidin-3-*O*-sambubioside, although these were only present in trace amounts. According to Wu et al. (2004) and Veberic et al. (2009) the two prevalent anthocyanins in *S. nigra* are cyanidin-3-*O*-glucoside and cyanidin-3-*O*-sambubioside, with the major anthocyanin cyanidin-3-*O*-sambubioside, accounting for more than half of all determined anthocyanins, approximately. In comparison to sweet cherry, which contains, on average, 100-120 mg total anthocyanins per 100 g FW (Usenik et al., 2008) elderberry fruit has significantly higher anthocyanin content as it reaches levels between 602.90 and 1265.30 mg per100 g FW (Veberic et al. 2009). While berries of *S. nigra* contain primarily four anthocyanins, berries of *S. canadensis* contain seven (Thole et al., 2006). In addition to cyanidin-3-*O*-sambubioside-5-*O*-glucoside, cyanidin-3,5-*O*-diglucoside, cyanidin-3-*O*-sambubioside and cyanidin-3-*O*-glucoside they also accumulate more stable acylated anthocyanins cyanidin-3-*O*-(6-*O*-*E*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl), cyanidin-3-*O*-(6-*O*-*Z*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl)-*β*-glucopyranoside-5-*O*-*β*-D-glucopyranoside, and cyanidin-3-*O*-(6-*O*-*E*-*p*-coumaroyl-2-*O*-*β*-D-xylopyranosyl)-*β*-D-glucopyranoside (Thole et al.,2006). Lee and Finn (2007) determined the presence of petunidin-3-*O*-rutinoside in berries of *S. canadensis* present only in trace amounts.

Both *S. nigra* and *S. canadensis* contain many polyphenolic compounds; among those cinnamic acids (neochlorogenic acid, chlorogenic acid, and cryptochlorogenic acid) and flavonol-glycosides (quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside, isorhamnetin-3-*O*-rutinoside and isorhamnetin-3-*O*-glucoside have been identified (Lee and Finn, 2007). The main quercetin in berries is quercetin-3-*O*-rutinoside (rutin), with values ranging from 35.6 mg per 100 g FW to 52.0 mg per 100 g FW in berries of *S. nigra* (Veberic et al., 2009). Similar values (15.0 to 41.9 mg per 100 g FW) are reported for S. canadensis berries (Lee and Finn, 2007). The other two quercetins are present in considerably lower amounts, especially the aglicon quercetin; the concentration of this was approximately 1/10 of the amount of rutin. Total quercetins are reported in range from 51.9 mg per100 g FW to 73.4 mg per 100 g FW(Veberic et al., 2009), total cinnamic acids in range from 28.7 to 42.8 mg per 100 g FW and total polyphenols in range from 85.7 to 140.3 mg per 100 g FW (Lee and Finn, 2007). A series of proanthocyanidins (dimers, trimers and tetramer) as well as gallocatechin, and other flavonols have been reported in *S. nigra* berries (Thole et al. 2006; Cejpek et al., 2009). There is a slight difference in polyphenolic composition of *S. canadensis* berries; mainly they contain quercetin based glycosides. Specifically, rutin, epigallocatechin, and quercetin monoglucoside (Thole et al., 2006) as well as neochlorogenic and chlorogenic acid (Lee and Finn, 2007) are the predominant constituents.

S. nigra and *S. canadensis* fruit have higher antioxidant capacity than vitamin C or E and are thus capable of enhancing immune system response through elevated production of cytokines (Thole et al., 2006). Dawidowitz et al. (2006) report the antioxidant activity (calculated by the DPPH method) of elder berries in range from 50.3 to 67.7, mainly attributed to the presence of flavonols and anthocyanins. Thus, their antioxidant capacity ranks high when compared to other well known small fruit, such as cranberry (Cejpek et al., 2009). Using the ORAC method, Wu et al., (2004) showed that especially *S. canadensis* berries posses a much higher potential than cranberry and blueberry, two fruits praised for their high antioxidant potential.

