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ABSTRACT

Only a small number of plants have been widely used regularly for food, fibers, shelter, industrial, cultural and medicinal purposes. The remaining plant diversity, terrestrial or marine, is underutilized. We summarize previous research done on the various aspects of North American native plant *Aronia,* which has many compounds in the berries and leaves that are potentially beneficial for human health. Berries can be processed into many value- added products giving a boost to the local economy. Micromorphological studies indicate three types of trichomes present on the leaves that are positive to diphenyl boric acid-β-ethylamino ester (DPBA) reaction suggesting presence of secondary metabolites. These features can help taxonomists to solve classification problems among various *Aronia* species and trichomes can be utilized for biotransformation studies. We conclude that *Aronia* berries and leaves are rich in total polyphenol and flavonoid content that enables the plant to register higher antioxidant activity in comparison to many other culinary herbs and medicinal plants in use. A systematic study covering agronomy, phytochemistry, product development and economic return potential is suggested based on a review of literature.

INTRODUCTION

In recent times, chokeberry (*Aronia* genus) has attracted attention as food and a nutraceutical crop. *Aronia* (Medik.), is a taxonomically complex and misunderstood genus currently seeing a revival in North America as both an ornamental and fruit crop with a rich source of antioxidants. *Aronia* fruit, a berry, can be eaten whole (they are usually canned), and are used for juice, jam, and wine production in addition to its use as an ornamental shrubs for its white flower clumps. Various group of chemicals present in the plants and plant products can play role in the prevention and treatment of diseases and unfavorable health effects caused by exposure to xenobiotic agents (Hao et al., 2015; Qader et al., 2014). This has led to growing interest in investigating plants for their active compounds beneficial for the human body (Borowska and Brzóska, 2016). The berries contain anthocyanins and procyanidins, well known for high antioxidative capacity leading to antibacterial, anticancer, antidiabetic, anti-inflammatory, antimutagenic, antiviral, cardioprotective, gastroprotective, hepatoprotective, immunomodulatory and radioprotective activities both *in vitro* and *in vivo* studies (Jurikova et al., 2017). *Aronia* juice is a good option to add to the other fruit juice beverages to enhance the levels of anthocyanins and flavonoids. Additionally, *Aronia* berries are immunomodulatory,

antibacterial, hepatoprotective, gastroprotective and anti-inflammatory, in nature and this potential has been evident in both *in vitro*, in cells or cell lines, and in *in vivo* studies in humans or animals (Kokotkiewicz et al., 2010).

Aronia melanocarpa became popular in Eastern European countries and Soviet Union in the 20th century and was used for the commercial production of fruits, juices and wines with the suggestion that there are no toxic effects of black chokeberry fruits, juice and extracts (McKay, 2001; Valcheva-Kuzmanova and Belcheva 2006). Later, black chokeberries gained popularity as herbal medicine in Russia and Eastern European countries as hypertensive and anti-atherosclerotic drug (Kokotkiewicz et al., 2010). Early research on chokeberries started with the focus on the prevention and treatment of non-communicable diseases (NCD) but now they are being studies for anti-xenobiotic activity as well (Borowska and Brzóska, 2016; Hao et al., 2015; Navaneethan and Rasool, 2014). *Aronia melanocarpa* fruits have one of the highest contents of polyphenols including flavonoids, anthocyanins, proanthocyanidins, flavanols, phenolic acids, and flavonols (Hwang et al., 2014; Kulling and Rawel, 2008; Malien-Aubert et al., 2001; Oszmiański and Wojdylo, 2005; Rugina et al., 2011; Seidemann, 1993; Slimestad et al., 2005; Wu et al., 2004; Zheng and Wang, 2003). Due to the high levels of various antioxidants, *Aronia* berries are one of the most effective fruits in treatment of chronic ailments pertaining to oxidative stress, diabetes, cardiovascular diseases, and cancer (Denev et al., 2012).

It serves as an alternative for non-native, invasive ornamental species and has been discovered to be a rich source of antioxidants. Genus *Aronia* is native to North America and is used in the Native American medicine to treat various ailments (Moerman, 1998). Berries of *A. melanocarpa* have been traditionally used by Potawatomi Native Americans to make tea to prevent and treat cold (Kokotkiewicz et al., 2010). Both the Potawatomi and Abnaki Indian tribes consumed the berries for food (Moerman, 1998). A nutritious food preparation with enhanced shelf-life 'pemmican' was prepared using animal fat, dried powdered meat, and the berries. North American settlers used berries and the bark tissues as an astringent (Kokotkiewicz et al., 2010). Although there are many research papers available on the bioactive compounds present in the berries, only a few studies investigate the bioactivity and micromorphology of leaves (Thi and Hwang, 2014).

The major contribution of this communication is to compile previous research done on *Aronia* to comprehend its commercial potential in the context of USA. Currently, Fort Valley State University (FVSU) research field contains 150 *A. melanocarpa* 'Viking' plants to study its adaptability in the middle Georgia conditions as a commercial crop by recording major traits like plant growth, flower and fruit setting. We report antioxidant capacity measurement of shade dried ripe berries and leaves for their possible use in value added products. A detailed account of leaf micromorphology using cleared leaf material and visible, fluorescent, and scanning electron microscopy (SEM) are also reported.

MATERIALS AND METHODS

All the available research covering various aspects of *Aronia* biology were collected and summarized.

