AGROFORESTRY IN ACTION

University of Missouri Center for Agroforestry

The First International Symposium on Elderberry

Symposium Proceedings

Scientific Program
Producers' Forum
Book of Abstracts

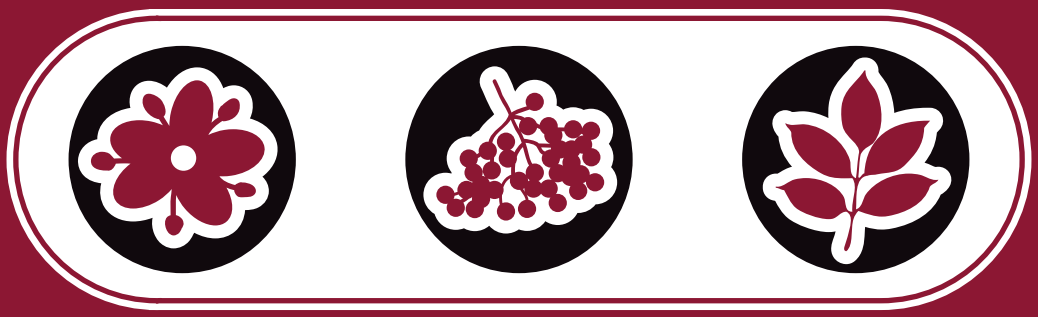
Columbia, Missouri, USA - June 9 - 14, 2013

Andrew L. Thomas, Denis Charlebois, C. Michael Greenlief, P. Leszek Vincent, Kevin L. Fritsche, Karl Kaack, *Editors*

centerforagroforestry.org

First International Symposium on

ELDERBERRY



PROCEEDINGS

Stoney Creek Inn • Columbia, Missouri USA
June 9-14, 2013

The First International Symposium on Elderberry

June 9-14, 2013 | Stoney Creek Inn | Columbia, Missouri, USA

Welcome to the First International Symposium on Elderberry (Sambucus), being held in Columbia, Missouri, USA, June 9–14, 2013. This is the world's first gathering of international scientists from multiple disciplines studying all aspects of the elderberry plant and fruit, and its use as a food and dietary supplement. Horticulturists, botanists, biochemists, food scientists, economists, and others are gathering in Missouri, USA during peak elderberry flowering season for several days of scientific exchange and fellowship. Together, we are raising elderberry to the scientific level it deserves. The Symposium is being organized under the auspices of the International Society for Horticultural Science, and research papers resulting from the Symposium will be published in a peer-reviewed stand-alone volume of Acta Horticulturae.

Andrew Thomas
Research Assistant Professor
University of Missouri – Southwest Research Center

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ORGANIZING COMMITTEE

University of Missouri (USA)
Andrew Thomas, Barbara Wills, Patrick Byers, Kevin Fritsche, Grace Sun, Dennis Lubahn, Mike Greenlief, Debbie Blaisdell, Leszek Vincent, Michael Gold, Jewel Coffman, Mihaela Cernusca, Julie Rhoads, James Quinn

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University of Aarhus (Denmark)
Karl Kaack

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Midwest Elderberry Growers Association (USA)
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Missouri Department of Agriculture (USA)
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Oregon State University (USA)
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University of Missouri, College of Agriculture, Food, and Natural Resources
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University of Missouri, Center for Botanical Interaction Studies*
University of Missouri, Food for the 21st Century
University of Missouri, Center for Agroforestry*
University of Missouri, Department of Biochemistry
University of Missouri, Mizzou Advantage
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Lincoln University*
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SURO*
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Rising Creek Nursery
Natures Organic Haven LLC
The Botany Shop*
Norm's Farms*
Riverhills Harvest*
Artemis International*
Wyldewood Cellars*

*Exhibitor Booth

Editors:

Andrew L. Thomas[1], Denis Charlebois[2], C. Michael Greenlief[3], P. Leszek D. Vincent[4], and Kevin L. Fritsche[5], Karl Kaak[6]

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SCIENTIFIC PROGRAM AT A GLANCE

Sunday, June 9, 2013
Stoney Creek Inn

5:00
Registration

6:00 – 7:30
Welcome Social

Monday, June 10, 2013
Stoney Creek Inn

7:15 – 9:00
Registration

8:30
Plenary Session & Keynotes
Salon A

10:00
Break

10:30
CONCURRENT SESSION 1:
Elderberry Botany and Ethnobotany
Lewis and Clark Room

CONCURRENT SESSION 2:
Elderberries and Human Health
Salon A

11:50
Lunch - Introduction of Sponsors
Salon C

1:00
CONCURRENT SESSION 3:
Elderberry Biochemistry
Salon A

CONCURRENT SESSION 4:
Elderberry Horticulture
Lewis and Clark Room

3:00
Break

3:30
CONCURRENT SESSION 5:
Workshop and Discussion on Approval of Elderberry Products by the European Safety Agency
Salon A

4:30
CONCURRENT SESSION 6:
Posters Session and Ice Cream Social
Salon B

5:30
Shuttles to MU Campus and Downtown Columbia; Dinner on own

Optional Walking Tour of University of Missouri Campus

Tuesday, June 11, 2013
Field Trips

Tour #1: 7:00 – 10:00
Missouri Botanical Garden (St. Louis), Watershed Farms (New Haven), Dinner Gala at Natures Organic Haven Farm (Hermann)

Tour #2: 8:30 – 10:00
University of Missouri Research Facilities (Columbia), Eridu Farms (Hartsburg), Stone Hill Winery (Hermann), Dinner Gala at Natures Organic Haven Farm (Hermann)

Option #3: 5:00 – 10:00
Dinner Gala only at Natures Organic Haven Farm (Hermann)

Wednesday, June 12, 2013
Stoney Creek Inn

8:00
Registration

8:50

SCIENTIFIC PROGRAM AT A GLANCE

Plenary Session
Salon A

10:00
Break

10:30
CONCURRENT SESSION 7:
Elderberry and Human Health
Salon A

CONCURRENT SESSION 8:
Elderberry Horticulture
Lewis and Clark Room

11:50
Lunch & Group Photo
Salon C

1:20
CONCURRENT SESSION 9:
Elderberry Horticulture
Lewis and Clark Room

1:30–3:00 & 3:30–5:30
CONCURRENT SESSION 10:
The Albert Y. Sun Memorial Colloquium: Berries and Brain Health
Salon A

3:00
Break

3:30
CONCURRENT SESSION 11:
Elderberry Marketing and Industry
Lewis and Clark Room

5:00–6:00
ISHS and Elderberry Working Group Business Meeting
Lewis and Clark Room

6:00–9:00
Closing Banquet: Scientists Meet the Farmers
Salon C

Awards and Recognition, Keynote Address

SCIENTIFIC PROGRAM

Sunday, June 9, 2013
Stoney Creek Inn

5:00 – 6:00
Registration open

6:00 – 7:30
Welcome Social
Music, hors-d'oeuvres, cash bar

Welcome addresses
Andrew Thomas, *University of Missouri*
Anson Elliott, *Missouri State University*
Patrick Byers, *University of Missouri*

Monday, June 10, 2013
Stoney Creek Inn

7:15 – 9:00
Registration

PLENARY SESSION

8:30 – 9:00
Welcome and Opening Remarks:
Rachel Mobley, *Missouri Department of Agriculture*
Dennis Lubahn, *Director, University of Missouri Center for Botanical Interaction Studies ("The Elderberry Center")*
Denis Charlebois, *Agriculture and Agri-food Canada*

9:00 – 9:30
Keynote Presentation
Ákos Máthé, *University of West Hungary (Hungary)*
The Versatile Elderberry: Research, Production, and Utilization

9:30 – 10:00
Keynote Presentation
Madeleine Bliah-Mumcuoglu, *Jerusalem (Israel)*
The Revival of the Elderberry

10:00 – 10:30
Break

CONCURRENT SESSION 1:
Elderberry Botany and Ethnobotany
Moderator: Patrick Byers

10:30 – 10:50
Wendy L. Applequist, *Missouri Botanical Garden (USA)*
An Elderberry by any other Name: Taxonomy and Nomenclature of* Sambucus nigra *sensu lato

10:50 – 11:10
Ivan Salamon, *Presov University (Slovak Republic)*
Elderberry (*Sambucus nigra *L.) – Natural Medicine from the Ancient Times or Protection against Witches in Middle Ages?

11:10 – 11:30
Frank Qiang Fu and **Wenchi Jin**, *Horticulture Institute of New Zealand (New Zealand)* and *University of Missouri (USA)*
Elderberry in China

11:30 – 11:50
Elizabeth Mudge, *British Columbia Institute of Technology (Canada)*
Flavonoid Content of Elderberry Collected in the Eastern United States

CONCURRENT SESSION 2:
Elderberries and Human Health
Moderator: Kevin Fritsche and Chi-Hua Lu

10:30 – 10:50
Glenn Jackson, **Dennis Lubahn**, et al., *University of Missouri (USA)*
Elderberry Juice Prevents Prostate Cancer* In Vitro *and in an* In Vivo *Mouse Model

10:50 – 11:10
Bjarne F. Knudsen and **Karl V. Kaack**, *EMPAS Consulting, and University of Aarhus, Aarslev (Denmark)*
Flowers of Elder (*Sambucus nigra*): Well-established and Traditional Human Disease Claims

SCIENTIFIC PROGRAM

11:10 – 11:30
Valentyna Lylo and **Irina Karpova**, *Ukrainian National Academy of Science (Ukraine)*
Lectins of* Sambucus nigra *in Regulation of Cellular DNA-Protective Mechanisms

11:30 – 11:50
Irina Karpova and **Valentyna Lylo**, *Ukrainian National Academy of Science (Ukraine)*
Lectins of* Sambucus nigra *as Biologically Active and DNA-Protective Substances

11:50 – 1:00 **Lunch**

Welcome
Tom Payne, *Dean, University of Missouri College of Agriculture, Food, and Natural Resources*

CONCURRENT SESSION 3:
Elderberry Biochemistry
Moderators: Susan McGuire and Grace Sun

1:00 – 1:20
Ivan Salamon, *Presov University (Slovak Republic)*
Extraction of Pure Anthocyanins from Fruits of* Sambucus nigra *L.

1:20 – 1:40
Hanzhi Wu, C. Michael Greenlief, et al., *University of Missouri (USA)*
Determination of Anthocyanin in a Variety of Elderberry Juices by UPLC/MS

1:40 – 2:00
Salvador, Ângelo, *University of Aveiro (Portugal)*
New Insights on the Elderberries and Elderflowers (*Sambucus nigra *L.) Metabolites

2:00 – 2:20
Chi-Hua Lu, et al., *University of Missouri (USA)*
Impact of Genotypes on American Elderberry Juice Chemistry and Anti-oxidant Capabilities

2:20 – 2:40
Wei Lei, Kevin Fritsche, et al., *University of Missouri (USA)*
In vitro Screening for Anti-inflammatory Bioactivity of Various American Elderberry Genotypes

2:40 – 3:00
Madeleine Bliah-Mumcuoglu, *Jerusalem (Israel)*
The Antiviral Effect of a Standardized Elderberry Extract

CONCURRENT SESSION 4:
Elderberry Horticulture
Moderators: Denis Charlebois and Sanjun Gu

1:00 – 1:20
Jill Bushakra and **Kim Hummer**, *USDA, Corvallis, Oregon (USA)*
Sambucus *Genetic Resources at the US National Clonal Germplasm Repository*

1:20 – 1:40
Ales Matejicek, et al., *Research and Breeding Institute of Pomology Holovousy Ltd. (Czech Republic)*
Elderberry Cultivars Growing in the Czech Republic

1:40 – 2:00
Michele Warmund, *University of Missouri (USA)*
Eriophyid Mites Inhabiting American Elderberry

2:00 – 2:20
Karl V. Kaack and **Bjarne F. Knudsen**, *University of Aarhus, Aarslev, and EMPAS Consulting (Denmark)*
Horticultural Production of Flowers and Berries of Elder (*Sambucus*) as Raw Material for Processing of Foods and Medicines (Pharmaceuticals)

SCIENTIFIC PROGRAM

2:20 – 3:00
Andrew L. Thomas, Abhinandya Datta, et al., *University of Missouri (USA)*
Occurrence of Polyphenols, Organic Acids, and Sugars among Diverse Elderberry Genotypes Grown in Three Missouri Locations

3:00 – 3:30
Break

3:30 – 4:30

SESSION 5:
Workshop and Discussion on Approval of Elderberry Products by the European Safety Agency
Bjarne Knudsen and Karl Kaack, *EMPAS Consulting and University of Aarhus (Denmark)*

4:30 – 5:30

SESSION 6:
Poster Session - Ice Cream Social

5:30
Dinner on your own and free evening
Optional walking tour of University of Missouri campus

Shuttles to Campus and Downtown Columbia, Restaurants

Tuesday, June 11, 2013
Field Trips

Tour #1: 7:00 am – 10:00 pm
Missouri Botanical Garden (St. Louis), Watershed Farms (New Haven), Dinner Gala at Natures Organic Haven Farm (Hermann)

Tour #2: 8:30 am – 10:00 pm
University of Missouri Research Facilities: Center for Translational Neuroscience, School of Medicine, and Animal Science Research Center (Columbia), Eridu Farms (Hartsburg), Stone Hill Winery (Hermann), Dinner Gala at Natures Organic Haven Farm (Hermann)

Option #3: 5:00 – 10:00
Dinner Gala only at Natures Organic Haven Farm (Hermann)

Wednesday, June 12, 2013
Stoney Creek Inn

8:00
Registration

PLENARY SESSION

8:50 – 9:00
Welcome

9:00 – 9:30
Keynote Presentation: **Sigrun Chrubasik**, *Institute of Forensic Medicine, and University of Freiberg (Germany)*
Pharmacological and Clinical Effects of Elderberry Fruit

9:30 – 10:00
Keynote Presentation: **Vivian Barak**, *Israeli Cytokines Standardization Laboratory, Hadassah-Hebrew University Medical Center, Jerusalem (Israel)*
The Effect of Sambucol on the Immune System (Cytokines Production)

10:00 – 10:30
Break

CONCURRENT SESSION 7:
Elderberry and Human Health
Moderators: Karl Kaack and Kevin Fritsche

