Hop and Medicinal Plants, Year XXX, No. 1-2, 2022
ISSN 2360 – 0179 print, ISSN 2360 – 0187 electronic

Evaluation of *Sambucus nigra* as a Natural Food Additive

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Abstract: Black elder (*Sambucus nigra* L.) has a lot of health benefits and is used for several purposes depending on the morphological part of the plant. Although the benefits of elderflower fruit have been known in Europe and America for a very long time, in recent years it has been of increasing interest to the food and pharmaceutical industry. The present paper shows the evaluation of the biochemical compounds of some extracts from elderberries with the aim of being used as natural food additives. Numerous studies demonstrate that due to its high nutritional value, elderberry can be used as a functional ingredient in a wide range of food products. These fruits contain a small amount of cyanogenic glycosides, which are potentially toxic, for this reason many consider it harmful to the human body, but this compound can be eliminated by applying thermal treatments. Depending on the degree of pollution in the harvesting area, the quality of elderberries may show deficiencies in the amount of biochemical compounds and an increase in the amount of heavy metals. Being rich in antioxidants, flavonoids and volatile compounds, it can be a suitable source for obtaining natural dyes and flavourings.

Keywords: antioxidants, colorings, elderberries, extracts, flavorings, *Sambucus nigra* L.

Introduction

Sambucus (Caprifoliaceae) is a genus of approximately 20 temperate and subtropical species of small trees, shrubs, and herbs (Atkinson and Atkinson, 2002). *Sambucus nigra* L. (Fig.1) has three subspecies: *S. nigra* L. ssp. *nigra, S. nigra* L. ssp. *canadensis, S. nigra* L. ssp. *cerulea* (Młynarczyk et al., 2017). *Sambucus canadensis*, the American elder is native to the eastern part of North America, it is found from Florida to the northern part of the Gaspe coast of Quebec in Canada, where the wild type and some varieties grow well, but the fruit hardly reaches full maturity because a short growing season. *Sambucus nigra*, the European elder has a distribution that extends further north than its American counterpart, reaching Norway. It reaches its latitudinal and altitudinal limits, where the average temperature in October is around 7°C, which is probably a period for ripening. *Sambucus nigra* has been introduced to various parts of the world, including Asia, North America, New Zealand, and southern Australia (Atkinson and Atkinson 2002). The parts of the plant material that are relevant for production are the fruit and flower extract, in many countries the larger amounts of raw material are collected from the wild. Total fruit production compared to other berry crops is quite small and therefore relevant statistical data are scarce or incomplete (Christensen et al., 2008). The largest producers are in Germany, where elderberry production amounted to 1,576 tons (580 ha) in 2013 and 1,759 tons (583 ha) in 2015 (Höhne, 2014). In Romania, the species is commonly found in fields, hills, and lower mountain areas, it is one of the most recognized medicinal plants in Bihor County, where in 2017 there was a documented production of 13,137 tons of elderflowers (Timiș-Gânsac et al., 2018).

Food colourings are organic, natural, or synthetic substances with a well-established physiological role in nature but also with the role of normalizing the colour of some foods and drinks or improving the appearance and attractiveness of food (Tofana and Socaci, 2014). Natural dyes can be classified according to their chemical structure: flavonoid derivatives (anthocyanins, flavones and flavonols), isoprenoid derivatives (carotenoids), nitrogen-heterocyclic derivatives (betalaines) or pyrrole derivatives (chlorophylls) (Sigurdson et al., 2017).

Anthocyanins are of particular interest to the food colouring industry because their ability to impart vibrant colours. Anthocyanins

are the most important pigments among flavonoids; are anthocyanidins linked to one or more sugar molecules (the glycoside form), which consist of an aromatic ring linked to an oxygen-containing heterocyclic ring, which is also linked by a carbon-carbon bond to a third aromatic ring (Albuquerque, 2020). Over the past 20 years, research has shown that anthocyanins are non-toxic natural pigments that have antioxidant and anti-inflammatory effect. Anthocyanins also have antimicrobial, antiviral, anti-allergic, anti-carcinogenic, anti-mutagenic and anti-proliferative and therefore can play an essential role in prevention of various degenerative diseases. Research data shows a reduction in disease incidence circulatory, nervous, endocrine, digestive, urinary diseases and beneficial effects in immunity against cancer by consuming foods rich in anthocyanins. Additionally, anthocyanins can be extracted from crops brightly coloured such as strawberries, black currants, grapes, blackberries, black raspberries, cherries, black beans, purple corn, and purple sweet potatoes.

