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Fireweed, roseroot, *Bergenia* and chokeberry – joint research for supporting the herb production

Sanna Kauppinen and Bertalan Galambosi (eds.)

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Sanna Kauppinen and Bertalan Galambosi (eds.)

[1]Natural Resources Institute Finland, Lönnrotinkatu 3, 50100 Mikkeli, Finland, sanna.kauppinen@luke.fi

[2]bertalan.galambosi@surffi.fi

Abstract

In this publication you find the main results of the project called ”Special crop education for economic development in North-West Russia and South-East Finland SPECICROP”. These results concern the fermentation methods of fireweed and *Bergenia* leaves, measurements of active substances of different roseroot strains, the effect of *Bergenia* leaf extract for the endurance of sportsmen, the effect of chokeberry for blood pressure and inflammatory factors and the development of herb production in Leningrad area and Finland.

Infusion from fermented *Epilobium angustifolium* L. leaves (Ivan tsay, Koporye tea) is traditionally consumed in Russia for the treatment of stomach ulceration, gastritis, and sleeping disorders. The traditional production method of Ivan tea includes of several labour-consuming steps. During 2012–2014 in the frame of SPECICROP project a semi-industrial fermentation process of shoots of fireweed was studied. On the base of these experiments we concluded that there is a possibility to produce Ivan tea commercially from a 40 cm shoot yield instead of leaves. The steps and the optimized parameters of the process are described on the proposed technological recommendation. The production process can be reproduced in the conditions of small-scale enterprises.

During the domestication experiments of *Rhodiola rosea,* a collection of roseroot strains from different geographical origins was created at Mikkeli, South-East Finland. The aims of this study was to compare the root biomass and salidroside and total rosavin contents of different accessions and to choose the best accessions for further propagation. Six accessions containing high salidroside and total rosavin contents were chosen for saving in the official Finnish medicinal plant gene collection.

The leaves of the commonly known decorative perennial, *Bergenia* species have several interesting biological activities. The green leaves contain arbutin up to 22% (d.w.), a compound, which is used in cosmetic industry as a skin-whitening agent in humans. Infusion prepared from the naturally fermented black leaves is used as a tea in Russian ethno medicine. For the utilization of new possibilities of *Bergenia* species during 2012–2014 several studies were carried out in SPECICROP project for elaborating a semi-industrial fermentation process of *Bergenia* leaf yield.

In the previous studies it was shown that supplementation of animals with the extract from fermented leaves of *Bergenia* enhanced the maximum swimming capacity of mice. In the SPECICROP project it was studied whether *Bergenia* leaf extract has any effects on endurance exercise capacity or muscle strength of a human. The study included two experimental designs: to investigate the acute effects and the effect of 7-day use of *Bergenia*. The results of the present study showed that the *Bergenia* supplementation with the current doses of 100–400 mg does not have almost any effect on endurance and maximal force characteristics in physically active men. *Bergenia* supplementation did not cause any negative effects on the present subjects either. Probably other dose ranges would be more correct for future human experiments.

In Specicrop project we studied effects of different dosages of Finnish chokeberry (X *Sorbaronia mitschurinii,* var. *Viking*) juice on blood pressure of the spontaneously hypertensive rats. We also measured the inflammatory cytokines in rat blood in order to see the potential beneficial effects of the chokeberry juice on chronic inflammation which is typical in hypertension. The study provided encouraging data.

During the last decade the interest for domestic grown herb raw material has increased among pharmaceutical companies in the Leningrad region, Russia. There are eight vocational agricultural colleges in the Leningrad region, and none of them has taught herb cultivation. There is a lack of cultivation expertise, also in herb cultivation education material. Due to the cross border cooperation with the support of EU Interreg and ENPI programs, during 1995–2014 several projects have resulted specialisation for herb education in the Begunitsky Agrotechnological College, in connections with Finnish institutes. Due to the similar northern climate conditions in regions of South-East Finland and North-West Russia, the cooperation focused on cold tolerant medicinal and berry and fruit plants. One of the aims was to transfer the methods of cultivation obtained in small-scale herb farms in Finland into Russia. In addition to an update, modern herb-cultivation teaching material was produced in Finnish and Russian.

Keywords: *Aronia mitschurinii, Bergenia,* chokeberry, cross-border cooperation, *Ebilobium angustifolium*, fireweed, herb cultivation, *Rhodiola rosea*, roseroot, X *Sorbaronia mitschurinii*

Horsma, ruusujuuri, vuorenkilpi ja marjapihlonia – yhteistutkimusta yrttituotannon tueksi

Sanna Kauppinen[1] ja Bertalan Galambosi[2] (toim.)

[1]Luonnonvarakeskus, Lönnrotinkatu 3, 50100 Mikkeli, sanna.kauppinen@luke.fi

[2]bertalan.galambosi@surffi.fi

Tiivistelmä

Tähän julkaisuun on koottu ”Special crop education for economic development in North-West Russia and South-East Finland SPECICROP” -hankkeen merkittävimmät tulokset. Ne käsittelevät maitohorsman ja vuorenkilven lehtien fermentointimenetelmiä, eri ruusujuurikantojen vaikuttavien aineiden pitoisuuksia, vuorenkilven vaikutusta ihmisen suorituskykyyn, marjapihlonian (aik. marja-aronia) vaikutusta verenpaineeseen ja tulehdustekijöihin sekä yrttiopetuksen kehittämistä Leningradin alueella ja Suomessa.

Maitohorsman (*Epilobium angustifolium* L.) fermentoiduista lehdistä valmistettua teetä käytetään Venäjällä perinteisesti vatsahaavojen, mahakatarrin ja nukkumisvaikeuksien hoitoon. Maitohorsmateen perinteinen valmistusmenetelmä sisältää useita käsityötä vaativia vaiheita. Vuosien 2012–14 aikana SPECICROP-hankkeessa tehtiin useita koesarjoja joissa fermentoitua teetä valmistettiin maitohorsman versoista. Tuloksien pohjalta todettiin, että tee voidaan valmistaa kaupallisessa mittakaavassa maitohorsman 40 cm pitkistä tuoreista versoista. Tuotantoprosessin eri vaiheet optimoitiin ja ne esitellään ehdotetussa tuotantomenetelmässä. Kuvattu menetelmä sopii pienen yrityksen olosuhteisiin.

Mikkeliin kerättiin aikoinaan kokoelma ruusujuuria (*Rhodiola rosea*) eri puolilta maailmaa. SPECICROP-hankkeen kokeissa vertailtiin ruusujuurikantojen eroja juuren biomassan ja kahden vaikuttavan aineen (salidrorosidi ja rosaviini) suhteen sekä valittiin parhaimmat kannat jatkoviljelyyn. Kuusi ruusujuurikantaa, joiden salidrosidi- ja kokonaisrosaviinipitoisuudet olivat korkeat, valittiin talletettavaksi Suomen viralliseen lääkekasvien geenivarakokoelmaan.

Puutarhojemme suositun perennan vuorenkilven (*Bergenia* sp.) vihreät lehdet sisältävät jopa 22 % kuivapainostaan arbutiinia, jota käytetään kosmetiikkateollisuudessa ihon vaalentamisaineena. Luonnollisesti fermentoiduista mustista lehdistä valmistetaan Venäjällä teetä, jota käytetään kansanlääkinnässä. Hyödyntääksemme vuorenkilven uusia mahdollisuuksia kehitimme sen lehtisadolle tuotantomittakaavaisen fermentointimenetelmän.

Aikaisemmassa tutkimuksessa on osoitettu, että käyneiden *Bergenia*-lehtien uutteella voidaan parantaa hiirien maksimaalista uintisuoritusta kontrolliryhmään verrattuna. SPECICROP-hankkeessa selvitettiin *Bergenia*-ravintolisän vaikutusta ihmisen kestävyys- ja voimaominaisuuksiin. Tutkimuksessa oli kaksi koeasetelmaa: *Bergenia*-ravintolisän akuuttivaikutus sekä yhden viikon päivittäisen käytön vaikutus. Tutkimuksen tulokset osoittivat, että käytetyillä 100–400 mg:n *Bergenia*-ravintolisäannoksilla ei ollut juuri minkäänlaisia vaikutuksia kestävyys- tai voimaominaisuuksiin liikuntaa harrastavilla miehillä. Myöskään haittavaikutuksia ei ilmennyt. Tulevaisuudessa pitäisi kokeilla muita annoskokoja.

SPECICROP–hankkeessa tutkittiin myös suomalaisesta marjapihloniasta (X *Sorbaronia mitschurinii*, lajike ‘Viking’) tehdyn mehun vaikutuksia erisuuruisina annoksina rottiin, joilla oli synnynnäinen verenpainetauti. Mittasimme myös rottien veren tulehduksen merkkiaineita, jotta saataisiin selville vaikuttaako aroniamehu verenpainetaudille tyypilliseen krooniseen tulehdukseen. Tutkimuksesta saatiin rohkaisevia tuloksia.

Viimeisen vuosikymmenen aikana venäläisten lääkeyritysten kiinnostus kotimaassa kasvatettua yrttiraaka-ainetta kohtaan on kasvanut Leningradin alueella. Tällä alueella toimii kahdeksan maatalousoppilaitosta, mutta yksikään niistä ei opeta yrttiviljelyä. Yrttiviljelyasiantuntemuksesta

samoin kuin yrttiviljelyn opetusmateriaalista on puute. Rajat ylittävän yhteistyön avulla ja EU Interreg- ja ENPI-ohjelmien avustuksella vuosina 1995–2014 useat hankkeet kehittivät Begunitsyn maatalousoppilaitoksen yrttiviljelyopetusta suomalaisten organisaatioiden tuella. Kaakkois-Suomessa ja Luoteis-Venäjällä on samanlainen pohjoinen ilmasto, joten yhteistyö keskittyi talvenkestäviin yrtti- ja rohdoskasveihin sekä marjoihin ja hedelmiin. Yhteistyön yhtenä tavoitteena oli siirtää pienten suomalaisten yrtti- ja marjatilojen toimintamallia Venäjälle. Lisäksi SPECICROP-hankkeessa tuotettiin yrttiopetusmateriaalia suomeksi ja venäjäksi.

Avainsanat: *Aronia mitschurinii, Bergenia, Ebilobium angustifolium*, lähialueyhteistyö, maitohorsma, marja-aronia, marjapihlonia, *Rhodiola rosea*, ruusujuuri, vuorenkilpi, X *Sorbaronia mitschurinii,* yrttiviljely

Foreword

This publication introduces you research results that have been created in the long-term cross-border collaboration between Finnish and Russian researchers.

One of the strategies of Natural Resources Institute Finland (Luke), formerly MTT Agrifood Research Finland, in Mikkeli location was to integrate and research Russian medicinal plants in Finnish climate. During the years 2002–14 within five international projects a comprehensive and intensive cooperation was carried out between MTT and research institutes in St. Petersburg. The basis for this collaboration has been the geographical proximity and climatic similarity of South Savo and North-West Russia.

Plants like trifid bur-marigold, alfalfa, *Bergenia* and fenugreek that formerly have not known as medicinal plants in Finland have been tested in experimental fields in Mikkeli. Also berry species from Russia, like chokeberry and sea buckthorn, have been under investigation. These research results were published in 2007 (Kivijärvi,P. & Galambosi, B. 2007. Uutuusrohdoskasvit sekä tyrni ja marja-aronia terveyden edistäjinä (*Novel medicinal plants and sea buckthorn and chokeberry as health promoters, in Finnish*). Maa- ja elintarviketalous 105. http://urn.fi/URN:ISBN:978-952-487-117-4).