10:30 – 10:50
Lars P. Christensen, *University of Southern Denmark (Denmark)*
Bioactive Compounds of Elder (Sambucus nigra L.) with Focus on their Potential Anti-diabetic Effects

10:50 – 11:10
Bjarne F. Knudsen and **Karl V. Kaack**, *EMPAS Consulting, and University of Aarhus, Aarslev (Denmark)*
Elderberries (Sambucus nigra): Human Health and Disease Claims

SCIENTIFIC PROGRAM

11:10 – 11:30
Vivian Barak and **Madeleine Mumcuoglu**, *Israeli Cytokine Standardization Laboratory, Hadassah - Hebrew University Medical Center, Jerusalem (Israel)*
Clinical Studies on the Effects of Sambucol - a Review

11:30 – 11:50
Sigrun Chrubasik, *Institute of Forensic Medicine, University of Freiburg (Germany)*
Usefulness of Elderberry Fruit as Functional Food

CONCURRENT SESSION 8:
Elderberry Horticulture
Moderator: Patrick Byers

10:30 – 10:50
Robert Martin, et al., *USDA, Corvallis Oregon (USA)*
Identification of Two Carlaviruses in Elderberry

10:50 – 11:10
Ivan Salamon, *Presov University (Slovak Republic)*
Some Aspects of Environmental Condition Determination and Quality of Special Crop Products (with the Elder Flowers) in Slovakia

11:10 – 11:30
Denis Charlebois, *Agriculture and Agri-food Canada, Quebec (Canada)*
Physicochemical Changes in American Elder (Sambucus canadensis L.) Fruits during Development

11:30 – 11:50
Denis Charlebois, *Agriculture and Agri-food Canada, Quebec (Canada)*
Micropropagation of Common Elder (Sambucus canadensis L.) with Emphasis on Disinfection of Explants

11:50 – 1:00
Lunch
Introduction of Sponsors

1:00 – 1:10
Group Photo

CONCURRENT SESSION 9:
Elderberry Horticulture
Moderator: Andrew Thomas

1:20 – 1:40
Bjarne F. Knudsen and **Karl V. Kaack,** *EMPAS Consulting, and University of Aarhus, Aarslev (Denmark):*
Guidelines for Pharmaceutical Qualities of Elder (Sambucus) Herbal Remedies with Human Disease Claims

1:40 – 2:00
Ahmed Hammad, *National Authority for Remote Sensing and Space Science (Egypt)*
The Color Management Classification of Several Fruits Using the Chromatic Pigments Pixels of the Epicarp - [OCR] Operation with Standard Gray Vision

2:00 – 2:20
Patrick L. Byers, et al., *University of Missouri (USA)*
'Marge': A European Elderberry for North American Producers

2:20 – 2:40
Ivan Salamon, *Presov University (Slovak Republic)*
Health from the Root to the Flower

2:40 – 3:00
Ivan Salamon, Presov University (Slovak Republic)
Environmental Determination of Heavy Metals, Radioactivity, and Pesticide Residues in Selected Raw Materials in Slovakia

3:00 – 3:30
Break, visit Sponsors

SCIENTIFIC PROGRAM

CONCURRENT SESSION 10:
The Albert Y. Sun Memorial Colloquium: Berries and Brain Health
Moderator: Dennis Lubahn

1:30 – 1:45
Dennis Lubahn, *University of Missouri (USA)*
Welcome and Brief Tribute to Albert Y. Sun (1932 – 2012)

1:45 - 2:10
Shibu Poulose, *USDA and Tufts University, Boston, Massachusetts (USA)*
Effect of Berries on Cognitive and Neurochemical Functions

2:10 – 2:35
Susan O. McGuire, *U.S. Veterans Administration, Hines, Illinois (USA)*
Neuroprotection by Berry-enriched Diets: Panacea or Methodological Conundrum?

2:35 – 3:00
Grace Sun, et al., *University of Missouri (USA)*
Probing Action of Botanical Polyphenols on Oxidative and Inflammatory Signaling Pathways in Microglial Cells

3:00 – 3:30
Break

3:30 – 3:55
Dennis Chuang, et al., *University of Missouri (USA)*
Protective Effects of Elderberry against Cerebral Ischemic Injury

3:55 – 4:20
Jennifer M. Walker, et al., *University of Missouri (USA)*
Beneficial Effects of Botanical Polyphenol Diets and Exercise on Behavior in Alzheimer's Disease Mouse Models

4:20 – 4:45
Zezong Gu, *University of Missouri (USA)*
Quantitative Proteomic Analysis for the Action of Botanicals on Nitrosative-oxidative Stress Signaling

4:45 – 5:30
Discussion

CONCURRENT SESSION 11:
Elderberry Marketing and Industry
Moderator: Michael Gold

3:30 – 4:00
Jan Mills, *Artemis International (USA)*
An Industry's Perspective on Elderberry

4:00 – 4:30
Mihaela Cernusca, et al., *University of Missouri (USA)*
Breaking Down Market Barriers for Elderberry Growers and Producers

4:30 – 5:00
Chung-Ho Lin, et al., *University of Missouri (USA)*
Exploring the Health Benefits and Economic Opportunities of the Bioactive Phytochemicals - An Overview of Phytochemical Research at the University of Missouri Center for Agroforestry

5:00 – 6:00
ISHS and Elderberry Working Group Business Meeting

6:00 – 9:00
Closing Banquet: Scientists Meet the Farmers
Moderator: Leszek Vincent

Awards and Recognition

Keynote Address: **Karl Kaack**, University of Aarhus, Aarslev (Denmark)
Exchanging Elderberry Knowledge among Farmers and Scientists

ELDERBERRY PRODUCERS' FORUM

Thursday, June 13, 2013 Stoney Creek Inn, Columbia, Missouri				
7:15	**Registration**			
8:00	**Plenary Sessions: Elderberry Around the World** Salon A			
	Presenter		**Title**	
9:00 - 9:30	**Jan Mills**, *Artemis International*		**An Industry Perspective on Elderberry**	
9:30 - 10:00	**Karl Kaack**, *University of Aarhus*		**How we Grow, Process, and Market Elderberries in Europe**	
10:00 - 10:30	**Denis Charlebois**, *Agriculture and Agri-food Canada*		**Did You Say Elderberry? Some Pitfalls to Avoid**	
10:30 - 11:00	**Morning break -- visit vendors**			
11:00 - 12:00	**Sandor Pethes** (Hungary) and **Stefan Lampl** (Austria), *European Elderberry Grower Cooperatives*		**Panel discussion: How Elderberry is Grown and Marketed in Europe**	
12:00 - 1:30	**Lunch -- visit vendors** Salon C			
	Track I: Elderberry Production Salon A		**Track 2: Elderberry Marketing and Development** Lewis and Clark	
	Presenter	**Title**	**Presenter**	**Title**
1:30 - 2:00	**Terry Durham**, *River Hills Producers*	**Elderberry Production in Missouri**	**Ina Cernusca**, *University of Missouri Center for Agroforestry*	**Elderberry Marketing**
2:00 - 2:30			**Michael Gold**, *University of Missouri Center for Agroforestry*	**Elderberry Financial Decision Making Tool**
2:30 - 3:00	**Patrick Byers**, *University of Missouri*	**Elderberry Cultivars**	**Misha Kwasniewski**, *Enology Program Leader, University of Missouri*	**Opportunities and Challenges in Production of Wines from Elderberries and Other Fruits**
3:00 - 3:30	**Afternoon break -- visit vendors**			
3:30 - 4:00	**Joe Wilson**, *Sano Springs*	**Our Experiences with Growing Elderberries in West-central Missouri**	**Rodger Lenhardt**, *Norm's Farm, North Carolina*	**Elderberry Marketing in North Carolina**

ELDERBERRY PRODUCERS' FORUM

4:00 - 4:30	**Michele Warmund**, *University of Missouri*	**Elderberry Rust Disease**	**John Brewer**, *Wyldewood Cellars, Kansas*	**Considerations for Sourcing Elderberry Juice- Europe vs. North America**
4:30 - 5:00	**Sebastian** and **Cathy Hanus**, *Nebraska*	**Mycorrhizae in Elderberries**	**Sylvain Mercier**, *SURO*	**SURO: How We Market Elderberry Products Across Canada**
5:00 - 5:30	**Patrick Byers**, *University of Missouri*	**'Marge' European Elderberry for North America**	**Terry Durham**, *River Hills Producers*	**Becoming a River Hills Harvest Producer**
5:30 - 6:00	**Conclusion / Discussion**			
6:00 - 6:30	**Meeting of Midwest Elderberry Producers** Salon A			

Friday, June 14, 2013
Eridu Farms, 19010 S. Mackie Lane, Hartsburg, Missouri*

8:30 - 12:00	**Rotating Workshops**	
	Presenter	**Title**
	Michele Warmund, *University of Missouri*	**Elderberry Insects, Mites, and Diseases**
	Jacob Wilson, *Lincoln University, Missouri*	**Managing Japanese Beetles in Elderberry**
	Joe Klinefelter, *Eridu Farms*	**Elderberry Field Equipment and Post-harvest Handling of Fruit**
	Terry Durham, *Eridu Farms*	**Elderberry Orchard Tours**
	Lupé Rios, *Forrest Keeling Nursery*	**Elderberry Propagation**
12:00 - 3:00	**Lunch, Networking, Music**	

*Maps to Eridu Farms are available at the conference registration desk.

LIST OF POSTERS

Poster Session Monday
4:30 – 5:30

Milena Vespalcova, et al., *Institute of Food Science and Biotechnology, Brno University of Technology (Czech Republic)*
Elder (Sambucus nigra L.) as a Source of the Flavonoid Rutin

Michael Traub, et al., *Lokahi Health Center, Kailua Kona, Hawaii (USA)*
Pilot Study on the Use of European Elderberry for Prevention of Influenza

Penelope Perkins-Veazie, **Andrew Thomas**, et al., *North Carolina State University, Kannapolis, North Carolina (USA), University of Missouri (USA)*
Fruit Composition of Elderberry (*Sambucus canadensis *and* S. nigra*) Genotypes Grown in Oregon and Missouri, USA

Andrew L. Thomas, et al., *University of Missouri (USA)*
Horticultural Performance of Eight Promising American Elderberry Genotypes at Three Missouri Locations

Ahmed Hammad, *National Authority for Remote Sensing and Space Science (Egypt)*
The Color Management Classification of Several Fruits Using the Chromatic Pigments Pixels of the Epicarp - [OCR] Operation with Standard Gray Vision

Patrick L. Byers, et al., *University of Missouri (USA)*
Effect of Season, Genotype, and Tissue Type on American Elderberry Leaf Elemental Status

Hui Zhou, **Zhe Qu**, et al., *University of Missouri (USA)*
Quantitative Proteomic Analysis for the Effects of Elderberry on Global Cerebral Ischemia in Mice

Jaime Piñero and **Jacob Wilson**, *Lincoln University, Missouri (USA)*
Mass Trapping: A Potential Organic Management Option for the Japanese Beetle (Coleoptera: Scarabaeidae)

Mitch Johnson, et al., *University of Missouri (USA)*
Methods for Profiling and Quantification of Anthocyanin and other Polyphenols Present in Elderberry Juices

Dennis Y. Chuang, et al., *University of Missouri, Center for Botanical Interaction Studies (USA)*
Protective Effects of Elderberry and Sutherlandia against Cerebral Ischemic Injury

Jiri Kaplan, et al., *Research and Breeding Institute of Pomology Holovousy Ltd. (Czech Republic)*
Evaluation of Fruit Quality of Elderberry Cultivars

Note: Poster presentations indicated by (P) after title in following abstracts

ELDERBERRY BOTANY AND ETHNOBOTANY

An Elderberry by any other Name: Taxonomy and Nomenclature of *Sambucus nigra* sensu lato

Wendy L. Applequist

Missouri Botanical Garden, St. Louis, Missouri, USA

The taxonomy and nomenclature of *Sambucus nigra* and related species will be briefly reviewed. Traditionally, the European elderberry, the American elderberry of the eastern U.S., and the Western blue elderberry have been treated as three different species, all of which are used as food and medicine. In 1994, Bolli published a revision in which all three, as well as other South American and island taxa, were treated as subspecies within a greatly expanded *Sambucus nigra*. Though the taxonomic characters used in that revision did not show enough consistent variation to distinguish these taxa at a species level, growers may observe variation in habit and vigor that suggests significant genetic differentiation, and chemistry also differs among taxa. Since the decision to distinguish taxa at the species or subspecies level is to some extent subjective, neither classification can be seen as definitive, and either may be used according to author preference. Literature using *S. nigra* without qualification should make clear whether it is used in the traditional sense or sensu Bolli; non-European populations should never be referred to as "*S. nigra*" without a following subspecific epithet. The legal requirement in the U.S. dietary supplement market that these three taxa be referred to by three different Standardized Common Names is unaffected by the classification chosen.

Elderberry Germplasm (*Sambucus* spp.) in Iran: Distribution and Applications in Iranian Traditional Medicine

Mohammad Hasan Assareh[1]*, Mohsen Naseri[2], Kianoush Nikoumanesh[3] , Mahmoud Mohammadi[1] , Ali Ebrahimi[4]

[1]Department of Natural Resources Biotechnology, Research Institute of Forests and Rangelands, Tehran, Iran.
[2]Traditional Medicine Clinical Trial Research Centre, Shahed University, Tehran, Iran.
[3]Agriculture Biotechnology Research Institute of Iran (ABRII), Seed and Plant Improvement Institutes Campus, Mahdasht Road, 31535-1897 Karaj, Iran.
[4]Agricultural Research and Education Organization (AREO), Tehran, Iran
* Corresponding Author: asareh@rifr-ac.ir

Key words: geographical distribution, botanical characteristics, Iranian traditional medicine

Elderberry (*Sambucus* spp. L.) is a genus of between 5 and 30 species of shrubs, small trees or herbs in the Caprifoliaceae or Adoxaceae family. The genus is native to temperate and subtropical regions of both northern and southern hemispheres. Iran, due to a diverse variability in geographical regions and climates, has been known as one of the hotspots for biodiversity and is considered as the origin of several fruit species. Elderberry or "Palam" (in Farsi) has been known as a functional food with lots of applications in Iranian Traditional Medicine for millennia. Both native (*Sambucus ebulus*, *S. nigra*) and introduced (*S. canadensis*, *S. racemosa*) species of elders are grown in Iran. In traditional medicine, extracts from the root and aerial organs of elderberry are frequently used for the treatment of wounds and inflammatory diseases such as inflammatory joint diseases, rheumatic pain and sore throat. These properties have also been proved by allopathic studies and together with high antioxidant activities of its flower's extract, make elderberry a valuable source for herbal medicine industries. In this study we will take a close look at the distribution of elderberry throughout Iran, its botanical characteristics and its applications in Iranian traditional medicine.