General aspects regarding the elderberry

Elderberry can be grown on a wide variety of soils. Excellent growth and yield can be expected in organic soil (mud). Mineral soil will also provide good conditions for elderberry production. Sandy soils, although capable of supporting limited growth and production, provide few nutrients and insufficient water retention. The most favourable environment for development includes soils rich in bases, nitrogen, and phosphate (Atkinson and Atkinson, 2002). Nitrogen can be applied in any form. When the cuttings are placed, all that is required is to manually apply the equivalent of 0.30 kg N near, but not touching, the base of each cutting (Charlebois et al., 2010).

Almost every part of the elder plant has some uses: fruits, flowers, leaves, roots, meds, and bark (Valles et al., 2004). Researchers have documented that elderberry is valued primarily as a food and medicinal plant, and descriptions of such uses are part of Native American history and European culture.

The leaves, flowers, but especially the fruits were used by Americans and Europeans to produce dyes for a wide variety of objects and leather. The fruits give a brown dye. Elder bark can be used as a source of a black dye; the flowers are still used in the perfume industry to produce a yellow dye (Allen et al., 2002).

Figure 1. *Sambucus nigra*
Source: https://gradinahobby.blogspot.com/

The American elder has been distributed by humans beyond its native range and is cultivated in central Mexico and Costa Rica, El Salvador, but can be found up to an altitude of about 1,500 m. *Sambucus canadensis* has been reported in the Himalayas at an altitude of 2,200 m. Distribution along the east coast of America from Nord is probably limited by its relative sensitivity to salt (Griffiths, 2006). The plant is intolerant to salt. This is an important point to consider if the elderberries are to be planted along roads, where salt is used in winter, or if they are to be irrigated with water from bad quality. American elder tolerates air pollution and can be used as an ornamental in areas urban (Charlebois et al., 2010).

Ripe elderberries contain carbohydrates, including dietary fiber, mainly hemicelluloses and polygalacturonic acid, pectins and simple sugars (Przybylska-Balcerek et al., 2021). Other carbohydrates found in *Sambucus nigra* are pectic acid, protopectin, Ca-pectate and cellulose (Młynarczyk et al., 2017). In terms of carbohydrates, elderberries contain 7.86–11.50% of total sugar and 2.8–8.55% from the reducing sugar (Przybylska-Balcerek et al., 2021). The main sugars identified by Veberic et al. (2009), were glucose and fructose, while only small amounts of sucrose were present in fruits.

The chemical composition of elderberries depends on various factors, including variety, conditions of environment, processing method and storage conditions. Ripe elderberries contain carbohydrates, including dietary fiber, mainly hemicelluloses and

polygalacturonic acid, pectins and simple sugars (Przybylska-Balcerek et al., 2021). Other carbohydrates found in *Sambucus nigra* are pectic acid, protopectin, Ca-pectate and cellulose (Młynarczyk et al., 2017). In terms of carbohydrates, elderberries contain 7.86–11.50% of total sugar and 2.8–8.55% from the reducing sugar (Przybylska-Balcerek et al., 2021). The main sugars identified by Veberic et al. (2009), were glucose and fructose, while only small amounts of sucrose were present in fruits.

