Chapter 4
Successful Cultivation and Utilization of *Aronia melanocarpa* (Michx.) Elliott (Black Chokeberry), a Species of North-American Origin, in Poland and the Biosynthetic Potential of Cells from In Vitro Cultures

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Abstract *Aronia melanocarpa* is a medicinal, culinary, and ornamental plant known for many years in the Central, Eastern, and Southern European countries, in Scandinavia and Russia, but is native to North America. At the end of the eighteenth century, it was introduced to Europe and Asia where it has become naturalized and successfully cultivated on an increasingly large scale. This species is a source of the raw material, i.e., fruits rich in antioxidants, most of all anthocyanins, procyanidins, phenolic acids, catechins and flavonoids, as well vitamins and bioelements. This article reviews basic information on the morphology, ecology, and distribution of *A. melanocarpa* in natural habitats. The requirements for cultivation of this species are also characterized. Much attention has been paid to the chemical composition of the fruits and their consequent therapeutic, health-promoting, culinary and cosmetic applications as confirmed by scientific studies. The current state of the art in biotechnological studies of this species is described, with a special focus on the investigations of the biosynthetic potential of cells cultured in vitro. The study aimed to establish the most beneficial culture conditions for the accumulation of phenolic acids, which are well-known strong antioxidants showing also many other important directions of biological activity. The optimization of culture conditions comprised testing the basal media, concentrations of plant growth regulators, supplementation of biosynthetic precursors, as well as examination of the impact of light conditions (monochromatic lights, white light, darkness, UV-A irradiation), and culture type (agar callus cultures and agar, agitated and bioreactor shoot cultures). In addition, the biotransformation potential of cells from agitated shoot cultures and high production of arbutin from exogenous hydroquinone were presented. Finally, the evaluation

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of efficacy and potential applications of biotechnological studies have been outlined. The obtained biotechnological results have documented that shoot cultures of *A. melanocarpa* could be a rich potential source of phenolic acids and arbutin, which are valuable products with therapeutic, health-promoting, and cosmetological values.

Keywords Black aronia · Botanical characteristics · Chemical composition · Biological activities · In Vitro cultures · Endogenous production of phenolic acids · Biotransformation potential

Abbreviations

BAP	6-benzylaminopurine
DW	Dry weight
FW	Fresh weight
HPLC-DAD	High-pressure liquid chromatography with diode array detector
LS	Linsmaier and Skoog
MS	Murashige and Skoog
NAA	1-naphthaleneacetic acid
PGRs	Plant growth regulators

4.1 Introduction

Aronia melanocarpa (Michx.) Elliott (black chokeberry, black aronia), a shrub (Fig. 4.1) of the *Spiraeoideae* (earlier *Pomoideae*) subfamily (*Rosaceae* family) is a North American autochthonous species. It is distributed along the east coast of Canada and the USA, and from the Great Lakes as far as to Florida. This species was introduced to Scandinavia and Russia at the end of the eighteenth century and was successfully naturalized in Europe and Northwestern Asia. It is a long known popular medicinal, culinary, cosmetic, and ornamental plant, cultivated mostly in Central, Eastern, and Southern European countries and in Scandinavia and Russia (Kulling and Rawel 2008; Valcheva-Kuzmanova and Belcheva 2006; Walther and Schnell 2009; Wawer 2006).

The fruits are the essential part of this plant (Fig. 4.1). They are an exceptionally rich source of a variety of subgroups of antioxidants, including polyphenols (anthocyanins, procyanidins, phenolic acids, catechins and flavonoids), carotenoids and vitamin (among others C and E) and numerous bioelements. Many professional studies of biological activity of fresh and dry fruits, fruit extracts and juice have proven their very numerous valuable properties—antioxidant, anti-inflammatory, hepatoprotective, gastroprotective, antimicrobial, and anticancer. They could be used in the prevention of ophthalmologic and circulatory diseases and in diabetes

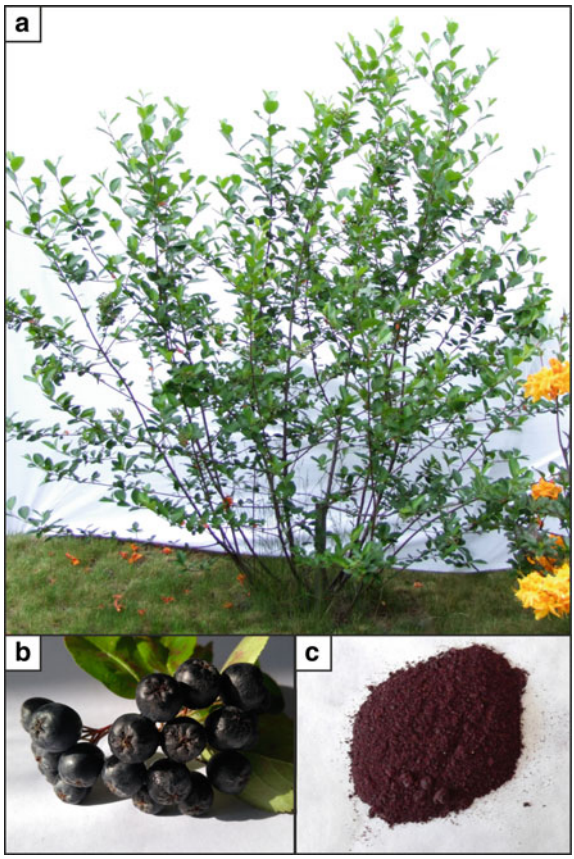


Fig. 4.1 *A. melanocarpa;* the appearance of the shrub cultivated in Poland in spring (**a**), fresh fruits of Viking cultivar (**b**), powdered dried fruits used in industry (**c**)

(Augustyniak et al. 2010; Brand 2010; Brewer 2011; Chrubasik et al. 2010; Kattappagari et al. 2015; Kokotkiewicz et al. 2010; Kulling and Rawel 2008; Oszmianski et al. 2005; Szopa et al. 2017a).

Great interest in this species, its importance and popularity in the European medicine and food industry can be corroborated by review article which were published in the last decade (e.g., Sidor et al. 2019; Szopa et al. 2017a; Denev et al. 2012; Kokotkiewicz et al. 2010) and the newest reviews of phytochemical and pharmacological studies (Borowska and Brzóska 2016; Denev et al. 2012, 2019; King and Bolling 2020; Kokotkiewicz et al. 2010; Sidor et al. 2019; Szopa et al. 2017b).

The raw material, i.e., the fruit is sourced from plants, especially of the cultivars "Nero", "Galicjanka", and *Aronia mitschurinii*, commercially cultivated with great success in Central and Eastern European countries, including Poland. Recently, these crops have become very popular and are cultivated on a mass scale, also as organic farming (Michalak 2015).

The conspicuously growing interest in *A. melanocarpa* fruits and the constantly rising demand for this raw material result principally from the rich composition of antioxidants important in the prevention and treatment of different civilization diseases. These compounds can be useful in hypertension, angina, stroke prevention, neurodegenerative disorders, and neoplastic diseases and control of lipid and cholesterol levels. Moreover, these compounds show antiaging effect. They are a focus of interest of the pharmaceutical, health food, and cosmetic industries (Cai et al. 2004; Chrubasik et al. 2010; Denev et al. 2019; Heleno et al. 2015; Kakkar and Bais 2014; Sidor et al. 2019; Szopa et al. 2018a).

A substantial need for the search for new natural sources of antioxidants, natural phenomenon of chemical variability as well as environmental pollution and current rapid climate changes have also inspired interest in the morphological and biosynthetic potential of in vitro cultures of this species. Earlier biotechnological studies of *A. melanocarpa* carried out by other research groups concentrated on the development of micropropagation protocols (Brand and Cullina 1992; Litwinczuk 2002; Petrovic and Jacimovic-Plavšic 1992; Ruzic 1993). The wide-ranging studies of our team have aimed to optimize favorable conditions for the synthesis of one of group of antioxidants, namely phenolic acids. Optimization has involved agar (callus and shoot) cultures, agitated shoot cultures, and shoot cultures carried out in commercially available bioreactors—RITA and PlantForm (temporary immersion systems) (Rugină et al. 2012; Szopa et al. 2018a, 2020; Zheng and Wang 2003). Some studies examined biotransformation potential of cells cultured in vitro. They were concentrated on biotransformation of exogenous hydroquinone into its β-D-glucoside, arbutin (Kwiecień et al. 2013).

4.2 Synonyms and Names in Other Languages

Black chokeberry and black aronia are the most popular and commonly used English names of *Aronia melanocarpa* (Michx.) Elliott. This species possesses several other Latin synonymous names: *Aronia arbutifolia* (L.) Pers. var. nigra (Willd.) Seymour, *Aronia nigra* (Willd.) Koehne, *Photinia melanocarpa* (Michx.) K.R. Robertson and Phipps., *Pyrus arbutifolia* (L.) L. f. var. nigra Willd., *Pyrus melanocarpa* (Michx.) Willd. and *Sorbus melanocarpa* (Michx.) Heynh (University of Maine 2020). Other foreign names of the species are as follows: aronia czarna, aronia czarnoowocowa (Polish), schwarze Apfelbeere (German), aronie, aronia à fruits noirs, aronie noire, (French).

4.3 Morphology

Aronia melanocarpa is a perennial shrub growing to ca. 3 m tall and up to ca. 2.5 m wide (Fig. 4.1). It develops an extensive but shallow root system within the

perimeter of the crown. The taproot penetrates to 1.5 m deep. The lateral roots are thin and spread horizontally. The plant grows vigorously and spontaneously develops numerous suckers, thus forming dense colonies. Shrubs assume a compact form, bushier during fruiting. The shrubs are densely branched and are capable of regeneration and thickening (Celka and Szkudlarz 2010; Kleparski and Domino 1990; Kokotkiewicz et al. 2010; Rumińska 1984).

Both leaf buds and flower buds closely adhere to the shoots. They are ca. 1 cm long and 3–4 mm wide. In European climate conditions, the flower buds blossom much later than in other fruit trees and shrubs. Blooming begins most often in April when the temperature exceeds 5 °C (Celka and Szkudlarz 2010; Kleparski and Domino 1990; Kokotkiewicz et al. 2010; Rumińska 1984).

Young one-year twigs are thin, flaccid, slightly hairy, dark gray in color, not branched. Older shoots are dark brown. Leaves are elliptic, leathery, glossy. Leaf blade top is glabrous, while the bottom is covered by whitish delicate tomentum. Midrib is conspicuous. Leaves have toothed margins. Leaves are borne on short stalks with two bracts. Leaves on vegetative shoots are ca. 6–8 cm long and ca. 4–6 cm wide. In European conditions, in September leaves turn yellow-orange and red and fall relatively quickly (Celka and Szkudlarz 2010; Kleparski and Domino 1990; Kokotkiewicz et al. 2010; Rumińska 1984).

Aronia flowers are bisexual, with five petals. Flowers are small, inconspicuous, ca. 1 cm across, white or pinkish-white. They are gathered in corymbs. Freshly opened flowers are distinguished by violet anthers. In Europe, flowering starts at the end of June and lasts ca. 10 days. In general, flowers are entomophilous, however, in unfavorable circumstances, they can self-pollinate (Kleparski and Domino 1990; Rumińska 1984).

Unripe fruits are green, during ripening they turn dark red, and then almost black, from matt they change into glossy. Most often they ripen at the beginning of September. Fully ripe aronia fruits are black or dark blue, and they are covered by thick wax coating. One fruit is ca. 6–15 mm across and weights ca. 1 g. Each fruit contains ca. 5 seeds. Aronia fruits do not fall and can remain on plants to the first frost (Kleparski and Domino 1990; Rumińska 1984).

For medicinal and culinary purposes, chokeberry fruits should be harvested when fully ripe, i.e., almost black. They are usually harvested in September. The fruits are first dried on sieves at the beginning at a temperature of 30 °C and then slightly higher but not exceeding 45 °C (Rumińska 1984; Senderski 2004) (Fig. 4.1).

4.4 Natural Habitats in North America and Ecology

Aronia is a species native to North America. Its natural habitats are located in the eastern part of North America from Great Lakes extending south as far as to Florida. Its natural locations can be found both in Canada and the USA (Wawer 2006).

Aronia is cold hardy, and it tolerates temperatures even below −30 °C. It does not have special soil requirements. Since it develops a shallow root system, it can be

cultivated even in areas unsuitable for other more demanding fruit crops. It is tolerant both to drought and excessive humidity (Kleparski and Domino 1990; Kokotkiewicz et al. 2010).

4.5 Successful Cultivation in European Countries

The first *A. melanocarpa* plants were introduced to Europe at the end of the eighteenth century. Cultivation of black chokeberry first developed in Scandinavia and Russia. Farm cultivation on industrial scale became popular especially in the Altai Mountains and in the area of Moscow and Petersburg (Kulling and Rawel 2008; Valcheva-Kuzmanova and Belcheva 2006; Walther and Schnell 2009).

At present, chokeberry is a commonly grown shrub in Central, Eastern, and Southern European countries and in Scandinavia. It is suitable as well for amateur cultivation in backyards as for industrial-scale crop production. Commercial plantings are based on most popular cultivars, such as the Czech cultivar "Nero" and Polish cultivar "Galicjanka". These cultivars owe their popularity to a high yield and high resistance to harmful environmental conditions. The Russian cultivar *Aronia mitschurinii* Amit is another known and popular cultivar which was obtained by grafting *Aronia melanocarpa* on a rootstock of mountain ash (*Sorbus aucuparia*). This cultivar is distinguished by especially high cold-tolerance level. Its fruits are very large and sweet (Kleparski and Domino 1990).

Due to its attractive appearance, in particular changeable leaf color, aronia is also cultivated as an ornamental plant. For its esthetic beauty, it is planted in gardens and parks. The cultivars available in Sweden—"Viking" (originating from Finland), "Aron" (from Denmark), and "Hugin" (from Sweden) are appreciated not only as medicinal but also as ornamental plants (Jeppsson 1999). There are also other cultivars of black chokeberry bred in various countries. Few of them were developed in Poland: "Albigowa", "Dąbrowice", "Egerta", "Kutno", and "Nowa Wieś". Another was developed in Hungary—"Fertödi". The hybrid between Russian and Finnish plants named "Rubina" is the next cultivated cultivar. The most popular cultivars in the USA include "Autumn Magic", "McKenzie", and "Morton" (University of Maine 2020).