Elderberry in China

Frank Qiang Fu[1] and Wenchi Jen[2]

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Most Chinese horticulturists and farmers consider elderberry a wild plant. It is regarded as a plant of little value because elderberry can be found everywhere among hills and mountains. There are about nine species of elderberry in China. This article has full details of these *Sambucus* species groups, including the botanical names, Chinese common names, physical locations, and full descriptions of the elderberry. In southwest China, where the climate is mildly warm, there are two species of elderberries; one is *Sambucus adnata*, local residents name it "blood-red herb elderberry" as the roots and rhizomes are red and the branches exude red juice when broken. The other is *S. javanica* which is also named *S. chinensis*, commonly called "herb elderberry". In northeast China where the climate is cold, there are seven species of elderberry, however most scientists recognize only two species: *S. williamsii*, commonly called "woody elderberry", and *S. sibirica*, commonly called "Siberian woody elderberry". Few scientists in the world know that there are five additional species of elderberry in the cold climate of northeast China. *Sambucus sieboldiana* is significantly different from *S. williamsii*, and is commonly called "hairy woody elderberry". The others are *S. foetidissima,* commonly called "hook-tooth leaf woody elderberry", *S. coreana*, "Korean woody elderberry" which may have originated in Korea and whose unique seeds contain 18.8% oil, *S. latipinna*, "broad-leaf woody elderberry", and *S. manshurica,* "northeast woody elderberry", whose leaves and shoots have been used as traditional Chinese medicines to treat human bone fractures.

Elderberry (*Sambucus nigra* L.): Natural Medicine from the Ancient Times or Protection against Witches in the Middle Ages?

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Key words: magic, medicinal plants, mythology

In ancient times man understood and knew that some plants are able to have certain effects to the human body. Because of this fact, the original inhabitants could not explain the magical power of plants; they attributed supernatural forces to them. Elderberry seeds were found by archaeologists and assigned to the Stone and Bronze Age. At that time people used fruits, bark and branches of mentioned species. Naturalists, healers and philosophers of Ancient Greece and Rome described this mysterious healing plant. Hippocrates devoted special attention to the elderberry fruits and Dioscorides recommended wine made from its roots as a medicine after snake bite. In the Middle Ages elder was a plant used as protection against witches. In another period, its effects were attributed to devils power. According to mythology, the spirit dwells in its trunk. While the Germanic people believed that it was an evil spirit, in Denmark and Latvia it was a good spirit. Unpleasant odor and the presence of parasitic fungus *Auricularia auricula Judae* is associated with the biblical story, which states that Judas did not satisfy his treason and hanged just on this shrub. We now know that different parts of the elder have positive and beneficial effects on the human body, if used correctly.

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ELDERBERRY BIOCHEMISTRY

Impact of Genotypes on American Elderberry Juice Chemistry and Anti-oxidative Capabilities

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European elderberry products have been widely marketed as immune supporting products based on anti-oxidative capability. However, supporting evidence for American elderberry products falls short on that perspective, and this research is exploring the chemistry and anti-oxidative capabilities of various American elderberry genotypes. Elderberries from nine genotypes including Bob Gordon, Dallas, Marge, Ocoee, Ozark, Ozone, Sperandio, Wyldewood, and York were harvested from the same location in the year of 2011 at Mt. Vernon, Missouri (USA). The juices were French-pressed, centrifuged, filtered through 0.22-micrometer filters, and stored in an -80 °C freezer for further analysis. Chemistry profiles were evaluated by high performance liquid chromatography (HPLC), and anti-oxidative capabilities are evaluated by the oxygen radical absorbance capacity (ORAC) assay. Preliminary results showed that juices from different genotypes carry different anthocyanin and polyphenol profiles as well as ORAC values. Further research will be needed to elucidate the link between chemistry and biological functions and the difference between American elderberry products and European elderberry products.

Methods for Profiling and Quantification of Anthocyanin and other Polyphenols Present in Elderberry Juices (P)

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The phenolic content of elderberry juices is expected to vary with cultivar and growing region. In this study, elderberry juice was obtained from a number of different cultivars and locations. The juice was then filtered through a 0.45 µm filter. We describe a method for determining the total phenolic content for these juice samples. Cation exchange solid-phase extraction (SPE) was used to separate anthocyanins and other polyphenols. Samples were tested for total phenolic concentration using a modified version of the colorimetric Folin-Ciocalteu reagent

(FCR) method. The results from the colorimetric method showed a range of total phenol concentrations with elderberry cultivar. Reversed-phase ultra-performance liquid chromatography (UPLC) was used in conjunction with tandem mass spectrometry for identification and quantification of anthocyanins and other polyphenols. This presentation will focus on the development of the analytical methods for the determination of polyphenols in elderberry juice.

Determination of Anthocyanin in a Variety of Elderberry Juices by UPLC/MS

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The juice from elderberry fruit is known to contain a variety of polyphenols, the concentration of which can change with elderberry variety. In this study, reversed-phase chromatography was optimized to achieve separation of analytes. Positive-ion electrospray mass spectrometry was then used for compound detection. Juice samples are examined in a profile mode to obtain an overall chemical "fingerprint." Multiple reaction monitoring (MRM) is then used. MRM was selected for the reduction of false positives, maximizing selectivity, and reliable quantification. The quantitative performance of the method was validated. The results suggest that this method will help to characterize and profile the polyphenol composition of elderberry juices for quality control, assessment of dietary intake, and polyphenol biochemical studies. From these results, we hope to be able to correlate the polyphenol content of elderberry juices within different regions and varieties.

Flavonoid Content of Elderberry Collected in the Eastern United States

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American elderberry (*Sambucus nigra* subsp. *canadensis*) is traditionally consumed as wine, jams, pies, etc. and has garnered more interest as a medicinal product in recent years. There is limited information on the medicinal properties of this less researched subspecies compared to the better known European elderberry (*Sambucus nigra* subsp. *nigra*). Several phytochemicals have been identified in American elderberry including phenolic acids, flavonols, and

anthocyanins. The flavonols and phenolic acids could potentially be bioactive compounds in elderberry and can be used in quality assessments of elderberry and related products. These are rutin, chlorogenic acid, isoquercetin and quercetin. In this work, 110 samples of wild elderberry fruits were collected in 2010 and 2011 from different locations in the Eastern United States ranging as far west as Kansas and Mississippi and north to Maine, Vermont, and New York. The fruits were freeze-dried and analyzed for the four individual flavonoids using UPLC-UV. Separation was achieved on a Kinetex C18 (4.6 x 100mm, 2.6µm) column with a mobile phase consisting of A: 0.1% phosphoric acid in water and B: tetrahydrofuran: acetonitrile: isopropyl alcohol (4:4:1) in a run time of 31 minutes. The average flavonoid content of the 110 collected fruit samples was 107 mg/kg chlorogenic acid, 525 mg/kg rutin, 21.6 mg/kg quercetin and 65.2 mg/kg isoquercetin on a fresh weight basis. The variability of the flavonoid content in the fruits was compared based on geographical locations of the collections.

Extraction of Pure Anthocyanins from Fruits of *Sambucus nigra* L.

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Natural products contain numerous matrix components, such as sugars, phenols, organic acids, proteins, salts, and other flavonoids that will be extracted along with anthocyanins during solid-liquid extraction. Liquid-liquid extraction and solid phase extraction can be used to further purify a solution containing anthocyanins. In this study, we have used adsorbent C18 which was reported as optimal in several studies. During SPE purification experiments we experienced the significant loses of anthocyanins (up to 83 %). The loses can be reduced to 15 % by increasing the volume of 70 % ethanol-water 1 % citric acid solvent which was used for flashing of solid adsorbent. Our experiments were carried out to find the number of extractions needed for the same plant sample to extract the main part of anthocyanins. Total amount of anthocyanins was determined by differential spectrophotometry. We have studied extraction of anthocyanins from *Sambucus nigra* L. Totally, after three extractions we have obtained anthocyanins in an amount of near 0.98 % of fresh plant weight (expected 0.664 – 1.816 % wt.).

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Elder (*Sambucus nigra* L.) as a Source of the Flavonoid Rutin (P)

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Wild elder is the most common bush in the Czech Republic. It grows almost on each balk or any unplowed strip of land. Cultivated elderberry is still not a recognized fruit in the Czech Republic, however, elderberry growing has just started and elder orchards are planted. Our studies have recently focused on biologically active substances contained in the elder and elderberries. Elder branches left after winter pruning could be a valuable source of a pharmaceutical active substance – flavonoid rutin. Therefore, this paper is focused on determining rutin and its aglycone quercetin in waste branches, in berries and in leaves of 17 selected elderberry cultivars. Flavonoids were extracted by a new technique called PSE (pressurized solvent extraction), and determined by HPLC (high performance liquid chromatography). The highest amount of rutin was determined in leaves of cultivar ´Pregarten´ (26.34 ± 0.50 $mg.g^{-1}$). Analyzing dry ground branches, the highest rutin content was found in ´Samyl´ cultivar (4.87 ± 0.01 $mg.g^{-1}$). The highest amount of rutin has been extracted from berries of ´Albida´ (6.7 $mg.g^{-1}$). Content of quercetin was insignificant in leaves, branches, and berries of all cultivars except for the wild elderberry (0.24 $mg.g^{-1}$). Based on these findings, we may conclude that most of the valuable active substance in elderberry occurs in the form of rutin, which can be easily isolated.

New Insights on the Elderberries and Elderflowers (*Sambucus nigra* L.) Metabolites

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Translation of plant metabolism into products that may promote health benefits or prevent illnesses in humans is a starting point to integrate the advances in metabolomics in several applications. At the moment, the study of elder bioactive compounds is mainly focused on phenolic compounds; however other chemical families may also present relevant biological properties. Thus, valorization of elderberries and elderflowers (*Sambucus nigra* L.), as raw materials, should comprise an in-depth bioprospection of new potential bioactive compounds

from other families, as well as their evolution during maturation and storage. In the present study the volatile and lipophilic fractions of elderberries and flowers were analyzed by HS-SPME/GC × GC-ToFMS and GC-qMS respectively, and the impact of the maturation state and storage on the expression of the volatile terpenic and lipophilic compounds was evaluated. This study allowed us to identify 55 new volatile compounds in berries, and several families of lipophilic compounds, namely fatty acids and alcohols, triterpenoids and phytosterols with the two last families being particularly abundant in flowers. The follow up of composition (particularly in terms of bioactive compounds) during the maturation process will allow the producers to select the best harvesting period to obtain berries with the highest quality. An insight into the impact of processing and storage on the volatile and lipophilic composition of elder fruits and flowers was also carried out, and some strategies were tested in order to prevent their losses/degradation.

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ELDERBERRY AND HUMAN HEALTH

Pharmacological and Clinical Effects of Elderberry Fruit

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In traditional German medicine, the dried ripe or fresh berries of *Sambucus nigra* were used for the treatment of constipation, to increase diuresis, as a diaphoretic in upper respiratory tract infections, for the alleviation of low back and/or neuropathic pain, headache and tooth ache. In the meantime, there are data on the antibacterial, anti-inflammatory, analgesic and antiproliferative effects available, but these need further support. Several *in vitro* studies together with two exploratory studies in humans and one open study in chimpanzees indicate that the aqueous elderberry extract Sambucol^{R} may be useful for the treatment of viral influenza infections or other viral infections. The active principle of elderberry, a mixture of procyanidins, anthocyanins and phenolic acids, has a potent antioxidative effect that may be useful for the treatment of the metabolic syndrome (hyperlipidemia, diabetes, hypertonus) associated with obesity. Although the evidence of effectiveness is poor due to the poor quality of the studies, the outcome measures (blood lipids, blood glucose, and blood pressure) provide promising results. Though laboratory and clinical data indicate that elderberry products may be a “functional food” for disorders or diseases related to oxidative stress, these promising indications need to be confirmed in more rigorous studies.

Usefulness of Elderberry Fruit as Functional Food

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Pharmacological and clinical studies indicate that products from elderberry fruit are useful for the treatment of diseases and the maintenance of health. This, however, requires an adequate daily dose of the active principle of elderberry fruit. Dose-finding studies have not yet been carried out for the different indications. Most promising are elderberry products for viral infections and diseases associated with oxidative stress including arteriosclerosis, diabetes, hyperlipidemia, cancer etc. Temperature, oxygen, and light are destroying the active principle in elderberry juice. This has to be taken into consideration when preparing elderberry functional food. Addition of vitamin C may help to protect from oxidative degradation. The starting material has to be free of leaves and stems because these contain toxic lectins causing intoxications (acute gastrointestinal and neurologic symptoms). Unlike intoxications with solanin, elderberry intoxications had a benign outcome so far, poisoning from cyanide had not

been involved (www.cdc.gov/mmwr/preview/mmwrhtml/00000311.htm). For practical reasons, chokeberry (*Aronia*) cultures may replace elderberry cultures, because *Aronia melanocarpa*, for example, is easier to harvest and provides an active principle similar to elderberry but even more potent.