Elderberry is a source of protein – its content is 2.7–2.9% in the fruit (Młynarczyk et al., 2017; Przybylska-Balcerek et al., 2021), 2.5% in flowers and 3.3% in leaves. This protein includes sixteen amino acids, nine of which are essential; the total content of essential amino acids is approx. 9% in flowers and 11.5% in leaves. Glutamic acid, aspartic acid and alanine were reported as amino acids dominant (Młynarczyk et al., 2017). They are also rich in fatty acids, mainly acid linoleic, linolenic acid, and oleic acid (Przybylska-Balcerek et al., 2021). More fat accumulates chosen in elderberry seeds (fat content: 22.4%) and seed meal (fat content: 15.9%) (Dulf et al., 2013; Młynarczyk et al., 2017). The main fatty acids are polyunsaturated fatty acids, which constitute 75.15% of the total fatty acids in the seeds and respectively 21.54% of the seed flour, while that monounsaturated fatty acids (14.21% and 4.21%) and saturated fatty acids (10.64% and 4.81%) make up a significantly smaller part. Veberic et al. (2009), detected four organic acids in elderberries. The most abundant of them was citric acid. In another study, the amount of citric acid varies between 6-25 mg/100g (Młynarczyka, 2020), 6.11 -16.7 mg/100 g FW (Ferreira et al., 2020) or 34.10 mg/100g (Vulić et al., 2008), whose content was quite high compared to other fruits. Next were malic acid (0.97–1.31 g/kg FW) and the remaining two acids were shikimic acid (0.14–0.93 g/kg FW) and fumaric acid (0.10–0.29 g/kg FW). The mineral content is 0.90–1.55% of the fruit mass and includes K, P, Ca, Na, Mg, Fe, Zn, Mn, and Cu. In addition, berries also contain heavy metals such as lead (0.016–0.098 mg/kg FW) and cadmium (0.039–0.053), as well as nitrate (V) (2.63–3.21 mg/kg FW) and nitrate (III) (0.69–0.86) (Dulf et al., 2013; Kołodziej et al., 2012; Veberic et al., 2009; Vulić et al., 2008). According to the study by Przybylska-Balcerek et al. 2021, besides that, the elderberries contain 0.01% essential oil, which is composed of over 30 different compounds, including phenyl aldehydes (3–25.8% of the oil composition) and furfural (18%). Another recent study shows that

essential oils consist of approx. 53 compounds in berries and 58 compounds in flowers (Kaack, 2008).

The bioactive compounds found in elderberries are mainly polyphenols and anthocyanins. Due to its rich phenolic composition, *S. nigra* extracts show significant antioxidant activity. The content of polyphenols and anthocyanins also depends on the growing season. The main polyphenols in elderberries are chlorogenic acid, neochlorogenic acid, acid cryptochlorogenic, quercetin, quercetin-3-rutinoside, quercetin-3-glucoside (isoquercitrin), kaempferol-3-rutinoside, kaempferol-3-glucoside, isorhamnetin-3-rutinoside and isorhamnetin-3-glucoside. The content of polyphenols change at different stages of fruit ripening and each compound shows its own pattern of individual change during this process. (Młynarczyk et al., 2017). A total of 16 phenolic compounds, like those previously described were also identified by Uzlasir et al., (2020).

Sambucus nigra fruit contains anthocyanins such as cyanidin-3-glucoside and cyanidin-3-sambubioside. Two other (minor) anthocyanins are cyanidin-3,5-diglucosides and cyanidin-3-sambubioside-5-glucoside. In addition, traces of cyanidin-3-rutinosides, pelargonidin-3-glucosides and delphinidin-3-rutinosides have been identified in the fruits of certain elderberry varieties (Młynarczyk et al., 2017). Lee and Finn (2007), found the same 11 anthocyanins present in each of the S. canadensis that tested them: cyanidin 3-(E)-p-coumaroylsambubioside-5-glucoside (major pigment present) cyanidin 3-sambubioside-5-glucoside (second major pigment present), cyanidin 3,5-diglucoside, cyanidin 3-sambubioside, cyanidin 3-glucoside, cyanidin 3-rutinoside, delphinidin 3-rutinoside (traces present),cyanidin 3-(Z)-p-coumaroyl-sambubioside5-glucoside, cyanidin 3-p-coumaroyl-glucoside, petunidin 3-rutinoside (traces present), and cyanidin 3-p-coumaroyl-sambubioside.

Alcoholic fermentation of elderberries causes changes in the content of phenolic compounds and anthocyanins, which also leads to colour changes (Młynarczyk et al., 2017). Although the fruit elderberry and elderflowers are rich sources of phenolic compounds, exposure to changing oxygen, light, temperature and pH, the presence of metal ions, water, and enzyme activity, can enhance degradation and the loss of their activity (Ferreira et al., 2020).