After this research the focus was directed into the main medicinal plants where the aim was to support their commercialization by developing the processing methods and bringing up the health effects. During the years 2012–14, by the project called "SPECICROP", several results were achieved that are now published. Fermentation methods of traditional fireweed tea (Ivan tea, Koporye tea) and *Bergenia* tea (Siberian or Mongolian tea) from Altai mountain range were optimized to suit to the small-scale companies. By the result of international cooperation 26 roseroot strains from all over the world could be analyzed by their quality characters and the best of those were chosen for further production. In addition to these field experiments the chokeberry juice was tested for blood pressure and inflammatory factors in the laboratory and the effect of *Bergenia* leaf extract was evaluated for endurance of sportsmen.

In addition to herbs in the SPECICROP project also foreign, mainly Russian, fruit cultivars were tested and evaluated for Finnish climate. Preliminary results can be found from the publication published earlier (Nissinen, M., Kauppinen, S. & Kinnanen, H. 2016. Ulkomaisia hedelmälajikkeita Suomen oloihin – alustavia viljelykokemuksia ja suosituksia. (*Foreign fruit cultivars suitable for Finnish climate – preliminary experiences and recommendations, in Finnish*.) Luonnonvara- ja biotalouden tutkimus 9/2016. http://urn.fi/URN:ISBN:978-952-326-184-6).

During the SPECICROP project we had a chance for valuable interaction: Russian experts got familiar with small-scale farms and their efficient berry and herb production in South Savo and correspondingly Finnish experts got to visit Russian fruit institutes in Moscow and herb industry in Altai region.

In addition to theoretical research the cooperation gained also practical results. Thanks to the projects Begunitsy agrotechnological college has got better premises for teaching the herb cultivation (demonstration garden, processing machinery, teaching material) and the use of black plastic as a mulch has been introduced for herb and berry production in North-West Russia.

The long term cooperation has been fruitful also what comes to the amount of publications. Number of scientific and professional articles is altogether 75 pieces during the years 2003–2015. The list of publications can be found from the annex of article 6. The SPECICROP project enabled the compilation of Bertalan Galambosi's herb research results of several decades: Finnish National Board of Education published in November 2016 a text book called Yrttien viljely (*Cultivation of herbs, in Finnish*), where a comprehensive information of 69 herbs can be found. Similar Russian text book with 22 herbs is into works, but manuscript is already compiled. Bertalan Galambosi retired in year

2011 from the vacancy of research scientist in MTT Agrifood Research Finland and that is why he acts as a private person in this publication.

The project ”Special crop education for economic development in North-West Russia and South-East Finland SPECICROP” was enabled by the co-financier South-East Finland – Russia ENPI CBC 2007-2013 program and its administrator Regional Council of South Karelia. The lead partner of project was MTT (currently Luke) and other partners were St. Petersburg Institute of Pharmacy, Begunitsy Agrotechnological College, South-Savo Vocational College and Euro-Rahoitus Oy.

This publication is an example of good spiritual and successful cross-border cooperation. We are grateful for all the people and organizations that have been part of and given their contribution to the SPECICROP project and this publication.

We hope that the research work now published the other hand shows our belief for the importance of the development of this special business area and on the other hand gives basis and stimulus for the continuity of the work!

December 2016
Editors

Contents

1. Optimization of the fermentation of fireweed (*Epilobium angustifolium*) shoot 11

1.1. Fireweed (*Epilobium angustifolium*) as a medicinal plant 11

1.2. Previous research in MTT Agrifood Research Finland with *Epilobium sp.* 12

1.3. Fermentation experiment in Specicrop project 2012–2014 12

1.4. Material and methods 13

1.5. Results 13

1.6. Summary 20

2. Evaluation of Finnish roseroot (*Rhodiola rosea*) gene collection from different geographical origins 23

2.1. Introduction 23

2.2. Aim of the research 24

2.3. Material and methods 24

2.4. Results 27

2.5. Discussion 30

2.6. Evaluation of the groups and the choice of the best accessions 31

3. Optimization of the fermentation of *Bergenia sp*. green leaves 34

3.1. Bergenia as an interesting research object of Finnish-Russian cooperation 34

3.2. The aims of the experiments 35

3.3. Material and methods 36

3.4. Results 36

3.5. Conclusion 41

Appendix 1. Recommendation for the fermentation process of Bergenia leaf yield 44

4. Clinical trial design for *Bergenia* samples, double blind placebo controlled crossover study 46

4.1. Aim of the study 46

4.2. Material and methods 46

4.3. Results 48

4.4. Conclusion 49

5. Chokeberry (X *Sorbaronia mitschurinii*) for health 55

5.1. Study of effects of chokeberry juice in Specicrop project 55

5.2. Chokeberry and risk of widespread diseases 56

5.3. Conclusions 57

6. Development of herb education in Begunitsky Agrotechnological College 60

6.1. Lack of herb educational expertise in North West Russia 60

6.2. Some achievements of the projects between 2002–2011 61

6.3. Activities in the SPECICROP project 62

6.4. Summary 66

Appendix 1. Participation in different congresses with posters and lectures between 2003–2010 68

Appendix 2. List of scientific publications between 2003–2012 71

Appendix 3. Scientific papers, congress abstracts and popular articles in SPECICROP-project 2012–2014 74

Appendix 4. The content of Finnish and Russian herb cultivation book 77

1. Optimization of the fermentation of fireweed (*Epilobium angustifolium*) shoot

Galambosi, B.[1], Galambosi, Zs.[2], Shikov, A.N.[3], Kosman, V. M.[3], Pozharitskaya, O.N.[3], Kauppinen, S.[2], Makarov, V.G.[3]

[1]bertalan.galambosi@surffi.fi

[2]Natural Resources Institute Finland (Luke), Lönnrotinkatu 3, 50100 Mikkeli, Finland

[3] St.-Petersburg Institute of Pharmacy, 56, Bolshaja Porochovskaya, POBox 16, 195248, St-Petersburg, Russia

Abstract

Infusion from fermented *Epilobium angustifolium* L. leaves (Ivan tsay, Koporye tea) is traditionally consumed in Russia for the treatment of stomach ulceration, gastritis, and sleeping disorders. The traditional production method of Ivan tea includes of several labour-consuming steps. During 2012–2014 in the frame of SPECICROP project a semi-industrial fermentation process of shoots of *Epilobium angustifolum* L. was studied. The experiments were carried out in MTT Agrifood Research Finland Mikkeli in cooperation with St.-Petersburg Institute of Pharmacy, St-Petersburg, Russia. On the base of these experiments we concluded that there is a possibility to produce Ivan tea commercially from a 40 cm shoot yield instead of leaves. The steps and the optimized parameters of the process are described on the proposed technological recommendation. The production process can be reproduced in the conditions of small-scale enterprises.

Key words: Epilobium angustifolium, shoot yield, fermentation parameters, technological recommendation

1.1. Fireweed (*Epilobium angustifolium*) as a medicinal plant

Epilobium angustifolium L. (fireweed) is a medicinal plant that has been used to treat diarrhea, mucous colitis, irritable bowel syndrome, skin problems, prostate problems, menstrual disorders, asthma, whooping cough, and hiccups. In Finland it belongs to the official natural herbs (7). Infusion from fermented *Epilobium angustifolium* L. leaves (known as Ivan tsay or Koporye tea) is traditionally consumed in Russia for the treatment of stomach ulceration, gastritis and sleeping disorders. It has been used in folk medicine to treat a variety of ailments such as benign prostate hyperplasia and the associated problems of micturition. The traditional production in North-West Russia includes several labour consuming steps (1, 8, 15).

In the oral tradition and in the literature several methods have been mentioned, in which the important parameters (preparation before fermentation, fermentation temperature, time, equipments) are described differently and inaccurate.

According to a Russian agronomist (15) the peasants of Kopor'ye village in North-West Russia produced fermented Ivan tsaj during the XIX century by the following method:

"Preparation of tea was done as follows: first leaves were poured by boiled water and stewed in a stew pot on hot stones. After that every leaf was rolled between the palm, subsequently shaped by wooden boards until small bales became wet and strong-smelling. Bales were spread thinly on shingle sheets and put in oven with mild heat so that they didn't get roasted or swollen. A new tea got a name "Tsaj Koporski" after the Finnish village Koppiorja."

According to a personal communication (Georgy Kirakosian, Expert Bio Ltd, St-Petersburg) the leaves have to be withered at room temperature for 4–5 hours, then partly desiccated leaves have to be crushed by hand or by meat mincer. The fermentation is conducted at room temperature in plastic bags during 12 hours. The fermented plant masses finally have to be dried at 40 °C.

A Russian research team described the fermentation of *Epilobium angustifolia* leaves in commercial scale as follows: The botanical material was (pre)dried in a dehydration tunnel at 40°C for 5–6 h. After comminution, the tea was placed in heaps on outside platforms, wetted and bruised to initiate "fermentation", and fermented for 6 h at 40–41 °C. After this the fermented tea was dried in a dehydration tunnel at 40 °C for 4–6 h (12).

According to experimental series of a Finnish research team during the 80's, the fermentation method was proposed for several herb teas, including *Epilobium* leaf tea, as follows: 1. The leaves are withered for some hours. 2. The plant material is crushed by hand for destroying the plant cells until the plant balls are wet from their own moisture. 3. The fermentation is carried out in 0.5–1 litre glass jars, filled tightly with the wet plant balls. The jar is not totally closed, so that some oxygen can move into the plan mass. The fermentation is carried out at 40 °C for 2 days. The fermented, black leaves have to be dried at 35 °C with good ventilation. (6).

1.2. Previous research in MTT Agrifood Research Finland with *Epilobium sp.*

The above mentioned methods are suitable for small scale tea production and are quite labour intensive. During 2006–2008 several experiments were carried out in the frame of Tacis/Interreg project (No. 2007/134-881.) in MTT in cooperation with Interregional Center "Adaptogen" St.Petersburg, Russia. In the project "New herbs for rural development" we focused on the phytochemical and agrobiological questions of several *Epilobium* species. We have carried out preliminary fermentation experiments of *E. angustifolium* as well, which aimed semi-industrial production of fermented *Epilobium* tea. The experiments took into consideration the above mentioned traditional methods of fermentation, but there was one important difference: instead of leaves we used 40 cm long shoot tips, as a precondition for mechanical harvest in industrial production.

The results of those experiments have been published in several papers and lectures (1, 2, 3, 9, 13, 14). As a conclusion, we could state, that instead of leaves the young plant shoots can be used for fermented Ivan tea preparation. The optimum harvest time is the budding phase or the start of flowering. For destroying the plant cells before fermentation we developed a mechanical method, using a meat mincer. For fermentation we used the proposed temperature region (21–31° C), but the fermentation started quite slowly and its period was long. We concluded that additional experiments seem to be necessary.

1.3. Fermentation experiment in Specicrop project 2012–2014

In the case of industrial scale production of *Epilobium* leaf raw material the shoot yield has to be harvested effectively by hand or mechanically. Due to its different characteristics, the technological process has to be studied and developed in detail. On the base of our previous experiences, these aspects were studied during 2012–2013 in Specicrop project. (5).

Aim of the experiment

The aim of the experiment was to optimize the technological parameters of a semi-industrial fermentation process of *Epilobium angustifolium* shoot and give recommendation for industrial production in small enterprises or specialized farms.

In these experiments the following parameters were studied:

- optimum harvest time (phenological phase for optimization of effective substances)
- characteristics of raw material (leaf: stem ratio, dry matter content, differences in the contents between leaves and stems

- mincing the material (laboratory size and industrial size machinery)
- equipment for fermented plant mass (bag, box, size)
- optimum temperature of fermentation, time of fermentation in hours
- markers for fermentation process, checking the fermentation process
- drying the fermented mass –drying time
- post drying operations of the dry material
- storability of the dry, fermented tea
- effect of the technological elements on the quality of fermented plant material

1.4. Material and methods

Experimental site

The experiments were conducted in MTT Agrifood Research Finland Mikkeli, Karila experimental station during 2012–2014.