Antioxidant studies. The leaves from greenhouse-grown plants and fruits from nursery were used for antioxidant capacity studies. Three replicates weighing one gram each of fresh leaves and fruits were shade dried at room temperature (RT) (25 ± 2 ºC) till weight became constant. Dried samples were weighed and methanolic extraction was carried out (Vaidya, 2013). Each extract was evaluated for total polyphenol content (TPP), total flavonoid content estimation, and antioxidant capacity estimation using TROLOX Equivalent Antioxidant Capacity (TEAC) assay as optimized in our lab (Vaidya et al., 2013). The data obtained is presented as the mean of three replicates and no statistical analysis was performed.

Clearing of plant material. Leaf morphology was studied in cleared plant material. Leaves were collected from greenhouse and field grown plants and washed under running tap water with a drop of detergent for 30 minutes to remove spores and debris. Leaves were kept in 90 % ethanol overnight to remove chlorophyll. After washing two times in water, leaves were transferred to a 5 % commercial bleach (Clorox®) solution for one hour. Leaves were washed three times for 15 min each in water and transferred to 1N sodium hydroxide (NaOH) solution for 3-6 h, periodically checking that plant material do not become too soft. Once the NaOH treatment was over, leaves were washed in water 3-4 times and stained with 0.1 % aqueous safranin 'O' for two h. Samples were destained in water to remove excess stain and were observed for trichome morphology under microscope (Soundappan et al., 2018).

Light and fluorescent microscopy. The protocols optimized earlier (Vaidya, 2013; Soundappan et al., 2018) were followed for light microscopy observations. The leaf samples were processed as per Soundappan et al. (2018) using natural product reagent Diphenyl boric acid-β-ethylamino ester (DPBA) stain (Sigma-Aldrich, MO, USA) for fluorescent microscopy as outlined in Heinrich et al. (2002). A fluorescent microscope (Olympus BX-43 fitted with Olympus DP-72 camera; Olympus America, PA, USA with ultraviolet (UV) light source from X-Cite series 120 Q; Lumen Dynamics, MA, USA) was used to capture images.

Scanning electron microscopy. In brief, juvenile and mature leaves were fixed in 2 % glutaraldehyde (Electron Microscopy Sciences, PA, USA), then secondary fixation was carried out in 1 % osmium tetroxide (Electron Microscopy Sciences, PA, USA). Fixation was followed by critical point drying using Leica EM CPD300 (Leica microsystems, MA, USA) and finally sputter coated with gold using Denton vacuum Desk V (Denton, NJ, USA) and viewed under Variable Pressure Hitachi 3400N II scanning electron microscope (Vaidya et al., 2016).

RESULTS AND DISCUSSION

Botany and distribution. The *Aronia* genus is a member of Rose family (Rosaceae) and includes two cold-hardy deciduous species of native North American shrubs: *Aronia melanocarpa* (Michx.) Ell. (black chokeberry) and *A. arbutifolia* (L.) Pers. (red chokeberry). Chokeberries are classified under the genus *Aronia* with 16 species (Engels and Brinckmann, 2014). According to Hardin (1973), black chokeberry is native from Newfoundland south to northern Georgia and Alabama, and north to Minnesota and southern Ontario, and that red chokeberry is native to Newfoundland south to central Florida, west to east Texas. 'Viking' and 'Nero' cultivars of *Aronia* are self-fertile and popular for fruit production in North America (McKay, 2001). DNA fingerprinting studies revealed that there is very little difference between the two cultivars that perform about the same commercially (McKay, 2001; Smolik et al., 2011). Some other cultivars that were bred outside USA include Rubina (cross of Russian and Finnish plants), 'Kurkumäcki' (Finland), 'Hugin' (Sweden), 'Fertödi' (Hungary) and 'Aron' (Denmark) (Kulling and Rawel, 2008). In Poland, two commonly found cultivars of chokeberry are 'Nero' and 'Galicjanka' (Ochmian et al., 2012).

Black chokeberry is a multi-stemmed deciduous shrub that reaches 1-3 m in height, but with selection of hybrids, many cultivars available for commercial production reach only 1-2 m of height. Flowering begins in May-June and pollinated by bees. Black chokeberry flowers that are arranged in umbel inflorescence, have five white petals, and pink stamens (Figure 1A). Leaves are petiolate and have both surfaces glabrous (smooth) and are alternate on the stems, simple, 2.5-7.5 cm in length and 1.9-5.0 cm in width. They are obovate in shape (oval, but narrower at the base than near the tip), with fine and regular teeth along the edges (Figure 1B, I, L). Adaxial leaf surfaces are dark green and lustrous, with dark glands arranged on the midrib (Figure 1 B, C) whereas abaxial leaf surfaces are lighter green and elongated trichomes (Figure 1 E, F). Black chokeberry fruits are 0.8-1.25 cm in diameter, glossy and black when ripe and hang down in clusters. The fruits are pomes, and each contains 1-5 seeds. Fruits mature in August and shortly after that, shrivel, and

then drop. *Aronia* berries weigh 0.5-2 g on an average (Seidemann, 1993). Many cultivars introduced for ornamental purposes, develop excellent fall colors.

The natural distribution of black Chokeberry in the USA is predominantly in the North East states that stretches in the south up to north Georgia. Natural habitat of chokeberries is in moist woods but can also survive in drier areas (Knudson, 2005). In the first half of the 20th century, black chokeberry cultivars were introduced to the Soviet Union and other European countries for their fruits (Ochmian et al., 2012). Chokeberries were brought to Russian botanical gardens in the beginning of 20th century. From Russia, *Aronia* species spread to the Central and Eastern European countries and developed into a commercial crop (Seidemann, 1993). Chokeberries were introduced for fruit production in Poland during 1970s (Kleparski and Domino, 1990). In the USA, commercial acceptance came when 'Wildman' brand launched it as juice blends (McKay, 2001).