4.6 Cultivation Requirements

As already mentioned, aronia is a cold-hardy species. However, it applies to its aboveground parts while the roots can be damaged by frost below −11 °C, and this is why snow cover is vital (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

Chokeberry is best grown on fertile humus, high-humidity, medium-cohesive soils, on permeable substrate, rich in nutrients. It is important to choose an area free of frost basins, unflooded in the spring, with a low level of groundwater. Light

conditions should also be taken into account because aronia is a sun-loving plant and should not be shaded by other plants (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

The best sites for chokeberry plantings are those where root crops were grown on manure-amended soils or after horticultural crops. If plantations are to be established on fallow land, the application of manure is indispensable. If manure is not available, it can be replaced by compost. Presowing tillage procedures should include deep plowing and harrowing (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

Plantations can be established both from seeds and from plantlets. The vegetative propagation is based on three methods: division of old shrubs, root suckers, and rooted softwood or hardwood stem cuttings. One parent plant several years old can yield ca. 20 rooted plantlets. Vegetative propagation accelerates plant growth and boosts yield. Vegetatively propagated plants begin yielding already after 2–3 years while shrubs grown from seed require 3 or even 5 years. In the case of propagation by seeds, they have to be stratified for 3–5 months (by storage in humid coarse sand at a temperature of 2–3 °C) (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

The plantlets used for establishing aronia crops should be 50 cm tall and should have 2–3 lateral shoots. The stem at the neck should be 1 cm across. The root system should be highly branched, reaching ca. 20 cm in length (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

Planting can be done both in autumn and early spring. Spring planting is preferred in the regions which lack the snow cover in winter. Planting should be carried out immediately after the soil thaws—not later than till mid-April. If autumn planting is planned and when planting material has to be stored, mulching or covering with snow is recommended before frost occurs (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

The raw material, namely aronia fruits, is harvested when the fruits are fully colored and almost black. Depending on atmospheric conditions in a given year and plantation age, yields can reach from several tens of kilograms to several tons per hectare. A 3-year well-growing plantation can yield ca. 3 tons of chokeberry fruits per 1 ha. A plantation can be used for 10–15 years, and then it should be liquidated (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

Aronia is a species resistant to plant diseases. Brown spot disease and brown rot are observed the most often. In addition, the following insects can feed on chokeberry: apple aphid (*Aphis pomi de Geer*), woolly aphid (*Eriosoma lanigerum* Hasm.) and leaf blister mite (*Eriophyes piri* Pgst.)—a mite species (Gruszczyk et al. 2018; Rumińska 1984; Senderski 2004).

4.7 Chemical Composition

Aronia fruits are the most valuable part of this species and are used as the raw material for pharmaceutical, culinary, and/or cosmetic purposes as they are a rich source of polyphenols—anthocyanins, procyanidins, phenolic acids, catechins, and

Table 4.1 Maximal contents of antioxidant compounds in fresh [mg/g FW] and dried [mg/g DW] fruits and fruit extracts of *A. melanocarpa*

Group of metabolites	Compound	Fresh fruits	Dried fruits	Dried fruit extracts
Anthocyanins	Cyanidin-3-galactoside	9.9	12.8	314.0
	Cyanidin-3-arabinoside	4.0	5.8	159.6
	Cyanidin-3-xyloside	0.5	0.5	40.0
	Cyanidin-3-glucoside	0.4	0.4	14.5
	Pelargonidin-3-arabinoside with pelargonidin-3-galactoside	nd[a]	nd	0.5
Proanthocyanidins	Procyanidin B_1	nd	nd	25.4
Phenolic acids	Hydroxycynnamic acid	0.01	nd	nd
	Chlorogenic acid	2.8	3.02	79.0
	Neochlorogenic acid	1.8	2.9	44.7
	3,4-Dihydroxyphenylacetic acid	0.1	nd	nd
	Protocatechuic acid	0.1	nd	nd
	Rosmarinic acid	0.1	nd	nd
	Caffeic acid	1.4	nd	0.7
Catechins	(+)-catechin	nd	nd	19.9
	(−)-epicatechin	nd	0.2	12.8
Flavonoids	Quercetin	0.1	nd	1.8
	Quercetin-3-rutinoside	nd	18.0	18.3
	Quercetin-3-galactoside	0.3	0.4	8.9
	Quercetin-3-glucoside	0.3	nd	21.5

[a]nd—no data

flavonoids (Table 4.1) (Bijak et al. 2013; Brzóska et al. 2015; Jakobek et al. 2012; Jodynis-Liebert et al. 2014; Jurgoński et al. 2008; Kim et al. 2013; Pérez-Jiménez et al. 2010; Rugină et al. 2012; Ryszawa et al. 2006; Wangensteen et al. 2014).

Phytochemical studies have concentrated on the chemical composition of fresh and dried fruits, dry fruit extracts, and juice (Benvenuti et al. 2006; Bijak et al. 2013; Brzóska et al. 2015; Jakobek 2007; Jodynis-Liebert et al. 2014; Jurgoński et al. 2008; Kim et al. 2013; Pérez-Jiménez et al. 2010; Rugină et al. 2012; Ryszawa et al. 2006; Taheri et al. 2013; Vlachojannis et al. 2015; Wang et al. 1996; Wangensteen et al. 2014; Wu et al. 2004; Zheng and Wang 2003) (Table 4.1).

Anthocyanins in aronia fruits have been confirmed to comprise mostly cyanidin glycosides with dominating cyanidin-3-galactoside and cyanidin-3-arabinoside while cyanidin-3-xyloside and cyanidin-3-glucoside are present at lower amounts. Cyanidin glycosides are accompanied by glycoside compounds of pelargonidin: arabinoside and galactoside. The group of procyanidins includes mostly procyanidin B_1 (Table 4.1).

Phenolic acids are represented by caffeic acid and hydroxycinnamic acid and depsides—chlorogenic acid and neochlorogenic acid (Fig. 4.2). The catechin group has been shown to include (+)catechin and (−)epicatechin. On the other hand, among flavonoids present in aronia fruits, mostly quercetin glycosides (rutinoside, galactoside, and glucoside) and an aglycone, namely quercetin, were identified (Table 4.1) (Kulling and Rawel 2008; Sidor and Gramza-Michałowska 2019; Szopa et al. 2017a). Other flavonoid compounds confirmed to be present in chokeberry fruits include: isorhamnetin 3–O–: -galactoside and –glucoside; isorhamnetin–3–: -galactoside, -glucoside, -neohesperidoside, and –rutinoside; kaempferol 3–O–galactoside; kaempferol-3-: -galactoside and –glucoside; myricetin 3–O–: -galactoside and –glucoside; and quercetin–3–: -robinobioside and –vicianoside (Gramza-Michałowska et al. 2017; Sidor et al. 2019).

Aronia fruits are also a rich source of vitamins, in particular, vitamin C, E, K, folic acid, many vitamins of B complex (B_1, B_2, B_6), niacin (vitamin B_3) and pantothenic acid (vitamin B_5). They also contain β-carotene (provitamin A) as well as other carotenoids—β-cryptoxanthine and violaxanthine. In addition, the fruits are a generous supply of bioelements, including zinc, magnesium, potassium, sodium, calcium, copper, selenium, and iron (Andrzejewska et al. 2015; Benvenuti et al. 2006; Kulling and Rawel 2008; Razungles et al. 1989; Sikora et al. 2009; Stralsjoe et al. 2003; Tanaka and Tanaka 2001) (Table 4.2).

The above-mentioned metabolites and bioelements are accompanied by organic acids (citric acid and malic acid), fiber, pectins, fatty acids, and sterols.

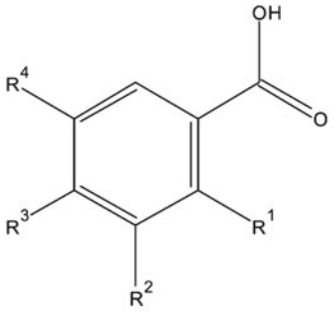
Phytochemical studies of the dried fruits and leaves of aronia specimens acquired from the Arboretum of the Warsaw University of Life Sciences (SGGW) in Rogów (central part of Poland), as carried out by our team, have proven that the leaves of this species are very rich in polyphenols while some polyphenolic compounds were demonstrated in fruits for the first time. The quantitatively dominating group in the analyzed leaves was identified as flavonoids. The presence of quercetin and its two glycosides—quercitrin and rutoside was confirmed. The group of phenolic acids was documented to include two depsides: chlorogenic and neochlorogenic acids and additionally 3,4-dihydroxyphenylacetic and protocatechuic acids. Among anthocyanins, cyanidin arabinoside, and galactoside were identified (Szopa et al. 2017a).

The studies of other authors (Tian et al. 2017) underlined also the biosynthetic potential of the leaves of various berry plants, among them also black chokeberry. The leaves extracts of some berry plants could also be a potential rich source of phenolic compounds (Tian et al. 2017).

Our analysis of fruit extracts confirmed the presence of chlorogenic acid and neochlorogenic acid, while rosmarinic acid, protocatechuic acid, and 3,4-dihydroxyphenylacetic acid were identified for the first time. The anthocyanin group was shown to be represented by three cyanidin glycosides, namely galactoside, arabinoside, and glucoside. On the other hand, only the aglycone quercetin was found among flavonoids (Szopa et al. 2017a).

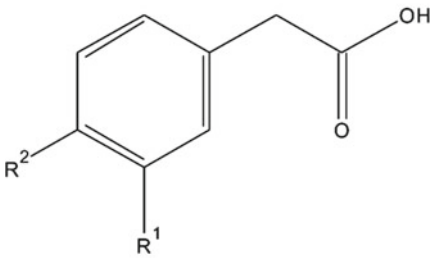
Fig. 4.2 Chemical structures of the main subgroups of phenolic acids

Benzoic acid



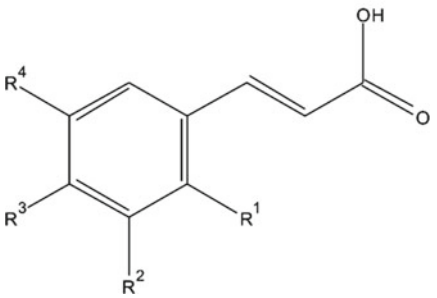
Gallic acid	R^1=H, R^2=OH, R^3=OH, R^4=OH
Protocatechuic acid	R^1=H, R^2=OH, R^3=OH, R^4=H
Salicylic acid R^1=OH,	R^2=H, R^3=H, R^4=H
Syringic acid R^1=H,	R^2=OCH_3, R^3=OH, R^4=OCH_3
Vanillic acid R^1=H,	R^2=OCH_3, R^3=OH, R^4=H

Phenylacetic acid



3-Hydroxyphenylacetic acid	R^1=OH, R^2=H
3,4-Dihydroxyphenylacetic acid	R^1=OH, R^2=OH

Cinnamic acid



Ferulic acid	R^1=H, R^2=OCH_3, R^3=OH, R^4=H
Caffeic acid	R^1=H, R^2=OH, R^3=OH, R^4=H
o-Coumaric acid	R^1=OH, R^2=H, R^3=H, R^4=H
p-Coumaric acid	R^1=H, R^2=H, R^3=OH, R^4=H
Sinapic acid	R^1=H, R^2=OCH_3, R^3=OH, R^4=OCH_3

Table 4.2 Content of vitamins and bioelements in fruits of *A. melanocarpa*

Vitamins and bioelements		Content
Vitamins	C	0.013–0.27 mg/g DW
	B_1 (thiamine)	0.0002 mg/g DW
	B_2 (riboflavine)	0.0002 mg/g DW
	B_3 (niacin)	0.003 mg/g DW
	B_5 (pantothenic acid)	0.0028 mg/g DW
	B_6 (pirydoxine)	0.0003 mg/g DW
	B_9 (folic acid)	0.0002 mg/g DW
	E	0.008–0.31 mg/g DW
	K	0.0002 mg/g DW
Carotenoids	β-carotene	0.0077–0.0168 mg/g FW
	β-cryptoxanthin	0.0046–0.0122 mg/g FW
	Violaxanthin	0.013 mg/g FW
Bioelements	Zinc	0.0015 mg/g DW
	Magnesium	0.162 mg/g DW
	Potassium	2.18 mg/g DW
	Sodium	0.026 mg/g DW
	Calcium	0.322 mg/g DW
	Iron	0.0093 mg/g DW

The extraction procedures of chokeberries are decisive for the quantities of individual subgroups of phenolics and for their percent content in total phenolics. Methanolic extracts are prepared most often (Vázquez-Espinosa et al. 2019).

According to the newer investigations of the Finland team (Tian et al. 2017), anthocyanins in fresh chokeberries collected in Finland (2013–2014) represented 50% of total phenolics with two main cyanidin glycosides: 3-*O*-galactoside (222 mg/100 g FW) and 3-*O*-arabinoside (159 mg/100 g FW). 3-*O*-Caffeoylquinic acid (23%) and 5-*O*-caffeoylquinic acid (11%) were the next dominant group among the total phenolics. Flavonoid glycosides made up ca. 10% of total phenolics. The quantitatively dominant compounds include quercetin 3-*O*-galactoside and quercetin 3-*O*-glucoside. These results were obtained by the authors after extraction of fresh plant material with acidic aqueous ethanol (Tian et al. 2017).

Other authors after extraction of the fresh fruits with 0.1% hydrochloric acid in methanol documented a lower anthocyanin content in chokeberries (481 mg/100 g FW), which included also cyanidin-3-*O*-galactoside (65% of total anthocyanins) and cyanidin-3-*O*-arabinoside (30%), as the main compounds (Slimestad et al. 2005).

Some scientific investigations documented differences in the quantity of secondary metabolites between individual aronia cultivars. Five cultivars (“Aron”, “Fertödi”, “Hugin”, “Nero”, and “Viking”) grown in an experimental orchard in Zlin

(Czech Republic) in 2008–2010 (three vegetation periods) were shown to differ in total content of phenolic compounds and total antioxidant activity. The highest values were found for cultivars "Viking" and "Nero". Those cultivars were proposed by the authors as the most promising for further use in food and pharmaceutical applications (Rop et al. 2010).

On the other hand, three different cultivars—"Nero", "Viking", and "Galicjanka" and wild chokeberry, all grown in the Slavonia region (Croatia) in 2010–2011, had the same profile of phenolic compounds, but the contents of subgroups of compounds were different. The total phenolic and total anthocyanin contents were higher in "Nero" and "Viking", while in "Galicjanka" the lowest content was confirmed. Flavonoid content was comparable in all chokeberries. Phenolic acid contents were the highest in "Viking" and wild chokeberries. The antiradical activity was the strongest (in DPPH and ABTS tests) also for "Viking" and wild-chokeberry berries (Jakobek et al. 2012).

The more recent research on this issue documented that qualitative composition of anthocyanins did not differ between the wild-grown and cultivated cultivars, but, in general, their contents were different (Veberic et al. 2015).

The quantitative content of bioactive compounds in aronia fruits is affected by weather conditions during the growth and ripening of the fruits, which is obvious. The investigations by another Croatian team (fruits from experimental orchard, Donja Zelina, 2012–2014) documented that the mean monthly temperature and bright sunshine hours (May–September) had a positive impact on the concentrations of phenolic substances (total phenolics and total flavonoids) and, therefore, on the antioxidant activity of juice from aronia fruits (FRAP test) (Tolić et al. 2017).

Phenolic acids—the object of our biotechnological approach

Our biotechnological studies presented below in this article focused specifically on phenolic acids. Therefore, we decided to present this group in more detail specifying their characteristics—chemical division, biogenesis, and biological activity. These compounds are antioxidants widespread in the plant kingdom. Structurally they are polyphenols and are divided into derivatives of cinnamic acid, benzoic acid, and phenylacetic acid (Fig. 4.2). The derivatives of cinnamic acid are the most prevalent in the plant kingdom. They include mostly *p*-coumaric acid, *o*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid. Benzoic acid derivatives include principally salicylic acid, *p*-hydroxybenzoic acid, protocatechuic acid, and vanillic, syringic, and gallic acids. On the other hand, phenylacetic acid derivatives are represented predominantly by 3-hydroxyphenylacetic and 3,4-dihydroxyphenylacetic acid.