Elderberry Juice Prevents Prostate Cancer *In Vitro* and in an *In Vivo* Mouse Model

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Anti-cancer botanical compounds are proposed to suppress tumor growth by disrupting signaling pathways which are involved with cancer cell growth. Here we investigate the potential for elderberry juice (*Sambucus nigra* L. subsp. *canadensis* (L.) Bolli) to inhibit Gli/hedgehog (Gli/Hh) signaling, a pathway previously found to be important for the growth of many cancers, including prostate cancer. We hypothesized that the claimed anti-cancer effects of elderberry are due to its inhibition of the Gli/Hh signaling and that elderberry would prevent prostate cancer in the TRAMP (Transgenic Adenocarcinoma Mouse Prostate) model. *In vitro*, measuring the Gli/Hh pathway activity, we observe comparable dose-dependent inhibition of the Gli-reporter activity in Shh Light II cells treated with dilutions of either elderberry juice or elderberry pulp extract. Importantly, 100 to 250 fold dilutions of elderberry juice or pulp/pomace extracts are effective at suppressing Gli/Hh signaling by 50%. *In vivo*, diets containing freeze-dried elderberry juice solids at 3 concentrations (low=0.09%, middle=0.45%, and high=2%) were fed to TRAMP mice to determine if elderberry could delay or inhibit prostate cancer progression. *[The Middle elderberry diet in mice is the equivalent dose of a 60-kg human drinking 2 tablespoons of elderberry juice per day.]* In comparison to the control group, fed AIN-93G diet without any freeze-dried elderberry solids, these 3 concentrations of elderberry in the AIN-93G diet exhibited potent dose dependent inhibition of cancer formation with the Middle and High dose elderberry diets being statistically significant at the $p = 0.03$ and $p = 0.006$ level, respectively. These results support our hypothesis that elderberry juice is capable of preventing prostate cancer. Additional research is clearly warranted on elderberry's mechanism(s) of action in prostate and other cancers that are potentially dependent upon Gli/Hh signaling.

Bioactive Compounds of Elder (*Sambucus nigra* L.) with Focus on their Potential Anti-diabetic Effects

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Preparations of elder (*Sambucus nigra*) are used in traditional medicine as diuretics and to treat colds, influenza, inflammation, and diabetes. Elderberries and elderflowers are rich in polyphenols such as phenolic acids, flavonol glycosides, and anthocyanins. Polyphenols are known for their antioxidant activity and are believed to prevent oxidative stress, a condition that may lead to serious diseases such as cancer, cardiovascular diseases, inflammation and type-2 diabetes (T2D). Hence, polyphenols have been pointed out as beneficial agents in both elderflowers and elderberries. Epidemiological investigations tend to confirm the protective effects of polyphenols against cardiovascular diseases and T2D but the mechanisms by which they exert their protective effects are far from understood. The reported beneficial effects of polyphenols against cancers and inflammation are mainly based on *in vitro* studies. The disparity between an excellent *in vitro* activity related to a specific disease(s) and a weaker *in vivo* effect of polyphenols may be due to pharmacokinetic properties such as poor bioavailability, low absorbability, and/or metabolism. Therefore the health effects of elder could be due to (1) polyphenols whose modes of action differ from the traditional one proposed for exogenous antioxidants or (2) other types of biomolecules not identified yet.

Extracts of elderflowers have been found to exert insulin-like and insulin-releasing actions *in vitro* and to activate PPARγ as well as to stimulate insulin-dependent glucose uptake. This indicates that elderflowers may be used in the prevention and/or treatment of insulin resistance. In the search for an explanation of the anti-diabetic effects of elderflowers we undertook an investigation of a methanol extract of elderflowers. A bioassay-guided fractionation of the extract by reverse phase flash column chromatography and semi-preparative HPLC resulted in the isolation of several compounds being able to activate PPARγ, including linoleic acid and linolenic acid, and the flavanone aglycone naringenin. However, major elderflower metabolites such as quercetin-3-O-rutinoside, quercetin-3-O-glucoside, kaempferol-3-O-rutinoside, isorhamnetin-3-O-rutinoside, isorhamnetin-3-O-glucoside, and 5-O-caffeoylquinic acid were unable to activate PPARγ *in vitro*. Several compounds with structures similar to that of naringenin have been identified previously as activators and/or agonists of PPARγ. These include among others the flavonol aglycone kaempferol, which indicates that flavonoid aglycones are relatively good ligands for PPARγ in contrast to their respective glycosides. Further investigations are, however, needed to demonstrate the bioavailability, absorption, and metabolism of elderflower flavonoids in order to determine their potential anti-diabetic or other health-promoting effects.

Lectins of *Sambucus nigra* as Biologically Active and DNA-protective Substances

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The human genome is affected by diverse environmental pollution factors that are particularly significant for Ukraine, which suffered from Chernobyl radiation disaster. At the same time many natural compounds, especially from medicinal plants, are known as agents preventing DNA lesions which often result in increasing mutagenesis and carcinogenesis. The aim of our work is to present a review of own results and literature data concerning DNA-protective potential of *Sambucus nigra* biologically active compounds named lectins, a very large group of universally occurring proteins that recognize and specifically bind to carbohydrates /glycoconjugates. Various physiological activities of lectins based on protein-carbohydrate interactions have been demonstrated in all living forms. For example, lectins participate in host defense in plants against stress-related conditions, the attack of phytopathogens and phytophagous insects, as well as modulation of immune response, mitogenic stimulation or induction of apoptosis in animals. Antiviral, immunomodulating, antioxidant and insulin-stimulating properties of *S. nigra* fruit and flower extracts have been described in scientific literature. Also, the elderberry lectins were found in roots, leaves, bark, seeds and fruits, with SNA-IV being the predominant protein in the juice. In Ukraine, various parts of the *S. nigra* plant have been used in folk medicine for ages. Using methods of isoelectric focusing and chromatography we succeeded in finding that elderberry flowers and pollen contain rather high levels of lectins agglutinating animal and human erythrocytes (3-4 mg of 100 g dry material), which differ from commercial preparation of *S. nigra* bark lectin (SNA-I). The major lectin found in inflorescences named SNAflu-I is GalNAc specific and is supposed to be a heterotetramer with subunits of about 30 and 33 kDa. *Sambucus nigra* lectins demonstrated the protective and antimutagenic effects against heavy metals (nickel ions) in the soil bacteria *Bacillus subtilis.* Also it was shown that lectins under study can modulate in a concentration-dependent manner the frequency of mutations and genotoxic activity of alkylating agents in eukaryotic cell cultures. Studies focused on elucidation the cell targets sensitive for the *S. nigra* lectins action demonstrated that at least one of the targets in *B. subtilis* may be the DNA-dependent synthesis of RNA – the way to modulate gene expression. The results obtained give the reason to conclude that the protective functions of lectins both in pro- and eukaryotes involve complex mechanisms including components of DNA repair system.

Lectins of *Sambucus nigra* in Regulation of Cellular DNA-protective Mechanisms

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Genomes of living organisms are constantly affected by exogenous and endogenous factors, which lead to generating cytotoxic, carcinogenic, and/or mutagenic DNA lesions. However cells possess a number of protection mechanisms directed against DNA damage. The repair enzyme O6-methylguanine-DNA methyltransferase (MGMT) plays a key role in the repair of primary damages of DNA caused by alkylating compounds, which are widely used in industry and medicine. In humans MGMT protects the integrity of the genome, but also contributes to the resistance of tumors to DNA-alkylating chemotherapeutic agents. Therefore, modulation of MGMT expression is a possible strategy to improve the efficiency of cancer therapy and defend normal cells from toxicity of alkylating drugs. Development of medicinal plants for this purpose is highly desirable, due to their safety, low toxicity, and ease of oral intake. The aim of our work was to study the potential of *Sambucus nigra* biologically active compounds, such as lectins, in modification of DNA damage repair process and induction of the MGMT expression. *S. nigra* is widely used in Ukrainian folk medicine. It contains a variety of biologically active substances of pharmacological interest and high levels of lectins as well. Lectins are the carbohydrate-recognizing proteins with regulatory function. Protective and antimutagenic activity of *S. nigra* lectins against some DNA-damaging factors with different mechanism of action (nickel ions and alkylating agents) have been studied in mammalian cells *in vitro*. Lectins are known to exhibit no enzymatic activity, but can up- and down-regulate the activity of different enzymes and other regulatory proteins such as cytokines. That is why we have been studying the modulating effect of *S. nigra* lectins on MGMT gene expression in mammalian cell culture. These lectins were shown to be able to affect in concentration-dependent manner the genotoxic activity of damaging agents and to modulate the MGMT gene expression at the protein level. The obtained results give us the reason to assume that one of the protective mechanisms of the lectin acting is stimulating DNA repair in a cell, including direct reversal repair with the help of the MGMT enzyme.

Health from the Root to the Flower

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Key words: elderberry, essential oil, colors, natural components, medicinal plant

Elderberry (*Sambucus nigra* L.) is one of the oldest medicinal plants. All parts could be usefully applied in phytotherapy. It is usually used as a dried drug and its healing power is enhanced in a mixture of herbs. Leaves (*Folia sambuci*) are collected before flowering. Flowers (*Flores sambuci*), with wonderful fragrance, are collected from May to June. They are the most famous parts of this species for preparing tea. Young sprouts provide the bark (*Cortex sambuci*) for medicinal uses. A toxic substance, sambunigrine, occurs in immature fruits of elderberry, but it is possible to deactivate it by higher temperature. Attention has to be paid in use of extracts from bark and roots of elder. Study of this species is necessary because not all components and their therapeutical effects are known in this modern age.

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The Effect of Sambucol on the Immune System (Cytokines Production)

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Natural remedies, including *Sambucus nigra*, were shown *in vitro* and *in vivo* to have activating effects on the immune system and to posses anti viral properties, as published in former studies. Sambucol products (Sambucol Black Elderberry Extract, Sambucol Active Defense Formula, and Sambucol for Kids), based on a standardized black elderberry (*Sambucus nigra* L.) extract, were studied in comparison to two other natural products (Protec and Chizukit N) containing Propolis and Echinacea. Those five herbal remedies which are sold as food additives and believed to have immune enhancing properties, have been evaluated for their effects to stimulate production of cytokines - one of the main components of the immune system activation. The production of inflammatory (IL-1ß, TNFa, IL-6 and IL-8) and anti-inflammatory (IL-10) cytokines, was tested using blood derived monocytes from 15 healthy donors, as published earlier. The production of all five cytokines was significantly increased by the Sambucol preparations, mostly by the BE type (2 - 45 fold), as compared to controls.

Protec induced only a moderate production of IL-8 (1.6 fold) and of IL-10 (2.3 fold), while Chizukit N caused only a moderate increase in IL-10 (1.4 fold). Both Protec and Chizukit N induced moderate decreases in IL-1ß, TNFa and IL-6 production. LPS, a known activator of monocytes, used as a positive control, induced higher levels of cytokine production (3.6 - 10.7 fold), but sometimes even less than Sambucol. Sambucol formulations demonstrated activation of the healthy immune system by increasing inflammatory and anti - inflammatory cytokines production, while the effect of Protec and Chizukit N is much less. Sambucol could therefore have immuno-stimulatory properties when administered to patients suffering from influenza or to immuno depressed cancer / AIDS patients, in conjunction with chemotherapeutic or other treatments.

Clinical Studies on the Effects of Sambucol - a Review

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Sambucol, a standardized elderberry extract, was evaluated in several clinical studies: 1) Reduction of symptoms during an outbreak of influenza B/Panama, 2) Efficacy and safety of oral elderberry extract in the treatment of influenza A and B virus infections – a randomized study, and 3) Reduction in flu-like symptoms in chimpanzees – prophylactic and symptom-dependent treatment.

1) A placebo-controlled double blind study was carried out on a group of individuals living in an agricultural community (kibbutz) during an outbreak of influenza B/Panama in 1993. Fever, feeling of improvement, and complete cure were recorded during 6 days. Sera obtained in the acute and convalescent phases were tested for the presence of antibodies to influenza A, B, respiratory syncytial and adenoviruses. Convalescent phases serology showed higher mean hemagglutination inhibition (HI) titers to influenza B in the group treated with Sambucol, than in the control group. A significant improvement of the symptoms, including fever, was seen in 93.3% o f the cases in the Sambucol treated group within 2 days, whereas in the control group 91.7% of the patients showed an improvement within 6 days ($p < 0.001$). A complete cure was achieved within 2 to 3 days in nearly 90% of the Sambucol-treated group and within at least 6 days in the placebo group ($p < 0.001$). Considering the efficacy of the extract *in vitro* on all strains of influenza virus tested, the clinical results, its low cost, and absence of side effects, this preparation could offer a possibility for safe treatment for influenza A and B.
2) The efficacy and safety of oral elderberry syrup for treating influenza A and B was tested. Sixty patients suffering from influenza-like symptoms for 48 h or less, were enrolled in this randomized double-blind, placebo-controlled study during the influenza season of 1999 – 2000 in Norway. Patients received 15 ml elderberry or placebo syrup four times per day for five days, and recorded their symptoms using a visual analogue scale. Symptoms were relieved on average four days earlier and use of rescue medication was significantly less in those receiving

elderberry extract, compared with placebo. It was concluded that the Elderberry extract offered an efficient, safe and cost-effective treatment for influenza.
3) The effects of Sambucol treatment on flu like symptoms in chimpanzees were evaluated, and a reduction in those symptoms and the duration of illness was reduced in the treated primates.

Flowers of Elder (*Sambucus nigra*): Well-established and Traditional Human Disease Claims

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Key words: elder flowers, WHO monograph, THMP, clinical studies, disease claim, adverse effects

European elder is traditionally used as a medicinal plant by many native peoples and herbalists alike. Stem, bark, leaves, flowers, fruits, and root extracts are used to treat bronchitis, cough, upper respiratory cold infections, and fever. Several pharmacopoeias have for centuries included *Flos Sambuci* or dried flower of elderberry, and even recognized textbooks and monographs have described the medicinal use of elderberry flower. Thus in 2002, WHO (World Health Organization) published a monograph, which stated that although no controlled clinical data are available, the descriptions in pharmacopoeias and traditional systems of medicine justify elder flower herbal preparation being used as a diaphoretic for treatment of fever and chills, and as an expectorant for treatment of mild inflammation of the upper respiratory tract, and also for symptomatic treatment of the common cold. In 2008 EMA (European Medicinal Agency) issued the Community herbal monograph on *Sambucus nigra* L., Flos. With the following approval: Herbal medicinal product traditionally used for the relief of early symptoms of common cold. The product is a traditional herbal medicinal product for use in specified indications exclusively based upon long-standing use.