Propagation, yield and pests. Aronia plants grow well in the neutral to slightly acidic soils. It is cold hardy to about -30 °C and is not sensitive to the spring frost because of its delayed flowering (Seidemann, 1993). A 3 m plant to plant and between rows spacing has been suggested for optimum fruit production as it allows for adequate sunlight. Bare root seedlings can be planted in the spring if there is no danger of frost whereas containerized stocks are planted from spring to the mid-summer with providing required moisture (Knudson, 2005). Plants can be raised using following methods:

Seeds. Plant production of chokeberries is primarily done by seeds. The seeds are small and on average, one pound of seeds can be extracted from about 100 pounds of berries. Chokeberry seeds have internal dormancy and stratification in moist peat for three months at 1 ºC to 5 ºC have been suggested to overcome it (Knudson, 2005; McKay, 2001). Nursery grown seedlings establish readily if they are planted in an environment free of weeds and 38 cm or more of annual precipitation.

Cuttings. *Aronia* can be propagated using softwood or semi-softwood cuttings rooted under mist. A two-year plant can provide as many as 25 cuttings that are about five cm long containing 3-4 nodes. These cuttings are placed in the planting medium with or without rooting hormones. Pinching off apical buds of these cuttings help in breaking apical dominance and promotes better rooting and early establishment.

In vitro propagation. There are a few reports on the micropropagation studies from the US and Europe. Most of the studies have used Murashige and Skoog, 1962 (MS) media supplemented with cytokinin, 6-benzyl amino purine (BAP) in combination with an auxin at a lower concentration, e.g. indole 3-acetic acid (IAA) or indole 3-butyric acid (IBA) to trigger adventitious shoot induction (Brand and Cullina, 1992; Litwinczuk, 2013; Petrova et al., 2019; Rusea et al., 2018).

Aronia shrubs yield five to ten tons fruits per hectare (McKay, 2001) that are harvested mechanically during August to September. Application of fertilizers enhance yield and growth with little loss in pigmentation (Skupień and Oszmiański, 2007). Preliminary studies have shown that fertilizers may alter the chemical composition of *Aronia* berries, whereas low fertilizer rate (50 kg N/ha, 44 kg P/ha, 100 kg K/ha) resulted in maximum anthocyanin content (Skupień and Oszmiański, 2007). Plants respond with high fruit yield when mulched properly to avoid competing weeds (McKay, 2001). Leaf eating beetles and aphids on shoots are the possible pests. Plants require adequate sunlight and air circulation to contain potential fungal diseases (Knudson, 2005).

Chemical composition and health benefits of Aronia berries. Major research on *Aronia* phytochemicals in the past has been on *A. melanocarpa* berries (Hwang et al., 2014; Kulling and Rawel, 2008; Oszmianski and Wojdylo, 2005; Rugina et al., 2011; Slimestad et al., 2005; Zheng and Wang, 2003). These studies mainly explored polyphenols present and their value as strong antioxidants. A summary of extensive

research on polyphenolic compounds is presented in Table 2.

Sugar and organic acid content. Twenty-three samples of the *Aronia* berries that were evaluated for the organic acids (mg/100 g FW) and sugar content, exhibited presence of quinic, malic, ascorbic, shikimic, citric, oxalic, and succinic acids (Denev et al., 2018). The major sugars present in the *Aronia* berries were sorbitol (6.55-12.99 g/100 g FW), glucose (1.53-3.02 g/100 g FW), sucrose (0.06-0.41 g/100 g FW) and fructose (2.2-3.69 g/100 g FW) (Denev et al., 2018). It is interesting to note that berries are particularly low in sucrose content and sorbitol is the most abundant sugar.

Nutritional profile and antioxidant activity. Berries are rich in bioactive compounds that result in high antioxidant potential (Table 1 and 2). Twenty-seven polyphenolic compounds were identified in chokeberry products, including 7 anthocyanins, 11 flavonols, 5 phenolic acids, 3 flavan-3-ols and 1 flavanone. Three anthocyanin derivatives were reported for the first time from chokeberry fruit (Oszmianski and Wojdylo, 2005; Table 2). In addition, the pomace with much lower moisture content was a more suitable material to produce dry powders, compared to fresh chokeberry fruits (Oszmianski and Lachowicz, 2016). In terms of antioxidant levels and activity, *A. melanocarpa* berries register very high among many berries that were compared (Zheng and Wang, 2003). Acetone extracts of black chokeberries have shown five times more antioxidant activity than those of blueberries (*Vaccinium corymbosum*), eight times more than cranberries (*V. macrocarpon*), and four times more than lingonberries (*V. vitisidaea*), using oxygen radical absorbing capacity (ORAC) assay (Zheng and Wang, 2003). An extended study to compare *A. melanocarpa* with other berries (blackcurrant, redcurrant, gooseberry, and elderberry) revealed that chokeberries have the highest level of antioxidant activity (Wu et al., 2005). *Aronia melanocarpa* have shown greater antioxidant activity than blackberry (*Rubus fruticosus*), raspberry (*R. idaeus*), and strawberry (*Fragaria x ananassa*) in other studies as well (Espin et al., 2000; Jakobek et al., 2007).