Phenolic acids occur in the plant kingdom in the free form or often as depsides. They may also be a part of glycosidic linkages. Depsides are compounds built of two or rarely more of molecules of phenolic acids linked by an ester bond. They often contain caffeic acid or its derivatives. The most commonly known depsides include chlorogenic acid and its numerous isomers—e.g., isochlorogenic acid, neochlorogenic acid, and cryptochlorogenic acid. Rosmarinic acid, ellagic acid, and *m*-digallic acid are other well-known and popular representatives of this group. Chicory acid,

cinarine, cetraric acid, and lecanoric acid are less common depsides in the plant kingdom.

Biogenesis of phenolic acids is closely associated with the shikimic acid pathway (Fig. 4.3). Shikimic acid gives rise to chorismic acid, and in the next step, to prephenic acid. Prephenic acid is a precursor of the path leading to phenylalanine and tyrosine—aromatic amino acids. Deamination of phenylalanine yields cinnamic acid, the parent compound of one subgroup of phenolic acids. The next stage involves the formation of hydroxy and methoxy derivatives of cinnamic acid—*p*-coumaric acid, caffeic acid, ferulic acid, and sinapic acid. Phenylalanine is the main direct biogenetic precursor of phenolic acids. *p*-Coumaric acid can also be formed from tyrosine.

Benzoic acid derivatives can be formed *via* chorismic acid transformations. This path leads to salicylic acid formation. Salicylic acid and other benzoic acid derivatives can be produced also from cinnamic acid by shortening of the side chain of this compound by 1–3 carbon atoms.

In addition to strong antioxidant properties phenolic acids show a multitude of therapeutically important biological actions. They exhibit cholagogic, choleretic, hypolipemic, hypocholesterolemic, and hepatoprotective activities. They also cause spasmolytic effect. They were proven to have anxiolytic, chemoprotective, and immunostimulating properties. Their antiviral, bacteriostatic, fungistatic, cytotoxic, and anti-inflammatory activities are also known. However, these are antioxidant and anticancer effects documented for some phenolic acids that have attracted the greatest interest. It is a group of plant metabolites which receives tremendous attention of many scientific centers worldwide (Brewer 2011; Kattappagari et al. 2015; Krishnaiah et al. 2011; Szopa et al. 2018a; Willcox et al. 2004; Zhang and Tsao 2016).

A wide range of biological activities and resulting possible therapeutic applications of phenolic acids outlined above prompted us to choose this group of compounds as the object of our biotechnological studies.

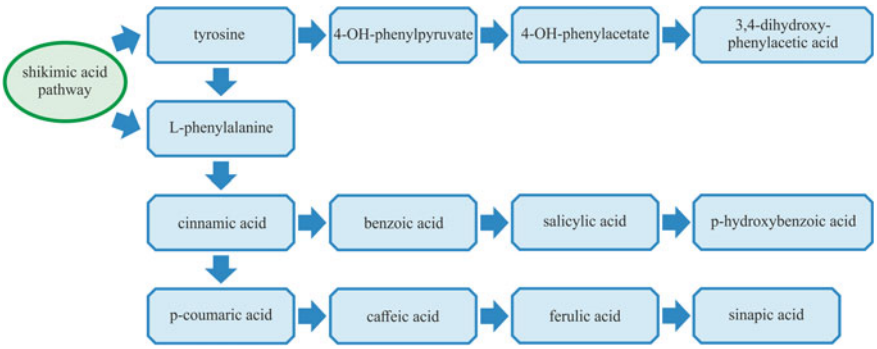


Fig. 4.3 Biosynthesis of chosen phenolic acids

4.8 Medicinal and Health-Promoting Properties

The above-presented chemical composition of chokeberry fruits underlies their multidirectional health-promoting properties and efficacy in supportive treatment of many of civilization diseases (Wawer 2006). Most of the directions of their biological activity were confirmed by scientific studies.

In the last years, three valuable review articles (Sidor et al. 2019, Sidor and Gramza-Michałowska 2019; King and Bolling 2020) presented the current knowledge about different directions of biological activity of different forms of fruits (fresh, dry, juice, extract) based on black chokeberry fruits, as evidenced by animal experiments and/or human trials. The first article paid a special attention on health-promoting activity of those products in cardiovascular diseases, hyperlipemia, hypercholesterolemia, hypertension, and diabetes (Sidor et al. 2019). The second article documented most of all the antioxidant activity of aronia products measured with use of different tests (DPPH, FRAP, ABTS, CUPRAC) (Sidor and Gramza-Michałowska 2019). Objective of the third article is to analyze aronia berry composition, including polyphenols nutrients, summarize available studies on the polyphenol bioavailability and health benefits (King and Bolling 2020). The most important biological activities of chokeberry products confirmed by scientific research are presented below.

Antioxidant action

Both fresh, dried, and/or powdered fruits and their products show a strong antioxidant activity, which is associated with a high content of polyphenols, known antioxidants, which include anthocyanins, proanthocyanidins, phenolic acids, catechins and flavonoids. Some other ingredients present in fruits, such as vitamin C and E, β-carotene, and bioelements—zinc, copper, and selenium, have also antioxidant properties.

A comparison of antioxidant actions of fresh *A. melanocarpa* fruits with other fruits—blueberries, apples, strawberries, cranberries, and even grapes demonstrated ca. four times stronger action of aronia fruits. Antioxidant activity of chokeberry fruits was documented in in vitro tests (ABTS, DPPH, FRAP, CUPRAC, ORAC), in vivo experiments on laboratory animals and in clinical trials (Denev et al. 2012, 2019; Kokotkiewicz et al. 2010; Kulling and Rawel 2008; Oszmianski et al. 2005; Tolić et al. 2015).

The studies carried out by our research team also proved (by DPPH and FRAP tests) a high antioxidant activity of both fruit and leaf extracts (Szopa et al. 2017a).

Beneficial effect on the cardiovascular system

Scientific studies confirmed the protective and stimulating effect of aronia polyphenol compounds on the cardiovascular system function, in particular their anticoagulant, vasoprotective, cardioprotective, hypotensive and blood triglyceride, and cholesterol-lowering effects. In vitro studies have proven the protective and regenerative actions of polyphenols present in aronia products on the endothelial cell function due to their

antioxidant and anti-inflammatory potential. Supplementation of the diet with chokeberry juice in men with mild hypercholesterolemia resulted in a marked reduction of the total cholesterol, LDL cholesterol, and triglyceride levels, which was accompanied by a rise in HDL cholesterol. Moreover, a decrease in both systolic and diastolic blood pressure was documented. Similar effects of the fruit extract were observed in patients with type II diabetes, after myocardial infarction and co-treated with statins (Bell and Gochenaur 2006; Broncel et al. 2010; Kokotkiewicz et al. 2010; Kulling and Rawel 2008; Naruszewicz et al. 2007).

Diabetes prevention and treatment

Numerous studies have documented a beneficial effect of aronia fruit extracts on prevention and outcomes of type II diabetes. Administration of aronia products to patients reduced blood levels of glucose, glycated hemoglobin (HbA1c), cholesterol, and lipids (Badescu et al. 2015; Jurgoński et al. 2008; Kokotkiewicz et al. 2010; Kulling and Rawel 2008; Valcheva-Kuzmanova et al. 2007). It was shown that chlorogenic acid present in chokeberry juice and fruit extracts stimulated glucose and lipid metabolism. On the other hand, cyanidin-3-arabinoside inhibited the activity of α-glucosidase, which is the enzyme participating in carbohydrate breakdown (Meng et al. 2013).

A Japanese research team has recently documented the beneficial effect of the identified cyanidin-3,5-*O*-diglucoside from aronia juice on hyperglycemia in mice-administered aronia juice. This diglucoside is a dipeptidyl peptidase IV inhibitor. The investigations in male and female adult healthy Japanese who consumed aronia juice demonstrated the reduction of postprandial blood glucose levels. In addition, the authors documented that activities of dipeptidyl peptidase IV, α-glucosidase, and angiotensin-converting enzyme were reduced by aronia juice. Probably aronia juice suppresses the elevation of postprandial blood glucose levels through the inhibition of those enzyme activities and could be useful for prevention of metabolic disease in adult healthy Japanese (Yamane et al. 2017).

Hepatoprotective activity

Studies on experimental animals (rats) have demonstrated the protective action of both juice and nectar from aronia fruits on hepatocytes. In animals, lipid peroxidation was induced by the administration of CCl_4, aminophenazone, and sodium nitrite which caused hepatocyte death while anthocyanins and other phenolic compounds from aronia fruits were proven to suppress lipid peroxidation. Moreover, efficiency of chokeberry fruit extracts was evidenced in the treatment of non-alcoholic hepatic steatosis (Park et al. 2016; Pool-Zobel et al. 1999; Valcheva-Kuzmanova et al. 2004).

Recently, another research team has evaluated the hepatoprotective activity of aronia juice and silymarin in the rat model of fibrosis (induced by CCl_4). After administration of aronia juice, the peroxidation of lipids was suppressed. The beneficial effect of aronia juice was also confirmed during histological examination. In this model, the effect of silymarin used as a positive control was very limited (Piotrowska-Kempisty et al. 2020).

Gastroprotective activity

Animal studies in the rat documented antiulcer activity and protective action on the gastrointestinal mucosa after the administration of aronia juice. Animals which did not receive the juice more often showed indomethacin-induced stomach injury (Valcheva-Kuzmanova et al. 2005).

Ophthalmological applications

Aronia anthocyanins accelerated regeneration of rhodopsin in rods of the retina of the eye and improved color vision, mesopic vision, and image registration. On the other hand, flavonoids present in chokeberry fruits improved elasticity of the capillary walls in the eyeball by interacting with collagen. In this way, they reduced fragility and permeability of the vascular wall. The complex of antioxidants in aronia preparations shows a beneficial effect in the case of progressing cataract, glaucoma, and macular degeneration (Wawer et al. 2012; Wolski et al. 2007).

Anti-inflammatory activity

Anti-inflammatory activity was evidenced for dry extract from aronia fruit. This activity was multidirectional. Flavonoids and anthocyanins present in the extract inhibited the activity of cyclooxygenase (COX-2) and inflammatory reactions in which it participates. On the other hand, research on mouse macrophage cultures documented anthocyanin-induced inhibition of mastocyte degranulation and reduction of tumor necrosis factor (TNF-α) level (Valcheva-Kuzmanova et al. 2005).

Antibacterial and antiviral activity

Bacteriostatic activity of aronia fruit juice was proven against *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Antiviral activity was demonstrated for influenza A virus (Valcheva-Kuzmanova and Belcheva 2006).

The mechanisms of antiviral activity of chokeberry extract against influenza viruses have been presented recently. It was shown that this effect is connected with the presence of anthocyanins, proanthocyanidins, and other classes of flavonoids and phenolic acids in fruits. Polysaccharides could also play an important role. The components of fruits could inhibit replication of the virus directly, e.g., by blocking surface glycoproteins of influenza virus or indirectly by stimulating the immune system of the host. Similar properties were documented for other popular berry fruits, elderberry, goji berry, cranberry, and black currant (Gramza-Michałowska et al. 2017; Sidor et al. 2019).

Chelation of heavy metals

Chelating actions of anthocyanins was revealed in experimental animals (rats) intoxicated with cadmium. Aronia juice supplementation was shown to decrease the accumulation and toxicity of this element (Kokotkiewicz et al. 2010). A similar chelating action was also proven in lead poisoning in which a drop in the concentration of lead compounds in serum and internal organs was observed. This effect was also attributed to aronia anthocyanins (Jan Niedworok 2001).

Radioprotective activity

It has been evidenced that anthocyanins contained in aronia fruits have a beneficial effect on the outcomes of acute radiation syndrome experimentally induced in rats. Anthocyanins suppressed the generation of free radicals and rapid drop in leukocyte count. The enhanced regenerative potential of cell was also documented (Wawer et al. 2012).

Another study confirmed the radioprotective activity of cyanidin and its glycosides as well as the extract containing aronia anthocyanins. This effect was observed on monkey renal cell line exposed to the radioactive complex of technetium and 2,3-dimercaptosuccinic acid (Wawer et al. 2012).

In addition, gel from chokeberry fruits applied to the skin protected it from the harmful effect of UV-B radiation (Niedworok et al. 1999; Pratheeshkumar et al. 2014).

Antimutagenic activity

Studies on human lymphocyte culture exposed to mutagenic compounds (benzopyrene and 2-aminofluorene) have demonstrated antimutagenic action of aronia fruit extracts. Anthocyanins present in the studied extracts produced antigenotoxic effect which resulted from the ability of anthocyanidins to neutralize free radicals and to inhibit enzymes activating mutagenic substances (Gasiorowski et al. 2000).

Anticancer activity

Anticancer action of products from aronia fruits is closely linked with their strong antioxidant potential (Lala et al. 2006; Thani et al. 2014). The presence of various groups of polyphenols is decisive for cell protection against oxidative stress and apoptosis leading to carcinogenesis. Furthermore, these compounds suppress cell cycle of abnormal cancer cells (Sharif et al. 2012; Zhao et al. 2004).

In vitro studies proved the inhibition of human colon adenocarcinoma cells HT29 and Caco-2 by extracts and juice from chokeberry fruit (Bermúdez-Soto et al. 2007a, b; Jing et al. 2008; Lala et al. 2006; Malik et al. 2003; Saruwatari et al. 2008; Zhao et al. 2004). In addition, acetone extracts from aronia fruits and extracts from its leaves were observed to inhibit the development of leukemia cell line L1210 and HL60 (Skupień et al. 2008; Sueiro et al. 2006).

Safety

Despite a common use of the processed and unprocessed aronia fruits, there are no data on effective and safe dosages. No records are kept on undesired reactions and possible toxicity of the chokeberry fruits and products. It is only known that procyanidins (mostly procyanidin B_1) and anthocyanins (mostly cyanidin-3-arabinoside) are inhibitors of cytochrome P450 3A4 (CYP3A4) which participates in biotransformations of some drugs. Therefore, it is important not to take some drugs with aronia fruits and their products.

4.9 Significance in the Production of Dietary Supplements and in Food Industry

A. melanocarpa is an ingredient of numerous dietary supplements in Europe produced with the addition of dried fruits, powdered fruits, aqueous extracts, juice or juice concentrate. They are manufactured by European, American, Canadian, and even South Korean companies. Products of Polish, German, Swiss, Italian, Ukrainian, and Turkish enterprises dominate in European countries. Table 4.3 presents important data on some chosen chokeberry-based dietary supplements.

According to the opinion of the European Food Safety Authority (EFSA) presented at the Panel on Dietetic Products, Nutrition and Allergies (NDA), *A. melanocarpa* possesses antioxidant properties and is a source of anthocyanins and polyphenols with antioxidant activity. It states that *A. melanocarpa* "helps to protect cells from the free-radical damage and oxidative stress" (EFSA 2011).