Plant material

The studied plant material was obtained from a natural population of *Epilobium angustifolium* near the forest of institute, every year from the same place. The upper shoots of plants were harvested in different phenological phase, presented correctly in each experiment.

Machinery used:

Plant cutting machine:	Hege 44 Wintersteiger
Meat mincer:	Kenwood Gourmet 1600
Vegetable mixer:	Kenwood Gourmet 1600
Industrial meat mincer:	type KT 32, owned by Pennan Liha Ltd, Juva
Drying machines (for drying and fermentation):	WTB type, Binder, /8532.,Tuttlinger, Germany
Equipment:	small plastic boxes (size 12 x 14 x 5 cm)
	bigger plastic boxes (size 21 x 28 x 8 cm)
	black plastic bags
	screen with 3 mm hole

Chemical analyses

The analyses of non-fermented dried shoots (at 40° C), and the fermented whole shoots (at 28–35–40 °C) were carried out in the laboratory of St. Petersburg Institute of Pharmacy, St-Petersburg, Russia. The content of tannins, flavonoids, hyperoside and oenothein B were determined.

1.5. Results

1.5.1. Characteristics and quality of the plant material

The hand harvested plant material consisted of 30–40 cm long upper parts of cc.1– 1.5 m long shoots. In 2012 the budding time was at 11th of June, flowering started at 5th of July and full flowering was at 10th of July. The composition of shoots in leaf and budding time is soft and juicy, later the stems become thicker and woodier. The dry matter content soon after the harvest is variable between 15–22%, depending on the weather conditions.

According to our cultivation experiments the harvest of upper parts of shoots can be carried out mechanically (3).

The proportion of leaves of the whole plant mass was 57% before budding, and increased up to 71% after start of flowering. During full flowering the leaves reached their maximum size and their proportion was increased up to 80%.

The analyses of separate dried leaves and stems demonstrated significant quality differences between leaves and stems. According to the results, total tannin content in the stems was lower by 43%, flavonoid content 79% lower and hyperoside content 74% lower than in leaves (Table 2).

Contrary and surprisingly oenothein B content was similar or 5–10% higher in stems than in leaves. From practical point of view this result seems quite positive in the case of using the whole stem for fermented tea production. Several chemical studies suppose that oenothein B is one of the most important compound in *Epilobium* preparations (11).

1.5.2. Optimum phenological phase

In the experimental year 2012 we studied the effect of phenological phases of *Epilobium angustifolium* to the contents of studied compounds of the dry shoots (Table 1).

Table 1. Contents of the studied compounds (mg/g) in the whole dried shoots. Mikkeli, 2012

Harvest time	Phenological phase	Total tannins	Total flavonoids	Hyperoside	Oenothein B
June 11th	leaves, before budding	76±1	17±1	4.8±0,2	33.8±1.6
July 5th	start of flowering	85±2	19±1	7.8±0,5	38.3±0.2
July 10th	full flowering	78±2	22±1	9.5±0.3	25±1

According to the results, concentrations of tannin and oenothein B were maximal during the beginning of flowering, while the total flavonoids and hyperoside contents were at medium level. Thus, we concluded that the optimum harvest time seems to be in the start of flowering.

The flowering time may vary year to year. Due to warm spring on year 2013 the flowering started on 27th of June, which was two weeks earlier than on year 2012. Contrary, in our previous results, on 2007 the highest oenothein B content was measured similarly during the budding phase, even if this phase was quite late, on 1st of July (13).

1.5.3. Withering time

According to the traditional production method of Ivan tea (15), the leaves should be separated from stems, they are withered for several hours and crushed by hand for destroying the cells of the leaves, initiating the fermentation process by enzyme activity.

In the case of processing large amount of shoots, this process has to be mechanized. We carried out the following processing steps: withering, destroying the plant cells by machinery and using different equipment for fermentation.

The best harvesting time seemed to be late afternoon, cc. 2–6 pm, since during this time plantations were naturally dry. From practical point of view the length of the withering time was cc. 12 hours. The shoot yield were spread at 3–5 cm layer on the table or on the floor and withered until next morning at room temperature. After this the composition of shoots was soft and their dry matter content increased by 2–5 percentages being 23–27%.

According to the analytical results, the withering time had no effect on the contents of studied compounds (Table 2). Generally, no significant differences was observed in the tannin content (withered: non-withered = 80.1 : 80.9 mg/100 g, respectively). Regular, but small (14–18%) decreases were recorded in the total flavonoids, hyperoside and oenothein B contents.

Table 2. Contents of the studied compounds in the non-withered and withered plants on 2013.

Compound mg/g	Repetition	Non-withered			Withered for 12 hours		
		leaf	stem	mean*	leaf	stem	mean*
Total tannins	1	79±1	50±1	73.2	86±1	47±1	76.2
	2	98±1	50±1	87.0	95±1	48±1	85.6
	mean	88.5	50	**80.1**	90.5	47.5	**80.9**
Total flavonoids in rutin equivalents	1	24±1	5±1	20.2	23±1	4±1	18.17
	2	24±1	5±1	19.6	19±1	5±1	16.2
	mean	24	5	**19.9**	21	4.5	**17.18**
Hyperoside	1	9.7±0.1	2.5±0,5	8.26	8.5±0.1	1.5±0.1	6.7
	2	8.3±0,1	1.9±0.1	6.8	6.7±0.1	1.4±0.1	5.6
	mean	9.0	2.2	**7.53**	7.6	1.45	**6.15**
Oenothein B	1	30.9±1	38.3±1	32.4	27.7±1	26.8±1	27.5
	2	32.3±1	31.8±1	32.2	26.3±1	33.2±1	27.7
	mean	31.6	35.0	**32.3**	27.0	30.0	**27.6**

* calculation of the leaf:stem ratio

1.5.4. Destroying of the plant cells by different machines

For crushing the whole plant cells of fresh shoot mass, we tried a home kitchen size meat mincer (Kenwood Gourmet 1600), a home kitchen size vegetable mixer (Kenwood Gourmet 1600) and an industrial size meat mincer (type: KT 32).

By using the kitchen size meat mincer we obtained the optimum result: the plant mass was totally destroyed, resulting green, soft granulates. By using the vegetable mixer we reached quite good result: only some parts of the stems were not totally crushed.

At the same time, we failed in trying the industrial meat mincer. After some minutes the axle of meat mincer got stuck by plant parts and the juice containing polysaccharides made the crushing impossible. For a commercial purpose an industrial-size mixer or cutter is still to be found.

1.5.5. Equipment for fermentation

During the fermentation process we have tried application of equipment for packing the plant mass. The small plastic boxes, originally used for berry packaging contained 250–450 g of fresh minced plant material. We had negative results keeping plant mass in closed plastic bags, or without any covering. In the first case the plant material started to mold, in the second, the top layer was getting dry too quickly. The best result was achieved when the fresh material was covered by a paper. In this way some air could circulate through the gap of the paper, resulted equal fermentation, without too dry surface. No difference was detected in the quality of boxes filled with 250 and 450 g of fresh material.

The fermentation was successful, as well, when we used bigger plastic boxes containing 1.8–2.2 kg of fresh material. The plant material was covered by paper and the plant mass was not mixed during the fermentation. No difference was found in the analytical results between the small and bigger boxes.

1.5.6. The sensory parameters of fermentation

Presently the progress of fermentation process has no adequate markers and it needs some expertise with visual and sensory experience.

During the first 3–5 hours no alteration can be observed and the plant mass remains green. After cc.5 hours the clear green colour is getting grayish green and after 12–16 hours it turns into grey-yellowish colour. Simultaneously after 12–16 hours the original "green, chlorophyll-like" smell starts to change into a pleasant, so-called "pipe tobacco" smell.

After 24–36 hours the grey-yellowish colour turns into deep grey, mustard-yellow colour and the tobacco-like scent strengthens. The end of the fermentation seems to be in that phase, when the colour is not changing more deep and the specific pleasant scent is not strengthening anymore.

1.5.7. Optimization of fermentation temperature

In the traditional fermentation method no exact temperatures were proposed, only temperature range from room temperature to 40°C. In our previous fermentation experiment temperature range 20–31°C was used, but then the fermentation process started slowly and the plant material got easily mould with an acidic smell (2).

During experiments on 2012–2013 three temperature regimes were tested: 28–35 and 40 °C. The fermentation of same plant material was carried out simultaneously in three dryers.

1.5.8. The time of the whole fermentation process

The time we measured and calculated consisted of only the fermentation and the post drying time and didn't include the time for harvest, withering and equalization. According to the results of seven fermentation periods, the length of total fermentation process lasted in average 44 hours (1.8 days), in which the fermentation part was 27–33 hours and the post-fermentation drying was 13 hours (Table 3). In practice these times may vary, since the process is going on during the night, it may mean some additional hours.

Table 3. The length of fermentation processes in 2012–2013.

Year	Temperature	Experiment	Fermentation	Drying	Total length in	
	°C	n	hours (mean)	hours	hours	days
2012	28	2	43	16	59	2.4
	35 and 40	2	33	14	47	1.9
2013	35	1	33	12	45	1.9
	40	3	27	12	39	1.6
mean	35–40		31	12.7	44	1.8

1.5.9. The effect of fermentation temperature on the contents of compounds studied

The general trend was that the fermentation decreased the quantity of the studied compounds, but there were some differences between the components (Table 4 and figures 1–4). The fermentation resulted on the decrease of the total tannin content by 31–44% nearly similarly in both years compared to the non-fermented shoots (Figure 1). Similarly, the fermentation decreased the total flavonoid content as well, but the decrease was smaller, 14–24% (Figure 2). The hyperoside content was decreased in both years by more than half, by 51–68% after fermentation (Figure 3). The

oenothein B content in the fermented plants showed a larger variation than the other compounds. The fermentation decreased its content by 50–71%, the decrease being smallest at 40 °C (Figure 4).

Table 4. Content of the studied compounds in non-fermented and fermented *Epilobium* shoots at three temperatures on 2012–2013.

Compound	Fermentation temp. °C	Leaf 11/6/2012	Budding 5/7/2012	Budding 27/6/2013
Total tannins mg/g	Non-fermented	76±1	85±2	80.9±1
	28	48±1	50.7±1	
	35	49±1	55±1	55.3±3.9
	40	40±1	53±1	55.2±3.9
Total flavonoids mg/g	Non-fermented	17±1	19±1	17.2±1
	28	16±1	18±1	
	35	13.5±1	20±1	14.7±1
	40	12±1	19±1	13.2±0.84
Total hyperoside mg/g	Non-fermented	4.8±0.2	78±0.5	6.15±1
	28	1.9±0.1	3,0±0.1	
	35	2.7±0.1	3.1±0.1	2.97±0.1
	40	1.9±0.1	3.3±0.2	1.96±0.05
Oenothein B mg/g	Non-fermented	33.8±1.6	38.3±0.2	27.6±1
	28	traces	3.9±0.2	
	35	9.2±0.5	2.5±0.1	8±0.1
	40	25.3±1.2	9.6±0.4	9.76±2.56

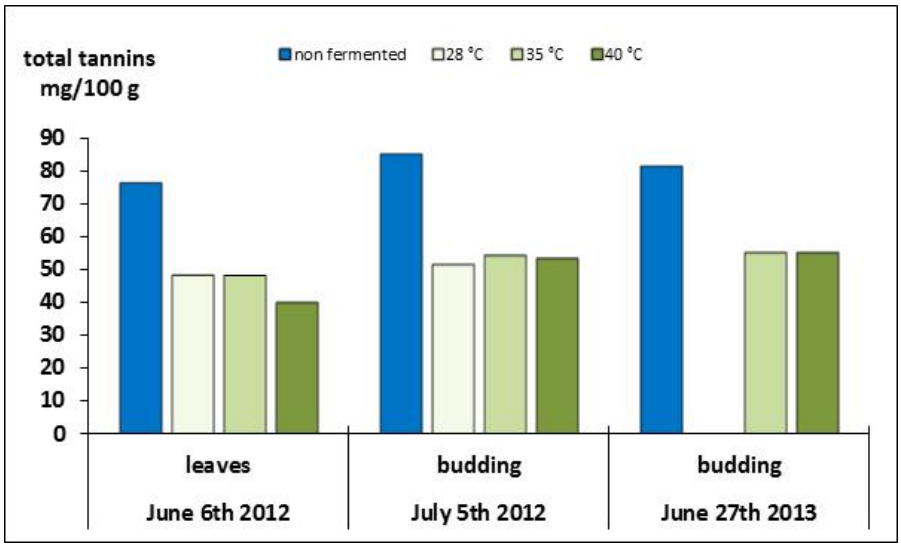

Figure 1. The effect of fermentation temperature on the total tannins during 2012–2013.