The number of extractable oils is significant (approx. 30% of dry weight) for this species, and agro-industrial wastes from various producers are used as feed supplements for animals or organic

fertilizer, cosmetic agents and in pharmaceutical industries. Waste from processing elderberries could probably be used in a similar way. In fact, the oil content of the pressing residues can reach up to 12%, and these residues are particularly rich in tocopherol. Amounts important amounts of anthocyanins can be extracted from elderberry (Seabra et al., 2010).

Elderberries are characterized by a high antioxidant activity, which ranges from 82.08 at 89.25% inhibition with respect to the DPPH radical. The antioxidant properties of elderberries are attributed primarily to the presence of phenolic compounds and largely depend on the chemical structure of molecules and individual fruit composition. Anthocyanins significantly influence the activity antioxidant in elderberries. As the concentration of anthocyanins increases, the activity also increases antioxidant, but only up to a certain level, after which this parameter begins to decrease (Pliszka et al., 2005).

Anthocyanins can present different chemical forms depending on the pH, being more stable under the acid condition, in conjugated forms (glycosylation and esterification), as well as complexed with other flavonoids (copigmentation). Anthocyanins could be significantly absorbed in the stomach due to the acidic pH (Fig. 2.), but anthocyanins present as glycosides are not absorbed in the small intestine, they must transform into aglycone which is more lipophilic and is absorbed by passive diffusion (Ferreira et al., 2020).

Sambucus nigra has been examined for its potential as a natural dye and supplement botanical for human nutrition by numerous researchers. The fruit products of *S. nigra* and *S. canadensis* were studied for their stability in response to heat and light. *Sambucus nigra* has higher number of acylated anthocyanins while *S. canadensis* contains more stable acylated anthocyanins. Due to the health benefits brought by elderberries, it is proposed to extract the compounds of color from fruit through different methods of extraction and their introduction into food products replacing the artificial colors used at this time, bringing a benefit to the nutritional value of the products in which it is entered.

Due to the presence of flavonoids, elderflowers primarily demonstrate diaphoretic properties and diuretics. They seal the capillary walls, improve their flexibility, and prevent the infiltration of red cells and plasma outside the vessels, due to the content of compounds (rutin) having the properties of vitamin P. May much, elderflowers show anti-inflammatory and antibacterial properties

and are therefore used for gargle to treat sore throats or as compresses to treat conjunctivitis. They are the most often used as dried flower infusions for internal or external application (Krawitz et al., 2011).

So far, the antimicrobial activity of aqueous ethanol extracts from fruit concentrates of freeze-dried elderberry was investigated on 13 pathogens, including *Staphylococcus aureus, Bacillus cereus, Salmonella poona* and *Pseudomonas aeruginosa*. Elderflower extracts have shown efficacy higher antimicrobial activity and larger zones of inhibition against a wide range of bacteria, especially *Staphylococcus aureus* (17 mm) or *Pseudomonas aeruginosa* (9 mm), than other extracts. An extract ten-fold aqueous solution of elder leaves showed moderate activity against the growth of *Bacillus cereus* and *Serratia marcescens* (6 mm) but failed to inhibit the growth of some pathogens (Hearst et al., 2010).

Cyanogenic glycosides are secondary metabolites of plants, which consist of an aglycone and a fragment of sugar. Amygdalin can be found in several plant families, including the family *Caprifoliaceae*, which also includes *Sambucus nigra*. An important characteristic of cyanogenic plants is the ability to generate toxic hydrogen cyanide. Although cyanogenic glycosides are not toxic when they are intact, they become toxic when plant enzymes (b-glucosidases and a-hydroxynitrile lyases) come in contact with the cyanogenic glycosides in the plant as a result of tissue damage after bruising or chewing (Bolarinwa, 2014).

