Bioactive compounds of *Aronia melanocarpa* berries and their potential anti-inflammatory and anti-atherosclerotic activity.

The main goal of our project is to find out, which constituents of *Aronia melanocarpa* berries are responsible for their biological activity. The correlation between chemical composition, antioxidant properties and anti-inflammatory activity in human endothelial cells with the support of advanced statistical analysis will be studied. We expect that the standardized formulations, prepared on the basis of our results, combined with a balanced diet, can be widely used for the prevention of cardiovascular diseases and diabetes type 2.

Aronia (chokeberry, Aronia melanocarpa) is a valuable fruit, now gaining popularity all over the world, combining the health benefits of green tea and red wine. A native of North America, it appeared in Poland in the '70s. Over several decades, an industrial variety was bred (Nero, Galicjanka/Eggert) with simultaneously ripening, juicy berries suitable for machine harvesting. As a cultivated species, aronia has little requirements, is resistant to diseases, pests and frost. It is recommended for organic production; hence, many aronia farms in Poland are ECO certified. Because raw fruits have an astringent taste, aronia berries are usually consumed in the form of products, such as juices, jams, juice concentrate or wine, having a beautiful, dark-red color. Unfortunately, it is difficult to find aronia juice in Polish shops, almost all production is exported. This Polish treasure is underestimated as a support of healthy diet &lifestyle of EU citizens. **Aronia products acting as "Polish paradox" could lower the risk of cardiovascular diseases, like red wine and known "French paradox"**.

Aronia has a unique chemical composition - a variety of valuable healthy polyphenolic compounds with strong antioxidant properties. These include: anthocyanins, catechins, procyanidins, phenolic acids (e.g. hydroxycinnamic acids, chlorogenic acid). Aronia berries can contain up to 10-20 g per 1 kg of polyphenols, including approx. 5g anthocyanins. [1]. This is one of the richest sources of anthocyanins in nature, that gives the fruits very intense, dark color. Polyphenolic antioxidants prevent atherosclerosis, improve microcirculation, reduce the fragility of blood vessels and exhibit anti-aggregative activity. Administration of aronia anthocyanins significantly lowers blood pressure and reduces oxidative stress [2, 3]. There was also found that Aronia extracts have regulated the concentration of several biomarkers, e.g. reduced serum total cholesterol, LDL cholesterol and triglycerides in patients with metabolic syndrome. Anthocyanins also have antimutagenic and radioprotective properties [4].

An important group of polyphenols are tannins. Although their high content in aronia fruits causes the astringent taste, but it makes them resistant to decay, so berries can be stored for relatively long time after harvesting. These compounds have the ability to bind metals and toxic alkaloids and showed anti-inflammatory properties. Oligomeric procyanidins exhibit antioxidant and antiradical activity, stronger than that of vitamin C and E. Procyanidins have antimicrobial, antiviral, anti-inflammatory, and anti-hypertensive properties. They play the protective role in the development of atherosclerosis, cardiovascular disease, diabetes type-2, and also improve platelet aggregation [5].

Phenolic acids are another group of antioxidants found in aronia berries. In comparison with other berries, Aronia contains significant amounts of biologically active hydroxycinnamic acids: chlorogenic acid and its isomer neochlorogenic acid. Chlorogenic acid can be found also in coffee, cocoa beans, blackcurrants, mulberries, and others. It exhibits strong antiradical, antioxidant and anti-inflammatory properties. Chlorogenic acid delays the absorption of glucose into the blood. Phenolic acids show relatively high bioavailability in human organism.

Usually, biological tests in vitro and in vivo were performed using juice or fruit extracts (administered to patients). Numerous studies were made for Aronox (currently not produced) descibed as "anthocyanins of aronia". However, it contained 25% anthocyanins, 50% oligomeric procyanidins and 9% phenolic acids [3]. **According to our knowledge, there is no study on single compound or groups of compounds present in aronia in relation to particular biological effect. In every studied preparation there was a complex mixture.** Inconsistent results of many clinical studies are due to the lack of standardization of plant extracts.

The novelty of our project: we would like to demonstrate which of the substances found in aronia berry are responsible for anti-inflammatory and anti-atherosclerotic activity, using modern analytical (NMR) and statistical methods (chemometrics).

We plan to not only examine the chemical compounds found in berries at the time of harvesting, but also **to determine their variability during ripening** (i.e. from green to frostbitten fruit), as well as to access the aspect of geographical location of the farm. The extracts and juices will be prepared, and analyzed using 1H/ 13C nuclear magnetic resonance (NMR) and high performance liquid chromatography methods (HPLC). Analysis by HPLC enables the separation of mixtures into individual components, their identification and quantification. The aim of NMR measurements is to determine the profile of bioactive compounds in the mixture, the identification of single compounds and spectra assignment. All samples will be also tested for their antioxidant properties with: ORAC (Oxygen Radical Absorbance Capacity) and radical scavenging ability test using the method of electron paramagnetic resonance (EPR). The advantage of EPR measurements is that all samples can be examined regardless of their density, opacity, color, whereas UV/Vis spectrophotometry requires clear liquid samples.

The results of HPLC, NMR and the antioxidant parameters will be used as an input data for the chemometric analysis. Chemometrics is applied to multivariate analysis of large data sets, its objective is to find the relationship between the variables using mathematical and statistical approach. In our research, linking results of NMR, HPLC, EPR with chemometric methods (principal component analysis (PCA), partial least squares method (PLS) and neural networks) enable to find the correlation between chemical composition and antioxidant properties, and identify the most active compounds.

An important part in our project will be **the study on the anti-inflammatory effect of compounds present in aronia berries on human endothelial cells**. Vascular cells play an active role in the biology of blood vessels, including processes such as the clotting of blood, blood pressure regulation and inflammation [5]. Malfunctioning of these cells is typical for many vascular diseases, leading to atherosclerosis. In our study we will assess a protective effect on the endothelium, e.g. inflammatory and antiplatelet activity of compounds of Aronia berries. Chemometric analysis helps correlating the chemical composition of the samples with their effects on epithelial cells and as a final result - the selection of samples containing the most biologically active substances.

[1] Oszmiański J., Wojdyło A. Aronia melanocarpa phenolics and their antioxidant activity. Eur. Food Res. Technol. 2005; 221:809–813. [2] Broncel M., i in. Wpływ antocyjanin z aronii czarnoowocowej na ciśnienie tętnicze oraz stężenie endoteliny-1 i lipidów u pacjentów z zespołem metabolicznym. Pol. Merk. Lek. 2007; 134: 116–119. [3] M. Naruszewicz, i in. Combination therapy of statin with flavonoids rich extract from chokeberry fruits enhanced reduction in cardiovascularrisk markers in patients after myocardial infraction (MI). Atherosclerosis 2007, 194: 179–184. [4] K. Gąsiorowski, K. i in., Antimutagenic activity of anthocyanins isolated from Aronia melanocarpa fruits, Cancer Lett. 1997: 119 37-46. [5] Zapolska-Downar D.i in. Aronia melanocarpa fruit extract exhibits anti-inflammatory activity in human aortic endothelial cells. Eur. J. Nutr. 2012, 51: 563-572.