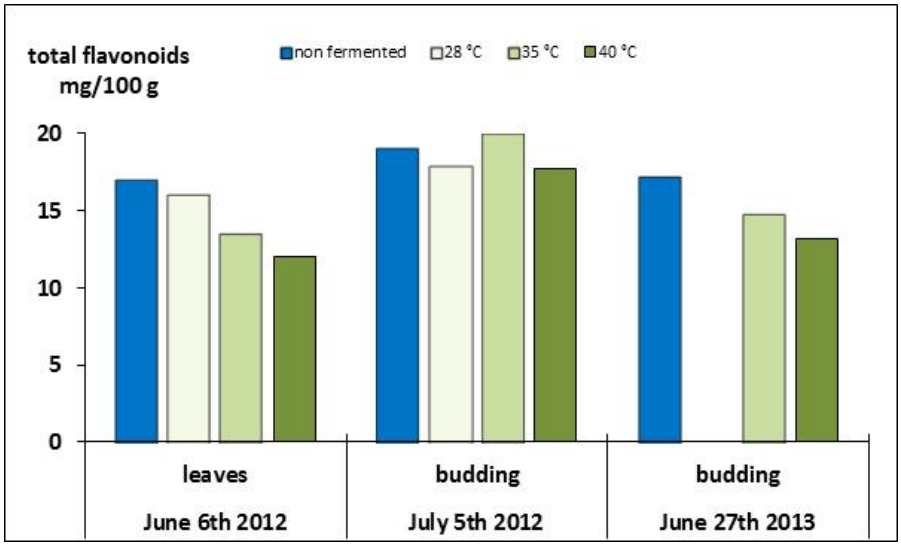

Figure 2. The effect of fermentation temperature on the total flavonoid contents during 2012–2013.

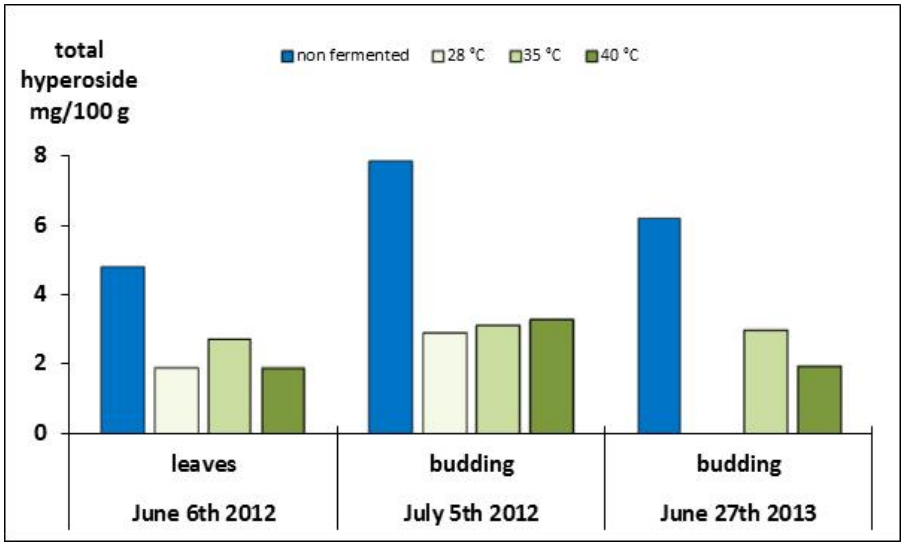

Figure 3. The effect of fermentation temperature on the hyperoside content during 2012–2013.

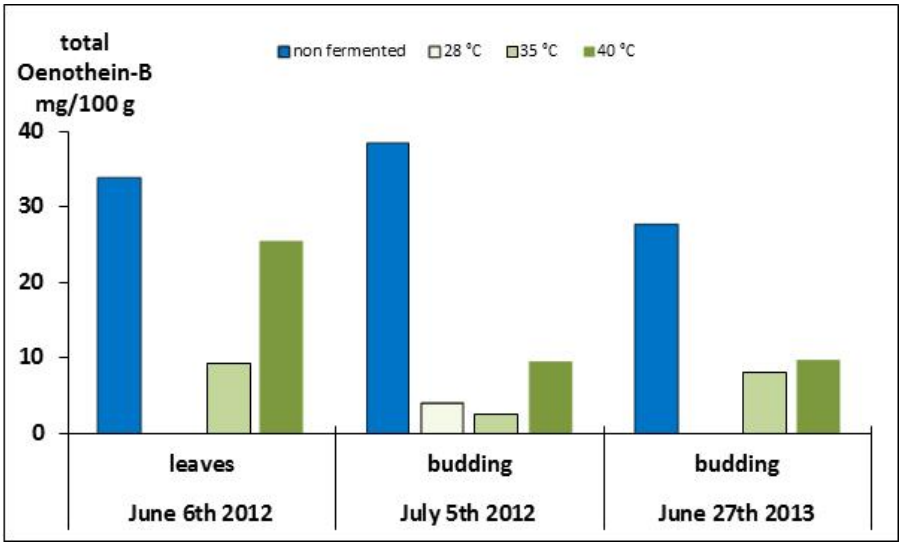

Figure 4. The effect of fermentation temperature on the oenothein B content during 2012–2013.

1.5.10. Drying and handling of the fermented plant mass

The fermented leaf mass was spread into drying boxes in thin layer for the quick drying. For the better ventilation it is preferable that the bottoms of the drying boxes are made of metal net with holes size of 0.5 mm. The drying temperature was 40 °C and the period was 8–12 hours depending on the thickness of the layers. During the end of drying the colour turned to deep brown or light black, like the real tea leaves.

The ratio of the fresh: dry material in leaf phase was 5–6:1, later 4–5: 1. From one kilogram of fresh fermented material in leaf phase could be obtained 160–180 g, later 210–220 g. To obtain one kilogram of dry fermented material is 4–6 kg of fresh milled plant material needed.

The fermented dry plant material consists of hard, small granulates and very small particles. These have to be broken and equalized. For crumbling metal nets of 3 mm hole were used, but coffee mills are suitable as well. After breaking the powder should be separated away, according to the requirement of tea material. The crumbling didn't have any negative effects on the quality of fermented tea (Table 5). Depending on the quality of the cutting of shoots, 0.5–1% of bigger leaf or stem particles may occur. After crumbling it is easy to separate them.

1.5.11. Storing the equalized plant material

Fermented tea material was stored in double packages (paper sack inside and plastic bag outside). The effect of storing time on the quality of the fermented tea material was studied after two and a half year storing at room temperature (Table 5).The change of the individual compounds during 2.5 years storage period was different:

- **Total tannin content:** During the storage period the content of tannins did not change. The differences were only 1–2%.
- **Total flavonoids:** The total flavonoid content in the non-fermented shoots increased significantly, by 47%, while no or minor changes were detected in the fermented samples.
- **Hyperoside**: The hyperoside content in the non-fermented sample increased nearly by two-fold. (For the explanation of these changes an additional research is needed.) The hyperoside contents of fermented samples were small already after fermentation and they dropped more after two years storage being 1.7 mg/g in average.
- **Oenothein B**: Its content in the non-fermented samples decreased 14%, but in the fermented samples the decrease was significant, 65%, in average.

Table 5. Content (mg/g) of studied compounds of *Epilobium angustifolium* after a 2.5-year storage period.

Sample No.	Processing in 2012	Total tannins		Total flavonoids		Hyperoside		Oenothein B	
		2012	2014	2012	2014	2012	2014	2012	2014
B0	Non-fermented, 40°C	76±1	79±1	17±1	25±1	4.8±0.2	7.7±0.1	33.8±1.6	29.1±0.2
B2**a**	Fermented at 35°C	48±1	49±1	13±1	13±2	3.2±0.2	1.4±0.1	10.5±0.5	3.2±0.1
B2**b**	Fermented at 35°C	49±1	51±1	14±1	14±1	2.2±0.1	1.3±0.1	8.0±0.4	0.9±0.1
C3**a**	Fermented at 40°C	57±1	56±1	19±1	19±1	3±0.1	2.1±0.1	2.7±0.1	2.5±1
C3**b**	Fermented at 40°C	50±1	50±1	15±1	16±1	3.2±0.1	2±0.1	6,0±0.3	2.8±1
	mean of all:	56	57.5	15.6	17.4	3.3	2.9	12.2	7.7
mean of fermented samples:		51	51.5	15.3	15.5	2.9	1,7	6.8	2.4

a=non-crumbled
b=crumbled

On the base of the results of storage experiment we concluded that the 2.5-year storage period had no “detrimental” effect on the quality of fermented samples. We observed regular, but small (14–18%) decreases in the total flavonoids, hyperoside and oenothein B contents.

1.6. Summary

On the base of above presented experiences and experimental results, we described the steps of the fermentation process of *Epilobium angustifolium* shoot yield. The machines and equipment should be used as presented in these experiments or to be obtained from special suppliers. The critical point is the mincing or grinding machine. The drying, packing and separating equipment is quite common in the food industry enterprises and these machines are usually also suitable for fermentation process described above. That is why no big machine investment is needed for adding a new fermented tea product into production.

Steps of the fermentation process:

- Harvest: Optimum phase during start of flowering, at dry weather, in the afternoon. The length of the shoots should be 40 cm (Picture 1).
- Withering: During the night (12 hours) at room temperature, on the floor, in 3–5 cm layer (Picture 2).
- Grinding: Using meat mincer or vegetable mixer (Picture 3).
- Package: Pack the grinded plant mass softly into plastic boxes, in quantity 2–5–10 kg/box. Cover the surface by paper (Picture 4).
- Fermentation: In room or in a dryer at 35–40 °C, for 24–36 hours. The fermentation ends when the colour of the plant material is deep yellow and the specific scent is intensive.
- Drying: The fermented plant material is spread out in thin layers and dried at 40°C with proper ventilation
- Equalization: The dry and hard tea material needs to be crushed, separated and equalized.
- Storing: In double paper-plastic sacks at room temperature for 2–2.5 years.

Picture 1. Budding phase of Epilobium shoots. Photo Bertalan Galambosi.

Picture 2. Withering the harvested shoots on the floor. Photo Bertalan Galambosi.

Picture 3. Mincing of the shoots. Photo Bertalan Galambosi.

Picture 4. Fermentation on plastic boxes in the dryer. Photo Bertalan Galambosi.