Sticks, roots, and leaves are not dangerous if properly prepared. Glycosides cyanogenic are not toxic in themselves, but the interaction between enzymes and cyanogenic glycosides in the microflora intestinal in the gastrointestinal tract or plant enzymes promote the cleavage of the glycoside portion of cyanohydrins and then later decompose to hydrogen cyanide (HCN) and aldehyde (Ferreira et al., 2020)

Extraction methods of flavouring and colourings

Considering the diversity of plants and essential tissues, selecting the optimal extraction process can ensure the successful extraction of bioactive substances from plant materials. Traditional methods of extraction, which basically include Soxhlet extraction, steam or hydro distillation, maceration, or solvent extraction, usually contain large amounts of hazardous organic solvents, are non-

selective, time extraction time is long and, in some cases, extirpates thermosensitive substances (Garavand et al., 2019).

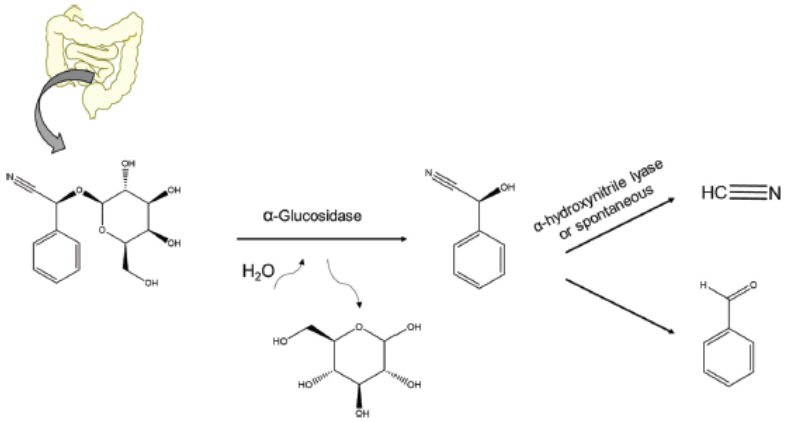

Figure 2. Degradation of sambunigrin (cyanogenic glycoside) in the gastrointestinal tract
(Source: Ferreira et al., 2020)

"Green" analytical chemistry aims to reduce the number of organic solvents during preparation samples, extraction, purification processes and detection. Minimizing the use of organic solvents, regardless of economic and environmental aspects, it can significantly reduce by-products in processes of extraction and analysis, affecting the quality of the final compounds (Ali Redha, 2021). Ecological solvents improve energy performance, given the high level of safety during work in laboratory, the minimum sample size to use and the use of friendly analytical methods environment (Gałuszka et al., 2013). Milena et al. (2019) reported a technique of efficient extraction using green solvents to isolate polyphenols from elderberries. Kowalska et al. (2021) evaluated a green chemistry approach through a possible use of glycerin to obtain extracts with anthocyanins from elderberries. They suggested glycerin as a safe alternative to other solvents such as ethanol and reported an optimal efficiency of water-glycerol solvents for the extraction of anthocyanins from fruits of shock Aspects of "green" analytical chemistry could be performed at each step (sample preparation, extraction, purification as well as identification) in this procedure.

Anthocyanins are polar in nature. The use of polar solvents like methanol and ethanol makes extraction of anthocyanins efficient. However, for food grade applications, ethanol is preferred over methanol. Often, organic (acetic, citric, or tartaric acids) or mineral

acids (hydrochloric acid or phosphoric acid) are added to the extraction solvent to stabilize the flavylium cation. The use of hydrochloric acid is restricted, as hydrochloric acid might break down acylated anthocyanins. The temperature of the extraction also affects the recovery of anthocyanins. Extraction processes are repeated for several rounds, depending on the extractability of the system, as well as the quantities of total anthocyanins present in the matrix. For commercial applications, the extracts are concentrated into a thick paste under a reduced vacuum in a rotavapor. The concentrated extract is freeze-dried to obtain a fine anthocyanin powder. UV/Vis spectrophotometric methods and HPLC methods using anthocyanin standards are the methods of choice for anthocyanin identification and quantification. Many anthocyanin pigments have characteristic absorption profiles in the UV-Vis spectral region, which makes UV-Vis an invaluable tool for the identification of molecules with B-ring substitution (Alappat and Alappat, 2020).