Elderberry flower is also described in folk medicine, but not supported by experimental or clinical data, for treatment of conjunctivitis, constipation, diabetes, diarrhea, dry skin, headaches and rheumatism. Elderberry flowers are thus sold in Ukrainian and Russian drugstores for relief of congestion, specifically as an expectorant to relieve dry cough and make it productive. The flowers can be used to make an herbal tea as a remedy for inflammation caused by colds and fever. Combination of elderberry flower and four other botanicals (Sinupret®) is approved by national drug authorities according to “well-established medicinal use” based upon pharmacological and clinical literature which suggest that this phytomedicinal preparation has a relatively significant level of safety and efficacy compared to many other botanical or otherwise natural medicinal preparations intended for use in “maintaining the health of sinuses and the upper respiratory tract”. The scientific and clinical literature on Sinupret® supports pharmacological mechanisms of mucolytic, secretolytic, anti-inflammatory,

antibacterial, antiviral, and immunological activity, some of which has been documented in open-label and randomized controlled human clinical trials. The overall safety of Sinupret® has been extensively documented in pharmacovigilance data based on widespread and long-term use in Germany and other European countries, as well as other post-market surveillance safety data, including safety during pregnancy.

Elderberries (*Sambucus nigra*): Human Health and Disease Claims

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Key words: elder berries, syrup, saft, THMP, clinical studies, disease claim, adverse effects

European elder fruit has been used in folk medicine for centuries, but not supported by experimental or clinical data. In recent years, however, dietary supplements, traditional herbal medicinal products (THMP), and other natural health products containing extracts, juices or syrups of European elder berry have become much more popular in the EEC and the US as remedies for treating cold and flu symptoms. They are marketed primarily through natural food stores alongside other popular herbal remedies used for treatment or prevention of upper respiratory tract infections and other symptoms associated with colds and flu. Several small clinical studies have been performed but only results from five small controlled studies on various elder berry preparations that included a total of 144 participants have been published. Two randomized, double-blind, placebo-controlled studies demonstrated that patients having received elder berry syrup (Sambucol®) recovered significantly faster symptoms associated with influenza than patients in the control group. Three other clinical trials investigated the effect on blood lipids like triglyceride and cholesterol levels. No significant effect could be determined.

The biological activity of European elder berry has also been evaluated in experimental laboratory studies. *In vitro* studies have demonstrated a significant antioxidant capacity as determined by oxygen radical absorbing capacity (ORAC). Antiviral capacity is also demonstrated by inhibition of viral replication. Furthermore, immunoprotective and immunostimulatory activity was detected. Elder berry also increased production of inflammatory and anti-inflammatory cytokines. There is no reporting on adverse effects of properly prepared European elder berry preparations at therapeutic dosages. Unripe or insufficiently cooked elder berry preparation can induce toxic effects in humans (e.g., diarrhea, nausea, vomiting) due to content of cyanide-inducing-glycosides. There are reports of poisonings from the fresh juice of other *Sambucus* species like the American (*S. canadensis*) and Mexican elder (*S. mexicana*). Also, very sensitive persons have responded positive in allergy tests. Only few testimonials on drug interactions have been recorded regarding an interaction with diuretics and laxatives. However, there is a preliminary report of a potential beneficial interaction between elder berry preparations and decongestants and antibiotics.

Does Elderberry Extract Decrease Incidence of Influenza-like Illness? (P)

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Elderberry extract has been shown to be safe and efficacious in the treatment of influenza A and B and to block the ability of H1N1 virions to infect host cells. To our knowledge, no clinical studies have been conducted to test the ability of elderberry to prevent influenza. The purpose of our study was to address this question by comparing the incidence of seasonal influenza during a 12 week period in 3 consecutive years between a treatment group receiving elderberry extract and a control group receiving placebo. A randomized, double-blind, clinical trial was conducted in Hawaii, Washington, Montana, Colorado and North Carolina. Participants were healthy children (aged 2 and older) and adults with no recent history of influenza and no annual influenza vaccination, randomly assigned to take elderberry syrup or placebo for 12 weeks during the flu seasons of 2009-2010, 2010-2011, and 2011-2012. Dosage was 1 teaspoon daily for the first two years and twice daily for the third year. Flu cultures were performed for participants who developed ILI. Over the 3 years, 113 participants were enrolled; 33 taking elderberry completed the trial, and 30 taking placebo. There were 2 cases of influenza reported, both in subjects taking placebo. In those taking placebo who completed the trial, there were 7 reports of other illnesses, and in those taking elderberry, 4 reports of other illnesses. The most common adverse effect reported was dislike for the taste of the placebo, which led to some participants withdrawing. This pilot study lacked power but these results provide some support for prophylaxis of influenza with elderberry extract. A larger trial, sufficiently powered, should be conducted to confirm or reject this finding. A trend was also found suggesting that elderberry may have preventive activity against other viral illnesses as well.

In vitro Screening for Anti-inflammatory Bioactivity of Various American Elderberry Genotypes

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This research sought to characterize the anti-inflammatory bioactivity of various American elderberry genotypes. Elderberries from nine genotypes including Bob Gordon, Dallas, Marge, Ocoee, Ozark, Ozone, Sperandio, Wyldewood and York were harvested from the same location in the year of 2011 at Mt. Vernon, Missouri (USA). The juices were French-pressed, centrifuged, filtered through 0.22-micrometer filters, and stored in a -80°C freezer prior to analysis. Juices were diluted 1:1000 in cell culture medium and then placed on top of THP-1 cells, a human monocytic cell line. After 18 hr. of treatment, cells were exposed to an inflammatory stimulus (i.e., 10 ng/mL LPS) and the activation of the NF-kB signaling pathway was measured using a luciferase reporter construct that contained two NF-kB promoters. Our results showed that juices from different cultivars carry substantially different anti-inflammatory bioactivity. Our chemical analyses of juices suggest that this anti-inflammatory activity correlates (r^2=0.605, $p<0.02$) with the concentration of rutin, a major polyphenol present in elderberries. Further research is needed to elucidate the relationship between the chemistry and biological activity of American elderberries.

The Revival of the Elderberry

Madeleine Bliah-Mumcuoglu, Jerusalem, Israel

Elderberry has been named the “country medicine chest” for its diverse health uses.
A wealth of folklore is centered on the black elderberry tree, which is common in England and Northern Europe. From the days of Hippocrates, it has been famous for its medicinal properties. Its uses are manifold and important. Almost from time immemorial, a juice made from elderberries simmered and thickened with sugar formed an invaluable cordial for colds and coughs. It is known as one of the best preventatives against the advance of influenza and the ill effects of a chill. During my Ph.D. research, we isolated active substances from the black elderberry fruit, the AntiVirins®. The mechanism of the antiviral action will be explained. As a result of more than 20 years of research on a standardized extract, laboratory and clinical studies confirmed the activity of elderberry on several viruses as well as its stimulating action on the immune system. Additional studies showed its antioxidant properties. Since the launch of Sambucol in the US in 1995, and its receipt of the Vity Awards in 1997, 1998, 1999, numerous products have appeared and the beneficial properties of elderberry are now known worldwide.

The Antiviral Effect of a Standardized Elderberry Extract

Madeleine Bliah-Mumcuoglu, Jerusalem, Israel

The mechanism of the antiviral effect of elderberry on different viruses is described.
Viruses, unlike bacteria, are unable to replicate themselves on their own. They behave like parasites and must invade a living cell. A very early stage of viral infection is viral entry, when the virus attaches to and enters the host cell, then the virus replicates using the cell machinery. Each invaded cell will release thousands of new viruses that will infect new host cells. In this process the host cell will die. The surface of the influenza virus is covered by tiny protein spikes with H and N antigens. When the H antigen, hemagglutinin, binds to receptors on the surface of our cells, the cells begin to produce new copies of the virus. Elderberry disarms the spikes of hemagglutinin and thus, stops the first step of invasion of the virus by blocking the entry of the virus into the cell. The virus will be eliminated from the body. In laboratory studies using monolayer cell cultures this process can be observed. This test is used to show the antiviral properties of plant extracts or drugs. The virus is pre-incubated with dilutions of the elderberry extract before being added to the cells. The reduction of infectivity in the presence of elderberry is determined. The standardized elderberry extract was shown to inhibit the replication of influenza viruses type A and B, including avian (H5N1) and swine flu (H1N1) viruses. In addition sensitive and Acyclovir–resistant Herpes HSV-1, HIV, Respiratory Syncytial virus (RSV) were found to be sensitive to elderberry.

ELDERBERRY HORTICULTURE

Some Aspects of Environmental Condition Determination and Quality of Special Crop Products (with the Elder Flowers) in Slovakia

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Key words: biodiversity, radioactivity, heavy metals, medicinal plants

Medicinal plants, as special crop products from different origins of the world, have various therapeutical effects and quality in the world markets. The highest quality (a low content of heavy metals and low radioactivity) of medicinal plant drugs are the main condition for their further production and processing by the Slovak pharmaceutical industry as well as their export abroad. A study of the heavy metal pollution into dry medicinal plant raw-materials (*Matricaria recutita* L., *Sambucus nigra* L., *Euquisetum arvense* L., *Agrimonia eupatoria* L., *Viscum album* L., *Taraxacum officinalis* Web. and *Urtica dioica* L.), which were cultivated and collected in Central Zemplin (Slovak Republic) was made in the period of the years 2000 – 2009. The contents of heavy metal in different plant parts (flowers, herbs, leaves and roots) were not higher than the maximum permissible concentration of these elements into food - the standard tea, which is enacted by the Slovak Ministry of Agriculture. In regard to medicinal plant species, the highest result of radioactivity, 1.96 $Bq.kg^{-1}$, was determined in the mistletoe herb, which is accepted on the market with medicinal plants in the world.

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Identification of Two Carlaviruses in Elderberry

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Cuttings of four elderberry cultivars, 'Bob Gordon', 'Marge', 'Adams 2' and 'Wyldewood' were sent from Missouri to the USDA-ARS laboratory in Corvallis, Oregon (USA) to be tested for the presence of viruses. Double-stranded RNA (dsRNA) extracted from the four cultivars showed the same dsRNA pattern, with multiple bands of about 8,000 base pairs plus several smaller bands. The dsRNA from 'Bob Gordon' was used as a template for sequence and further analysis. DsRNA was converted to cDNA using reverse transcription with DOP (degenerate oligonucleotide primers), the cDNA was then amplified using PCR and subjected to high throughput sequencing using the Illumina format with an 80 bp, paired end kit. Approximately 75 million reads were obtained and assembled into contigs using SCRAPE.pl followed by CAP3. Two large but distinct contigs of 8521 and 8425 were obtained, which showed significant levels of sequence identity to several carlaviruses and 51% nucleotide sequence identity between each other. From the nucleotide sequence data and translated amino acid data, it is clear that there are two distinct carlaviruses infecting elderberry and it appears that one is related to American hop latent virus and the other related to poplar mosaic virus. Examination of the serological relationships of these viruses to elderberry latent virus is currently underway and will be presented along with more detailed information on the two carlaviruses.

***Sambucus* Genetic Resources at the U.S. National Clonal Germplasm Repository**

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Key words: germplasm, elderberry, genebank

The US Department of Agriculture (USDA), Agricultural Research Service (ARS), National Clonal Germplasm Repository (NCGR) in Corvallis, Oregon, maintains an elderberry (*Sambucus* L.) germplasm collection representing 7 of the 9 major world *Sambucus* species. The priority emphasis of the collection is to maintain diverse species and edible fruited cultivars. The genebank collection includes about 50 cultivars, including selections for fruit, as well as ornamental gold, purple, and cut-leaf forms. Seed accessions have been obtained during recent USDA plant collection trips to Russian Far East, Republic of Georgia, Armenia, and Japan. The collection includes plant material from about 30 different countries, with the most accessions

from the US, Russia, China, Japan, Ukraine, the United Kingdom, and Canada. The primary clonal collection is preserved as plants in a field genebank, with seedlots of wild collected material stored at −20 ^{o}C, thus preserving species diversity. Horticultural morphological descriptors and phytochemical fruit components for selected American elderberry [*S. nigra* subsp. *canadensis* (L.) Bolli] genotypes have been determined. Origin and evaluation information is described on the publicly accessible Germplasm Resources Information Network (GRIN) database. Orders for limited amounts of propagation material for research purposes can be placed through GRIN.

Eriophyid Mites Inhabiting American Elderberry

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With expanding commercial production of elderberry, identification and control of pests is important. Two genera of eriophyid mites, *Phyllocoptes wisconsinensis* (Keifer) and *Epitrimerus trilobus* (Nalepa) have been described taxonomically and are known to cause foliar damage on elderberry plants. However, mites have not been examined in commercial plantings in Missouri. Thus, specimens were collected from American elderberry (*Sambucus canadensis* L.) leaflets at two locations, Mt. Vernon and Hartsburg, Missouri, USA, from April 2012 to spring 2013. Mite induced damage varied by location. Those collected from Mt. Vernon induced curling of leaflet margins with erinea (i.e., enlarged leaf trichomes). In contrast, mites from Hartsburg reduced leaflet size and induced interveinal puckering of plant tissue. Examination of these mites using environmental scanning electron microscopy revealed a previously undescribed fusiform eriophyid mite species. Key features used to identify mites included prodorsal shield anatomy, leg morphology, and genital cover flap structure. Overwintering mites remained on plants under bud scales at Hartsburg and were quantified by bud position. As many as 26 mites per bud were found in early December.

Micropropagation of Common Elder (*Sambucus canadensis* L.) with Emphasis on Disinfection of Explants

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Key words: *In vitro* propagation, silver nitrate, elderberry, American elder

Over the last decade there has been a renewed interest in the fruits and flowers of the common elder (*Sambucus canadensis* L.) which hold an interesting potential as a source of food and medicine. Consequently, an increasing number of commercial orchards are appearing in North America. Research to produce improved cultivars and identify promising specimens from wild populations is currently underway and the demand for large quantities of elite plants is increasing. While elderberries are relatively easy to propagate using softwood cuttings, this multiplication technique has its limitations. It is therefore important to develop efficient multiplication methods to satisfy the demand. A detailed procedure for the micropropagation of common elder is presented for the first time. Various disinfection techniques have been tested in order to control the high contamination level usually observed with elderberry explants. Whole nodal sections containing two buds proved to be the most suitable material giving a fast growth and a satisfactory multiplication rate. The best results were obtained when stem sections are disinfected in a 3% solution of sodium hypochlorite (NaClO). Nodal sections are then soaked in a 0.5% silver nitrate ($AgNO_3$) solution followed by a final bath in a solution of citric ($C_6H_8O_7$) and ascorbic acid ($C_6H_8O_6$). The need for a strong disinfection procedure resulted in a low survival rate with some of the cultivars tested. However, subsequent subculture allowed for a multiplication rate varying between 4 and 5 every 6 weeks with simultaneous rooting. For the various cultivars tested, a MS medium containing 100 or 50% macro salts supplemented with 20 g L^{-1} sucrose, 8 g L^{-1} agar, 1 mg L^{-1} BAP, and 5 µg L^{-1} NAA gave excellent results. Explants readily acclimate to field conditions with 100% survival rate. More work is needed to fully evaluate long term field behaviour of micropropagated common elder and the performance of the proposed micropropagation technique on other cultivars.