Among elderberry products, elderberry juice, spreads and wine are most widely marketable. Elderberry juice pressed from S. nigra berries are characterized by the two major anthocyanins (cyanidin-3-*O*-sambubioside and cyanidin-3-*O*-glucoside) as well as high levels of catechins and phenolic acids such as chlorogenic acid, neochlorogenic acid, rutin and other flavonols (Kaack et al., 2008; Cejpek et al., 2009). The average content of cyanidin-3-*O*-sambubioside in elder berry juice pressed from different *S. nigra* cultivars is 379 mg per 100 ml, 331 mg per 100 ml cyanidin-3-*O*-glucoside, 81.7 mg per 100 ml cyanidin-3-*O*-sambubioside-5-*O*-glucoside and 24.9 mg per 100 ml cyanidin-3,5-*O*-diglucoside (Kaack et al., 2008). Fruit juice pressed from fresh or frozen elder berries has a concentration of soluble solids above 13% w/w, indicating a satisfactory content of sugars, and titratable acidity of between 0.6 and 1.7 (Kaack et al., 2008), an important parameter for a balanced taste. Comparing the beneficial effects of fresh fruit with processed ones, it should be kept in mind that especially anthocyanin stability is affected by numerous factors during processing and an almost total destruction of these pigments as well as chlorogenic acid is reported during production of elderberry spread (Cejpek et al., 2009).

During the elderberry fruit wine making process, significant changes take place in the composition and content of polyphenolic compounds resulting from fruit disintegration as well as fermentation and aging. Alcoholic fermentation of elderberry fruit yields an intensely purple-red colored elderberry wine with a high content of anthocyanic pigments and total phenolic content similar to red wines (Schmitzer et al., 2010). The beneficial compounds present in elderberry juice, have also been determined in elderberry wine. The compositional analysis of organic acids revealed malic and citric acids the predominant organic acids in fruit wine from *S. nigra* berries (Schmitzer et al., 2010). Ten phenolic compounds were identified in elderberry must and wine; chlorogenic acid and neochlorogenic acid, quercetin-3-*O*-rutinoside, quercetin-3-*O*-glucoside, kaempferol-3-*O*-rutinoside and five cyanidin based anthocyanins. Anthocyanins were the predominant polyphenols in elderberry must and wine. Cyanidin-3-*O*-sambubioside is the major anthocyanin in elderberry must; however, in elderberry wine cyanidin-3-*O*-glucoside is detected in highest concentrations (Schmitzer et al., 2010). During elderberry wine making process, significant transformations in phenolics take place and the composition of anthocyanic pigments is altered. Vinification results in a minor decrease of anthocyanin content but, formation of new, more stable, polymeric pigments has been reported (Schmitzer et al., 2010). Quercetin-3-*O*-rutinoside is the most abundant quercetin followed by quercetin-3-glucoside, which is comparable to the compositional analysis of elderberry fruit as well as elderberry juice. Similar to compositional analysis of elderberry juice, neochlorogenic acid and chlorogenic acid have been detected in elderberry must and wine; the latter was significantly higher in elderberry wine compared to must and aged wine (Schmitzer et al., 2010).

As a result of chemical reactions of monomeric and dimeric phenolics during processing from musts to wine as well as aging, many polymeric compounds are formed causing differences in both total phenolic content (TPC) and antioxidative potential (AP). Both parameters are significantly higher in elderberry wine compared to elderberry must (Schmitzer et al., 2010). TPC of elderberry wine is reported to be 2004.13 mg GAE per L, comparable to TPC measured in different red wines, which on average contain 2000 mg GAE L (Minussi et al., 2003). AP of elderberry must is reported to be 8.18 mM trolox per L and increases significantly in elderberry wine to 9.95 mM trolox per L (Schmitzer et al., 2010). Elderberry wine is therefore an appealing alcoholic beverage not only because of its intense red coloration but mostly due to its high total phenolic content. It could be used as a potential additive to other alcoholic beverages, with inadequate coloration.

CLINICAL STUDIES ON POTENTIAL HELTH EFFECTS OF ELDERBERRY

Elder flowers

In folk medicine, *Sambucus nigra* flower was traditionally suggested as a remedy for diabetes (Atkinson, 1979). Researchers in Northern Ireland conducted an in vitro study to evaluate the effect on blood sugar. In the two-armed study, aqueous extract of elder flower significantly increased glucose uptake, glucose oxidation, and glycogenesis in rat abdominal muscle. Elder flower extract incubated with rat pancreatic cells also had a dose-dependent stimulatory effect on insulin secretion. The researchers concluded elder flowers contain water-soluble constituents capable of direct stimulation of insulin secretion and glucose metabolism (Grey et al., 2000). Further clinical studies are required before elderberry can be recommended for use in diabetes.