While using phosphatidylcholine oxidation in a peroxidating liposome system, the phenolics present in black chokeberry juice were found to be more efficient in regenerating or protecting α-tocopherol than ascorbic acid or the phenolics in the blackcurrant (*Ribes nigrum*) juice (Graversen et al., 2007). The concentration of total phenolics in black chokeberries was six times higher than in blackcurrant juice. A comparison of antioxidant potential of 92 phenolic extracts from edible and non-edible plants (fruits, vegetables, herbs, cereals, and plant sprouts) and various berries (*A. melanocarpa*, bilberry blueberry, rabbiteye blueberry, blackcurrant, and elderberry) indicated one of the highest antioxidant activity in *Aronia* berry extracts (Kahkonen et al., 1999; Nakajima et al., 2004).

Aronia berries are rich in B1, B2, B3 (niacin), B5 (pantothenic acid), B6, C, K vitamins and in addition contain tocopherols (Tanaka and Tanaka, 2001) and carotenoids, β-carotene, β-cryptoxanthin, and violaxanthin (Razungles et al., 1989). The antioxidant potential of carotenoids is of particular significance to human health as epidemiological data strongly suggest that adequate carotenoid supplementation may significantly reduce the risk of several disorders mediated by reactive oxygen species (ROS) (Razungles et al., 1989). Folic acid that is required during periods of rapid growth is also present is *Aronia* berries at 0.20 µg/g (FW) (Stralsjo et al., 2003). A recent study exhibited production of depside compounds (a type of polyphenolic compound comprised of two or more monocyclic aromatic units joined by an ester bond) in callus cultures of *A. arbutifolia* and *A.* x *prunifolia* (a natural hybrid between *A. melanocarpa* and *A. arbutifolia*.) that are valuable in both pharmaceutical and cosmetic industry (Szopa et al., 2018 a, b).

Antibacterial properties. As high phenolic content is associated with antimicrobial activity, *A.*

melanocarpa extracts were tested on various bacterial genera as potential antimicrobial agent (Liepiņa et al., 2013). Valcheva-Kuzmanova et al. (2006) studied the bacteriostatic activity of the *A. melancocarpa* fruit juice against *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853). *Aronia melanocarpa* fruit juice inhibited the growth of *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922) whereas it had no such effect on *P. aeruginosa.* Aqueous and ethanolic extracts of *A. melanocarpa* obtained from fresh, dried and frozen fruits were studied for bacteriostatic activity. The extracts showed activity against *Bacillus cereus, S. aureus* and *P. aeruginosa* but failed to show any activity against *E. coli* (Liepiņa et al., 2013). These results were in contradiction with previous studies where *A. melanocarpa* extract was inhibitory to *E. coli* (Valcheva-Kuzmanova and Belchev, 2006).

Anticancer and antimutagenic activity. In vitro as well as *in vivo* studies have revealed that *A. melanocarpa* berries have the potential to reduce certain types of cancer (Pool-Zobel et al., 1999; Table 3). *In vitro* experiments have proved the efficiency of *A. melanocarpa* on the growth of human breast, leukemia, colon and cervical tumor cell lines (Jurikova et al., 2017). *Aronia melanocarpa* anthocyanins have shown antimutagenic activity. The study concluded that the antimutagenic influence of anthocyanins is mainly due to their free radical scavenging action, as well as by the inhibition of enzymes activating promutagens and converting mutagens to the DNA-reacting derivatives (Gasiorowski et al., 1997). In an *in vitro* model system employing platelets from patients with invasive breast cancer, administration of black chokeberry extracts significantly reduced the oxidative/nitrative stress. It was followed by a decrease of glutathione in platelets, a biomarker of oxidative stress (Kedzierska et al., 2012).

Anti-xenobiotic properties. Environmental contaminants or pollutants due to industrial and other anthropogenic activities are serious human health issues. Niedworok et al. (1997) reported that anthocyanins present in the chokeberry extract can protect from the cyclophosphamide induced oxidative stress in the erythrocytes of rabbit. Since then many reports have been published on the anti-xenobiotic property of the polyphenolic compounds present in the products of *A. melanocarpa* berries (Borowska and Brzoska, 2016; Hwang et al., 2014; Prabu et al., 2011; Qader et al., 2014;). Exposure to heavy metal, lead (Pb), can cause renal failure, osteoporosis, and hypertension (Rastogi, 2008; Vupputuri et al., 2003) and has been implicated in the development of Alzheimer's disease (Bakulski et al., 2012). Administration of quercetin protected against the Pb-induced oxidative stress in the liver of rats. There are reports suggesting consumption of *Aronia* berry products can prevent Pb-induced oxidative stress and damage to the liver (Borowska and Brzoska, 2016; Liu et al., 2010).

Tobacco smoke is one of the main sources of mutagens and carcinogens to the humans (Abdou and Elsaerei, 2004; Talio et al., 2010). Administration of 17.5 % extract of black chokeberry for eight months during and after the exposure to cigarette smoke in mice prevented histopathological alterations in the lungs, liver degeneration, weight loss and damage to erythrocytes (Balansky et al., 2012). Consumption of alcoholic beverages is common all over the world and have been associated with liver damage and depression of central nervous system (Niedworok et. al., 1997; Valcheva-Kuzmanova et al., 2013). Role of ethanol consumption in animal models have shown that administration of *A. melanocarpa* berry products and the compounds present therein protected gastric mucosal damage and hepatotoxic and neurotoxic action (Niedworok et. al., 1997). Administration of *A. melanocarpa* crude extract at 2 g/kg of body weight prevented ethanol induced gastric damages in rats (Matsumoto et al., 2004). Compounds present in chokeberry juice have been reported to protect from the hepatotoxic action of carbon tetrachloride that was used to cause acute liver damage in rats (Valcheva-Kuzmanova et al., 2004).