Effect of Season, Genotype, and Tissue Type on American Elderberry Leaf Elemental Status (P)

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Key words: *Sambucus*, fruit, yield, management, element

Leaf nutrient analysis is a useful monitoring tool to evaluate the efficacy of a fertilizer application program. Tracking seasonal variations in leaf element concentrations can help producers in scheduling foliar and soil nutrient applications. The American elderberry [*Sambucus canadensis* L.; *Sambucus nigra* L. subsp. *canadensis* (L.) Bolli] is increasingly cultivated in North America, yet information on sufficiency levels of leaf element concentration and seasonal variation in element concentration data is lacking. A study was conducted in 2006 at two locations in Missouri (USA) to measure the leaf element concentration of N, P, K, Ca, Mg, Zn, Fe, Mn, Cu, and B. Foliage from shoots arising from woody tissue, as well as foliage from current season's shoots from three genotypes was sampled at three growth stages (first flush, anthesis, and fruit harvest). Leaf element levels of B, Ca, and Mg increased significantly over the collection period, while levels of N, P, K, and Zn decreased. Significant differences were noted among the three genotypes for all elements except Cu. In several cases (N, P, K, Zn, Mn) significant differences in element content were noted between the tissues collected from woody shoots and from current season shoots. Significant differences among leaf element levels were also noted between the two sites for N, P, K, B, Ca, Mg, Zn, and Mn. A number of significant interactions were noted among the variables. Additional work is needed to further develop the usefulness of foliar nutrient analysis as a tool for elderberry producers.

Elderberry Cultivars Growing in the Czech Republic

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Key words: *Sambucus*, elderberry, cultivars, evaluation, growing

Elderberry (*Sambucus nigra* L.) is a neglected crop in commercial and organic fruit growing systems in the Czech Republic. Basically, elderberry has not been grown commercially in our country, although it is a crop with great potential of use. Currently, an increase in interest of organic growers in growing elderberry as a complementary crop has been noted. An interest in elderberry blossoms and fruits has also been shown from domestic as well as foreign processing industry. A unique, experimental collection of elderberry in terms of cultivar numbers was created in the Research and Breeding Institute of Pomology Holovousy Ltd. In 2009, cultivars ´Albida´, ´Allesö´, ´Aurea´, ´Bohatka´, ´Dana´, ´Haschberg´, ´Heidegg 13´, ´Körsör´, ´Mammut´, ´Pregarten´, ´Riese aus Voßloch´, ´Sambo´, ´Sambu´, ´Samdal´, ´Sampo´, ´Samyl´ and ´Weihenstephan´ were planted. In following years, this collection has been gradually supplemented with other cultivars including ´Juicy´, ´Tulbing´ and ´Weisser Holunder´. Elderberry cultivars were obtained from neighbouring countries, which have been growing and researching this crop for a long time and have similar natural and climatic conditions. Vegetative and fruit characters were evaluated in the framework of the project. Based on our findings, elderberry cultivars suitable for climatic conditions of the Czech Republic were recommended for growers.

Fruit Composition of Elderberry (*Sambucus canadensis* and *S. nigra*) Genotypes Grown in Oregon and Missouri, USA (P)

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Elderberry plants were grown in three locations west of the Mississippi River (USA) to determine suitability for production in diverse climates. Ten of the twelve *Sambucus canadensis* genotypes were selected from the wild in Missouri and Oklahoma whereas two cultivars originated east of the Mississippi River. An additional 24 genotypes, both *S. canadensis* and *S. nigra*, were planted in Oregon. Fruit from these genotypes was harvested and fruit composition evaluated in 2004, 2005, and 2006. Soluble solids content ranged from 8.6 to 12.5% and % titratable acid (as citric acid) was 0.5 to 1.6%. Ferric ion reducing antioxidant power (FRAP) trolox and ferric values were 13 to 31 µmol/g trolox equivalents and 14 to 68 µmol/g ferric reducing equivalents, respectively. Total anthocyanin content ranged from as little as 60 to as much as 360 mg/100 g cyanidin-3-glucoside equivalents (CGE) and total phenolic content from 450 to 670 mg/100 g gallic acid equivalents. Of the genotypes sampled in one or three locations, 'Adams2' was very high in total anthocyanin (>350), ten genotypes were high (250-350) and eight were very low in total anthocyanin (<150 mg/100 g CGE). Further phenolic analysis of selected genotypes indicated that *S. canadensis* genotypes were high in acylated anthocyanins while *S. nigra* had no acylated anthocyanins, regardless of total anthocyanin content. Our results show that while total estimations of antioxidants can be useful for rapid genotype screening, detailed analysis of elderberry pigments may be needed if cross species evaluation is done. In addition, *S. canadensis* fruit composition changes with production environment depending on genotype.

Evaluation of Fruit Quality of Elderberry Cultivars

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Key words: elderberry, cultivars, comparison, evaluation

The aim of this study was to evaluate fruit characters of elderberry cultivars ´Albida´, ´Allesö´, ´Aurea´, ´Bohatka´, ´Dana´, ´Haschberg´, ´Heidegg 13´, ´Körsör´, ´Mammut´, ´Pregarten´, ´Riese aus Voßloch´, ´Sambo´, ´Sambu´, ´Samdal´, ´Sampo´, ´Samyl´ and ´Weihenstephan´. The collection (3 plants representing each cultivar) was planted at an altitude of 320 m at a spacing

of 3 x 2 m. In two consecutive years, the following characters were evaluated: appearance, juiciness, aroma, flavour and overall taste. A nine-point evaluation scale was used with one as the lowest and nine the highest level of the character. The overall evaluation was based on the sum of points of evaluated traits. The highest score was obtained for the Austrian cultivar ´Heidegg 13´, which showed satisfactory values of all evaluated traits. Two Slovak cultivars ´Dana´ and ´Bohatka´ followed. ´Dana´ differed from ´Heidegg 13´ in appearance, which was lower only by 0.5 points. Danish cultivar ´Samdal´, Austrian cultivar ´Haschberg´ and German cultivar ´Weihenstephan´ also obtained high evaluations from the point of view of fruit quality. In contrast, the lowest quality fruit was produced by cultivars ´Albida´ and ´Aurea´.

Physicochemical Changes in American Elder (*Sambucus canadensis* L.) Fruits during Development

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Key words: Anthocyanin, antioxidant capacity, American elderberry, phenolic compounds, *Sambucus canadensis*

For two consecutive years, total soluble solids, anthocyanins, phenolics, and antioxidant capacity of the fruit of five cultivars and one wild ecotype of American elder (*Sambucus canadensis* L.) were evaluated during development. All parameters increased significantly over the ripening period and reached a plateau at the purple stage. Significant yearly and cultivar differences were noticed. Cultivars 'Kent' and 'Nova' showed significantly higher amounts of anthocyanins and phenolics along with a high antioxidant capacity. At maturity, anthocyanins, phenolics, and antioxidant capacity were strongly correlated with each other (R^2 = 0.82 to 0.97). All cultivars and the wild ecotype showed higher amounts of anthocyanins and phenolics and a higher antioxidant capacity than most commonly available small fruits such as blueberry. These compounds were more abundant in cultivars 'Scotia' and 'York' when grown in L'Acadie (Quebec) than in Corvallis (Oregon). Optimal elderberry quality can be obtained by selecting appropriate harvest date. Periodic determination of total soluble solids content could be used to support visual observation, given that this parameter tends to stabilize when the berries reach full maturity. Our results indicate that there are significant differences in phytochemical profiles and antioxidant activity among the tested cultivars. Selection should be based on adequate chemical characterization in parallel with field performance.

Horticultural Production of Flowers and Berries of Elder (*Sambucus*) as Raw Material for Processing of Foods and Medicines (Pharmaceuticals)

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Key words: elder bushes, propagation, cultivars, irrigation and fertilization, soil, GACP, economy

This research in Denmark includes knowledge about horticultural growing of elder bushes (*Sambucus nigra* L.) with the aim to improve the qualities of flowers and berries according to the requirements from producers of food and medicine. When cultivars are selected, woody cuttings can be obtained in January and February, while green cuttings are optimally made in June. The cuttings are planted in small pots, and kept in greenhouse until defoliation in the autumn. Supply of nutrients with irrigation water is absolutely necessary and a spell of drought may result in dead cuttings or non-homogeneity of elder plants. Row distance in the field may be from 2 to 5 m depending on the size of tractors and machinery utilized in the farm, whereas the distance in the plant row may be from 1 to 2 m. Nutrients (N, P, K) may be supplied according to the content of these plant nutrients in dry matter of leaves taken during the autumn. The flowers must be completely harvested in the first year of growth, in order to ensure maximum leaf and stem growth. The primary requirements for optimization of raw material production of flowers and berries must include selection of cultivars, optimized soil characteristics, proper microclimate in the orchard and delivery of raw materials without presence of foreign materials and absence of mechanically damaged flowers or berries. Both flowers and berries may be harvested gently without mechanical damage. It is very important to cool the flowers and keep the temperature low until either freezing or processing at cool temperature, in order to avoid browning or blackening of the flowers. It is recommended that the data for this research are combined with *"Guideline on Good Agricultural and Collection Practice (GACP) for Starting Materials of Herbal Origin"* adapted by the *"Committee on Herbal Medicinal Products (HMPC)"* in order to produce raw materials with sublime qualities and added value for processing of medicinal products. However, these guidelines are not necessarily required by production of raw materials for foods.

Guidelines for Pharmaceutical Qualities of Elder (*Sambucus*) Herbal Remedies with Human Disease Claims

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Key words: elder flower and berries, GACP, EMA, API, disease claims

This research includes knowledge about horticultural growing of elder bushes (*Sambucus nigra* L.) with the aim to improve the qualities of flowers and berries according to the requirements for producers of dietary supplements and medicine. In order to ensure appropriate and consistent quality of medicinal plant/herbal substances – such as elder flowers and berries, it is essential to establish good agricultural and collection practice (GACP) for herbal starting materials. The concept of Good Manufacturing Practice for the manufacture, processing, packaging and storage of Active Pharmaceutical Ingredients (API's) also applies to medicinal plants/herbal substances. In the case of herbal preparations of elder - such as extraction, distillation, expression, fractionation, purification, concentration, or fermentation, which also include comminuted or powdered herbal substances, tinctures, extracts, essential oils, expressed juices, and processed exudates, the production and primary processing of the medicinal plant/herbal substance has a direct influence on the quality of the API. Due to the inherent complexity of naturally-grown medicinal plants/herbal substances and the limited analytical techniques to characterize constituents solely by chemical or biological means, reproducible quality of starting materials of herbal origin requires an adequate quality assurance system for the collection and/or cultivation, harvest and primary processing. Collection in wild habitats of both elder flowers and berries may present special problems, especially with regard to confusion with similar plants, environmental damage, lack of control and poorly qualified personnel. The GACP was effective within the European Community since 2006 – issued by EMA (the European Medicinal Agency), but it does not fall directly under GMP (good manufacturing practices of medicine) guidelines in the traditional sense. However, these considerations should be used as a basis for the establishment of such an appropriate quality assurance system, if added value elder products are aimed for medicinal use with the medicinal claim *"against common cold and alike".*

The Color Management Classification of Several Fruits Using the Chromatic Pigments Pixels of the Epicarp - [OCR] Operation with Standard Gray Vision (P and Oral)

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Plant pigments have been used throughout history for the extraction of colors. In this study a number of different fruit varieties and species were examined. The digital chromatography images of plant tissues and fruits were analyzed based on a snapshot of the pigment coloring. The color images of digital fruits and tissues and the captured pixels were divided into pigment groups and coded based on their color vision. The digital chromatic pigments were then converted to gray shadows in order to distribute them on the standard gray grade scale using a hexadecimal color code (#HTML). The natural color pixels of the digital images can then be matched with gray pixels from plant tissue samples or the fruit peel. The pixel pigments under study were digital pigments such as anthocyanins, carotenoids, xanthophylls, chlorophylls, and polyphenoloxidase.

Occurrence of Polyphenols, Organic Acids, and Sugars among Diverse Elderberry Genotypes Grown in Three Missouri Locations

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Elderberry (*Sambucus* spp.) is an emerging horticultural crop in North America used in a variety of foods, wines, and dietary supplements. A better understanding of the elderberry juice complex, including its putative health-promoting compounds in relation to genetic and environmental parameters is needed. A multi-location planting of nine elderberry genotypes was established in 2008 at three geographically-diverse sites in Missouri (USA). One of the genotypes, 'Marge' is of European origin (*S. nigra* L.; *S. nigra* L. subsp. *nigra*), whereas the eight other genotypes are North American [*S. canadensis* L.; *S. nigra* L. subsp. *canadensis* (L.) Bolli]. Fruits were harvested from replicated plots at all three sites in 2009, 2010, and 2011 and immediately frozen. Berries were later thawed, de-stemmed, juiced, and re-frozen before laboratory analysis. Polyphenols, organic acids, and sugars each were quantified in separate HPLC analyses, and results evaluated for response to genotype, site, and year. The genotype

'Ozark' was consistently high in chlorogenic acid (328 ppm), neo-chlorogenic acid (140 ppm), and crypto-chlorogenic acid (49 ppm) compared to most other genotypes, whereas the European 'Marge' was significantly higher in rutin (418 ppm) compared to all North American genotypes. Significant differences in polyphenols were also detected among sites and production years. The organic acids citric (mean 1.23 mg/g), tartaric (mean 4.71 mg/g), malic (mean 3.24 mg/g), and succinic (mean 3.00 mg/g) varied significantly among sites, years, and genotypes, whereas shikimic (mean 0.123 mg/g) and fumaric (mean 0.022 mg/g) did not. Levels of lactic, acetic, and propionic acids were negligible in most samples. The European genotype 'Marge' was significantly lower in citric (0.29 mg/g) and higher in tartaric acids (5.28 mg/g) compared with all North American genotypes. The sugars glucose (mean 21.4 mg/g) and fructose (mean 23.7 mg/g) responded significantly to site and genotype, but not year. 'Ozark' and 'Marge' perform very well in Missouri horticulturally and appear to have additional potential as cultivars based on their unique juice dynamics.

