

## 2001 MAFMA Final Report

Project Title **Bioactivity and active compounds from aronia and elderberry fruits of commercial value**

PI **David Seigler**

Co-PI **Mary Ann Lila (M. A. L. Smith)**

Academic  
Institution **University of Illinois**

Award Date **10 January 2001 – 30 June 2003**

### 1. Objective Summary

We proposed to establish the identity of the natural products from fractions and mixtures of bioflavonoid-rich aronia and elderberry fruits that prove active in anticancer assays. Identity of the components of these fruits was to be established by bioassay-guided fractionation. Our bioassays were to be carried out in collaboration with Dr. John Pezzuto of the University of Illinois Chicago.

### 2. Objective Accomplishments

It is now widely known that many fruits and vegetables contain natural phytochemical compounds with antioxidant, antimicrobial, anti-inflammatory, cardioprotective, and cancer-chemopreventive properties. Berry fruits are particularly rich in flavonoid compounds (including anthocyanins and proanthocyanidins) although other secondary compounds (iridoid glycosides, sesquiterpenes, triterpenes, and phytosterols) from berries also have demonstrated ability to counteract or interfere with the progress of many chronic disease conditions.

In the work supported by MAFMA, we have investigated the compounds responsible for biological activity, in particular cancer chemoprevention, in a species of elderberry native to Europe (*Sambucus nigra*) and one native to North America (*Sambucus Canadensis*) and both domestic and European cultivated chokeberries or aronia fruits. We were able to fractionate and to find active fractions of both aronia and elderberry in bioassays for anti-inflammation assays (COX-2 and COX-1 inhibitory activity), assays for induction of phase 2 enzymes (QR inhibition), and assays for cancer chemoprevention (ODC), and to fractionate these mixtures.

Locally collected *Sambucus canadensis* (elderberry) was extracted and the extract fractionated over Toyopearl. One of these fractions (3) inhibited the COX-2 assay (inhibition stage of carcinogenesis) and another (4) led to the induction of phase two enzymes (QR assay) (t also inhibition stage of carcinogenesis) . These two Toyopearl fractions were combined and again fractionated over Silica Gel 60 by vacuum chromatography. Fractions 5-13 proved active in the COX-2 assay and fractions 7 and 8 in the QR assay. Fraction 8 had borderline activity in an assay evaluating the promotion stage of cancer (ODC) assay. Of these, fractions 5-13 were combined and fractionated by MPLC on a reversed phase (RP-18) column. In this final fractionation, Fractions 2 and 3 (QR), fractions 3 and 8 (ODC), and fractions 4 and 5 (COX-2) all demonstrated bioactivity.

European elderberry, *Sambucus nigra* (Artemis International Inc., cultivated in Austria), was extracted and fractionated over Toyopearl as above. One fraction (3) was active in the COX-2 assay and a second (4) was active in the QR assay. Fraction 3 was again fractionated on Silica Gel 60 by vacuum chromatography. Fractions 5-14 were active in the COX-2 assay and fractions 9-12 and 14 were active in the QR assay. Fractions 5-14 were combined and again fractionated, this time by MPLC on a silica gel column. Fractions 4 and 5 proved active in the COX-2 assay. The results of QR assays are not yet available.

Local collections of *Aronia melanocarpa* (chokeberry, Iroquois Co., Illinois) were collected, fractionated by vacuum chromatography on Silica Gel 60 and assayed for bioactivity. One fraction proved active for inhibition of the COX-2 system (16). Another group of fractions was active in the quinone reductase system (QR) (17-18). Fractions isolated in the same manner from European *Aronia melanocarpa* fruits (Artemis International Inc.) were also inhibitory for the COX-2 system (12, 15-17). Interestingly, none of the fractions active in the COX-2 assay were active in the COX-1 system.

Although we have followed the biological activity through three stages of purification for *Sambucus* species and for two stages for *Aronia*, we have not isolated and characterized pure compounds responsible for this activity. This work is currently in progress and it appears likely that we will reach this goal in the coming year.

One of the complications that we encountered was the timely performance of bioassays in the laboratory with which we were collaborating. In one instance, the ODC assays, there were extensive delays because of reasons beyond the control of either laboratory. In future work, we plan to conduct as many of these assays as possible in house to avoid these problems.

### **3. Practical impacts of research efforts.**

#### **a. Short Term Impacts.**

The types of biological activity found in both *Aronia* and *Sambucus* species confirm the value that these fruits can have as functional foods for humans. Although specific compounds responsible for the activity remain to be fully characterized, this work provides support for recommending these fruits for their health-giving properties.

#### **b. Long Term Impacts.**

Based on this diverse base of anecdotal, epidemiological, and laboratory-based experimental evidence, the black elderberry is well established as a medicinal species in Europe and has been the subject of intensive breeding research (to amplify anthocyanin content) and controlled cultivation in plantations harvested for both the pigment and purported health benefits of the berries. The native American species of elderberry, *Sambucus canadensis*, has not been cultivated, bred, and promoted as a medicinal plant like its better-characterized European cousin. Most native elderberry currently exists in the wild, although it has been incorporated to some extent into landscape settings as a plant which attracts birds and other wildlife, and the fruits of this species have also been used in wines, jams, and pastries. Both species contain bioactive compounds of several types, and should be useful as functional foods in this country. Although

the European *Sambucus nigra* has been introduced and cultivated, the American species *Sambucus canadensis* has much promise as a domestic food crop for the future.

**Publications resulting from this research.**

**Thole, J.M., T.F.B. Kraft, L.A. Sueiro, H. Kang, J.J. Gills, M. Cuendet, J.M. Pezzuto, D.S. Seigler, and M.A. Lila. 2006.** A comparative evaluation of the anti-cancer properties of European and American elderberry fruits. *Journal Medicinal Food* 9 (4), 498-504.