Elder berries

As mentioned before, the fruit of *Sambucus nigra* and *Sambucus canadensis* contains several constituents responsible for pharmacological activity. Among these are the flavonoids quercetin and rutin, anthocyanins identified as cyanidin-3-glucoside and cyanidin-3-sambubioside (Veberic et al., 2009), the hemagglutinin protein Sambucus nigra agglutinin III (SNA-III) (Mach et al., 1991), cyanogenic glycosides including sambunigrin, (Buhrmester et al., 200) viburnic acid, and vitamins A and C (Duke, 1974). Due to limited research, the pharmacokinetics of many constituents of elderberry is not completely understood. Available research has focused on the absorption and urinary excretion of the anthocyanin constituents. Historically, researchers were uncertain whether anthocyanins were absorbed unless they were first hydrolyzed in the gastrointestinal tract. Recently, however, several small pharmacokinetic studies of elderberry extract in healthy volunteers demonstrated elder berry anthocyanins are indeed absorbed and excreted in an intact form. Within four hours of consuming 12 g elderberry extract containing 720 mg total anthocyanins, the two major anthocyanins in elderberry extract were identified in the urine of four elderly women (Wu et al., 2000). A similar study involving 16 healthy volunteers confirmed the presence of the same two anthocyanins in the urine of study subjects after oral administration of elderberry extract (Mulleder et al., 2007). One study investigated the absorption of elderberry anthocyanins in a single male subject given 25 g elderberry extract (1.5 g total anthocyanins); high-performance liquid chromatography (HPLC) analysis detected two anthocyanin peaks in his plasma collected 30 minutes after consuming elderberry extract (Cao and Prior, 1999), and this was later confirmed in a study by Milbury et al. (2002).

While there are several mechanisms responsible for the beneficial effects of *Sambucus nigra* and *S. canadensis* and extracts of their berries, perhaps the most important and best studied are the antiviral effects. A powerful antioxidant activity *in vitro* has been demonstrated and Youdim et al., (2000) have reported that elderberry anthocyanins can be taken by endothelial cells, which are subsequently effectively protected against oxidative stress. Sambucol®, a syrup containing 38-percent standardized extract of elder berries, was developed and studies have shown it to neutralize and reduce the infectivity of influenza viruses A and B (Zakay-Rones et al., 2004), HIV strains and clinical isolates (Sahpira-Nahor et al., 1995), and Herpes simplex virus type 1 (HSV-1) strains and clinical isolates. It probably does so in the same manner as with influenza viruses, via neutralization of the virus resulting in reduced infectivity.

Elderberry extracts also have immune-modulating activity in healthy individuals as well as in those with viral infections or other diseases characterized by immunosuppression. Production of certain cytokines leads to activation of phagocytes and facilitates their movement to inflamed tissues (Janeway et al., 2001) Elder berries also contain several anthocyanin flavonoids known to possess significant antioxidant properties. Research has demonstrated that even low-level concentrations of elderberry anthocyanins can efficiently regenerate alpha-tocopherol from alpha-tocopheroxyl radicals in models of copper-mediated LDL oxidation (Abuja et al., 1998). Since it has been observed that anthocyanin glycosides are indeed absorbed in humans, it is likely that supplementing with elderberry extracts containing anthocyanins provides significant antioxidant benefit in increased protection against oxidative stress.

Numerous disease conditions are characterized by oxidative stress, including cardiovascular disease, cancer, neurodegenerative disease, peripheral vascular disease, autoimmune diseases, and multiple sclerosis. Bioactivity relevant to inhibition of both the initiation and promotion stages of carcinogenesis was detected in fruit extracts from *S. nigra* and *S. canadensis* (Thole et al., 2006). The ability of elderberry extract to provide antioxidant protection via inhibition of LDL-oxidation and scavenging of free radicals makes it a potentially valuable tool in the treatment of disease resulting from oxidative stress (Abuja et al., 1998).

Thus, we can conclude that elderberry, its extracts and products can be regarded as potent medicinal additives in the future as many traditional applications have been proven with clinical and in-vitro studies. Recent findings promote the use of elderberry mostly due to its high antioxidative status and favorable phenolic profile for novel pharmacological studies and later on for clinical applications.

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