Aronia berries in cosmetic industry. Aging processes are most evident on skin as a product of the cellular oxidative stress of the organism. Various environmental factors such as UV radiation, electromagnetic fields, chemicals, and climate expedite aging. In these cases, ROS are produced that damage DNA telomeres, enzymes, and cell membranes (Gonzalez et al., 2008). With the popularity of plant-based products and their use, terms like phytosome-complex of a plant based natural active ingredient and a phospholipid - mostly lecithin (Gupta et al., 2007), and cosmeceutical (cosmetic with therapeutic action) have been coined (Pieroni et al., 2004).

Human skin cells absorb chokeberry extract very well and to make process efficient nanoparticles made with lecithin and encapsulated chokeberry extract have been used with much better absorption due to their small size (Young and Young, 2015). In addition, extract of *A. melanocarpa* berries is known to possess anti-inflammatory action on human dermal cell line that makes it suitable additive for cosmetic products (Cujic et al., 2017). Studies based on the animal models have shown that polyphenols present in the chokeberries prevent from the UV radiation (Gabrielska et al., 1999; Pratheeshkumar et al., 2014). Cosmetics made from the *Aronia* berries are recommended for the decrease in inflammation and drying and for the improvement of microcirculation that delays skin aging (Borowska and Brzóska, 2016). Most of these products are available in the market in the form of lip balms, facial creams, soaps, body lotions and hand creams. Considering the interest of the people reverting to the natural and herbal products, the use *A. melanocarpa* in the cosmetology is expected to grow (Borowska and Brzóska, 2016). *In vitro* cultures of *A. melanocarpa* could efficiently biotransform exogenously supplemented hydroquinone into arbutin (hydroquinone β-D-glucoside), a plant-based compound known for urinary tract disinfection and skin whitening cosmetic properties (Kwiecien et al., 2013).

Safety of using Aronia products. Despite their beneficial roles and many commercial products in the market, no regulations on the recommended doses for the *Aronia* products are available. Certain studies on animal models have shown a few chokeberry compounds pose health hazards when consumed at high doses (Dunnick and Hailey, 1992; Hagiwara et al., 1991). A study conducted on rat and mice models showed that consumption of caffeic acid at a dose of 2000 mg/kg diet for 104 weeks in rats and for 96 weeks in mice caused condition such as forestomach squamous cell papilloma, carcinoma, adenoma in these animals (Hagiwara et al., 1991). A high dose of quercetin (approximately 1900 mg/kg /24 h) delivered in a diet for 2 years caused chronic nephropathy in rats (Dunnick and Hailey, 1992). Since such high intake of phenolic compounds with standard diet is not possible in humans, therefore, *Aronia* products are generally regarded as safe to use (Valcheva-Kuzmanova and Belcheva, 2006). Polyphenols have ability to form stable complexes with divalent metals including Fe^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+} (Liu and Guo, 2015) and prolonged use can cause essential metal deficiency in the body (Zijp et al., 2000). Thus, rational use of *Aronia* dietary supplements should be recommended by nutritionists (Borowska and Brzóska, 2016).

Product diversification of Aronia berries. The harvesting of black chokeberries was common among Native Americans. Black chokeberries possess sour and astringent taste therefore the berries are consumed after being processed. Black chokeberries are consumed in many different forms by humans including dried, purees, concentrate and juices (Jurikova et al., 2017). Moreover, *Aronia* berries can be used to make jams, jellies, sauce, toppings, gravy, syrup, icing, dietary supplements and to flavor and color yogurt and milk (Wolski et al., 2007). *Aronia melanocarpa* berries are used commercially to produce *Aronia* wine that is either purely made by *Aronia* berries or blended with other fruits and berries (Everhart, 2009). Black chokeberries are high in anthocyanins and are consumed as a health promoting beverage such as juices, teas or as an addendum to any beverage (Kokotkiewicz et al., 2010). *Aronia* berries must be

dried by fast and preservative drying methods to retain their antioxidant potential as well as to make them shelf-stable as exposure of black chokeberries to 60 °C for 8 h decreased the anthocyanins levels by 30 % and antioxidant potential by >50 % (Kasparaviciene and Briedis, 2003). Factors such as cultivar, genotype, geographical location, cultivation techniques, ripening stage, processing, and storage have a bearing on the total phenolic compounds of *Aronia* berries. The optimum storage temperature for chokeberries is 3 °C, however, the total polyphenolic content starts decreasing after six months at this temperature (Misiak and Irzyniec, 2009). According to an interesting study, flavone-rich Baikal skullcap (*Scutellaria baicalensis*) root is a suitable addition during black chokeberry juice production to stabilize the color and anthocyanins, a process known as co-pigmentation (Kokotkiewicz et al., 2010; Oszmianski, 2002;). *Aronia* berries can be used as an additive to jams to enhance their taste, color, and nutritional values (Scott and Skirvin, 2007). It has also been used as a food colorant (Oszmianski, 2002; Plocharski et al., 1989).

At FVSU, situated in middle Georgia, USA, we are screening this multipurpose plant for fruit production and a potential source for generating revenue and invigorating the rural economy. There has been research related to phytochemical profile of berries and biomedical research but very little research on the micromorphology of this genus is available that could have immense bearing as a taxonomy tool, on the adoption of the plant in different climates, improved physiology and identifying suitable candidates for breeding program (Figure 1). We present light, fluorescent, and scanning electron microscopic studies on micromorphology and staining for secondary metabolites in trichomes using established protocols (Vaidya et al., 2013, 2016). We are also studying reproductive biology of plants that will be helpful in future plant breeding programs to improve cultivars for more southern climate.