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2. Evaluation of Finnish roseroot (*Rhodiola rosea*) gene collection from different geographical origins

Galambosi, B.[1], Galambosi, Zs.[2], Kirakosian, G.M.[3], Alexandrova, O.A.[3], Morozova, M.A.[3], Trusov, S.A.[3], Enikeev, A.H.[4]

[1]bertalan.galambosi@surffi.fi

[2]Natural Resources Institute Finland (Luke), Lönnrotinkatu 3, 50100 Mikkeli, Finland

[3]Expert Bio LtD, Institutckiy Street 21/2, 194021 St.-Petersburg, Russia

[4] Research Institute "Rosbio" JSC, 12A Melnichnaya Street, 192019 St.-Petersburg, Russia

Abstract

During the domestication experiments of *Rhodiola rosea,* a collection of roseroot strains from different geographical origins was created at Mikkeli, South-East Finland (61° 44‘ N, 27° 18‘ E). The aims of this study was to compare the root biomass and salidroside and total rosavin contents of eleven Finnish, eight Scandinavian, five Central European and two Russian (Altai) accessions and to choose the best accessions for further propagation. The analyses were carried out in the laboratory of Expert Bio, St. Petersburg, Russia. The fresh weight of *Rhodiola rosea* roots ranged between 0.32 and 2.74 kg/plant. The Central European accessions developed the biggest roots, with an average of 1.93 kg/root (1.30–2.74 kg), followed by the Scandinavian accessions with the mean of 1.17 kg/plant (0.78–1.66 kg) and the Finnish accessions with the mean of 0.79 kg/plant (0.32–1.30). Altai accession was similar (x=0.76 kg/plant). The average salidroside content of the Scandinavian and Altai accessions were below 1.0 %, while the Finnish and Central European accessions contained 1.3–1.4% of salidroside. No statistical differences between the geographical groups were found. The Scandinavian and Central European accessions contained 1.0–1.15 % of total rosavins on average. The Finnish accessions contained higher quantities, 1.5 %, of total rosavins. Significantly higher contents were measured in the Altai accession, 2.4 % (P<0.001). Six accessions containing high salidroside and total rosavin contents were chosen for saving in the official Finnish medicinal plant gene collection.

Keywords: Gene collection, *Rhodiola rosea*, roseroot, strain, salidroside, total rosavins

2.1. Introduction

Rhodiola rosea can be classified as one of the youngest medicinal crop, of which domestication process is going on in our time. Its cultivation history covers only about 40 years. This species has got significant attention by elaborating the theory of adaptogen medicinal plant in the former Soviet Union after the Second World War (1, 2). Presently several modern phytochemical evaluations emphasize its adaptogen properties (12). The first cultivation experiments started during the 70's and during the last four decades nearly all European countries, where roseroot is an endemic species in the flora, the domestication experiments have been started. Those countries are for example Sweden, Finland, Norway, Poland, Italy, Germany and Switzerland (4). During the last decade intensive R & D activities and cultivation was carried out in Canada (17).

The studies of *Rhodiola rosea* domestication were started at MTT Mikkeli research station in Finland on 1990 (5). The process is quite slow, since the life cycle of this perennial plant is 4–5 years. In the first 10 years observation experiments explored basic questions of propagation biology, growth and biomass accumulation. Several agronomical issues affecting the quantity of root yield were studied. The results of this phase have been summarised in the first growing instructions (3, 15).

In the second phase the research was focused on quality questions: comparison of wild and cultivated *Rhodiola rosea* strains (6) and the comparison of male and female plants (7). One

comparative research showed a great variation in the quality of roseroots strains of different origin (8). Comparative research was challenging also because studied plants were in different age (3–8 years) and analysis were done in different laboratories.

Due to the intensive international interaction with the researchers, in whose countries roseroot is an endemic species, a large collection of roseroot strains was created at Mikkeli, Finland (Picture 1). The Finnish collection which included 26 accessions, gave a unique possibility to compare the quality of different *Rhodiola* strains. The plants were grown in the same place for 6–16 years by using similar growing technologies, the accessions were nearly at the same age and the analyses could be carried out by one laboratory.

2.2. Aim of the research

The main aim of this research was to evaluate the chemical characteristics of 26 accessions from different geographical origins. The second aim was to choose the best accessions for further propagation.

2.3. Material and methods

2.3.1. Plant material

The collection of different rose root accessions started 1995. Different plants were received through collaboration with different research institutes and by own collection in the Finnish nature. By the year 2012 the collection contained eight accessions from Scandinavia, five accessions from Central-Europe, two accessions from Russia (Tomsk, origin from Altai) and 11 accessions from Finland (Table 1, Picture 2). Each accession consisted of 4–6 plants.

The plants were propagated mainly vegetatively (by root division) to save the original gene pool. Cultivation of some of the accessions started originally from seeds, but later vegetatively, as marked in the table 1.

The experimental field was situated in the research station of MTT Agrifood Research Finland, Mikkeli (61° 44‘ N, 27° 18‘ E). The soil was finesandy till, pH 5.9, and the plots were fertilized by 20 t/ha of composted chicken manure (Biolan, N-P-K 0.5–0.5–1) before transplantation. In every second spring 20 g of composted chicken manure (Biolan) was given to each plant.

The one-year-old seedlings were transplanted into bed covered by black plastic mulch with plant distance of 40 cm. The plots were cleaned regularly from the weeds by hand and the weeds between the rows were cut by a lawn mower. No diseases and insects occurred during the cultivation period.

Table 1. *Rhodiola rosea* accessions in the gene collection at MTT Mikkeli on 2012.

No.	Origin and experimental code	Plant age (year) at harvest	Propagation from seedlings	Grown at Mikkeli (years)
	SCANDINAVIA			
1	Finland, A1, Hirvas	8	x	16
2	Norway, B1/97	8	x	16
3	Norway, B3, (Särkä)	8	x	16
4	Sweden, B4, Impecta	8	x	16
5	Iceland, Reykjavik, Bot. Garden, No 200	8		15
6	Iceland, Reykjavik, Bot. Garden, No 831/1	8		15
7	Iceland, Reykjavik Bot. Garden, No 832/3	8		15
8	Iceland, C4	8	x	16
	CENTRAL EUROPE			
9	Italy, Adanello	9		9
10	Italy, Bondolo	9		9
11	Italy,Valle d'aporte	9	x	9
12	Germany, C2, Kiel	9	x	16
13	Switzerland, Mattmark	8		8
	RUSSIA			
14	Tomsk Bot. Garden, original 2004 (n=2)	8		8
15	Tomsk Bot. Garden, from own seeds	5	x	5
	FINLAND			
16	Finland, Kilpisjärvi 1, Jeähkojärvi	9		9
17	Finland, Kilpisjärvi 2, Saana north part	9		9
18	Finland, Kilpisjärvi 3, Saananmaja	9		9
19	Finland, Kilpisjärvi 4, Tsahkaljärvi south shore	9		9
20	Finland, Kilpisjärvi 5, Tsahkaljärvi bridge	9		9
21	Finland, Kilpisjärvi 5, Tsahkaljärvi bridge (6)	6		6
22	Finland, Kilpisjärvi 6, Marjajärvi	9		9
23	Finland, Halti 1, Somaslompolo	9		9
24	Finland, Halti 2,Valtijoki	9		9
25	Finland, Halti 3, Jogasjärvi	9		9
26	Finland, Utsjoki, 2006	6		6

2.3.2. Plant and sample preparation

In the year 2012, between September 3rd and 17th well developed individuals were chosen from each accession and dug out (one individual plant from each accession). The age of most of the plants was 8–9 years, except two accessions being 5 and 6 year old (Table 1). The shoots and soil were taken away, and roots were sliced, washed and the total weight was recorded.

The dominating parts (cc. 85–90%) of the fresh roots were rhizomes, therefore we used only rhizomes for chemical analyses. Some plants showed the marks of aging, with red/brownish parts in the rhizomes (Table 2), therefore for analyses only the white parts of the rhizomes were chosen. 2 x 150 g of fresh rhizome samples were dried at 40–45° C in Orakas type drier, and the dry matter contents were calculated (Table 2). The dried samples were stored in paper bags for the analyses until November 2012. Additionally we obtained one commercial sample from Russia, originally from Altai.

Table 2. Root weight and dry matter content of 26 accessions at MTT Mikkeli on 2012.

No. Code	Origin	Fresh weight kg/plant	Dry matter content %	Dry weight kg/plant	Dead part %
	SCANDINAVIA				
1	Finland, A1, Hirvas	0,72	28,8	0,207	
2	Norway, B1/97	1,02	30,1	0,307	5 - 8 %
3	Norway, B3, (Särkä)	0,78	27,6	0,215	0 - 2 %
4	Sweden, B4, Impecta	0,84	28,5	0,239	
5	Iceland, Reykjavik, Bot. Garden, No 200	1,48	25,6	0,380	1 - 3 %
6	Iceland, Reykjavik, Bot. Garden, No 831/1	1,56	28,5	0,444	1 - 3 %
7	Iceland, Reykjavik Bot. Garden, No 832/3	1,36	28,7	0,390	5 %
8	Iceland , C4	1,66	30,6	0,507	1 - 3 %
	Mean	**1,17**	**28,6**	**0,319**	
	CENTRAL EUROPE				
9	Italy, Adanello	2,08	25,8	0,536	5 %
10	Italy, Bondolo	1,42	27,1	0,384	1 - 3 %
11	Italy,Valle d'aporte	2,12	24,3	0,515	
12	Germany, C2, Kiel	1,30	29,2	0,379	0 - 3 %
13	Switzerland, Mattmark	2,74	23,7	0,649	0 - 1 %
	Mean	**1,93**	**26,0**	**0,490**	
	RUSSIA				
14	Tomsk Bot. Garden, original	0,79	29,5	0,230	0 - 1 %
15	Tomsk Bot. Garden, from own seeds	0,72	30,1	0,216	0 - 1 %
	Mean	**0,76**	**29,8**	**0,223**	
	FINLAND				
16	Finland, Kilpisjärvi 1, Jeähkojärvi	0,52	30,0	0,156	
17	Finland, Kilpisjärvi 2, Saana north part	1,30	26,1	0,339	
18	Finland, Kilpisjärvi 3, Saananmaja	0,38	23,9	0,093	
19	Finland, Kilpisjärvi 4, Tsahkaljärvi south shore	0,80	22,0	0,176	30 %
20	Finland, Kilpisjärvi 5, Tsahkaljärvi bridge	1,17	28,4	0,332	
21	Finland, Kilpisjärvi 5, Tsahkaljärvi bridge (6)	1,04	28,8	0,299	
22	Finland, Kilpisjärvi 6, Marjajärvi	0,56	28,6	0,159	
23	Finland, Halti 1, Somaslompolo	0,32	27,8	0,089	
24	Finland, Halti 2,Valtijoki	0,52	30,3	0,157	
25	Finland, Halti 3, Jogasjärvi	0,64	27,2	0,174	
26	Finland, Utsjoki, 2006	1,26	27,8	0,350	
	Mean	**0,79**	**28,4**	**0,211**	

2.3.3. Chemical analyses

The analyses were carried out in the laboratory of Expert Bio Ltd, in St-Petersburg, Russia. The dry root samples were diluted into ethanol. The used equipments were Shimadzu LC-10 AVp and Shimadzu LC-20A liquid chromatographs equipped with UV detector. The standard samples for salidroside (CAS 10338-51-9) and for rosavin (CAS 84954-92-7) were purchased from LGC Standards.

Compounds were separated in Phenomenex Luna C-18 (2 µm; 250 X 4.6 mm, i.d.) column by using gradient eluation. The mobile phase consisted of (A) aqueous acetic acid (pH 2.5) and (B) methanol. Gradient elution was started with 10% methanol followed by a linear gradient to 90% methanol in 30 min.

Compounds were detected and quantified by UV detector in vawe lengths λ =278 nm (0–15 min) and λ =245 nm (15–20 min).

The quantities of salidroside and the sum of total rosavins and cinnamyl alcohol (further: total rosavins) were determined in two repetitions and presented as mean values (Table 4).

2.3.4. Statistical analyses of the analytical results

The contents of salidroside (%) and total rosavins were analysed by One-Way Analysis of variance, by the Anova Procedure. The t-test was carried out by Waller-Duncan K-ratio t-test.