Bioactive chemicals must be extracted from various plant compartments to be used in health-promoting foods and nutraceutical goods. In comparison to traditional techniques, it is claimed that a suitable extraction pathway can capture the target bioactive up to 5 times. Extraction strategies, ranging from classic to sophisticated, should be tailored based on the type of bioactive chemicals present in the examined plant and inherent plant characteristics such as tissue complexity and heat sensitivity (Asbahani et al., 2015).

Traditional methods of extracting volatile components are often time-consuming and prone to the loss or degradation of volatile components, in addition to low yields and the use of large amounts of solvents. Therefore, modern scientists are devoted to finding extraction techniques that use low or even no solvent, thereby reducing the residual number of harmful solvents in natural extracts. SPME is a relatively new extraction technique that is simpler than traditional methods, fast, solvent-free, environmentally friendly, does not thermally degrade or hydrolyse samples and inexpensive. Additional advantages without the need for time-consuming sample preparation are still needed, as well as strategies to reduce the harm caused by solvents to humans and the environment (Yeh et al., 2022).

As a result, several methods have emerged, but MAE and UAE extractions have a unique position in scientific study in the process of

separating bioactive chemicals from plants (Vujanovi et al., 2020). These approaches improve extraction efficiency by using specific phenomena that occur throughout the processes in the UAE, cavitation occurs, while in MAE, microwaves interact with polar molecules in the medium. Both UAE and MAE effectively extract plant bioactive components like polyphenols, resulting in extracts with higher active chemical concentrations and more excellent biological activity. Various studies have already proven the advantages of these approaches over conventional ones (Belwal et al., 2017).

Kowalska et al. (2021) evaluated a green chemistry approach by possibly using glycerin to attain anthocyanin extracts from elderberry. They suggested glycerin as a safe alternative for other solvents such as ethanol and reported an optimal efficiency of water-glycerol solvents to extract anthocyanins from elderberry. The green analytical chemistry aspects could be performed in every step (sample preparation, extraction, purification, as well as the identification) in this procedure.

Kaack et al. (2006) reported that the difference in quality attributes of elderberry extracts may enhance the quality of existing elderberry products and develop the diversity of new products of great quality. Development of the health-promoting features of elderberry extracts by optimizing and improving the content of bioactive compounds in their extracts is one of the quality components of such elderberry products that may be developed. Some researchers evaluated the elderberry extracts and their compounds using different chromatographic methods, including high-performance liquid chromatography (HPLC), thin-layer chromatography (TLC), and paper chromatography. This research has confirmed that elderberry is a valuable source of possible bioactive flavonoids (quercetin, kaempferol, rutin) and phenolic compounds (anthocyanins) (Ferreira et al., 2020; Domínguez et al., 2021; Uzlasir et al., 2021; Vladimir-Knežević et al., 2021). Flavonoids and phenolic acids have been associated as promising compounds in various diseases, such as cancer and cardiovascular disorders (Gutierrez-Grijalva et al., 2017).

Conclusions

➢ Because of their enormous antioxidant capacity, health-promoting qualities, and solubility in aqueous solutions, anthocyanins are regarded as the most significant category. However, their extraction capacity from the plant tissue is influenced by several factors. Consumers are inclined to choose natural components as are considered to have fewer adverse effects then synthetic/artificial ingredients. As more individuals grow interested in global environmental concerns, consumers are willing to the pay a higher price for products that contain natural ingredients. In the decision-making process of switching from synthetic to natural chemicals, consumers base their choices on persuasive facts and factual data. Industries must transform scientific findings into products and fund related research. Because of the additional advantages provided, using anthocyanin as a natural food colorant is more enticing to consumers. In conclusion, elderberry products have viable uses in the food industry since using elderberry is a promising method for creating functional foods and lengthening their shelf life. Additionally, using elderberries in food preparation might lessen the need for synthetic chemicals. Based on the product manufacturing strategy of food products, different quantities and techniques employed for elderberries' inclusion in production, can have a beneficial impact on the nutritional and technical characteristics of meals, while improving or at least not hurting the sensory quality of the foods. The usage of elderberry is a viable method for creating functional foods and lengthening their shelf-life, therefore as a general conclusion; the inclusion of elderberry products has prospective uses in the food sector.

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