In food industry and cuisine, fresh aronia fruits are used to prepare jams, marmalades, jellies, juices, infusions, and wines. On the other hand, chokeberry juice is used to colorating other products as a very intense natural colorant. Aronia fruit juice is a much richer source of colorants than grapes and black currant fruits (Jeppsson 1999; Wawer et al. 2012).

A new interesting proposal is to use not only aronia juice as a source of colorants for food industry, but also aronia juice pomace, the by-product in juice processing. Total anthocyanin levels in the pomace is affected mostly by enzyme treatment followed by maceration temperature (2 °C and 50 °C were tested). Cold maceration of frozen berries without enzyme addition yielded the highest concentrations of pigments in the pomace (Kitrytė et al. 2017).

Currently, also the possibility of black chokeberry pomace uses as a source of food ingredients, not only as a source of colorants, is intensively explored. With the use of commercially available cellulolytic and xylanolytic enzyme preparation, the cell walls in the pomace are broken down resulting in an increase in the yield of water

Table 4.3 Chosen diet supplements based on *A. melanocarpa,* and their description in accordance with the data provided by the manufacturers (according to web sites of producers and online stores)

Manufacturer and country of production	Trade name and form	Composition	Activity profile recommended by manufacturer
Pharmovit, Poland	Aronia Gold (capsules)	Black chokeberry extract (*A. melanocarpa*) standardized for 25% anthocyanins	Antioxidant
Herbapol Poznań, Poland	Aronia żel active 100 g (food gel)	Water extract composed of cistus, lemon verbena and lemongrass, chokeberry juice, gelling agent: xanthan gum; zinc gluconate, stevia extract	Support the body's immunity and vitality

(continued)

Table 4.3 (continued)

Manufacturer and country of production	Trade name and form	Composition	Activity profile recommended by manufacturer
Herbapol Kraków, Poland	Aronia (capsules)	Aronia fruit powder (194 mg/caps.), potassium chloride (130 mg/capsule) (equivalent to 68 mg of potassium)	The preparation contains powdered chokeberry fruit enriched with potassium chloride. Potassium is an element that helps in maintaining proper blood pressure and in the proper functioning of muscles and the proper functioning of the nervous system
Pure nature, Poland	Aronia berry—black chokeberry—Superfood (capsules)	*A. melanocarpa* fruit, microcrystalline cellulose, magnesium stearate	Support for immune system, antioxidant
URSAPHARM Arzneimittel GmbH, Germany	Aronia + Immun (drinking ampoules)	Aronia juice (from 87.3% concentrate), sucrose, aronia juice concentrate (2.7%), acid stabilizers (citric acid), preservatives (sodium benzoate, potassium sorbitate), zinc gluconate, natural flavor, niacin, pantothenic acid, vitamin B_6, vitamin B_2, sodium selenate, vitamin D_3.	Allow to maintain proper, high resistance to diseases and colds
Aronia ORIGINAL, Germany	Aronia Original: Zellschutzkapseln Bio Aronia + Acerola (capsules)	76% powder from pressed, dried aronia berries, 6.4% acerola extract, coating agent (capsule shell): hydroxypropylmethylcellulose, separating agent: talc	Protects cells from oxidative stress, source of vitamin C
Sanoctua GmbH & Co. KG, Germany	BASIS 7 GRÄSLER PLUS (drinking ampoules)	Water, extract from multifloral mountain blossom pollen, bee honey, orange juice concentrate, wheat germ extract, royal jelly, barley malt extract, acidifier citric acid, aronia juice concentrate, mountain ash fruit juice concentrate, blueberry juice concentrate, mixed juice, rose fruit concentrate, concentrated elderberry juice preservative potassium sorbate, vitamin B_1, vitamin B_2, vitamin B_6, vitamin B_{12}, niacin NE, pantothenic acid, folic acid, biotin, vitamin C, L-carnitine	Help to restore the physicological balance

(continued)

Table 4.3 (continued)

Manufacturer and country of production	Trade name and form	Composition	Activity profile recommended by manufacturer
Aronia-Swiss, Switzerland	Aronia Kapseln (capsules)	Capsules with aronia pomace (the residue that remains after pressing or juice production)	High concentration of antioxidant ingredients
Vegetal Progress, Italy	Vedyben® (capsules)	Aronia (*A. melanocarpa* L., EU origin) concentrated berry micronized powder, elderberry (*Sambucus nigra* L., EU origin) berry concentrated juice dehydrated micronized powder, blueberry (*Vaccinium myrtillus* L., EU origin) berry concentrated juice dehydrated micronized powder, currant black (*Ribes nigrum* L., EU and/or Asian origin) berry juice concentrate dehydrated micronized powder, aronia (*A. melanocarpa* L., EU origin) berry juice concentrated dehydrated micronized powder, maize maltodextrin, calcium carbonate	High content of polyphenols, anthocyanins, and vitamin C. Valuable aid to support the physiological functionality of the microcirculation and sight
Liktravy, Ukraine	*A. melanocarpa* fructus herbal tea, Ashberry blackheaded fruits (tea)	Ashberry blackheaded fruits	In complex therapy with hypo- and avitaminosis, hemorrhagic diathesis, bleeding of different origins, initial stages of arterial hypertension, thyrotoxicosis, atherosclerosis
L'ACTONE, Turkey	Life Besleyici Set Shake Karışım Thermo Çay Shaker, Aronia thermo (powder)	Powered aronia fruits	Activates the metabolism and supports fat burning
Eclectic Institute, USA	Aronia berry freeze-dried, 450 mg, 90 VegCap, great antioxidant (capsules)	Organic freeze-dried aronia berries	Antioxidant
Eclectic, USA	Eclectic Aronia Cog O, red, 1 fluid ounce (drops)	Organic freeze-dried aronia berry (*A. melanocarpa*), organic grain-free alcohol, filtered water. Dry herb strength 1:4 (250 mg/ml)	Antioxidant

(continued)

Table 4.3 (continued)

Manufacturer and country of production	Trade name and form	Composition	Activity profile recommended by manufacturer
Brownwood Acres Foods, USA	Pure aronia berry juice concentrate (juice)	*A. melanocarpa* fruit juice	Antioxidant
Swanson, USA	Full spectrum aronia (chokeberry)	Black chokeberry fruit (*A. melanocarpa*) 400 mg	Antioxidant
Nutridom, Canada	Nutridom Aronia 2000 300 Vcaps—powdered fruit of *A. melanocarpa* (capsules)	*A. melanocarpa* (Black chokeberry) 500 mg (4:1 concentrated, equivalent to 2000 mg)	Provides antioxidants
Natural One, Canada	Aronia extract 4:1 (capsules)	*A. melanocarpa* 500 mg Extract 4:1 DHE: 2000 mg dry	Provides antioxidants
GNM dignity of Nature, South Korea	GNM pure aronia/aronia juice/aronia extract/fruit juice (powder)	Aronia concentrate mix 100% (aronia, apple, grape, cranberries)	Good at antioxidative effect

soluble fractions (max. 113%), monosaccharide content (max. 140%), total phenolic content (max. 41%), and radical scavenging capacity (max. 39%). Solid residues from the enzyme-treated berry pomace possess also high antioxidant potential. These investigations indicated that all fractions after juice pressing could be utilized as a low-cost source of highly valuable functional food ingredients (Vagiri and Jensen 2017).

4.10 Cosmetic Applications

Constituents of *A. melanocarpa* fruit are decisive for their growing popularity in the manufacture of cosmetics.

In the European Cosmetic Ingredients (CosIng) database developed by the European Commission, as many as five forms of aronia have been authorized for use in cosmetic production, namely fruits extract, juice, fruit and leaf extracts, filtrates of products obtained by fermentation with *Acetobacter* and *Saccharomyces*. It is noteworthy that callus cultures can be used for the production of cosmetics (Table 4.4) (CosIng 2020).

Some cosmetic preparations are based on oil derived from the seeds; they have not yet been listed in the CosIng database. It is a rich source of essential unsaturated fatty acids. This oil is well absorbed through the skin and prevents comedone formation by suppressing the blockade of the sebaceous gland ducts. The oil is used in cosmetics as an emollient, i.e., a product moisturizing and strengthening the lipid barrier of the skin and inhibiting the transepidermal water loss (TEWL). It is also used in

Table 4.4 *A. melanocarpa* in cosmetic products according to CosIng

Form	Activity
Aronia melanocarpa fruit extract	Skin conditioning
Aronia melanocarpa fruit juice	Skin conditioning
Aronia melanocarpa fruit/leaf extract	Skin conditioning
Aronia melanocarpa callus extract	Antioxidant Hair conditioning Skin protecting
Acetobacter/Saccharomyces/(Aronia melanocarpa/Pyrus serotina) fruit juice extract ferment filtrate *(Acetobacter/Saccharomyces/(Aronia melanocarpa/Pyrus serotina)* fruit juice extract ferment filtrate is a filtrate of the product obtained by the fermentation of the extract of the juice obtained from the fruit of *Aronia melanocarpa* and *Pyrus serotina* by the microorganisms *Acetobacter* and *Saccharomyces)*	Antioxidant

medicine because it accelerates wound and burn healing and inhibits degenerative changes (BIOnly 2020).

The characteristics of chokeberry-derived raw materials particularly useful in cosmetics include their antioxidant actions and the ability to strengthen the vascular wall and to reduce erythematous changes. Anthocyanins are decisive for their UV protection property and anti-photoaging activity. Antibacterial actions are used in the treatment of acne lesions. Vitamin C and zinc contribute to strengthening the skin. Aronia preparations also reduce the sebaceous gland activity (BIOnly 2020).

Table 4.5 presents examples of the cosmetic products manufactured in different countries. In Europe, the main manufacturers are based in Poland, Austria, Germany, Switzerland, Italy, and Turkey. In the global market, the products of manufacturers from the USA and Korea also appear and can be bought via the internet.

4.11 Biotechnological Studies

4.11.1 Micropropagation

Micropropagation studies have been carried out by agricultural and horticultural research institutions. *A. melanocarpa* micropropagation protocols were developed, indicating that the standard Murashige and Skoog (MS) medium supplemented with indolebutyric acid (IBA), 6-benzylaminopurine (BAP), and gibberellic acid (GA_3) can create the conditions stimulating the development of microseedling (Brand and Cullina 1992; Murashige and Skoog 1962; Petrovic and Jacimovic-Plavšic 1992; Ruzic 1993).

Table 4.5 Chosen cosmetics based on *A. melanocarpa,* and their description in accordance with the data provided by the manufacturers (according to web sites of producers and online stores)

Manufacturer and country of production	Trade name and form	Used form of *A. melanocarpa*	Activity profile recommended by manufacturer
Produkty Naturalne Berezińscy Sp. Jawna, Poland	Naturalis Beauty Aronia—Łagodzące mleczko do twarzy (face milk)	*A. melanocarpa* anthocyanins	Aronia anthocyanins—natural strong antioxidants, strengthen capillary walls and reduce swelling
AJEDEN Sp. z o.o., Poland	Olej z pestek aronii (*A. melanocarpa* seed oil)	Cold-pressed chokeberry seed oil	For sensitive skin with a tendency to rosacea, vascular, mature, mixed skin. Soothing, healing, anti-inflammatory, and regenerative properties protect the skin from exposure to free radicals and antioxidant properties. Restores the hydrolipidic balance of the skin, protects it from dehydration
Aronialand, Austria	Chokeberry eye balm	*A. melanocarpa* fruit powder	Daily care for the eyes, contains numerous vitamins and minerals
Gerlinde Hofer—Florex GmbH, Austria	Flüssige Schafmilchseife Aronia (liquid soap)	*A. melanocarpa* fruit extract	–
Original Florex®, Austria	Aronia Badesalz (bath salt)	–	–
RAUSCH Ges.mbH, Austria	Aronia anti-grau intensiv-fluid (hair fluid)	–	Promotes melanin production, thus preserving and reactivating the natural hair color
Dr. Eckstein, Germany	Ultimate supreme aronia concentrate	*A. melanocarpa* fruit extract	Reduces and protects the visible signs of age, protects against the visible consequences of stress and negative environmental influences, supports the natural functions of the skin, supports the skin's own regeneration, especially after the sun

(continued)

Table 4.5 (continued)

Manufacturer and country of production	Trade name and form	Used form of *A. melanocarpa*	Activity profile recommended by manufacturer
Bioksama, Switzerland	Extra-rich body cream without palmoil, organic edelweiss, organic chokeberry (body cream)	*A. melanocarpa* fruit extract	–
Alma Briosa, Italy	Filler Riempirughe contorno occhi e labbra (eye and lip contour filler)	Aronia fruit extract	Antioxidant Moisturizer
MOR—Miracle Optimum Result, Turkey	MOR Aronia Özlü El Kremi (hand cream)	*A. melanocarpa* extract	Antioxidant
Farmhouse Fresh®, USA	Vitamin berry facial tonic—Instant pore-refining & replenishing facial toner	*A. melanocarpa* fruit juice	Antioxidant
Bianca Rosa, USA	Black chokeberry cream	*A. melanocarpa* fruit	–
ReinPlatz, South Korea	Hydro aid moisturizing aronia berry essence facial sheet mask	*A. melanocarpa* fruit extract rich in anthocyanins	Soothing and moisturizes antioxidant

4.11.2 Endogenous Production of Phenolic Acids in Various Types of in vitro Cultures

Studies on the biosynthetic potential of *A. melanocarpa* cells in in vitro cultures have been conducted very intensively since 2011 by our team representing the Chair and Department of Pharmaceutical Botany, Jagiellonian University, Medical College, Kraków (Poland). These studies were focused on the optimization of in vitro culture conditions favoring the accumulation of one group of antioxidants, namely phenolic acids. The optimization involved testing the basal media—Linsmaier and Skoog—LS (Linsmaier and Skoog 1965), and Murashige and Skoog—MS (Murashige and Skoog 1962), concentrations of PGRs (NAA and BAP), supplementation of biosynthetic precursors, light conditions (monochromatic lights, white light, darkness and UV-A irradiation), type of culture—agar callus culture, agar, agitated, and bioreactor shoot culture of aronia (Fig. 4.4) (Szopa et al. 2013, 2018a, 2020; Szopa and Ekiert 2014). A separate research direction regarding biotransformation potential of cells cultured in vitro concentrated on β-D-glucosylation of hydroquinone into arbutin (Kwiecień

et al. 2013). The phenolic acids and arbutin contents were estimated by DAD-HPLC methods.