2.4. Results

2.4.1. Yield characteristics of the accessions

Fresh weight: The fresh weights of *Rhodiola rosea* roots ranged between 0.32 and 2.74 kg/plant (Table 2). There were some differences between the accessions originated from different regions: The Central European accessions developed the biggest roots, with an average of 1.93 kg/root, (1.30–2.74 kg, picture 3). The average root weight of the Scandinavian accessions was 1.17 kg/plant (0.78–1.66 kg). The lowest root weights were measured in the natural accessions of Finland with average of 0.79 kg/plant (0.32–1.30 kg/plant). The weight of the Siberian origin Tomsk accessions were similar (0.76 kg/plant).

Dry matter content: The dry matter content ranged between 25.6–30.3% (Table 2). The lowest values were measured in the Central European accessions (x=26.02%). During the harvest time these accessions had nearly green shoots, which means that the dormancy had not started. The other accessions (Finnish, Scandinavian and Tomsk) had already started their dormancy period during the first half of September.

Dry weight: The calculated dry weight of the accessions ranged between 0.089 and 0.649 kg/plant. The highest dry weights were measured in the Central European accessions, with an average of 0.490 kg/plant (0.379–0.649), followed by the Scandinavian accessions: average: 0.319 kg/plant (0.207–0.507). The lowest dry weights were detected in the natural Finnish accessions, with an average of 0.211 kg (0.089–0.339). The dry weight of the Tomsk accession was 0.223 kg/plant.

The visual quality of 8–9 year old rhizomes: Generally, the quality of rhizomes was good. The rhizomes of about the half of the accessions were white in color, without red colored dead parts (Picture 4). Only one accession (No 19 Finland, Kilpisjärvi 4) had roots in bad quality, cc. 30 % of the rhizome was dead. Five to eight % of red parts were observed in three accessions and 0–3 % of red parts were observed in eight accessions (Table 2). For the analyses the red rhizome parts were avoided.

2.4.2. Comparison of the chemical quality of different geographical groups

Salidroside content

The average of salidroside contents of the Scandinavian and Altai accessions were below 1.0 %, while the Finnish and Central European accessions contained a bit higher amount of salidroside (1.3–1.4%). According to the statistical analyses, there was no statistical difference between the geographical groups. (Table 3, Figure 1).

The content of salidroside in individual accessions showed variation between 0.28 and 2.14 % (Table 3). The highest salidroside contents were measured in the Finnish natural accessions, Kilpisjärvi 5. (no. 20: 2.14 % and 2.02 %) and Halti 2 (no 24:1.85 %). The other Kilpisjärvi natural accessions contained low amount of salidroside, below 1.0 %. (no. 18, 19, 22).

The content of salidroside was over 1.0 % in all Central European accessions, with the highest values in accessions of Italian Adanello (no.9: 2.20 %) and Switzerland Mattmark (no. 13: 1.69 %). In the Scandinavian accessions the salidroside content was significantly lower, ranged between 0.28 % and 1.83 %. The salidroside content of the 8-years old Tomsk accession was very low (no.14: 0.64 %), but the younger plants of this accession (no. 15) contained 1.26 % of salidroside. The commercial Altai sample (no. 27) contained a medium level of salidroside, 1.13 % (Table 3).

Table 3. The content of the studied compounds of *Rhodiola rosea* accessions (d.w.) in 2012.

No.	Origin	Salidroside %	Total rosavins + Cinnamyl alcohol %
	SCANDINAVIA		
1	Finland, A1, Hirvas	0,65	0,56
2	Norway, B1/97	1,21	0,96
3	Norway, B3, (Särkä)	0,79	0,99
4	Sweden, B4, Impecta	1,83	1,30
5	Iceland, Reykjavik, Bot. Garden, No 200	0,28	0,94
6	Iceland, Reykjavik, Bot. Garden, No 831/1	0,64	1,03
7	Iceland, Reykjavik Bot. Garden, No 832/3	1,25	1,23
8	Iceland, C4	1,20	0,99
	Mean	**0,98**	**1,00**
	CENTRAL EUROPE		
9	Italy, Adanello	2,20	1,26
10	Italy, Bondolo	1,06	0,64
11	Italy,Valle d'aporte	1,07	0,97
12	Germany, C2, Kiel	1,41	1,09
13	Switzerland, Mattmark	1,69	1,81
	Mean	**1,49**	**1,16**
	RUSSIA		
14	Tomsk Bot. Garden, original plant	0,64	2,11
15	Tomsk Bot. Garden, from own seeds	1,26	2,60
	Mean	**0,95**	**2,35**
	FINLAND		
16	Finland, Kilpisjärvi 1, Jeähkojärvi	1,41	1,02
17	Finland, Kilpisjärvi 2, Saana north part	1,72	1,44
18	Finland, Kilpisjärvi 3, Saananmaja	0,78	0,87
19	Finland, Kilpisjärvi 4, Tsahkaljärvi south shore	0,77	1,66
20	Finland, Kilpisjärvi 5, Tsahkaljärvi bridge	2,02	1,32
21	Finland, Kilpisjärvi 5, Tsahkaljärvi bridge (6)	2,14	1,44
22	Finland, Kilpisjärvi 6, Marjajärvi	0,81	1,42
23	Finland, Halti 1, Somaslompolo	1,01	1,05
24	Finland, Halti 2,Valtijoki	1,85	1,98
25	Finland, Halti 3, Jogasjärvi	1,19	1,74
26	Finland, Utsjoki, 2006	1,44	2,77
	Mean	**1,38**	**1,52**
27	Altai (commercial, control 2012)	1,13	1,79

Total rosavin content

The Scandinavian and Central European accessions contained 1.0–1.15 % of total rosavins in average. Finnish accessions contained total rosavins more than that, an average being 1.52 %. Significantly higher contents were measured in the Tomsk accessions, 2.35 %.(Table 3, Figure 2). The result was significant at the 0.05 level.

The total rosavin content in the individual accessions ranged between 0.56 and 2.77 % (Table 3). The total rosavin content in the Tomsk accessions was 2.35 % in average. Its content in the 8 years old plants (no. 14) was 2.11 % and in the 5 years old plants (no. 15) was 2.60 %. The total rosavin content of the commercial Tomsk samples (no. 27) was quite high (1.79 %), but not as high as in the cultivated ones.

The total rosavin content of the natural Finnish accessions compared to the Central European and Scandinavian groups were higher by 32–52 % (x=1.52 %). The highest total rosavin content (2.77%) of this group was detected in Utsjoki accession (no. 26), in the accession that was collected from the northmost growing site of the country (69 N°).

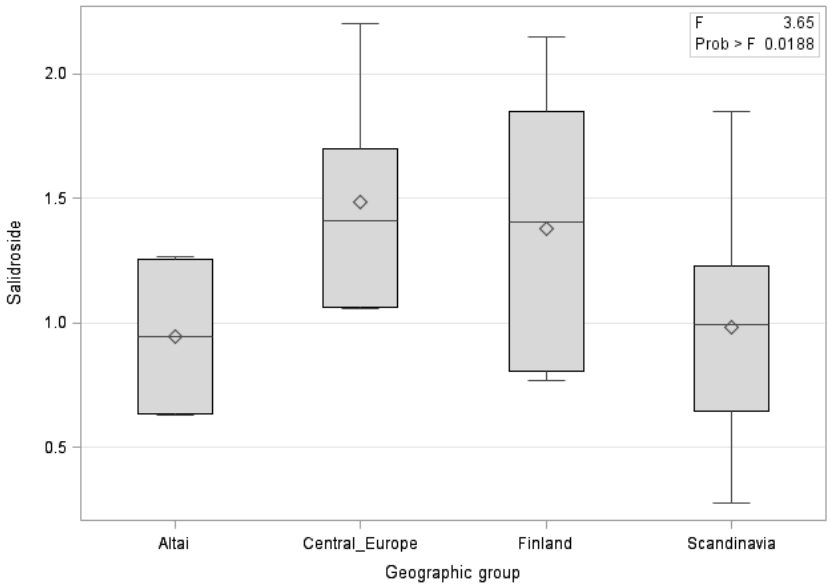

Figure 1. Salidroside content in the geographical groups of *Rhodiola rosea* accessions.

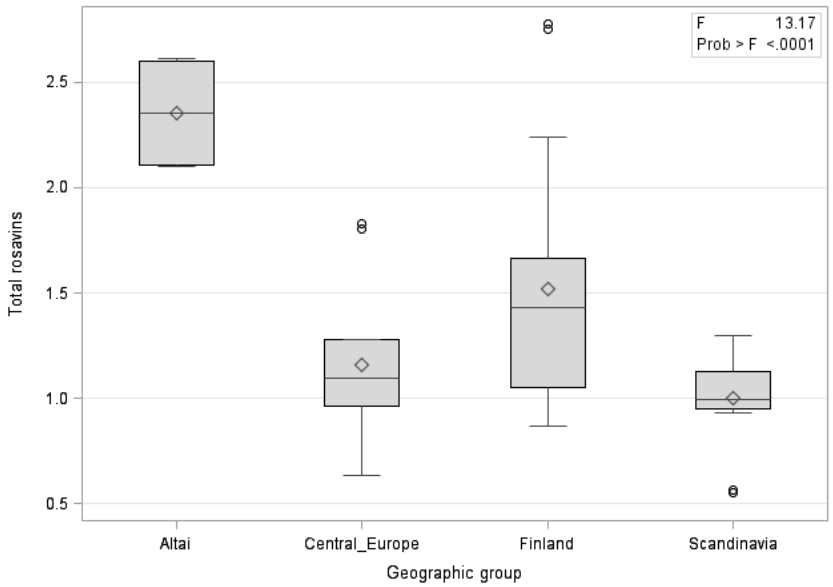

Figure 2. Total rosavin content in the geographical groups of *Rhodiola rosea* accessions.

2.5. Discussion

In this experiment we compared 26 accessions of *Rhodiola rosea* from four geographical regions that are natural habitats of *Rhodiola rosea* (Scandinavia, Northern Finland, Central Europe and Russia). This roseroot collection had served for several agronomical studies, seed production, education and gene collection, and the discontinuation of the collection gave possibilities for the analytical comparison. The value of this comparison may be due to this fact, that the accessions were grown at the same place and by similar growing methods for 8–16 years.

The data of this experiment are only informatory, since due to cost factor, only one plant per accession was analyzed. Our results do not show the real intraspecific quality potential of the given accession. E.g. in the case of the seed propagated German Kiel accession C2 (no.12), the average content of salidroside in three plants was 1.41%, but the variation in those three individuals was high: 1.93–1.72–0.58 %. A recent research has demonstrated large intraspecific variation in the individual compounds of roseroot plants. The individual component, like rosavin, has ranged between 0.005 and 0.70% and that of rosarin between 0.24 % and 5.30 % (17).

The age of the compared plants in this study was generally 8–9 years, which is not the best age for the *Rhodiola* harvest. Several authors describe that the optimum age for harvest of *Rhodiola* root yield is 4–6 years (7, 13). We observed as well the clear effect of the age factor in the case of Tomsk (no.14) and Kilpisjärvi 5 accessions. The contents of both studied compounds of the 5–6 year old plants (no. 15, no. 21) were higher than that of the 8–9 year old plants (no. 14, no.20) (Table 3).

Furthermore we like to emphasize that these results were obtained in Southern Finland, Mikkeli, at the latitude 61° 44' N, 27° 18' E, which is situated 800–900 km south from the natural habitats of *Rhodiola rosea* in Lapland, Kilpisjärvi (69° 02' N) (872 km) and in Utsjoki(69° 55' N) (916 km). Some newest research data indicates that the more southern sites the roseroot was cultivated, the lower the content of the secondary metabolites was. E.g. three *Rhodiola rosea* clones high in rosavin content were cultivated for four growing seasons at different locations in Norway, between 60°– 64° Nordic latitudes (14). The average total rosavin content was higher in the northern parts of the country (x= 7.09 %), than in the southern parts (4.17–5.04 %).