Micromorphology. Trichomes are unicellular or multicellular appendages originating from epidermal cells (Werker, 2000). Light, fluorescent, and scanning electron microscopy revealed the presence of trichomes on the serrated margins of the leaves and midveins of the adaxial surface (Figure 1B-D). Leaf margin is serrated and each denticle contains sessile trichome that are 200-300 micron long (Figure 1 I, J, and L). These are long, unicellular curved trichomes (Figure 1 I, J). These trichomes are connected with veins that widen towards margin, probably supplying water and secretory substances (Figure 1 K). A cleared and safranin stained trichome is visible in Figure 1J which implies the presence of lignified cells in the trichome and sculpturing on the trichome is evident in the inset picture. Similarly sculptured but longer trichomes (500-700 micron) were also found on the midvein on the adaxial surface of the leaf (Figure 1 B, C). Clearing and staining with safranin further shows the presence of long, thin trichomes on the abaxial surface of the leaf. Thin trichomes are up to 2 mm long (Figure C, E-H). Staining with DPBA, showed presence of secondary metabolites in all three types of trichomes (Figure 1 D, H, L). The trichomes on the midvein and leaf margin exhibited green fluorescence with DPBA staining (Figure 1D, L) which might be attributed to its differential binding with flavonoid compounds with a spectra ranging from green to yellow (Peer et al., 2001).

Antioxidant potential. Biochemical evaluation of total polyphenols, total flavonoids, and total antioxidant capacity revealed higher levels of antioxidants in *A. melanocarpa* dried berries than dried leaves (Table 1). Nevertheless, overall antioxidant potential was found high in *A. melanocarpa* tissues. Total polyphenolic content (TPP) of dried berries (1401.32 mg/ g GAE) was significantly higher when compared to that of dried leaves (765.63 mg/ g GAE). The total flavonoid content for dried leaves and berries is 96.16 µg/mL and 128.39 µg/mL, respectively (Table 1). Similar pattern was evident for the total flavonoid content as well. TROLOX equivalent antioxidant capacity (TEAC) of dried *Aronia* berries (2382.03 µM/ g) was also higher than dried leaves (1610.61 µM/ g).

Strong correlations have been observed between total polyphenol and total flavonoid contents in our earlier studies (Soundappan et al., 2018; Vaidya et al., 2013, 2019).

In many parts of the world, people are malnourished and thus suffer from multiple nutrient-deficiency disorders and this usually stems from ignorance about importance of fruits and vegetables in their diets. Lack of knowledge about surrounding biodiversity is usually main factor in inadequate harnessing of resources. A wide gap is thus formed between health and optimal use of natural sources of nutrients that can be filled up with the consumption of underutilized crops. These crops have advantages like easier to grow and hardy in nature, and production under adverse soil and climatic conditions. It is also an established fact that seasonal, locally available, and cheap fruits and vegetables can help population stay healthy and nutritionally fortified instead of consuming costly off-season ones. Further, the underutilized crops have the potential to provide economic security by giving employment and by fetching good returns from their sale in raw form as well as value-added products. *Aronia melanocarpa* is one of the lesser known and underutilized fruits though it has been known to have highly nutritional and bioactivity profiles. It is one of the richest plant sources known for phenolics such as procyanidins and anthocyanins. Substantial research in the past suggest that a positive correlations between higher amounts of TPP, TEAC, and flavonoid content can be associated with potential medicinal property of a plant extract (Nijveldt et al., 2001; Vaidya et al., 2013). Many herbs like peppermint (*Mentha* x *piperita*), rosemary, sage (*Salvia* spp.), spearmint (*Mentha spicata*), thyme (*Thymus* spp.), and many species of *Scutellaria* exhibit high TPP and TEAC and possess anti-tumorigenic and other medically desirable constituents (Parajuli et al., 2011; Vaidya et al., 2013; Yi and Wetzstein, 2010, 2011). A comparison of TPP, flavonoids, and TEAC values of *Aronia* berries and leaves with culinary herbs and medicinal plants indicates higher values in the case of former, suggesting more in-depth studies are required. Modern pharmacological research has presented chokeberry as a plant with multiple health promoting activities. Its biological activities include but are not limited to antioxidative, antimutagenic, cardioprotective, anti-cancer, anti-inflammatory etc. A recent study on the role of *A. melanocarpa* juice concentrate (AJC) supplementation in mice raised on high-fat diet exhibited its potential to prevent weight gain (Baum et al., 2016). There is no scientific study conducted as yet that supports the use of *A. melanocarpa* by Native Americans to treat cold. Some studies have hypothesized about the ability of *Aronia* berries to counteract the unfavorable effects of the exposure to xenobiotics and this is an area that needs to be explored in detail. It is proposed that further studies are required to confirm the effectiveness of *A. melanocarpa* constituents and decipher the mechanism underlying its beneficial action. It is critically important to analyze the antioxidant potential of the dried product because drying is one of the most commonly used post-harvest techniques in industrial processing of green biomass and greatly affects various bioactive compounds qualitatively and quantitatively (Roshanak et al., 2016). Different drying methods affect the plants at various levels; physiologically and biochemically, hence, it is imperative to find a suitable drying method for *A. melanocarpa* leaves and berries to retain its maximum antioxidant potential and make it shelf stable (Kasparaviciene and Briedis, 2003). In addition, many *Aronia* berry products are already available in the market in the form of pills and capsules but are no scientific studies that establish the recommended intake of *Aronia* berry products. *Aronia* berry and leaves promise an important role in the area of 'green cosmetics', various food items, as a landscaping shrub, possible development of tea, and herbal supplements. All these activities can be successfully integrated to support rural 'bioeconomy'

Table 1. Total polyphenol and flavonoid content and resultant antioxidant capacity measurement of *A. melanocarpa* dried leaves and ripe berries (the data is presented as the mean of three replicates).