4.11.2.1 The Biosynthetic Potential of Agar Callus Cultures

Testing of LS media variants

Callus cultures (Fig. 4.4) maintained on five different variants of agar LS medium containing NAA and BAP as growth regulators (in the concentration range 0.1–3 mg/l; NAA/BAP [mg/l]: 0.1/0.1, 0.5/1.0, 1.0/1.0, 2.0/2.0, 1.0/3.0) was shown to be capable of producing 5 (of 19 tested) phenolic acids—caffeic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, syringic acid, and vanillic acid. Their contents varied depending on PGR concentrations. On the LS medium variants with 1:1 auxin/cytokinin ratio, the patterns of phenolic acids were very similar, just as on the LS media with 1:2 and 1:3 PGRs ratio, where the pattern of the estimated compounds was also similar. Syringic acid was the dominant compound with maximum amounts of 46.26 and 41.20 mg/100 g DW. p-Hydroxybenzoic acid contents were substantial and ranged from 17.41 to 25.60 mg/100 g DW. The maximum contents of the remaining compounds were as follows: 7.31 mg/100 g DW (caffeic acid), 6.65 mg/100 g DW (vanillic acid), and ca. 12 mg/100 g DW (*p*-coumaric acid). The

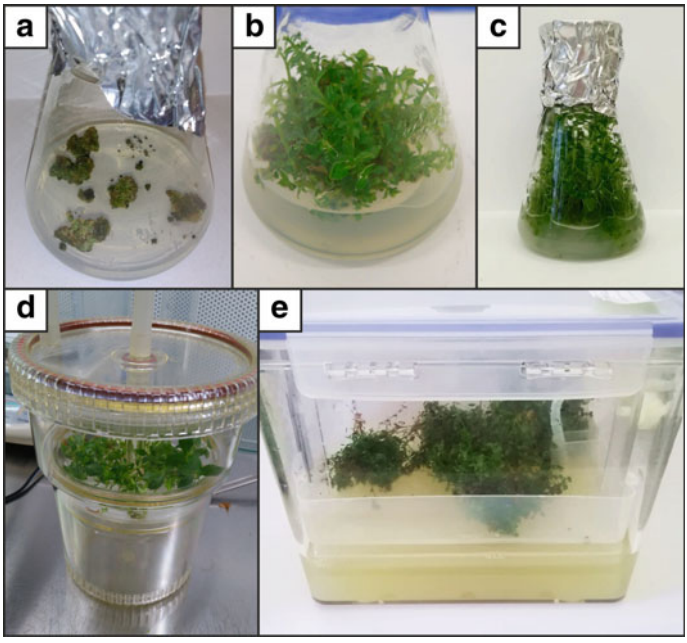


Fig. 4.4 *A. melanocarpa;* agar callus cultures (**a**), agar shoot cultures (**b**), agitated shoot cultures (**c**), and shoot cultures in RITA (**d**) and PlantForm (**e**) bioreactors

Table 4.6 Best media variants stimulating the biomass growth and phenolic acids production in *A. melanocarpa* in vitro cultures

Culture type	Medium	BAP/NAA [mg/l]	Total content of phenolic acids (mg/100 g DW)	Biomass increment
Agar callus culture	LS	1/0.5 and 3/1	78.82 and 81.56	3.5
Agar shoot culture	MS	0.5/2	83.84	5.0
	LS	1/0.5 and 1/1	150.95 and 145.49	15.3 and 10.0
Agitated shoot culture	MS	2/2 and 2/0.5	206.19 and 217.50	17.2 and 15.0
	LS	3/1	130.44	2.6
	MS	2/2	324.78	3.5

total content of the tested compounds was consistently dependent on LS medium variant and ranged from 50.23 to 81.56 mg/100 g DW. Biomass increments in 4-week growth cycles were relatively low in this type of culture (from 2.0- to 4.8-fold) (Szopa et al. 2013).

Two LS medium variants, containing 0.5 mg/l NAA + 1 mg/l BAP and 1 mg/l NAA + 3 mg/l BAP were selected as universal both "productive" and "growth-promoting" media (total phenolic acids was of about 80 mg/100 g DW and biomass increments were over 3.5-fold) (Table 4.6) (Szopa et al. 2013).

Testing of MS media variants

Callus cultures carried out on seven different variants of agar MS medium containing NAA and BAP in the concentration range 0.1–3 mg/l, BAP/NAA [mg/l]: 0.1/2.0, 0.5/2.0, 1.0/0.5, 2.0/0.5, 2.0/1.0, 2.0/2.0, 3.0/1.0 were demonstrated to be capable to producing the same five phenolic acids as in the case of LS media. Their contents were very diverse, differing by 1.8–4.0 times depending on PGRs concentration. On MS media with 2:1, 3:1 and 4:1 cytokinin/auxin ratio, the pattern of phenolic acids was similar. On MS media with 1:4 and 1:20 cytokinin/auxin ratio, analogous metabolite patterns were also obtained.

Syringic acid (max. 40.16 mg/100 g DW) and *p*-hydroxybenzoic acid (max. 23.59 mg/100 g DW) were the dominant metabolites. The contents of the remaining three phenolic acids did not exceed 11 mg/100 g DW. The total content of phenolic acids varied 1.78-fold from about 47 to 84 mg/100 g DW depending on the PGR concentration. Biomass increments during a 4-week growth cycle were relatively low and diverse (3.3–5.0-fold) (Szopa and Ekiert 2014).

The MS medium variant containing 0.5 mg/l BAP and 2 mg/l NAA was proposed as a "universal" medium (total content of phenolic acids was over 80 mg/100 g DW and the increase in biomass was one of the highest) (Table 4.6) (Szopa and Ekiert 2014).

In the callus cultures of aronia, grown on LS and MS tested media variants, predominant biosynthetic pathways were those of the benzoic acid derivatives. Syringic acid (dimethoxy-derivative of benzoic acid) and *p*-hydroxybenzoic acid were the metabolites accumulated in the greatest quantities (Szopa and Ekiert 2014).

The main compounds in the fruit extracts included salicylic acid (15.6 mg/100 g DW) and *p*-hydroxybenzoic acid—15.29 mg/100 g DW. Other four—caffeic, *p*-coumaric, syringic, and vanillic acids were present in quantities below 4.2 mg/100 g DW. The maximum total amounts of phenolic acids in callus cultures on the tested LS and MS medium variants were 2.51-times and 2.59-times higher than in the fruit extracts (32.43 mg/100 g DW), respectively (Szopa and Ekiert 2014).

The total amounts of phenolic acids in callus cultures cultivated on two LS and MS media variants with the same PGRs were almost the same. Only on one variant, namely that containing 3 mg/l BAP and 1 mg/l NAA, the total content was 1.22-times higher on MS basal medium than on LS basal medium (Szopa and Ekiert 2014).

4.11.2.2 The Biosynthetic Potential of Agar Shoot Cultures

Testing of LS medium variants

The production of phenolic acids was investigated in *A. melanocarpa* shoot cultures (Fig. 4.4) maintained on five agar variants of LS medium enriched in PGRs—NAA and BAP (in the concentration range 0.1–3.0 mg/l). The tested LS medium variants were identical as in the case of agar callus cultures. HPLC analysis was used to analyze 19 phenolic acids and cinnamic acid.

Methanolic extracts of biomass harvested after 4-week growth cycles were shown to contain five compounds: caffeic acid, *p*-hydroxybenzoic acid, syringic acid, vanillic acid, and salicylic acid. Salicylic acid (max. 78.25 mg/100 g DW) and *p*-hydroxybenzoic acid (max. 55.14 mg/100 g DW) were the main metabolites accumulated in the shoots (Szopa et al. 2013, 2018a).

The total content of the tested compounds ranged from 105.05 to 150.96 mg/100 g DW and was evidently dependent on the concentrations of PGRs in the LS media (Szopa et al. 2013, 2018a).

Biomass increments on the tested LS medium variants during 4-week growth cycles were very good (from 8.2- to 15.3-fold) (Szopa et al. 2013, 2018a).

Two LS medium variants were proposed as "universal" media, namely that containing 0.5 mg/l NAA and 1 mg/l BAP, and the second one supplemented with 1 mg/l NAA and 1 mg/l BAP (Table 4.6) (Szopa et al. 2013, 2018a).

Testing of MS medium variants

Shoot cultures of *A. melanocarpa* carried out on seven variants of MS medium containing NAA and BAP (0.1–3.0 mg/l), identical as in the case of agar callus cultures were demonstrated to produce 6 of 20 tested compounds. They included caffeic acid, *p*-coumaric acid, *p*-hydroxybenzoic acid, syringic acid, vanillic acid, and salicylic acid. Salicylic acid (max. 91.86 mg/100 g DW), *p*-coumaric acid (max.

62.39 mg/100 g DW), and *p*-hydroxybenzoic acid (max. 50.66 mg/100 g DW) were the quantitatively dominant metabolites. The amounts of individual compounds and consequently the total amounts of phenolic acids depended on the PGR concentrations in MS medium variants. The total amounts reached from 93.52 to 217.50 mg/100 g DW (Szopa et al. 2018a; Szopa and Ekiert 2014).

Increments of shoot biomass grown on the tested MS medium variants during 4-week growth cycles were very high (from 11.4- to 17.2-fold). Two variants were proposed as "universal" media—these supplemented with 2 mg/l NAA + 2 mg/l BAP and 2 mg/l NAA + 0.5 mg/l BAP (Table 4.6) (Szopa et al. 2018a; Szopa and Ekiert 2014).

The maximum total amounts of phenolic acids accumulated on the tested LS and MS medium variants were ca. 4.65-times and 6.69-times higher than in the fruit extracts (32.43 mg/100 g DW), respectively (Szopa et al. 2018a; Szopa and Ekiert 2014).

Three variants of LS and MS medium contain identical concentrations of PGRs. The obtained comparative results show that 1.6–2.0-times higher total amounts of phenolic acids can be obtained on MS medium variants. MS medium variants richer in various vitamins—co-enzymes of different enzymes can stimulate different reactions in the shoots grown in vitro (Szopa et al. 2018a; Szopa and Ekiert 2014).

The biosynthetic potential under different light conditions

Stationary shoot cultures were maintained on the MS medium variant with 1 mg/l NAA and 1 mg/l BAP in the presence of monochromatic light (far-red, red, blue lights, UV-A irradiation) in darkness and under multispectral white light during 4-week cycles (Szopa et al. 2018a, b).

In biomass extracts, the presence of four compounds (out of 20 analyzed) was confirmed, namely three depsides—chlorogenic, neochlorogenic and rosmarinic acids, and also protocatechuic acid. The total amounts of phenolic acids changed 3.2-fold, from 167.14 mg/100 g DW (UV-A irradiation) to 527.40 mg/100 g DW (blue light). Under control conditions (white light), the total content reached 339.45 mg/100 g DW while in the darkness it amounted to 289.64 mg/100 g DW. The total contents under red and far-red lights were similar—293.27 and 256.00 mg/100 g DW, respectively (Szopa et al. 2018a, b).

During 4-week growth cycles, biomass increased from 3.78-fold (darkness) to 8.06-fold (blue light), and in the control culture (white light)—4.77-fold (Szopa et al. 2018a, b).

The experiment evidenced the stimulating effect of blue light both on biomass growth and accumulation of phenolic acids. Under blue light, the total content of phenolic acids was 1.58-fold higher than under white light. The maximum content was ca. 16.3-fold higher than in the fruit extract (32.43 mg/100 g DW) (Szopa et al. 2018a, b).

4.11.2.3 The Biosynthetic Potential of Agitated Shoot Cultures

Testing of LS medium variants

Agitated cultures (Fig. 4.4) were maintained on three variants of LS medium (NAA/BAP [mg/l]: 2/0.5, 2/2, 1/3) during 4-week growth cycles (Szopa et al. 2015, 2018a).

Shoot extracts were shown to contain four phenolic acids—three depsides (rosmarinic, neochlorogenic, and chlorogenic acids) and also protocatechuic acid. Rosmarinic acid (max. 53.40 mg/100 g DW) was the main metabolite. The maximum amounts of two other depsides were also high (35.18 and 41.58 mg/100 g DW, respectively). The total amounts on three tested LS variants varied from 67.22 to 130.44 mg/100 g DW. The maximum amount was 4.02-times higher than the content in the fruit extract (32.43 mg/100 g DW) (Szopa et al. 2015, 2018a). In analyzed plant media, trace amounts of phenolic acids were confirmed.

The biomass increments were low and ranged from 2.33 to 2.55-fold. The LS variant containing 1 mg/l NAA and 3 mg/l BAP was proposed as "universal" medium, stimulating the growth and production of phenolic acids (Table 4.6) (Szopa et al. 2015, 2018a).

Testing of MS medium variants

Agitated cultures were maintained on three variants of MS medium supplemented with the identical concentrations of PGRs as those added of LS medium for agitated shoot cultures. The tested shoot extracts were evidenced to contain four compounds, the same as those accumulated on LS medium variants, namely three depsides and protocatechuic acid. Depsides including rosmarinic acid, chlorogenic acid, and neochlorogenic acid were accumulated in high amounts (max. 134.24, 105.45, and 82.00 mg/100 g DW, respectively) (Szopa et al. 2015, 2018a).

The total contents of phenolic acids synthesized on the tested MS medium variants ranged from 189.02 to 324.78 mg/100 g DW depending on PGR concentrations and were decidedly higher than those obtained on LS medium variants. The maximum amount was 10.1-times higher than in the fruit extract (Szopa et al. 2015, 2018a). In analyzed plant media, only trace amounts of phenolic acids were confirmed.

The biomass increments were relatively low but slightly higher than on identical LS medium variants and varied between 2.61- and 3.52-fold. The MS medium variant containing 2 mg/l NAA and 2 mg/l BAP was proposed as a "universal" medium (Table 4.6) (Szopa et al. 2015, 2018a).

Agitated cultures maintained with the addition of biosynthetic precursors

Agitated cultures of *A. melanocarpa* were maintained on MS medium with the addition of 1 mg/l NAA and 1 mg/l BAP for 20 days. Biosynthetic precursors of phenolic acids—phenylalanine, cinnamic acid, and benzoic acid at five concentrations 0.1, 0.5, 1.0, 5.0, and 10.0 mmol/l were added to culture flasks at the time "0" (at culture initiation) and on day 10th of culture. Moreover, the caffeic acid at the above-mentioned

five concentrations was used as a precursor in order to stimulate the production of depsides (Szopa et al. 2018a, 2020).

Out of the 26 tested phenolic acids (the analysis was extended by seven compounds, for which standards were available for purchase) assayed in the extracts from biomass harvested after 20-day growth cycles, seven compounds were identified—five depsides: neochlorogenic, chlorogenic, cryptochlorogenic, isochlorogenic, and rosmarinic acids, as well as caffeic and syringic acids (Szopa et al. 2018a, 2020).

In control cultures maintained without precursors, the total content of phenolic acids amounted to 290.10 mg/100 g DW. Precursor supplementation distinctly stimulated biosynthesis and accumulation of the tested compounds. The maximum total contents of phenolic acids after the addition of each of the precursors estimated at time "0" were as follows: 481.06 mg/100 g DW (phenylalanine—5 mmol/l), 543.35 mg/100 g DW (cinnamic acid—0.5 mmol/l), 439.43 mg/100 g DW (benzoic acid—1 mmol/l), and 660.63 mg/100 g DW (caffeic acid—1 mmol/l). The maximum total contents of phenolic acids after precursor administration assessed on the 10th day of culture were higher and reached: 592.27 mg/100 g DW (phenylalanine—0.1 mmol/l), 989.79 mg/100 g DW (cinnamic acid—5 mmol/l), 503.02 mg/100 g DW (benzoic acid—1 mmol/l), and 854.99 mg/100 g DW (caffeic acid—5 mmol/l), respectively (Table 4.7) (Szopa et al. 2018a, 2020). In all extracts from biomass growing with precursors, three depsides were the main metabolites, namely neochlorogenic acid (max. 127.00–163.97 mg/100 g DW), chlorogenic acid (max. 119.08–450.35 mg/100 g DW), and isochlorogenic acid (max. 168.23–249.88 mg/100 g DW) (Szopa et al. 2018a, 2020).