In another study Martinussen *et al*. (2011) simulated in phytotron the growing conditions for three adaptogen medicinal plants including *Rhodiola rosea* (11).The growing sites were Northern Norway (Buren at 69° 39'N), Northern Finland (Rovaniemi at 66° 20'N) and South-Eastern Norway (Kise, at 60° 77'). The growth parameters of roseroot (shoot number, root weight) were higher in the Southern growing site, but the contents of active metabolites were the highest in the northern site and decreased to the south. Therefore we are convinced that it is absolutely necessary to carry out quality analyses of a given *Rhodiola* accession in a new growing site, having data on the effects of the local environmental conditions to the quality parameters. Therefore additional studies are necessary in another location that is situated e.g. in more northern latitudes.

Nevertheless, although our results were obtained in South-East Finland at 61° Nordic latitude, the results may give some orientation for the biomass potential and quality features of *Rhodiola rosea* accessions originated from different geographical regions.

There are no officially accepted quality criterias for *Rhodiola rosea* root raw material, but generally high root yield, high salidroside and total rosavin contents are preferred. The Soviet Pharmacopeia 1989 standardized the root extracts to 3 % of rosavins and 0.8–1.0 % of salidroside (2). In Russia the content of rosavin was reported to be 2–2.5 % by several researchers, and in some cases much higher, up to 6 % (8, 9). Generally, the *Rhodiola rosea* accessions of the best quality are believed to be in Altai region in Russia. Although in this study we did not record as high rosavin content as mentioned previously (4–6 %), the Tomsk accession – mentioned to be from Altai – had one of the highest rosavin content (no: 15: 2.60 %). Only one Finnish natural accession had higher content of total rosavins (no. 26, Utsjoki, 2.77 %, Table 3).

2.6. Evaluation of the groups and the choice of the best accessions

On the base of the obtained results we tried to choose several high quality roseroot accessions from this collection. For comparison as a standard we used the results of the commercial Altai sample (no 27), which had salidroside content of 1.13 % and the total rosavin content of 1.79 %.

Scandinavian accessions: Although the root weight of these accessions in this study was the second biggest (1.17 kg/plant), the contents of the two compounds were generally lower, than that of Altai standard.

Central European accessions: These accessions had the biggest root weight (x= 1.93 kg/plant) and the quality of two accessions had nearly similar quality than the Altai standard. The no. 9 Italian Adanello accession had a bit lower total rosavin content (1.26 %), but its salidroside content was the highest of the whole collection (2.20 %). The Swiss Mattmark accession (no. 13) had very high root yield (2.74 kg/plant) and both the salidroside (1.69 %) and the total rosavin (1.81 %) contents were high. In the natural habitats in Swiss Alps, this accession have shown the medium content of total salidroside (1.56 %) but the highest salidroside content of five natural populations, 2.51 %. (10). On the base of this natural accession, the first breed roseroot variety "Mattmark" have been launched in Switzerland, having 2.89 % of salidroside and 2.0 % of rosavins (16).

Finnish natural accessions: Although the average root weight of this group had medium value (x=0.78 kg/plant), the contents of two compounds in several accessions were quite high. The contents of the studied compounds on Halti 2 (no. 24) and Utsjoki (no. 26) accessions exceeded the Altai standard significantly. Additionally very high salidroside content was detected in Kilpisjärvi 5 accession (no. 21: 2.14 %), although the total rosavin content of this accession was at medium level (1.32–1.44 %).

One of the main aims of this research was to choose the best quality accessions for further propagation. In the highlight of this study we have chosen the following accessions for further propagation: Russia: Tomsk, Italy: Adanello, Switzerland: Mattmark, Finland: Kilpisjärvi 5, Halti 2, Utsjoki. (Table 4). The mother plants of these accessions were deposited into the official Medicinal Plant Gene Collection of Finland.

Table 4. Selected *Rhodiola rosea* accessions in the Finnish Medicinal Plant Gene Collection in 2014.

Accession	Content (%)		Root weight kg/plant	
	Salidroside	Total rosavins	Fresh	Dry
Tomsk, Russia	1,26	2,6	0,75	0,22
Adanello, Italy	2,2	1,26	2,08	0,53
Mattmark, Switzerland	1,69	1,81	2,74	0,65
Kilpisjärvi 5, Finland	2,14	1,44	1,17	0,33
Halti 2, Finland	1,85	1,98	0,64	0,17
Utsjoki, Finland	1,44	2,77	1,26	0,35

Picture 1. View of the Rhodiola rosea gene collection. Photo Bertalan Galambosi.

Picture 2. Strains form Northern Finland. Photo Bertalan Galambosi.

Picture 3. Whole roots of Central European accessions. Photo Bertalan Galambosi.

Picture 4. Rhizome of a 9-years old Italian accession. Photo Bertalan Galambosi.

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3. Optimization of the fermentation of *Bergenia sp*. green leaves

Galambosi, B.[1], Galambosi, Zs.[2], Kosman, V. M.[3], Shikov, A.N.[3], Pozharitskaya, O.N.[3], Kauppinen, S.[2], Makarov, V.G.[3]

[1]bertalan.galambosi@surffi.fi

[2]Natural Resources Institute Finland (Luke), Lönnrotinkatu 3, 50100 Mikkeli, Finland

[3]St.-Petersburg Institute of Pharmacy, 56, Bolshaja Porochovskaya, POBox 16, 195248, St-Petersburg, Russia

Abstract

The leaves of the commonly known decorative perennial, *Bergenia* species have several interesting biological activities. The green leaves contain arbutin up to 22 % (d.w.), a compound, which is used in cosmetic industry as a skin-whitening agent in humans. Tea prepared from the naturally fermented black leaves is used as beverage called Siberian tea or Mongolian tea in Russian ethno medicine. The fermented green leaves of *Bergenia crassifolia* are appearing to meet the criteria of being adaptogen. For the utilization of these new possibilities of *Bergenia* species during 2012–2014 several studies were carried out in SPECICROP project for elaborating a semi-industrial fermentation process of *Bergenia* leaf yield. The experiments were carried out in MTT Agrifood Research Finland Mikkeli with cooperation of St.-Petersburg Institute of Pharmacy, St-Petersburg, Russia. On the base of these experiments we elaborated the optimized parameters of the fermentation process and described a technological recommendation suitable for small size farm entrepreneurs.

Keywords: *Bergenia*, arbutin content, fermentation parameters, technological recommendation

3.1. Bergenia as an interesting research object of Finnish-Russian cooperation

Bergenia species are popular perennials in Finland, due to their cold tolerance and decorative character. The large evergreen or purplish leaves and red/violet flowers, suit in rock gardens, as border plants or soil covering plant.

In cooperation with Russian medicinal plant experts, new dimensions have been opened for this decorative species. Rhizome of *B. crassifolia* L. (*Saxifragaceae*) has been included into the Russian State Pharmacopoeia and claimed as haemostatic, astringent, anti-inflammatory, antimicrobial agent and it is recommended to strengthen capillary walls, and to decrease arterial blood pressure. Its leaves has showed antimicrobial, immunostimulating and adaptogenic activities. The green leaves contain arbutin up to 22 % (d.w.), a compound, for which there is an increasing interest in cosmetic industry as a skin-whitening agent in humans. Tea prepared from the naturally fermented black leaves is used as beverage called Siberian tea or Mongolian tea in Russian ethno medicine. Bergenia crassifolia is appearing to meet the criteria of being adaptogen (14).

The above mentioned perspective possibilities have initiated number of R & D cooperative projects between Russia and Finland. Bergenia field production, fermentation, phytocemical characterization and pharmacological effects were studied in numerous experiments. The results of these projects have been published in several papers:

- During 2005–2006, in the project "*Development of new medicinal herb-based products in South-East Finland"* some agronomical questions of the field cultivation of *Bergenia* species were carried out (3, 4, 5, 6, 7). The main result of this project was the development of preliminary cultivation method and supplying raw material for further experiments.

- The first fermentation laboratory experiments were carried out during 2007–2008 in the project" *New herbs for rural development* "TACIS (8, 10). By modern *in vivo* experiments some positive effects of the fermented green leaf tee was demonstrated on mice and rats (11, 12).
- During 2010–2011 in the "HerbFruit" project detailed chemical analyses of *Bergenia* fermented leaves were carried out, focusing on the effects of fermentation process on the phenolic and volatile compounds of the leaves (1, 10). The project *(Commercialisation of special crop production in the Leningrad region, Russia (herbs, fruits, berries) 5.2.2010-31.3.2011*" was financed by Ministry for Foreign Affairs of Finland.
- In connection with the above described long-term research activities on *B. crassifolia,* a comprehensive phytochemical evaluation was carried out by Russian scientists giving deep information on this less known Russian medicinal plants, not included in the European Pharmacopaea. (13, 14, 15).

Before the industrial utilization of fermented *Bergenia* leaf tea, the conditions of fermentation process have to be standardized to be suitable for small industrial enterprises. In the previous experiments we were able to conclude that for the fermentation of *Bergenia* leaves the fermentation methodology of *Epilobium angustifolium* (Ivan tsay) generally can be adapted (9). Due to the morphological features of *Bergenia* leaves the proposed meat mincer machinery was not suitable for breaking the plant cells, but it may be substituted by selected cutting machinery. The proposed fermentation temperature regions (21–31° C) seemed not to be effective, since the fermentation process started slowly and some molding occurred.

The optimization of the fermentation process needed additional research which was studied in the "SPECICROP" project during 2012–2014.

3.2. The aims of the experiments

The first aim of these experiments was to standardize the fermentation process by studying the following questions:

- characteristics of raw material
- processing the leaf mass before fermentation by destroying the plant cells (laboratory size, industrial size machinery)
- equipment for fermentation of plant masses (bag, box, size)
- optimum temperature, time of fermentation
- markers for following the fermentation process
- drying the fermented mass
- post drying operations of the dry material
- storage of the dry, fermented tea
- effects of the technological elements on the quality of fermented plant material

The second aim was to create a production guide suitable for farm level production units to produce fermented green leaf raw material for further industrial processing (tea, pressed dry raw material, tablets, capsules, dry extract, etc).

3.3. Material and methods

Experimental site and time

The experiments were carried out at MTT Agrifood Research Finland (nowadays Natural Resources Institute Finland Luke), Mikkeli experimental station, during 2012–2014. The fermentation experiments were carried out during autumns 2012 and 2013.

Plant material

The selected plant material was obtained from cultivated populations of *Bergenia* accessions of different origin:

- Accession No 12 (*B. cordifolia*) was originated from a private garden (Outila garden, Mikkeli). This accession was cultivated in the experiment fields of MTT Mikkeli since 2003.
- Accession No 61 (*B. crassifolia).* Seeds of this accession were originated from Maatiainen Ltd Helsinki. This accession was cultivated in the experiment fields of MTT Mikkeli since 2003.
- Accession "Piha" *(Bergenia hybrida*) originated from the decorative garden of MTT Mikkeli experimental station grown in the same place from the 1960's. Its origin is unknown, probably from a private garden.

Machinery used:

Plant cutting machine:	Hege 44 Wintersteiger
Meat mincer and vegetable mixer:	Kenwood Gourmet 1600
Drying machines:	WTB type, Binder, /8532.,Tuttlinger, Germany.
Equipment:	small plastic boxes (size: 12 x 14 x 5 cm)
	big plastic boxes (size, 21 x 28 x 8 cm)
	black plastic bags, screen with 3 mm hole

Chemical analyses

The analyses of non-fermented dried leaves (dried at 40° C) and the fermented leaves (at 28–35–40 °C) were carried out in the laboratory of the St.-Petersburg Institute of Pharmacy, Russia. The contents of arbutin, gallic acid, hydroquinone, protocatechuic acid, bergenin and ellagic acid were determined.