Assays	Total Polyphenol Content (mg/g GAE)*	Total Flavonoid Content (µg/mL)	Total Antioxidant capacity measurement (TEAC assay, µmol TROLOX/g)
Dried leaf	765.63	96.16	1610.61
Dried berries	1401.32	128.39	2382.03

*mg/g Gallic Acid Equivalents

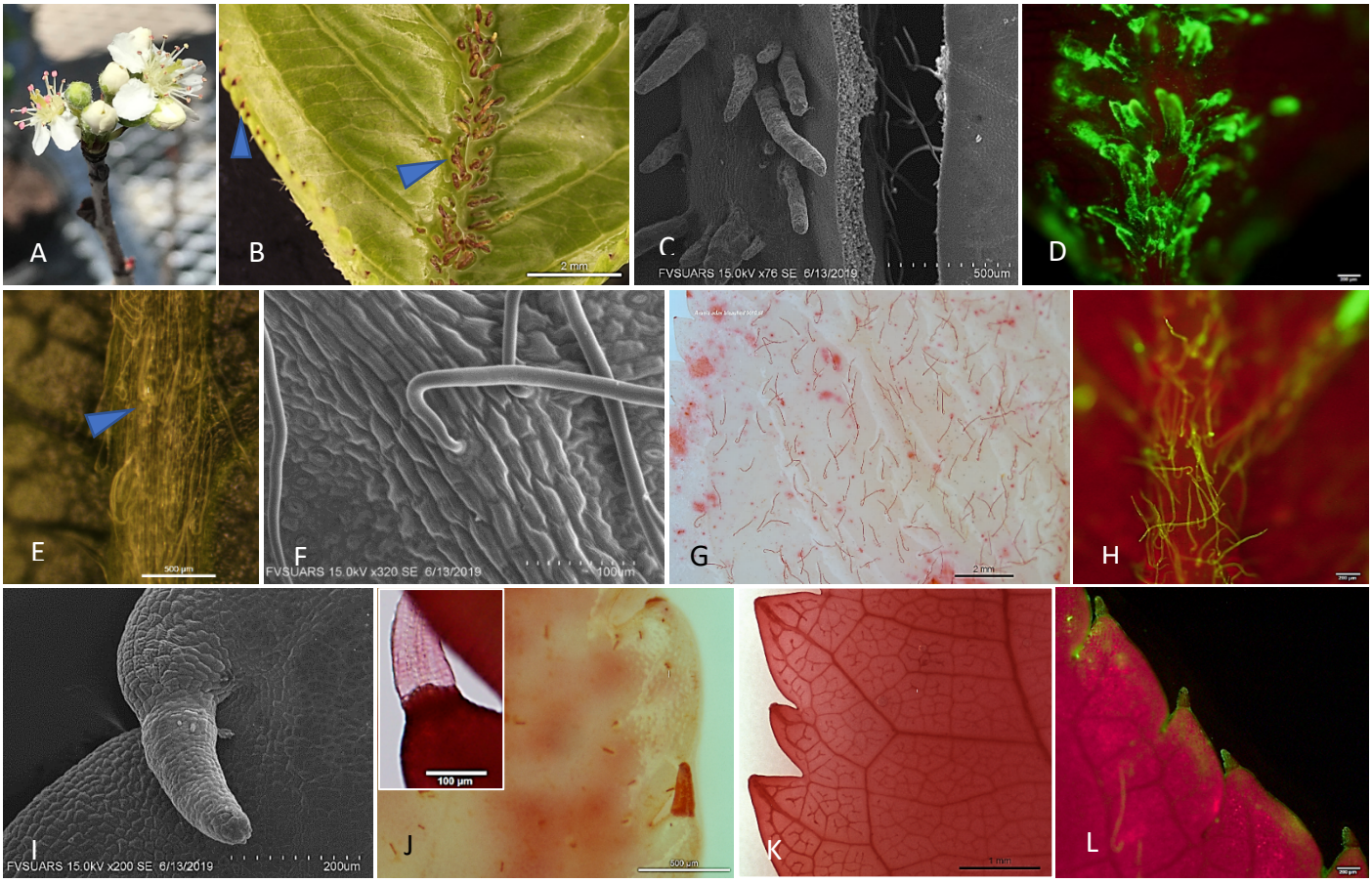

Figure 1. Micromorphological studies on *Aronia melanocarpa* cv. Viking. A. Flowering inflorescence in a greenhouse plant. B. *Aronia* leaf showing specialized trichomes on the adaxial midvein and serrated margin under light microscope. C. A fractured leaf under SEM to show thick, short trichomes on the adaxial and thin long trichomes on the abaxial surface. D. Fluorescent staining of adaxial trichomes present on the midvein for the presence of flavonoids, E- F. Long, thin, and smooth trichomes on the abaxial surface of the leaf under light, scanning electron microscope. G. Leaves were cleared and stained with safranin to reveal the structure of abaxial trichomes, H. Abaxial trichomes stained with DPBA, I. Magnified SEM view of trichomes on the leaf margin. J. Same as in I but in a cleared leaf, inset picture showing intricate sculpturing on the trichome surface, K. Cleared and safranin stained leaf margin showing veins ending at each serration with wide lumen, and L. Presence of secondary metabolites in trichomes present at the leaf margin. Arrows indicate three types of trichomes that are present on *Aronia* leaves.