Horticultural Performance of Eight Promising American Elderberry Genotypes at Three Missouri Locations (P)

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Key words: *Sambucus*, fruit, yield, cultivar, medicinal, phenology

American elderberry [*Sambucus canadensis* L.; *S. nigra* L. subsp. *canadensis* (L.) Bolli] is being increasingly cultivated in North America for its edible and medicinal fruit and flowers, yet remains largely undeveloped as a horticultural crop. Productive genotypes with desirable horticultural attributes, including disease and insect resistance, precocity, uniform fruit ripening, and large berry size are needed in order to advance the production of elderberries. A four-year study of eight elderberry genotypes was established in 2008 at three diverse Missouri (USA) locations. Harvest, field, and laboratory data were collected over three growing seasons, 2009 – 2011. Significant differences for most horticultural, phenological, and fruit juice characteristics were observed among the three sites, with genotype by site interactions generally significant for phenology, some insects and diseases, and yields, but not for fruit juice traits. The genotype 'Ozark' produced the highest fruit yields at two of the three sites, but not significantly higher than 'Bob Gordon' at one site and 'Ocoee' at an-other site. At the third site, the genotype 'Dallas' yielded numerically but not significantly higher than 'Ozark', 'Bob Gordon', and 'York'. None of the new genotypes being evaluated produced berries as large as or larger than the standard 'York' which is known for its large fruit. Some of the genotypes tested, especially 'Ozark' show promise as potential cultivars and as breeding stock for further development of elderberry as a commercially-viable horticultural crop.

'Marge': A European Elderberry for North American Producers

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Key words: *Sambucus*, fruit, yield, cultivar, genotype

Elderberries are being increasingly produced and consumed in North America for their edible and medicinal flowers and fruits. The American elderberry [*Sambucus canadensis* L.; *S. nigra* L. subsp. *canadensis* (L.) Bolli] is native to, and most often cultivated in North America. The European elderberry (*S. nigra* L.; *S. nigra* L. subsp. *nigra*) has been developed into a viable horticultural crop in Europe, but most European cultivars do not perform well in the midwestern USA. The genotype 'Marge', selected by and named after Marge Millican, Mulvane, Kansas (USA), is an open-pollinated seedling of *S. nigra* subsp. *nigra* 'Haschberg', which is one of the most popular elderberry cultivars grown in Europe. The phenotypic attributes of 'Marge' are identical to that of European elderberry in every respect except that it performs exceptionally well in the midwestern USA. The genetics of 'Marge' have not been fully studied; we cannot rule out that 'Marge' might be an inter-subspecific hybrid, but presume it to be 100% European. In a 4-year study (2008-2011) at three Missouri (USA) locations, 'Marge' significantly outperformed and out-yielded eight American elderberry genotypes under evaluation within and among the same field plots. Across three production years at all three sites, 'Marge' achieved budbreak significantly later, suffered significantly less Eriophyid mite damage, was significantly taller (mean height 70 cm), yielded significantly larger berries (mean individual berry size 176 mg), and yielded significantly greater amounts of fruit (mean yield 1.1 kg / plant) compared with all eight American elderberry genotypes in the study. At one site, 'Marge' produced three times the yield (1.89 kg / plant) compared with the next highest-producing American elderberry genotype (0.64 kg / plant). It is an exceptionally robust and drought-resistant elderberry genotype. As with most European genotypes, 'Marge' does not fruit on first-year wood, and will therefore require a very different management regimen compared with American elderberry if it is to be used successfully by North American producers. We do not yet know how 'Marge' will perform outside the midwestern USA, but it is so productive, unique, and mite resistant, that it merits introduction as a cultivar.

Mass Trapping: A Potential Organic Management Option for the Japanese Beetle (Coleoptera: Scarabaeidae) (P)

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The Japanese beetle, *Popillia japonica* (Coleoptera: Scarabaeidae), is a highly destructive plant pest of foreign origin. Grubs feed on the roots of grass and adults feed on the foliage producing complete defoliation of more than 300 plant species. In Missouri, damaged caused by this pest has been increasing as populations continue to become established and expand. Because no truly effective organic management options against this invasive pest are available, this study was aimed at evaluating the potential of mass trapping as an organic control method in elderberry and blueberry orchards. In 2012, we assessed the effectiveness of commercial and home-made traps baited with either one or two lures (a combination of a powerful sex pheromone and floral attractants) at capturing Japanese beetles in two Lincoln University (LU) farms and in one commercial elderberry farm in central Missouri. The level of damage produced by Japanese beetle to plants was estimated. Over the course of five weeks, approx. 1,550,000 Japanese beetles were captured by traps at the two LU farms, and approx. 1,120,000 beetles were captured at the commercial elderberry farm. The commercial trap baited with one lure proved to be the most cost-effective. Level of damage caused by Japanese beetle averaged 2.5% in elderberry in one LU farm, and it was minimal in the other LU farm. In the commercial elderberry farm, level of damage was minimal in some areas whereas for other areas not well protected by traps damage was greater but within limits acceptable by the farmer given that zero insecticides were applied. Further refinement of this technique is expected to contribute to more effective management of this pest not only in Missouri, but also in other U.S. regions where Japanese beetle is present.

ELDERBERRY MARKETING AND INDUSTRY

Breaking Down Market Barriers for Elderberry Growers and Producers

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Key words: market analysis, specialty crops, qualitative research, barriers to entry, NVivo, nascent industry.

Based on a previously performed national elderberry market research study and using qualitative data analysis tools, this paper focuses on understanding market barriers for elderberry producers and processors in a young and rapidly growing industry. Our research concentrated on understanding the motivation, challenges and unforeseen opportunities of the small number of entrepreneurial firms in the elderberry market and the way they start shaping a new industry and challenge market boundaries. Understanding all the market barriers can be used to create advantages for the future for current and potential market participants. Twenty in-depth phone interviews were analyzed with NVivo 8, a qualitative data analysis software tool, which facilitated a rigorous and efficient approach to data analysis. Results identified barriers to entry consistent with the ones existing in an established, competitive industry such as economies of scale, cost advantages for existing firms, the investment needed for start-up, the lack of necessary information, and steep learning curves. In addition, the high level of uncertainty that characterizes a nascent industry including the reluctance of banks to provide loans, existing prejudices, and the low awareness towards elderberry and its properties, pose extra challenges to entry and success in the marketplace. This research sheds some light on the challenges and opportunities that exist in a nascent industry and provides suggestions to overcome the challenges.

Exploring the Health Benefits and Economic Opportunities of the Bioactive Phytochemicals- An Overview of Phytochemical Research at the University of Missouri Center for Agroforestry

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The non-wood forest products in an agroforestry system can be a sustainable source of value-added bioactive secondary metabolites. To explore health benefits and economic opportunities of the bioactive compounds isolated from non-wood forest products, the Center for Agroforestry at the University of Missouri (MU) has developed a multidisciplinary phytochemical research program with participating MU scientists, international collaborators and industry partners. Interdisciplinary expertise ranges from natural product chemistry, bacterial molecular genetics, medical microbiology, analytical chemistry and structural biochemistry, to bioinformatics and biophysics. Many of the isolated phytochemicals not only have shown strong anti-microbial activities against a wide range of pathogenic bacteria, but also strong inhibitory activities against melanin biosynthesis. Recently, several isolated phytochemicals have been recognized as promising anti-inflammatory, anti-microbial, estrogenic and skin whitening agents by cosmetic and pharmaceutical industries. The main objectives of the research program are to: 1) isolate and characterize the biologically active phytochemicals, 2) elucidate chemical structures of active compounds and their associated mode of actions, 3) identify the immediate and potential commercial applications, and 4) develop cost-effective and environmentally-friendly pilot-scale bioprocessing production processes. The outcomes of this interdisciplinary effort will not only provide critical information for future drug design, but also provide the opportunity to turn the abundant, low value, renewable materials into a lucrative high tech industry in Missouri and the partnering countries.

THE ALBERT Y. SUN MEMORIAL COLLOQUIUM: BERRIES AND BRAIN HEALTH

Effect of Berries on Cognitive and Neurochemical Functions

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Berries are an excellent source of numerous, unique, bioactive compounds, which play a pivotal role in attenuating stress-associated biological dysfunctions. The protective effects of berries play even more significance in brain health as brain alone utilizes from 10-50% of the body's total oxygen supply. High rates of oxygen consumption result in excessive reactive oxygen species being produced, making the brain highly vulnerable to oxidative stress, inflammation, and neural dysfunction. A rapid rise in neurodegenerative diseases, an increasing aging population, and little success towards any cure for brain diseases compel us to explore strategies to alter the neuronal environment and reduce the impact of oxidative and inflammatory stressors. Numerous studies from our laboratory using various *in vitro* and animal models have indicated that supplementing the diet with berry fruits (blueberries, strawberries, blackberries, raspberries, cranberries and other colored fruits) significantly improves indices of cognition and behavior as a factor of age. Elderberry (*Sambucus* spp.), like blueberries, blackberries and other berry species, contains large amount of flavonoids, particularly catechins and anthocyanins. Our studies have shown that improvement in brain health by berry diets is a result of reduced oxidative stress and inflammation, and, at the same time, an upregulation of the endogenous defense system to ward off toxic protein accumulation, an improvement in neuronal signaling, and a promotion of adult neurogenesis in critical brain regions. We have shown that feeding aged rats (19-21 months old) with berries high in antioxidants is able to prevent and reverse the neurochemical and behavioral changes that occur in aging, such as loss of memory, cognition and motor functions. Therefore, it is critical to highlight the nutritional interventions of diets rich in nuts, berries, fruits and vegetables which may reverse or allay age-related motor or cognitive deficits, delay the onset of age-related neurodegenerative diseases and convey long-term health dividends in an aging population.

Neuroprotection by Berry-enriched Diets: Panacea or Methodological Conundrum?

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Multiple Sclerosis (MS), a neurodegenerative disease resulting from autoimmune destruction of myelin, is associated with progressive physical disability as well as fatigue, cognitive dysfunction and depression. Although MS has varied presentations and progressions, inflammation is a common element found in all disease states. Treatment strategies that decrease inflammation would be expected to improve symptoms and/or slow disease progression. Blueberries contain high levels of the antioxidant anthocyanin flavonoids and have been reported to possess immunomodulatory and anti-inflammatory properties in multiple models of aging and neurodegeneration. These properties provide an ideal treatment strategy through dietary supplementation with berries as they are easily administered, have low toxicity profiles, and can be combined with other medications. Experimental autoimmune encephalomyelitis (EAE) is a demyelinating autoimmune disease with pathological and clinical features very similar to those of MS, making it amenable for mechanistic and intervention studies. Immunization of C57BL/6 mice with myelin oligodendrocyte glycoprotein (MOG) yields a chronic monophasic disease which presents as an ascending spinal cord paralysis. Mice were fed NIH-31 rodent chow ± 1% whole, freeze dried blueberry powder (WBBP) beginning 3 days before induction of EAE and continued throughout the study. Disease incidence was decreased by 40% in blueberry-supplemented animals. Whereas no mortality was noted in the blueberry-supplemented mice, 16% of non-supplemented mice succumbed to EAE. Blueberry supplementation also was associated with decreased cumulative and final disease scores. Dose response studies in this model recapitulate the protection afforded by inclusion of 1% WBBP; however higher doses of WBBP provided no protection at all from disease. These data strongly suggest that dietary supplementation with WBBP can reduce disease incidence and severity but that the mechanism behind such protection is complex and must be determined before WBBP can be used in human MS trials.

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Probing Action of Botanical Polyphenols on Oxidative and Inflammatory Signaling Pathways in Microglial Cells

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Many berry species and fruits are enriched in polyphenols and their consumption has been shown to offer health effects including fighting against infectious and inflammatory diseases. In the central nervous system, microglial cells constitute a unique class of immune cells and they exhibit characteristic properties to carry out multifunctional duties in the brain. Our recent studies have focused on signaling pathways underlying microglial response to proinflammatory cytokines and bacterial endotoxin (lipopolysaccharides, LPS). Immortalized microglial cells (BV-2 from mouse and HAPI cells from rat) readily respond to LPS and interferon gamma (IFNγ) which activate transcriptional pathways (NF-κB and JAK-STAT) in releasing TNFα, IL-1β, and reactive oxygen and nitrogen species (ROS and RNS). In addition, there is evidence for a cross-talk mechanism among these pathways leading to activation of MAPK, e.g., ERK1/2 and p38. Interestingly, ERK1/2 activation is linked to a number of downstream pathways involving production of ROS from NADPH oxidase, phosphorylation of STAT1α, cross-talk with the NF-κB pathway through phosphorylation of IKK, and production of filopodia. Consequently, factors leading to ERK1/2 activation are important in regulating the oxidative and inflammatory responses in microglial cells. Using the microglial cell model, botanical phenolic compounds, such as epigallocatechin-gallate (EGCG) from green tea, honokiol from Magnolia bark, and even ethanol extract of *Sutherlandia frutescens* (a South African "cancer bush"), showed ability to inhibit oxidative and inflammatory responses in microglial cells through modulating pathways involving ERK1/2. Many species of berries including elderberries (*S. nigra subsp. canadensis*) are enriched in anthocyanins, compounds known to contribute to the purple color of the fruit. Cyanidin-3-glucoside is the most common anthocyanin in the elderberry fruit and has high antioxidant activities. Our study indicated ability for cyanidin-3-glucoside to inhibit IFNγ-induced NO and ROS production in microglial cells. Although more studies are needed to examine effects of other anthocyanins in the elderberry, these studies provide new insights of the multi-mode action of botanical polyphenols and underscore the important role of ERK1/2 pathways in mediating oxidative and inflammatory responses in microglial cells.