Cinnamic acid and caffeic acid supplementation at a concentration of 5 mmol/l on the 10th day of culture was the most efficient in stimulating the biosynthesis and accumulation of phenolic acids. Precursor supplementation raised phenolic acid contents by 3.41- and 2.95-fold, respectively, compared with precursor-free cultures. The obtained results have a potentially applicable nature (Szopa et al. 2018a, 2020).

In general, the addition of precursors did not suppress biomass growth. Only higher concentrations of cinnamic acid and benzoic acid (5 and 10 mmol/l) and

Table 4.7 Maximal total contents [mg/100 g DW] (MTC), precursor concentration [mmol/l] (PC) and increase versus control[a] (IC) of phenolic acids produced in the agitated shoot cultures of *A. melanocarpa* after feeding with the tested precursors

Precursor	Point "0"			10th day		
	MTC	PC	IC	MTC	PC	IC
Phenylalanine	481.06	5	1.66	592.27	0.1	2.04
Cinnamic acid	543.35	0.5	1.87	989.79	5	3.41
Benzoic acid	439.43	1	1.51	503.02	1	1.73
Caffeic acid	660.63	1	2.28	854.99	5	2.95

[a]Control culture—290.10 mg/100 g DW

the highest concentration of phenylalanine (10 mmol/l) added at time "0" reduced biomass increments (Szopa et al. 2018a, 2020).

Dynamics of accumulation of phenolic acids in agitated cultures—preliminary results

Agitated *A. melanocarpa* shoot cultures were maintained on MS medium enriched in 1 mg/l NAA and 1 mg/l BAP for 8 weeks (3 series). Biomass was harvested at 7-day intervals for determination of phenolic acid contents. Out of 26 assayed compounds, methanolic extracts were confirmed to contain 11 metabolites: 3-phenylacetic acid, 3,4-dihydroxyphenylacetic acid, syringic acid, caftaric acid, protocatechuic acid, caffeic acid and depsides—chlorogenic, cryptochlorogenic, neochlorogenic, isochlorogenic acids and rosmarinic acid (Kubica et al. 2019b).

The total content of phenolic acids, after an initial decrease (2nd week) gradually rose through 1128.25 mg/100 g DW (third week of the growth cycle) to reach the maximum value of 1237.62 mg/100 g DW in the 5th week. Beginning from the sixth week, the contents of the compounds under study drastically dropped to 710.84 mg/100 g DW in the sixth week, 383.02 mg/100 g DW in the 7th week and 75.15 mg/100 g DW in the eighth week of culture (Fig. 4.5) (Kubica et al. 2019b).

The dominant compounds quantified in the biomass included: 3-phenylacetic acid (max. 424.52 mg/100 g DW—third week), 3,4-dihydroxyphenylacetic acid (317.76 mg/100 g DW—fifth week), isochlorogenic acid (380.01 mg/100 g DW—fourth week), and cryptochlorogenic acid (228.73 mg/100 g DW—fourth week) (Kubica

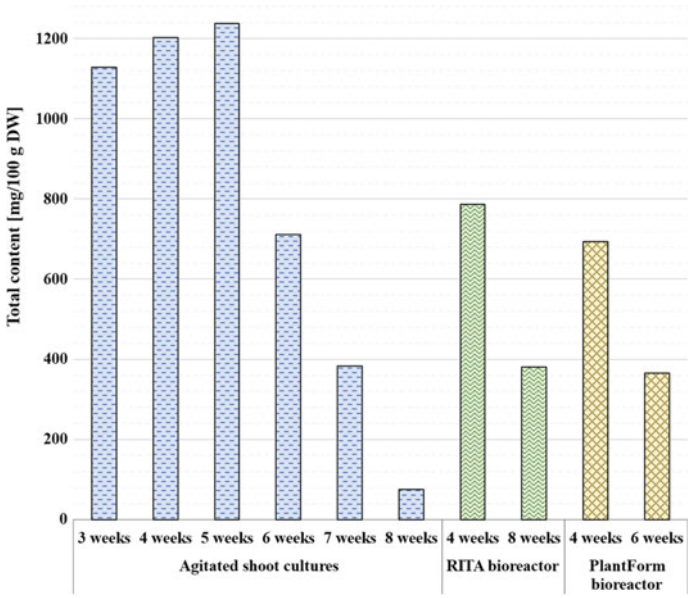


Fig. 4.5 Dynamic of phenolic acid accumulation in *A. melanocarpa* agitated and bioreactor in vitro cultures during the tested growth cycles

et al. 2019b). In analyzed plant media only trace amounts of phenolic acids were confirmed.

Dry shoot biomass increments (determined also at 7-day intervals) gradually increased by 4.27-fold (second week) and 6.59-fold (third week) to reach the maximum value of 9.08-fold (4th week), 8.31 (fifth week) and then steadily decreased to 6.84 (sixth week) and 5.44 (eighth week) (Kubica et al. 2019b).

The highest contents of phenolic acids were obtained during the stationary phase of the culture growth.

The obtained results are potentially applicable. They indicate that the maximum contents of the studied compounds are accumulated after 5-week culture growth cycles. However, reproducibility of these data is needed to be checked (Kubica et al. 2019b).

4.11.2.4 Shoot Cultures in Bioreactors—Preliminary Results

A. melanocarpa shoot cultures were carried out in two types of commercially available bioreactors—RITA (VITROPIC S.A., France) and PlantForm (Plant Form AB, Sweden) (Fig. 4.4), operating as temporary immersion systems, on MS medium containing 1 mg/l NAA and 1 mg/l BAP for 8 weeks (RITA) or 6 weeks (PlantForm). Biomass was harvested at 2 time points: after 4 weeks from both bioreactors, and after 8 weeks and 6 weeks, respectively (Kubica et al. 2019a, 2020).

HPLC analysis of methanolic extracts from the biomass growing in both types of bioreactors confirmed the presence of 11 metabolites of 26 tested compounds. They were the same compounds as those identified in the agitated cultures maintained with the aim to investigate the dynamics of phenolic acid accumulation (Kubica et al. 2019a, 2020).

In the RITA bioreactors, the total content of phenolic acids in extracts from the biomass growing for 4 weeks (786.88 mg/100 g DW) was over twice as high as their amount after 8-week growth cycles (380.66 mg/100 g DW) (Fig. 4.5) (Kubica et al. 2019a, 2020).

Three compounds were identified as the dominant metabolites, namely: isochlorogenic acid (max. 236.16 mg/100 g DW), cryptochlorogenic acid (max. 153.96 mg/100 g DW), and 3,4-dihydroxyphenylacetic acid (max. 151.80 mg/100 g DW). The maximum contents of the mentioned compounds were obtained after 4-week growth cycles (Kubica et al. 2019a, 2020). In analyzed plant media, no significant amounts of phenolic acids were confirmed. Dry biomass increments obtained during 4-week culture cycles were high (7.6-fold). The increments after 8-week cycle were lower but satisfactory (4.5-fold) (Kubica et al. 2019a, 2020).

These preliminary results indicate that it will be necessary to conduct quantitative analyses at other time points, in particular after 5-, 6-, and 7-week culture cycles (Kubica et al. 2019a, 2020). It would be also recommended to examine reproducibility of the data obtained so far. These studies are planned to be performed in the near term.

In the PlantForm bioreactor, the total content of phenolic acids in the extracts from biomass after 4-week culture cycles was almost double (693.29 mg/100 g DW) the content obtained after 6 weeks of culture (365.11 mg/100 g DW) (Fig. 4.5) (Kubica et al. 2019a, 2020).

The main compounds accumulated in shoots included: 3-phenylacetic acid (max. 374.87 mg/100 g DW), isochlorogenic acid (max. 77.84 mg/100 g DW), and dihydroxyphenylacetic acid (max. 58.64 mg/100 g DW). These maximum contents of the above-mentioned phenolic acids were documented after 4-week culture cycles. With regard to dry shoot biomass increments, the biomass increased over 5-fold (5.2-fold) after 4-week culture cycles; hence, the result was satisfactory. Extension of the culture cycle till 6 weeks resulted in a distinct reduction of biomass increments (3.3-fold biomass growth) (Kubica et al. 2019a, 2020).

To sum up, it appears that also in this type of bioreactor cultures, quantitative analyses will need to be performed after 5 weeks and reproducibility of study results should be checked, as well (Kubica et al. 2019a, 2020).

4.11.3 Biotransformation Potential of Cells in Agitated Shoot Cultures

A. melanocarpa agitated shoot cultures were investigated for the ability to transform exogenous hydroquinone into its β-D-glucoside, arbutin (Fig. 4.6). Arbutin has an important position both in medicine (as a urinary tract disinfectant) and in cosmetology (as a safe skin-lightening agent). In vitro cultures of numerous plant species representing very different taxa are able to produce arbutin via biotransformation by β-D-glucosylation of exogenous hydroquinone (Fig. 4.6). It is due to a common occurrence of enzymes belonging to β-glucosidases in the plant kingdom and the lack of substrate specificity of these enzymes.

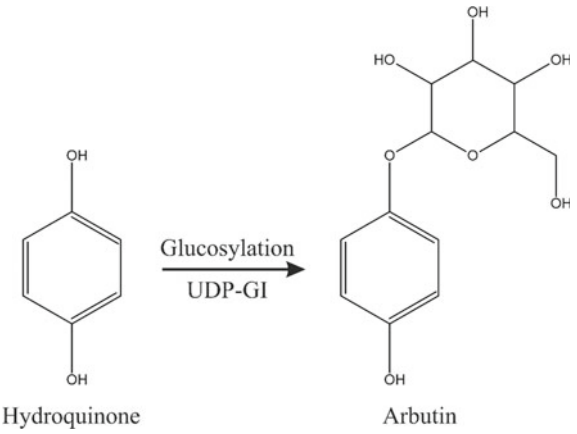


Fig. 4.6 Biotransformation of hydroquinone into arbutin

Cultures were maintained on MS medium supplemented with 2 mg/l BAP and 2 mg/l NAA. After a 2-week culture growth period, hydroquinone was added to culture flasks at different doses (100–400 mg/l) either as a single dose or divided into 2 or 3 doses given at 24-hour intervals. The contents of reaction products were determined by HPLC in methanolic extracts from biomass and in lyophilized media harvested at 24 h after the last precursor dose (Kwiecień et al. 2013).

The cells from in vitro cultures exhibited the ability to transform hydroquinone into arbutin. The product was accumulated mainly in the cultured biomass (72.09–93.42%) and was released to the medium at lower quantities. The total contents of the product were very diverse varying from 2.71 to 8.27 g%. Arbutin production gradually rose with increasing concentration of hydroquinone. The maximum contents of arbutin were obtained at the maximum concentration of hydroquinone (400 mg/l) and amounted to 7.94 g% (single dose), 8.27 g% (2 doses), and 7.76 g% (3 doses). The analyzed media contained arbutin in amounts ranging from 6.58% (150 mg, 3 doses) to 30.04% (400 mg, 2 doses) of the total content of this compound in biomass and medium (17.24 and 150.32 mg/l, respectively). The yield of the biotransformation process was very divergent ranging from 37.04% (400 mg/l, single dose) to 73.80% (100 mg/l, 3 doses). The identity of the product, i.e., arbutin was confirmed by spectral analysis (^{1}H-NMR spectrum) (Kwiecień et al. 2013).

The obtained maximum content of arbutin (8.27 g%) was higher than its minimum content in classical arbutin-containing raw material *Uvae ursi folium* (7.0 g%) required by the newest edition of the European Pharmacopoeia and higher than its minimal content in *Vitis idaeae folium* (4.0 g%), acc. to national monograph in the newest Polish Pharmacopoeia. This result is potentially applicable in practice (Kwiecień et al. 2013).

4.11.4 Evaluation of Our Biotechnological Investigations

- Stationary agar callus cultures of *A. melanocarpa* accumulate comparable amounts of phenolic acids on the tested variants of LS and MS medium. Culture on the MS media yielded slightly greater callus biomass increments (Table 4.8).
- Stationary agar and agitated shoot cultures accumulate larger amounts of phenolic acids growing on the MS medium variants compared with the LS medium variants. Shoot biomass increments are also higher on the MS medium variants (Table 4.8).
- The contents of PGRs—NAA (auxin) and BAP (cytokinin) and auxin/cytokinin ratio in the tested variants of LS and MS medium conspicuously influenced phenolic acid accumulation and biomass increments both in agar callus cultures and agar shoot and agitated shoot cultures.
- Both in agar callus and shoot cultures, cell metabolism was oriented toward the production of benzoic acid and/or cinnamic acid derivatives while the production of depsides prevailed in agitated shoot cultures (Table 4.9).
- Supplementation with biosynthetic precursors of phenolic acids (phenylalanine, cinnamic acid, and benzoic acid) and depside precursor (caffeic acid) of the

agitated shoot cultures evidently stimulated phenolic acid accumulation. The addition of cinnamic acid and/or caffeic acid was the most beneficial (Table 4.10).

- Among light conditions tested in stationary agar shoot cultures (monochromatic lights, UV-A irradiation, darkness, and multispectral white light), it was the blue light that clearly stimulated the accumulation of phenolic acids and biomass growth (Table 4.10).
- Callus biomass increments were relatively low. In contrast, shoot biomass increments in stationary agar culture were exceptionally high but in agitated cultures, they were low. The tested types of shoot culture, namely agar and agitated cultures, accumulated high amounts of phenolic acids. Particularly high metabolite contents were documented in agitated shoot cultures on the MS media. Callus cultures have proven to be a much less productive source of phenolic acids compared with shoot cultures (Table 4.10).
- The studies of the dynamics of phenolic acid accumulation in agitated shoot cultures over a period of 8 weeks demonstrated the maximum accumulation of phenolic acids after 5-week culture growth cycles.