Table 2. A summary of polyphenolic compounds and their quantitative range in chokeberries.

Compound	Content	Reference
Total Polyphenolic compounds	78.49 mg/g (DW)	(Oszmiański and Wojdylo, 2005)
	6.902 - 25.56 mg/g (FW)	
Proanthocyanidins	9.25 - 107 ± 6.6 mg/g (DW; catechin equivalent)	(Oszmiański and Wojdylo, 2005)
	6.64 mg/g	(Wu et al., 2004)
	3.55 ± 0.49 to 6.28 ± 0.55 mg/g (DW; epicatechin equivalent)	(Rugina et al., 2011)
Total anthocyanin	30.10 to 63.70 mg/g (FW)	(Seidemann, 1993)
	42.80 mg/g (FW)	(Zheng and Wang, 2003)
Cyanidin 3-galactoside	12.82 mg/g (DW)	(Oszmiański and Wojdylo, 2005)
Cyanidin-3-arabinoside	5.82 mg/g (DW)	(Oszmiański and Wojdylo, 2005)
Cyanidin-3-glucoside	0.42 mg/g (DW)	(Oszmiański and Wojdylo, 2005)
Cyanidin-3-xyloside	0.53 mg/g (DW)	(Oszmiański and Wojdylo, 2005)
	0.53 mg/g (DW)	(Malien-Aubert et al., 2001)
Kaempferol	0.0053 mg/g (FW)	(Oszmiański and Wojdylo, 2005)
Pelargonidin-3-arabinoside	0.023 mg/g	(Wu et al., 2004)
Pelargonidin-3-galactoside	trace	(Wu et al., 2004)
Flavanols		
Quercetin derivatives (sum)	0.71 mg/g (FW)	(Slimestad et al., 2005)
	1.01 mg/g (DW)	(Oszmiański and Wojdylo, 2005)
Querectin-3-galactoside	0.30 mg/g (FW)	(Zheng and Wang, 2003)
	0.37 mg/g (DW)	(Oszmiański and Wojdylo, 2005)
Querectin-3-glucoside	0.27 mg/g (FW)	(Zheng and Wang, 2003)
	0.22 mg/g (DW)	(Oszmiański and Wojdylo, 2005)
Querectin-3-rutinoside	0.15 mg/g (DW)	(Oszmiański and Wojdylo, 2005)
Other quercetin derivatives	0.27 mg/g (DW)	(Oszmiański and Wojdylo, 2005)
Total flavonoids	5.30 ± 0.8 mg/g (FW)	(Hwang et al., 2014)
	0.22 mg/g (DW)	(Oszmiański and Wojdylo, 2005)
Chlorogenic acid	3.00 mg/g (DW)	(Oszmiański and Wojdylo, 2005)
Neochlorogenic acid	2.90 mg/g (DW)	(Oszmiański and Wojdylo, 2005)

*DW = Dry Weight; FW: Fresh Weight

Table 3. A review of biomedical research testing various *Aronia* berry products for their bioactivity.

Medicinal property	Product	Experiment or test	Major findings
Anti-biofilm and Bacteriostatic activity	Fruit juice	*Aronia melanocarpa* extracts, subfractions and compounds tested	Several *Aronia* substances possess anti-biofilm activity, however, they were not inhibitory to the growth of *Escherichia coli* and *Bacillus cereus* (Bräunlich et al., 2013)
Hepatoprotective activity	Fruit juice	Rats with CCl_4-induced liver damage	Reduced histopathological changes such as necrosis, ballooning, degeneration, and inflammatory infiltration of lymphocytes in the liver (Valcheva-Kuzmanova et al., 2004)
Anti-inflammatory activity	Fruit extract	Effect of black chokeberry extract on rat paw swelling.	Significantly reduced swelling in rats evoked by administration of histamine or serotonin solutions (Borissova et al., 1994)
Antidiabetic activity	Fruit and leaf extract	Animal models with *in vitro* induced diabetes	Glucose level-reducing effect in rats with streptozotocin-induced diabetes (Jankowski et al., 1999)
Antidiabetic activity	Black chokeberry juice	human trials	Daily ingestion of black chokeberry juice over a 3-month period resulted in substantially lowered blood glucose levels in patients with non–insulin-dependent diabetes (Simeonov et al., 2002)
Hypotensive effect	Fruit extract	Treatment of hypertensive rats with commercial *Aronia* extract (*aronox*)	Lower systolic blood pressure compared to the control group of rats (Park and Park 2011; Hellstrom et al., 2010)
Cardioprotective effects	Fruit juice	Treatment of hyperlipidemia in rats	Reduced the dietary-induced elevation of plasma total cholesterol, LDL cholesterol and plasma lipids (Valcheva-Kuzmanova et al., 2007)
Anti-aggregatory	Commercial dried extract	Application on platelets of patients with cardiovascular risk	Produced anti-aggregatory effect on the Anti-aggregatory effect on human platelets (Ryszawa et al., 2006)
Radioprotective	Extract	Rats with experimentally induced radiation illness	Survival rate of γ-irradiated rats fed with chokeberry extract significantly increased (Andryskowski et al., 1998)
Antimutagenic	Dried extract	Ames test	Mutagenic activities of benzo[a]pyrene and 2-aminofluorene were significantly reduced in the presence of anthocyanins isolated from black chokeberry fruits (Gasiorowski et al., 1997)
Anti-cancer activity	Commercial extract	Experiment with human colon tumor HT29 clone 19A cells (Comet test)	H_2O_2-induced DNA strand breaks were significantly reduced in the presence of *A. melanocarpa* extract (Pool-Zobel et al., 1999)

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