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Quantitative Proteomic Analysis for the Action of Botanicals on Nitrosative-oxidative Stress Signaling

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Key words: botanical anti-oxidant, ischemia, quantitative proteomics; nitrosative-oxidative stress, S-nitrosylation, neuroinflammation

Consumption of anti-oxidants rich in herbal plants, including fruits, leaves, stems, roots and/or seeds may reduce the risk of stroke and other age-related neurodegenerative disorders. However, our understanding of the action of botanical anti-oxidants upon brain health is lacking. A convergent feature for most neurodegenerative disorders is to induce nitrosative-oxidative stress by excessive production of free radicals that contributes to neurovascular impairment and neuroinflammatory responses after brain injury. Nitric oxide (NO) is a signaling molecule that plays vital roles in regulating many biological processes. Protein *S*-nitrosylation, the addition of an NO group to the thiol of specific cysteine, occurs in a wide range of proteins. It has been increasingly recognized as a predominate mechanism of physiological NO-mediated signaling in cells, analogous to phosphorylation. Evidence indicates that NO dysfunctions via protein *S*-nitrosylation may exacerbate neurodegenerative disorders. Prompted by these diverse effects of *S*-nitrosothiol (SNO-) formation, there have been efforts to develop integrative proteomic strategies, including both gel- and mass spectrometry-based proteomic approaches to profile protein levels globally and quantify SNO-proteins specifically. These approaches enable us to investigate multi-target actions of botanicals on nitrosative-oxidative stress signaling in disease. In our studies, we applied these methods and examined the effects of anti-oxidants from botanicals, including *Sambucus nigra* subsp. *canadensis* (elderberry), *Camellia sinensis* (green tea), *Allium sativum* (garlic), and *Sutherlandia frutescens* (Sutherlandia) on either endotoxin lipopolysaccharides-induced activation of microglia and/or neurovasculature against ischemia in mice. We observed more proteins with significant alteration in the ischemic brains of mice fed the elderberry diet (2% of freeze-dried whole fruit) as compared to the AIN-93G control diet. We identified unique SNO-proteins and NO adduct on the specific cysteine of SNO-proteins. Characterization of these SNO-proteins and elucidation of the network of nitrosative-oxidative stress signaling provide valuable insights into molecular mechanisms of the botanical anti-oxidants on neuroinflammatory responses and against neurovascular impairment in the prevention of stroke.

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Beneficial Effects of Botanical Polyphenol Diets and Exercise on Behavior in Alzheimer's Disease Mouse Models

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Alzheimer's disease (AD) is one of the most debilitating age-related disorders and is the sixth-leading cause of death with an estimated global prevalence of 30 million, and more than 5 million currently in the United States. Preventing or postponing the onset of AD, as well as delaying or slowing its progression would lead to an improvement of health status and quality of life for the elderly. AD is characterized by progressive cognitive and functional impairments in areas such as problem solving, spatial navigation, attention, and memory. Amyloid plaques and neurofibrillary tangles are well-established diagnostic markers of AD, and other features commonly seen include oxidative damage and inflammation. Since the neuropathology is likely to occur at least decades prior to the emergence of clinical symptoms, prevention can be effective. Studies to explore the influence of psychological fitness, physical fitness, diet and environment to ameliorate the progression of AD are emerging. Epidemiological studies suggest that exercise and dietary antioxidants are beneficial in reducing AD risk. Rigorous nutritional modifications, which are pleiotropic by nature, may counteract chronic subclinical processes aiding the aging brain's endogenous defenses. To date, botanical flavonoids are most consistently associated with prevention of age-related diseases. Research from our laboratory, using animal models, has suggested that botanical polyphenols and/or exercise can be effectively used as a preventative measure for disease onset, as well as for slowing disease progression. We have shown that both dietary polyphenols and exercise are able to improve learning and memory performance in an AD animal model. The TgCRND8 mouse model of AD displays significant cerebral amyloid-beta plaque deposition as well as high levels of Aβ peptide by 3 months of age. These mice also show altered behavioral activity patterns, and mirror analogous cognitive symptoms of AD, such as spatial learning deficits, in a wide variety of behavioral tasks. Our studies have found age-dependent changes in cognitive and non-cognitive behaviors in these mice, and demonstrated that diet/exercise prevented or reversed some of these changes. These results, together with epidemiological and clinical studies in humans, suggest that dietary polyphenols and exercise may have beneficial effects on brain health and slow the progression of AD.

Protective Effects of Elderberry against Cerebral Ischemic Injury

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As one of the five National Institutes of Health-funded botanical centers in the U.S., this Center is interested in investigating how botanicals can contribute to the prevention and protection against major medical disorders, including stroke. Cerebral ischemia is the second leading cause of death in developed countries. In this study, we focus on the effects of *S. nigra* subsp. *canadensis* (elderberry), known to contain bioflavonoids and cyanidin-3-glucosides which are considered potent anti-oxidants. Previous studies have demonstrated protective effects of botanical polyphenols including resveratrol and curcumin on cerebral ischemia-reperfusion damage. In this study, we aimed to investigate whether supplementation with elderberry diet can similarly ameliorate brain damage using a transient global cerebral ischemia model in mice induced by a 30 min bilateral common carotid artery (BCCA) occlusion. C57BL/6J mice were fed with either the control AIN93 diet or AIN93 chow containing 2% elderberry in dried mass weight for 2 months. Rotarod behavioral assessment 24 hours after ischemia showed significant impairment in sensorimotor function in ischemic mice. Administration of elderberry diet partially ameliorated this deficit by prolonging the rotarod latencies. Quantitative digital pathology assessment of cresyl violet-stained stereological sections showed significant reduction in neuronal cell damage in the elderberry-treated group as compared with controls fed with the diet without elderberry. Immunohistochemical analysis documented pronounced microglia activation in the hippocampus and lateral striatum in animals with transient global ischemia compared with those that underwent sham operation. This microglia activation is partially prevented in animals on elderberry diet. Since we hypothesized that the botanical diets exert beneficial effects through mitigation of inflammatory and oxidative responses, we tested whether ischemia-induced production of reactive oxygen species (ROS) was associated with activation of NADPH oxidase, a superoxide producing enzyme complex comprised of cytosolic and membrane subunits. Assessment of immunoreactivity of p47phox, a cytosolic subunit of the NADPH oxidase complex, indicated co-localization with activated microglia, and suppression of its expression in the elderberry-fed animals. The same trend can be shown when brain sections were stained for phospho-ERK1/2, a MAPK known to be expressed in activated microglia. Taken together, our results demonstrated protective effects of elderberry diet in a mouse model of cerebral ischemic stroke and its potential translational value as dietary supplement against ischemic stroke.

Quantitative Proteomic Analysis for the Effects of Elderberry on Global Cerebral Ischemia in Mice (P)

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Stroke is the third leading cause of death and disability in aging adults. It is commonly caused by interference in blood flow to the brain that results in considerable brain damage. Elderberry has been found to exhibit benefits in human health through its antioxidant properties, which are assumed to offer beneficial effects on antagonizing inflammatory responses and preventing neurodegenerative diseases. Although interest in neuronal-related actions of elderberry is increasing, the molecular and cellular mechanisms underlying their mode of action have not been elucidated. In the present study, we selected a global cerebral ischemia mouse model. Mice were fed a semi-purified control diet (AIN-93G) or one of two experimental diets that contained elderberry (2% of freeze-dried whole fruit) for 2 months prior to 30-min bilateral common carotid artery (BCCA) occlusion followed by 3-day reperfusion. We then conducted a comprehensive proteomic analysis to investigate the overall changes in protein levels in various regions of ischemic injury mice brain (cortex, striatum and hippocampus regions). We used deuterium isobaric amine reactive tag (DiART) labeling, followed by liquid chromatography/tandem mass spectrometry (LC-MS/MS) analysis. Compared to the control diet for 2 months prior to global ischemia in mice, a total of 56, 62 and 94 proteins showed significant protein level changes ($p < 0.05$) in cortex, striatum and hippocampus, respectively. We further compared the proteomes of the three regions and identified 37 common proteins along with 12, 18 and 25 specific proteins for cortex, striatum and hippocampus, respectively. In addition, with the proteins exhibiting significant protein level changes, Ingenuity Pathway Analysis (IPA) was used to identify multiple molecular targets and predict signal transduction pathways affected by elderberry. Our study may provide important insights into the molecular events underlying the treatment of elderberry and allow the identification of novel therapeutic targets.

ELDERBERRY OVERVIEW

The Versatile Elderberry: Research, Production, and Utilization

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Elderberry, this legendary and valuable species, once called the "medicine chest of the country people" has been known, valued, and used for centuries. Since the very early times through history, this species has undergone a long path of research and development to reach its present state as a popular cultivated crop. *Sambucus* is a most versatile genus in many aspects. Based on its botanic traits, until recently the genus *Sambucus* was ranked to the Honeysuckle family (Caprifoliaceae) and it is only on the basis of new genetic evidence that it has been reclassified to the family Adoxaceae. Similarly, the taxonomic rank of American elder (*S. canadensis*) has been recently revised and ranked as subspecies (*S. nigra* L. ssp. canadensis (L.) R. Bolli) to *S. nigra* L. (European elder). The genus numbers some 5 to 30 or more species, all utilized to a different degree. *Sambucus nigra* is widespread in Europe, western Asia, and north Africa, and is also grown in North America, whereas the American elder and similar species (*S. mexicana*, *S. simpsonii*, *S. velutina*) are distributed mostly on the North American continent. Ethnobotanically, nearly all organs of the plant have been known to cure certain health conditions. Current traditional uses of elderberry fruit and flowers include flu, colds, fevers, constipation, and sinus infections. As unripe elderberries are toxic and cause nausea, vomiting, or diarrhea, only the ripe blue/black berries are edible. In traditional medicine, the leaves, inflorescences, and even the bark of green shoots (*Sambuci cortex*) have been used (e.g. to cure rheumatism, or as a diaphoretic). The known uses of elderberry are rather versatile: they include cordials, wines, and teas produced from both fruits and flowers. Other uses in food products include elderberry jam, elderflower fritters, and other baked goods. Due to their favorable ingredients the fruits have become important constituents in the so called reform-food for diabetics. The berries are also traditionally a rich source of natural colorants (dyes) used to improve both food and red wine. Recently, elderberry's antioxidants, its ability to fight heart disease, cancer, and aging, complemented by its reported antibacterial properties, have all increased the species' popularity among the public. Elderberry is also considered to be a useful range plant for domestic livestock, although it is not equally palatable during all seasons. Similarly wildlife can profit from it in many different forms for food and shelter.

The demand for elder flowers and fruits is apparently huge. Natural supplies are still abundant due to the weedy character of elder. Safety, efficacy, and quality requirements are becoming increasingly important not only for medicinal products, but also for other forms of utilization. The traceability of collection and production processes is expected to play an increasingly-important role with this crop. Best Collection, Production, and Manufacturing Practices (GCP, GMP) are used as tools to certify quality. Quality requirements for medicinal uses are becoming increasingly strict (e.g. according to both National Center for Complementary and Alternative

Medicine, and European Medicines Agency). Current scientific evidence is still not strong enough to support several forms of the reputed uses of elderberry, among them to relieve flu. The versatile character of elderberry and its most varied forms and ways of utilization seem to be a guarantee for fame and lore well into the future. New emerging value-added forms of utilization are expected to further increase demand, so that elderberry is likely to offer income and livelihood for stakeholders of the entire production verticum.

Professor ALBERT Y. SUN, Ph.D.

Professor Albert Y. Sun was born on October 13th, 1932 in Amoy, China and moved to Taiwan after junior high school. He received his B.S. degree in Agricultural Chemistry from the National Taiwan University. He then came to the U.S. where he pursued a Ph.D. degree in Biochemistry at Oregon State University, and further postdoctoral training in Biochemistry at Case Western Reserve University in Cleveland, Ohio. In 1969, Dr. Sun began his work as a research scientist in the Neurochemistry laboratory at the Cleveland Psychiatric Institute where he pioneered studies on Na, K-ATPase and synaptic membrane excitation, and mechanisms of bioenergetics (mitochondrial oxidative-phosphorylation) in the brain. In 1974, Dr. Sun was recruited as Research Professor to the Sinclair Comparative Medicine Research Farm at the University of Missouri in Columbia. Here, he furthered his research in neuroscience, focusing on mechanisms of free radical reactions and oxidative stress in brain damage, aging, and neurodegenerative disorders, using cell and animal models. He was recognized for his discoveries on the free radical hypothesis of ethanol and its oxidative damage in brain membranes. In 1989, Dr. Sun became an active faculty member in the Department of Pharmacology and initiated research linking neuropharmacology to age-related neurodegenerative diseases. He was recognized for his contribution on furthering the concept of the “French Paradox”, and subsequently demonstrated the neuroprotective effects of resveratrol, a polyphenol antioxidant found in grapes and red wine. Dr. Sun was also a pioneer faculty member of the MU Interdisciplinary Neuroscience Program and the Center for Translational Neuroscience, and in recent years, an Adjunct Professor in the Department of Pathology and Anatomical Sciences. During his career, Dr. Sun published more than 150 scientific papers. Although he retired and became a Professor *emeritus* in 2005, he continued to actively engage in research on botanical polyphenols as a member of both the MU Alzheimer’s Disease Research Program and the MU Center for Botanical Interaction Studies. For over 45 years, Dr. Albert Sun worked side by side with his wife, Dr. Grace Sun, to enhance the understanding of biochemical and biophysical mechanisms underlying neurodegenerative diseases and to develop novel therapeutic potentials to combat these diseases.

Dr. Sun was active in professional organizations including the Free Radical Society, the Society of Neurosciences, the International Society of Biomedical Research in Alcoholism (ISBRA), the American Society for Biochemistry and Molecular Biology, and the American and International Societies for Neurochemistry. Over the years, Dr. Sun chaired numerous international workshops and symposia including, for example, the “Workshop on Ethanol and Oxidative Stress” at the ISBRA International Meeting in Yokohama, Japan, 2000, and the International Symposium on Recent Advances in the Biomedical Research in Alcoholism in Taipei, Taiwan, 1988 and 2000.

Dr. Sun was a great mentor to many students, postdocs and junior faculty. He was also a devoted Christian, setting an example of faith, service, and humility for those who were blessed to be influenced by him. As an Elder of the Church, he devoted much time and energy to his ministry among Chinese students and families. He served as a board member of the Christian Witness Center in Warsaw, Missouri, for more than 30 years. Dr. Sun is the eldest of 3 brothers and 5 sisters. Besides the beloved husband of Grace, he is the loving father of Aggie Sun and grandfather of Joshua and Caleb Lin.

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