Table 4.8 Minimal and maximal total contents [mg/100 g DW] of phenolic acids estimated after testing of basal media and PGRs combinations in agar callus, agar shoot, and agitated shoot cultures of *A. melanocarpa*

Type of culture	Medium	Total content of phenolic acids	
		Minimal	Maximal
Agar callus culture	LS	50.23	81.56
	MS	47.00	84.00
Agar shoot culture	LS	105.05	150.95
	MS	93.52	217.00
Agitated shoot culture	LS	67.22	130.44
	MS	189.02	324.78

Table 4.9 Maximal contents [mg/100 g DW] of individual phenolic acids in different types of studied *A. melanocarpa* in vitro cultures

Phenolic acid	Agar callus culture		Agar shoot culture		Agitated shoot culture	
	LS	MS	LS	MS	LS	MS
p-Coumaric acid	12.15	10.93	-[a]	62.39	–	–
p-Hydroxybenzoic acid	25.60	23.59	55.14	50.66	–	–
Syringic acid	46.26	40.16	15.82	28.72	–	–
Vanillic acid	6.65	7.47	16.37	14.36	–	–
Salicylic acid	–	–	78.25	91.86	–	–
Rosmarinic acid	–	–	–	–	53.40	134.24
Neochlorogenic acid	–	–	–	–	35.18	82.00
Chlorogenic acid	–	–	–	–	41.58	105.45

[a]"-"—not confirmed

Table 4.10 Maximal total contents of phenolic acids [mg/100 g DW] in the tested types of *A. melanocarpa* in vitro cultures

Type of culture	Medium/conditions	Total content
Agar callus culture	LS	81.56
	MS	84.00
Agar shoot culture	LS	150.95
	MS	217.00
Agar shoot culture	Blue light	527.40
Agitated shoot culture	LS	130.44
	MS	324.78
Agitated shoot culture with precursor addition	MS medium supplemented with 5 mmol/l of cinnamic acid on 10th day	989.79
Bioreactors (preliminary results)	RITA bioreactor	787.88
	PlantForm bioreactor	693.29

A. melanocarpa fruits—32.43 mg/100 g DW

- *A. melanocarpa* bioreactor shoot cultures produced satisfactory amounts of phenolic acids and biomass increments. Decidedly greater amounts of phenolic acids were obtained after 4-week growth cycles compared with 8-week or 6-week cycles (in RITA and PlantForm bioreactors, respectively). The main metabolites in this culture type, besides depsides, were identified as phenylacetic acid derivatives. Slightly greater amounts of phenolic acids were obtained in the RITA bioreactors (Table 4.10).
- In all tested types of cultures, the total content of phenolic acids was greater than in fruit extracts—the pharmaceutical raw material (Table 4.10).
- At the present stage of research, agitated or agar shoot cultures of *A. melanocarpa* on the MS media can be proposed as a potential rich source of phenolic acids, including depsides (Table 4.10).
- Apart from the ability of cells from agitated shoot cultures to endogenously accumulate phenolic acids, they are also capable of exogenous hydroquinone biotransformation into its β-D-glucoside, arbutin. The obtained amounts of the product are interesting from a practical point of view.
- The types of *A. melanocarpa* in vitro cultures maintained in the course of these studies are very good model cultures for further biotechnological studies.

4.12 Summary and Prospects

A. melanocarpa, a species native to North America, was introduced to Northwestern Asia and Europe at the end of the eighteenth century and became naturalized in some European (Central and South Europe and Scandinavia) and Asian (Russia) countries. The fruits of this species are exceptionally rich in a wide variety of chemical

subgroups of antioxidants, vitamins and bioelements, hence in recent years they have been increasingly used for the production of dietary supplements and has important position in the food and cosmetics industries. This raw material has been documented by scientific research to exhibit invaluable directions of biological activity, including the prevention of civilization diseases. Tremendous interest in this raw material in Central, South European countries, including Poland and in Scandinavia, has contributed to its cultivation as a commercial crop on an increasingly wide scale in Europe. Recently, this species has been grown in ecological farming systems with great success.

Considering a very wide range of possible therapeutic applications, the raw material, *Aroniae fructus,* should be included into the list of pharmacopoeial plant raw materials in the European Pharmacopoeia so that is can be used in official medicine in European Union countries.

Phytochemical and pharmacological studies are paralleled by professional biotechnological investigations aimed at developing micropropagation protocols and examining the biosynthetic potential of in vitro cultures of this species. The results of the studies on the biosynthetic potential, so far focused on the endogenous accumulation of phenolic acids—one of the subgroups of antioxidants—clearly indicate that shoot cultures of this species can be a potential rich source of these metabolites and an alternative to plants growing in the open air. The biosynthetic potential of cells from in vitro cultures can be utilized for biotransformation of exogenous hydroquinone into its β-D-glucoside, arbutin, which is an important compound of high medicinal and cosmetic value. However, only the full phytochemical analysis of the biomass to estimate other subgroups (flavonoids, anthocyanins) of antioxidants will provide a full picture of the biosynthetic capabilities of the cells cultured in vitro.

Among a wide range of biotechnological approaches, the development of micropropagation protocols is of crucial significance. They can ensure the supply of high-producing genotypically identical plants. The plants from in vitro cultures could be used for establishing professional open-field plantations.

Considering the well-known natural phenomenon of chemical variability, still high environmental pollution and the rapid rate of climate change, the proposals of biotechnological solutions, most of all to use the biomass grown in large-scale bioreactor installations as a source of antioxidants, seems to be a very significant and future-oriented approach.

Currently the cosmetics industry makes use of the biosynthetic potential of the callus tissue cells of *A. melanocarpa* for the manufacture of cosmetics. Therefore, it can be expected that in the near future in vitro cultures of this species will also be used for the production of pharmaceuticals and food ingredients, as an alternative to field-grown crops.

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References

Andrzejewska J, Sadowska K, Klóska Ł, Rogowski L (2015) The effect of plant age and harvest time on the content of chosen components and antioxidative potential of black chokeberry fruit. Acta Sci Pol Hortorum Cultus 14(4):105–114

Augustyniak A, Bartosz G, Čipak A, Duburs G, Horáková U, Łuczaj W et al (2010) Natural and synthetic antioxidants: an updated overview. Free Radical Res 44(10):1216–62 https://www.bib.irb.hr/487622

Badescu M, Badulescu O, Badescu L, Ciocoiu M (2015) Effects of *Sambucus nigra* and *Aronia melanocarpa* extracts on immune system disorders within diabetes mellitus. Pharm Biol Informa Healthc 53(4):533–539

Bell DR, Gochenaur K (2006) Direct vasoactive and vasoprotective properties of anthocyanin-rich extracts. J Appl Physiol 100(4):1164–1170

Benvenuti S, Pellati F, Melegari M, Bertelli D (2006) Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of rubus, ribes, and aronia. J Food Sci 69(3):164–169

Bermúdez-Soto MJ, Larrosa M, García-Cantalejo JM et al (2007a) Transcriptional changes in human Caco-2 colon cancer cells following exposure to a recurrent non-toxic dose of polyphenol-rich chokeberry juice. In: Genes & nutrition. Springer, pp 111–113

Bermúdez-Soto MJ, Larrosa M, Garcia-Cantalejo JM et al (2007b) Up-regulation of tumor suppressor carcinoembryonic antigen-related cell adhesion molecule 1 in human colon cancer Caco-2 cells following repetitive exposure to dietary levels of a polyphenol-rich chokeberry juice. J Nutr Biochem 18(4):259–271

Bijak M, Saluk J, Antosik A, Ponczek MB, Zbikowska HM, Borowiecka M et al (2013) *Aronia melanocarpa* as a protector against nitration of fibrinogen. Int J Biol Macromol 55:264–268

BIOnly (2020) Aronia czarnoowocowa (*Aronia melanocarpa*) [Internet]. Kosmet. Nat. Org. bio. [cited 2020 Dec 27]. Available from: https://www.bionly.pl/pl/articles/details/72/porady

Borowska S, Brzóska MM (2016) Chokeberries (*Aronia melanocarpa*) and their products as a possible means for the prevention and treatment of noncommunicable diseases and unfavorable health effects due to exposure to xenobiotics. Compr Rev Food Sci Food Saf 15(6):982–1017

Brand MH (2010) Aronia: native shrubs with untapped potential. Aroldia 67:14–25

Brand MH, Cullina WG (1992) Micropropagation of red and black chokeberry (*Aronia spp.*). Hort Sci 27(1):81–81

Brewer MS (2011) Natural antioxidants: sources, compounds, mechanisms of action, and potential applications. Compr Rev Food Sci Food Saf 10(4):221–247

Broncel M, Kozirog M, Duchnowicz P, Koter-Michalak M, Sikora J, Chojnowska-Jezierska J (2010) *Aronia melanocarpa* extract reduces blood pressure, serum endothelin, lipid, and oxidative stress marker levels in patients with metabolic syndrome. Med Sci Monit Inter Sci Info Inc 16(1):CR28–CR34

Brzóska MM, Rogalska J, Galazyn-Sidorczuk M, Jurczuk M, Roszczenko A, Tomczyk M (2015) Protective effect of *Aronia melanocarpa* polyphenols against cadmium-induced disorders in bone metabolism: A study in a rat model of lifetime human exposure to this heavy metal. Chem Biol Interact 229:132–146

Cai Y, Luo Q, Sun M, Corke H (2004) Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Sci 74(17):2157–2184

Celka Z, Szkudlarz P (2010) Spontaneous occurrence and dispersion of *Aronia x prunifolia* (Marshall) Rehder (Rosaceae) in Poland on the example of the "Bagna" bog complex near Chlebowo (Western Poland). Acta Soc Bot Pol 79(1):37–42

Chrubasik C, Li G, Chrubasik S (2010) The clinical effectiveness of chokeberry: a systematic review. Phytother Res 24(8):1107–11014

CosIng (2020) CosIng—cosmetic ingredients database [Internet]. European Communications [cited 2 Dec 2020]. Available from: http://ec.europa.eu/growth/tools-databases/cosing/index.cfm?fuseaction=search.results

Denev PN, Kratchanov CG, Ciz M, Lojek A, Kratchanova MG (2012) Bioavailability and antioxidant activity of black chokeberry (*Aronia melanocarpa*) polyphenols: in vitro and in vivo evidences and possible mechanisms of action: a review. Compr Rev Food Sci Food Saf 11(5):471–489

Denev P, Číž M, Kratchanova M, Blazheva D (2019) Black chokeberry (*Aronia melanocarpa*) polyphenols reveal different antioxidant, antimicrobial and neutrophil-modulating activities. Food Chem 30(284):108–117

EFSA (2011) Scientific opinion part I on the substantiation of health claims related to various food(s)/food constituent(s) not supported by pertinent human data (ID 411, 559, 1174, 1184, 1197, 1380, 1409, 1656, 1667, 1670, 1763, 1767, 1806, 1884, 1908, 1997, 2141 2). EFSA J 9(6)

Gasiorowski KB, Brokos B, Kozubek A, Oszmianski J (2000) The antimutagenic activity of two plant—derived compounds. A comparative cytogenetic study. Cell Mol Biol Lett 2(05)

Gramza-Michałowska A, Sidor A, Kulczyński B (2017) Berries as a potential anti-influenza factor—a review. J Funct Foods 37:116–137

Gruszczyk M, Kołodziej B, Król B, Sugier D, Wiśniewski J, Nurzyńska-Widerek R et al (2018) Uprawa ziół. Poradnik dla plantatorów. wydanie II. Warszawa: Powszechne Wydawnictwo Rolnicze i Leśne

Heleno SA, Martins A, Queiroz MJRP, Ferreira ICFR (2015) Bioactivity of phenolic acids: metabolites versus parent compounds: a review. Food Chem 173:501–513

Jakobek L (2007) Antioxidant activity and polyphenols of aronia in comparison to other berry species. Agric Conspec Sci 72(4):301–306

Jakobek L, Drenjančević M, Jukić V, Šeruga M (2012) Phenolic acids, flavonols, anthocyanins and antiradical activity of "Nero", "Viking", "Galicianka" and wild chokeberries. Sci Hortic 147:56–63

Jan Niedworok FB (2001) The investigation of a biological and phytotherapeutical properties of the *Aronia melanocarpa* E. anthocyanins. Postępy Fitoter 1:20–24

Jeppsson NJ (1999) Evaluation of black chokeberry, *Aronia melanocarpa*, germplasm for production of natural food colourants. Acta Hortic Int Soc Hort Sci 484:193–198

Jing P, Bomser JA, Schwartz SJ, He J, Magnuson BA, Giusti MM (2008) Structure-function relationships of anthocyanins from various anthocyanin-rich extracts on the inhibition of colon cancer cell growth. J Agric Food Chem 56(20):9391–9398

Jodynis-Liebert J, Adamska T, Ewertowska M et al (2014) Effects of long-term administration of freeze-dried chokeberry juice to rats. J Pharm Nutr Sci 4(2):154–161

Jurgoński A, Juśkiewicz J, Zduńczyk Z (2008) Ingestion of black chokeberry fruit extract leads to intestinal and systemic changes in a rat model of prediabetes and hyperlipidemia. Plant Foods Hum Nutr 63(4):176–182

Kakkar S, Bais S (2014) A review on protocatechuic acid and its pharmacological potential. 2014, Article ID 952943, 9 pages http://dx.doi.org/10.1155/2014/952943

Kattappagari K, Ravi Teja C, Kommalapati R et al (2015) Role of antioxidants in facilitating the body functions: a review. J Orofac Sci Medknow Publications 7(2):75

Kim B, Ku CS, Pham TX, Park Y, Martin DA, Xie L et al (2013) *Aronia melanocarpa* (chokeberry) polyphenol-rich extract improves antioxidant function and reduces total plasma cholesterol in apolipoprotein E knockout mice. Nutr Res 33(5):406–413

King ES, Bolling BW (2020) View of composition, polyphenol bioavailability, and health benefits of aronia berry: a review. J Food Bioact 11:13–30

Kitrytė V, Kraujalienė V, Šulniūtė V, Pukalskas A, Venskutonis PR (2017) Chokeberry pomace valorization into food ingredients by enzyme-assisted extraction: process optimization and product characterization. Food Bioprod Process 105:36–50

Kleparski J, Domino Z (1990) Aronia. Państwowe Wydawnictwo Rolnicze i Leśne, Warszawa

Kokotkiewicz A, Jaremicz Z, Luczkiewicz M (2010) Aronia plants: a review of traditional use, biological activities, and perspectives for modern medicine. J Med Food 13(2):255–269

Krishnaiah D, Sarbatly R, Nithyanandam R (2011) A review of the antioxidant potential of medicinal plant species. Food Bioprod Process 89(3):217–233

Kubica P, Pałka A, Szopa A, Ekiert H (2019a) Production of phenolic acids in shoot cultures of black aronia (*Aronia melanocarpa*) cultivated in RITA bioreactors. In: 67th International Congress Annual Meeting of the Society for Medicinal Plant and Natural Product Research, Innsbruck

Kubica P, Szopa A, Żywko J, Pałka A, Ekiert H (2019b) Agitated and bioreactor's cultures of aronia species—investigations on accumulation dynamics of bioactive phenolic acids during the growth cycles. In: XI Conference "In vitro cultures in biotechnology plant physiology". 4–6 December 2019, Kraków (Poland)

Kubica P, Pałka A, Szopa A, Ekiert H (2020) Accumulation of phenolic acids in in vitro cultures of black aronia (*Aronia melanocarpa*) cultivated in PlantForm bioreactors. 3rd ICPMS Martin, Kraków, Szeged

Kulling SE, Rawel HM (2008) Chokeberry (*Aronia melanocarpa*)—a review on the characteristic components and potential health effects. Planta Med 74(13):1625–1634

Kwiecień I, Szopa A, Madej K, Ekiert H (2013) Arbutin production via biotransformation of hydroquinone in in vitro cultures of Aronia melanocarpa (Michx.) Elliott. Acta Biochim Pol 60(4):865–870

Lala G, Malik M, Zhao CW, He J, Kwon Y, Giusti MM et al (2006) Anthocyanin-rich extracts inhibit multiple biomarkers of colon cancer in rats. Nutr Cancer Int J 54(1):84–93

Linsmaier EM, Skoog F (1965) Organic growth factor requirements of tobacco tissue cultures. Physiol Plant 18(1):100–127

Litwinczuk W (2002) Propagation of black chokeberry (*Aronia melanocarpa* Elliot) through in vitro culture. Electron J Polish Agric Univ Ser Hortic 5(2)

Malik M, Zhao C, Schoene N, Guisti MM, Moyer MP, Magnuson BA (2003) Anthocyanin-rich extract from *Aronia meloncarpa* E. induces a cell cycle block in colon cancer but not normal colonic cells. Nutr Cancer 46(2):186–196

Meng S, Cao J, Feng Q, Peng J, Hu Y (2013) Roles of chlorogenic acid on regulating glucose and lipids metabolism: a review. Evidence-based Complement Altern Med Hindawi Pub Corp 801457

Michalak P (2015) Aronia planter's guide, 1st edn. Dąbie

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15(3):473–479

Naruszewicz M, Łaniewska I, Millo B, Dłuzniewski M (2007) Combination therapy of statin with flavonoids rich extract from chokeberry fruits enhanced reduction in cardiovascular risk markers in patients after myocardial infraction (MI). Atherosclerosis 194(2):179–184

Niedworok J, Gwardys A, Jankowski A, Kowalczyk E, Oszmański J, Skośkiewicz J (1999) Badania nad protekcyjnym wpływem żelu antocyjaninowego na fototoksyczne działanie promieni UV. Ochr Środ Zas Nat 18:83–87

Oszmianski J, Wojdylo A, Oszmiański J, Wojdylo A (2005) Aronia melanocarpa phenolics and their antioxidant activity. Eur Food Res Technol 221(6):809–813

Park H, Liu Y, Kim HS, Shin JH (2016) Chokeberry attenuates the expression of genes related to *de novo* lipogenesis in the hepatocytes of mice with nonalcoholic fatty liver disease. Nutr Res 36(1):57–64

Pérez-Jiménez J, Neveu V, Vos F, Scalbert A (2010) Identification of the 100 richest dietary sources of polyphenols: an application of the phenol-explorer database. Eur J Clin Nutr 64(3):112–120

Petrovic DM, Jacimovic-Plavšic MM (1992) *Aronia melancarpa* and propagation in vitro. Acta Hortic 300:133–136

Piotrowska-Kempisty H, Nowicki M, Jodynis-Liebert J et al (2020) Assessment of hepatoprotective effect of chokeberry juice in rats treated chronically with carbon tetrachloride. Mol MDPI AG 25(6):1268

Pool-Zobel BL, Bub A, Schröder N, Rechkemmer G (1999) Anthocyanins are potent antioxidants in model systems but do not reduce endogenous oxidative DNA damage in human colon cells. Eur J Nutr 38(5):227–234

Pratheeshkumar P, Son YO, Wang X, Divya SP et al (2014) Cyanidin-3-glucoside inhibits UVB-induced oxidative damage and inflammation by regulating MAP kinase and NF-κB signaling pathways in SKH-1 hairless mice skin. Toxicol Appl Pharmacol 280(1):127–137

Razungles A, Oszmański J, Sapis J-C (1989) Determination of Carotenoids in Fruits of Rosa sp. (*Rosa canina* and *Rosa rugosa*) and of Chokeberry (*Aronia melanocarpa*). J Food Sci 54(3):774–775

Rop O, Mlcek J, Jurikova T, Valsikova M et al (2010) Phenolic content, antioxidant capacity, radical oxygen species scavenging and lipid peroxidation inhibiting activities of extracts of five black chokeberry (*Aronia melanocarpa* (Michx.) Elliot) cultivars. J Med Plants Res 4(22):2431–2437

Rugină D, Sconţa Z, Leopold L, Pintea A, Bunea A, Socaciu C (2012) Antioxidant activities of chokeberry extracts and the cytotoxic action of their anthocyanin fraction on HeLa human cervical tumor cells. J Med Food 15(8):700–706

Rumińska A (1984) Poradnik plantatora ziół. Państwowe Wydawnictwo Rolnicze i Leśne—oddział w Poznaniu, Poznań

Ruzic D (1993) In vitro rooting and subsequent growth of black chokeberry (*Aronia melanocarpa*) plants *ex vitro*. J Fruit Ornam Plant Res. Research Institute of Pomology and Floriculture 1:1–8

Ryszawa N, Kawczyńska-Drózdz A, Pryjma J et al (2006) Effects of novel plant antioxidants on platelet superoxide production and aggregation in atherosclerosis. J Physiol Pharmacol 57(4):611–626

Saruwatari A, Isshiki M, Tamura H (2008) Inhibitory effects of various beverages on the sulfoconjugation of 17β-estradiol in human colon carcinoma caco-2 cells. Biol Pharm Bull Pharmaceu Soc Japan 31(11):2131–2136

Senderski ME (2004) Prawie wszystko o ziołach. Wydanie pi. Mateusz E. Senderski, Podkowa Leśna

Sharif T, Alhosin M, Auger C, Minker C et al (2012) *Aronia melanocarpa* juice induces a redox-sensitive p73-related caspase 3-dependent apoptosis in human leukemia cells. PLoS One 7(3)

Sidor A, Gramza-Michałowska A (2019) Qualitative composition, phenolic profile and antioxidant potential. Molecules 24(20):3710. https://doi.org/10.3390/molecules24203710

Sidor A, Drożdżyńska A, Gramza-Michałowska A (2019) Black chokeberry (*Aronia melanocarpa*) and its products as potential health-promoting factors—an overview. Trends Food Sci Technol 89:45–60

Sikora J, Markowicz M, Mikiciuk-Olasik E (2009) Rola i właściwości lecznicze aronii czarnoowocowej w profilaktyce chorób cywilizacyjnych. Bromat Chem Toksykol XLII(1):10–17

Skupień K, Kostrzewa-Nowak D, Oszmiański J, Tarasiuk J (2008) In vitro antileukaemic activity of extracts from chokeberry (*Aronia melanocarpa* (Michx) Elliott) and mulberry (*Morus alba* L.) leaves against sensitive and multidrug resistant HL60 cells. Phyther Res 22(5):689–694

Slimestad R, Torskangerpoll K, Nateland HS, Johannessen T, Giske NH (2005) Flavonoids from black chokeberries, *Aronia melanocarpa*. J Food Compos Anal 18(1):61–68

Stralsjoe L, Ahlin H, Witthoeft CM, Jastrebova J (2003) Folate determination in Swedish berries by radioprotein-binding assay (RPBA) and high performance liquid chromatography (HPLC). Eur Food Res Technol 216(3):264–269

Sueiro L, Yousef GG, Seigler D, De Mejia EG, Grace MH, Lila MA (2006) Chemopreventive potential of flavonoid extracts from plantation-bred and wild *Aronia melanocarpa* (black chokeberry) fruits. J Food Sci 71(8):C480–C488

Szopa A, Ekiert H (2014) Production of biologically active phenolic acids in *Aronia melanocarpa* (Michx.) Elliott in vitro cultures cultivated on different variants of the Murashige and Skoog medium. Plant Growth Regul 72(1):51–58

Szopa A, Ekiert H, Muszyńska B (2013) Accumulation of hydroxybenzoic acids and other biologically active phenolic acids in shoot and callus cultures of *Aronia melanocarpa* (Michx.) Elliott (black chokeberry). Plant Cell Tiss Org Cult 113(2):323–329

Szopa A, Setkiewicz A, Ekiert H (2015) *Aronia melanocarpa* (black chokeberry) in vitro cultures—potential source of bioactive phenolic acids for phytotherapy. In: Conference. Bonn

Szopa A, Kokotkiewicz A, Kubica P, Banaszczak P et al (2017a) Comparative analysis of different groups of phenolic compounds in fruit and leaf extracts of Aronia sp.: *A. melanocarpa, A. arbutifolia and A. x prunifolia*, and their antioxidant activities. Eur Food Res Technol 243(9):1645–1657. https://doi.org/10.1007/s00217-017-2872-8

Szopa A, Kubica P, Ekiert H (2017b) Ecology, chemical composition, health-promoting effects and biotechnological studies on black chokeberry (*Aronia melanocarpa*), red chokeberry (*Aronia arbutifolia*) and purple chokeberry (*Aronia* × *prunifolia*). Postępy Fitoter 18(2):145–157

Szopa A, Kubica P, Ekiert H (2018a) High production of depsides and other phenolic acids in different types of shoot cultures of three aronias: *Aronia melanocarpa, A. arbutifolia and A.* × *prunifolia.* In: Ramawat KG, Ekiert MH, Goyal S (eds) Plant cell tissue differentiation and secondary metabolites. Springer, Berlin Heidelberg, pp 309–336

Szopa A, Starzec A, Ekiert H (2018b) The importance of monochromatic lights in the production of phenolic acids and flavonoids in shoot cultures of *Aronia melanocarpa, Aronia arbutifolia* and *Aronia* × *prunifolia.* J Photochem Photobiol B Biol 179:91–97

Szopa A, Kubica P, Komsta Ł, Walkowicz-Bożek A, Ekiert H (2020) The effect of feeding culture media with biogenetic precursors on high production of depsides in agitated shoot cultures of black and red aronias. Plant Cell Tiss Org Cult 142:379–399

Taheri R, Connolly BA, Brand MH, Bolling BW (2013) Underutilized chokeberry (*Aronia melanocarpa, Aronia arbutifolia, Aronia prunifolia*) accessions are rich sources of anthocyanins, flavonoids, hydroxycinnamic acids, and proanthocyanidins. J Agric Food Chem 61(36):8581–8588

Tanaka T, Tanaka A (2001) Chemical components and characteristics of black chokeberry. J Jpn Soc Food Sci Technol 48:606–610

Thani NAA, Keshavarz S, Lwaleed BA, Cooper AJ, Rooprai HK (2014) Cytotoxicity of gemcitabine enhanced by polyphenolics from *Aronia melanocarpa* in pancreatic cancer cell line AsPC-1. J Clin Pathol 67(11):949–954

Tian Y, Liimatainen J, Alanne AL, Lindstedt A, Liu P, Sinkkonen J et al (2017) Phenolic compounds extracted by acidic aqueous ethanol from berries and leaves of different berry plants. Food Chem 220:266–281: http://dx.doi.org/10.1016/j.foodchem.2016.09.145

Tolić MT, Jurčević IL, Krbavčić IP, Marković K, Vahčić N (2015) Phenolic content, antioxidant capacity and quality of chokeberry (*Aronia melanocarpa*) products. Food Technol Biotechnol 53(2):171–179

Tolić MT, Krbavčić IP, Vujević P, Milinović B, Jurčević IL, Vahčić N(2017) Effects of weather conditions on phenolic content and antioxidant capacity in juice of chokeberries (*Aronia melanocarpa* L.). Polish J Food Nutr Sci 67(1):67–74

University of Maine (2020) Plant description and habitat of aronia (black chokeberry) Orono, Maine. https://extension.umaine.edu/agriculture/aronia/plant-description-and-habitat/

Vagiri M, Jensen M (2017) Influence of juice processing factors on quality of black chokeberry pomace as a future resource for colour extraction. Food Chem 217:409–417

Valcheva-Kuzmanova S, Belcheva A (2006) Current knowledge of *Aronia melanocarpa* as a medicinal plant. Folia Med (Plovdiv) 48(2):11–17

Valcheva-Kuzmanova S, Borisova P, Galunska B, Krasnaliev I, Belcheva A (2004) Hepatoprotective effect of the natural fruit juice from *Aronia melanocarpa* on carbon tetrachloride-induced acute liver damage in rats. Exp Toxicol Pathol 56(3):195–201

Valcheva-Kuzmanova S, Marazova K, Krasnaliev I, Galunska B, Borisova P, Belcheva A (2005) Effect of *Aronia melanocarpa* fruit juice on indomethacin-induced gastric mucosal damage and oxidative stress in rats. Exp Toxicol Pathol 56(6):385–392

Valcheva-Kuzmanova S, Kuzmanov K, Tancheva S, Belcheva A (2007) Hypoglycemic and hypolipidemic effects of *Aronia melanocarpa* fruit juice in streptozotocin-induced diabetic rats. Methods Find Exp Clin Pharmacol 29(2):101–105

Vázquez-Espinosa M, V. González-de-Peredo A, Espada-Bellido E et al (2019) Ultrasound-assisted extraction of two types of antioxidant compounds (TPC and TA) from black chokeberry (*Aronia*

melanocarpa L.): optimization of the individual and simultaneous extraction methods. Agronomy. MDPI AG 9(8):456
Veberic R, Slatnar A, Bizjak J, Stampar F, Mikulic-Petkovsek M (2015) Anthocyanin composition of different wild and cultivated berry species. LWT 60(1):509–517
Vlachojannis C, Zimmermann BF, Chrubasik-Hausmann S (2015) Quantification of anthocyanins in elderberry and chokeberry dietary supplements. Phyther Res 29(4):561–565
Walther E, Schnell S (2009) Black chokeberry (*Aronia melanocarpa*)-a special fruit crop. Zeitschrift für Arznei- & Gewürzpflanzen 14(4):179–182
Wang H, Cao G, Prior RL (1996) Total antioxidant capacity of fruits. J Agric Food Chem 44:701–705
Wangensteen H, Bräunlich M, Nikolic V et al (2014) Anthocyanins, proanthocyanidins and total phenolics in four cultivars of aronia: antioxidant and enzyme inhibitory effects. J Funct Foods 7(1):746–752
Wawer I (2006) Aronia Polski Paradoks. Agropharm, Warszawa
Wawer I, Eggert P, Hołub B (2012) Aronia super owoc. Wektor, Warszawa
Willcox JK, Ash SL, Catignani GL (2004) Antioxidants and prevention of chronic disease. Crit Rev Food Sci Nutr 44(4):275–295
Wolski T, Kalisz O, Prasał M (2007) Black chokeberry—*Aronia melanocarpa* (Michx.) Elliott—the rich source of antioxidants. Postępy Fitoter 3:145–154
Wu X, Gu L, Prior RL, McKay S (2004) Characterization of anthocyanins and proanthocyanidins in some cultivars of Ribes, Aronia, and Sambucus and their antioxidant capacity. J Agric Food Chem 52(26):7846–7856
Yamane T, Kozuka M, Wada-Yoneta M, Sakamoto T et al (2017) Aronia juice suppresses the elevation of postprandial blood glucose levels in adult healthy Japanese. Clin Nutr Exp 12:20–26
Zhang H, Tsao R (2016) Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Curr Opin Food Sci 8:33–42
Zhao C, Giusti MM, Malik M, Moyer MP, Magnuson BA (2004) Effects of commercial anthocyanin-rich extracts on colonic cancer and nontumorigenic colonic cell growth. J Agric Food Chem 52(20):6122–6128
Zheng W, Wang SY (2003) Oxygen radical absorbing capacity of phenolics in blueberries, cranberries, chokeberries, and lingonberries. J Agric Food Chem 51(2):502–509