3.4. Results

3.4.1. Characteristics of *Bergenia* raw material

The green leaves were harvested by hand from the cultivated plots. The harvest time in both years was nearly the same: in October 29th 2012 and October 21st 2013. In both years the harvest was after a week of the first light autumn frost. The leaves were green, the upper parts were purple. In the case of larger scale production, the harvest can be carried out mechanically. Several hay harvesters, like Haldrup-1500 or Wintersteiger, seem to be suitable for harvesting (Picture 1).

The raw plant material of *Bergenia* sp. contains leaves and leaf stalks, but bud and flower stems are not allowed. According to our previous results contrary to the 10.0–13.5 % of arbutin content of the leaves, the flowers contain arbutin only 2–3 % (3).

The surface of leaves during this time of the year is generally moist. The dry matter content in 2012 dried at 40° C was in average 25.4 % (23.5–26.9 %), dried at 100 °C was 29.9 % (28.0–31.2 %).

3.4.2. Pre-fermentation procedures

Before fermentation the leaves have to be withered, sliced, minced and packed into fermentation boxes.

Withering: The harvested leaves were withered for 12–14 hours at room temperature (20–22 °C). The harvest was carried out between 16–18 pm and the withering occurred during the night. The leaves were spread in 1–5 cm layers on the surface of clean floor (concrete or wood, not on plastic due to the moisture condensation). During spreading the possible foreign plant parts were taken away (leaves of trees, weeds, damaged Bergenia leaves).

Slicing: The easy and effective mincing/mixing of leather-like leaf mass requires small plant parts, therefore after withering the whole leaves and leaf stalks have to be sliced into 1–2 cm parts. For slicing suitable machinery is e.g. old-style forage cutter, or modern cutter like Wintersteiger vegetable cutter.

Mixing: The sliced leaves have to be minced/ mixed for destroying the leaf cells. The crushing of plant cells is necessary for promoting the enzyme activity in the plant mass.

Contrary to *Epilobium angustifolia*, any kind of meat mincer can't be used for mincing the *Bergenia* leaves. The traditional hand or electric mincers became full with the leather-like plant parts and blocked quickly (Picture 2). Instead mincing the crushing of leaves with a kitchen size cutting machine (Kenwood Gourmet 1600) seemed to be suitable. After some minutes of mixing the leaves were cut for very small particles (0.1–0.5 mm) with sufficiently destroyed plant cells (Picture 3).

Packing: The mixed green plant mass was put into plastic boxes. For the experiments smaller volume (250–450 g) and bigger volume (1.8–2.2 kg) plastic boxes were used. The plastic boxes were covered with the piece of paper, but not hermetically. Covering the surface with paper saved surface from drying and didn't prevent the air circulation inside the plant mass.

3.4.3. Fermentation

The fermentation was carried out in two WTB Binder type and one UT 5760 type drying chambers, in which the temperature can be regulated. For optimization the drying temperature we tested three temperature regimes: 28 °C, 35 °C and 40 °C. The fermentation of the same plant material was carried out simultaneously in three dryers with three repetitions.

Since there is no exact criterion for the *Bergenia* fermentation process we applied our previous sensory experiences in fermentation of *Epilobium*. The fermentation process was stated to be ended, when the green plant mass turned to deep tobacco brown color and it had a nice, specific smell (Picture 4). The fermentation periods in 2012 at 27–35–40 °C temperatures lasted 4.5, 4.0 and 3.5 days. In 2013 the fermentation periods at 35 and 40 °C temperatures lasted 3.3 and 2.9 days.

3.4.4. Chemical changes in the plant material effected by the fermentation

For the characterization of effect of fermentation, six selected compounds were analyzed both in the non-fermented and fermented leaf samples (Table 1, figures 1–6).

The trends of chemical changes were quite similar in both years. The arbutin content after fermentation decreased significantly by 20–60%, compared to the non-fermented leaves (Figure 1). At the same time the contents of nearly all other compounds increased significantly. The contents of gallic acid increased by 5–20 times (Figure 2), hydroquinone (Figure 3) and protocatechuic acid contents by 4–8 times (Figure 4), ellagic acid by 2–3 times (Figure 5). No changes were measured in bergenin contents after fermentation (Figure 6).

Among the three temperatures studied the biggest decrease of arbutin and biggest increase of gallic acid and hydroquinone contents were observed at the lowest temperature (27 °C). At this temperature the fermentation period was the longest (4.5 day). The fermentation process started

slowly and in the end some non-specific, acidic smell was observed as well. It seems that the long low temperature period is not favourable for the arbutin.

Among the accessions, the accession No 61 showed different features from the two others: its dry matter content was the highest, and arbutin, protocatechuic acid and bergenin content were lower compared to the others (Table 1).

Table 1. The contents of studied compounds of *Bergenia* leaves in the fermentation experiments.

Compound (mg/g)	**Acces-sion**	**Non- fermented**		**Fermentation temperature**				
		40°C	**40°C**	**27°C**	**35°C**		**40°C**	
		2012	**2013**	**2012**	**2012**	**2013**	**2012**	**2013**
Arbutin	No. 12	171.4±6,5	111.8±3.7	81.2±0.2	107.6±0.6	92.3±2.6	106.2±0.8	113.6±3.17
	No. 61	176.1±0.8	130.3±4.1	9±0.1	16.1±0.4	51.8±6.3	54.3±0.4	72.7±20.4
	Piha	150.7±0.7	154.3±1.2	90.8±0.1	91±0.1	89.5±17.1	109.1±0.7	102.8±23.1
	X	**167,0**	**132.1**	**60.6**	**71.6**	**77.9**	**89.9**	**96.4**
Gallic acid	No. 12	5.7±0.1	3.9±	288.6±1.0	94±1.6	46.4±4.6	128.1±1.7	44.2±0.08
	No. 61	12.8±0.1	8.9±	269.4±2.0	101.9±2.1	54±19.2	108.8±1.3	52.1±3.86
	Piha	13.4±0.2	13.1±	61.6±0.9	81.1±0.5	126.6±60.5	45.9±0.3	52.5±12.85
	X	**10.6**	**8.6**	**173.2**	**92.3**	**75.7**	**94.3**	**49.6**
Hydroquinone	No. 12	<0.35	<0.35	2.9±0.1	1.2±0.1	0.75±0.02	1.3±0.1	0.56±0.01
	No. 61	<0.35	0.35	4.1±0.1	1.9±0.1	1.8±0.15	2.4±0.1	1.4±0.12
	Piha	<0.35	0.35	1.9±0.1	1.2±0.1	0.68±0.35	0.8±0.1	0.96±0.31
	X	**0.35**	**0.35**	**2.96**	**1.4**	**1.06**	**1.5**	**0.98**
Protocatechuic acid	No. 12	<0.08	<0.08	5.6±0.1	6±0.2	0.55±0.16	<0.08	1.9±0.26
	No. 61	<0.08	1,0±	<0.08	<0.08	<0.08	<0.08	<0.08
	Piha	1.8±0.1	1.6±	6.9±0.1	8.1±0.2	1.3±0.5	<0.08	1.1±0.06
	X	**0.65**	**0.89**	**4.19**	**4.7**	**0.64**	**0.08**	**1.02**
Bergenin	No. 12	3.6±0.1	2.7±	4.5±0.1	4.7±0.3	3.9±0.32	4.3±0.1	4.8±0.1
	No. 61	2.5±0.1	1.3±	1.2±1.2	1±0.1	1.5±0.38	1.2±0.1	1.2±0.06
	Piha	3.9±0.1	3.5±	4.4±0.1	4.4±0.3	3.9±0.86	3.9±0.1	3.5±1.1
	X	**3.33**	**2.5**	**3.36**	**3.36**	**3.1**	**3.13**	**3.2**
Ellagic acid	No. 12	0.28±0.01	0.28±	0.44±0.01	0.65±0.02	0.77±0.05	1.3±0.10	0.89±0.06
	No. 61	0.42±0.02	0.78±	1.4±0.10	0.35±0.01	0.86±0.11	1±0.01	0.98±0.19
	Piha	0.28±0.01	0.62±	1±0.10	0.51±0.06	0.25±0.05	0.78±0.07	0.73±0.24
	X	**0.32**	**0.56**	**0.95**	**0.5**	**0.62**	**1.02**	**0.86**

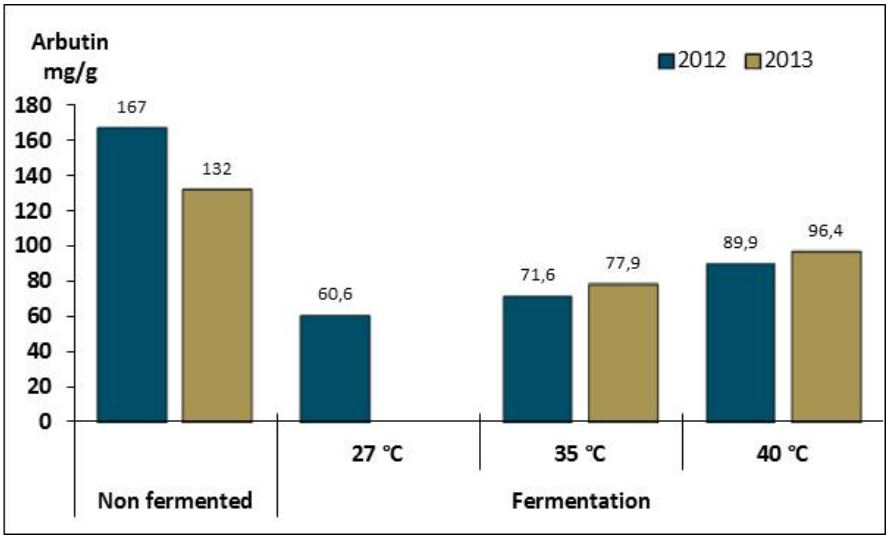

Figure 1. Effect of fermentation temperatures on the average arbutin content of three Bergenia accessions.

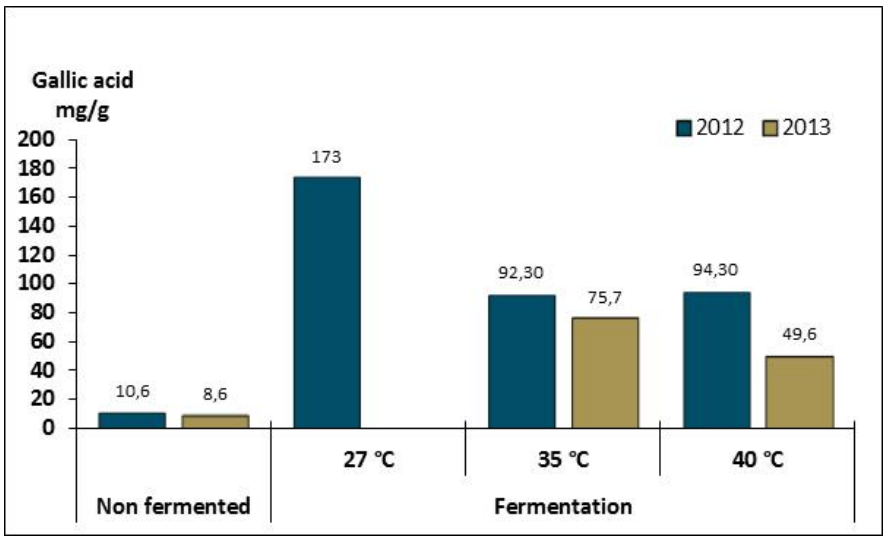

Figure 2. Effect of fermentation temperatures on the average gallic acid content of three *Bergenia* accessions.

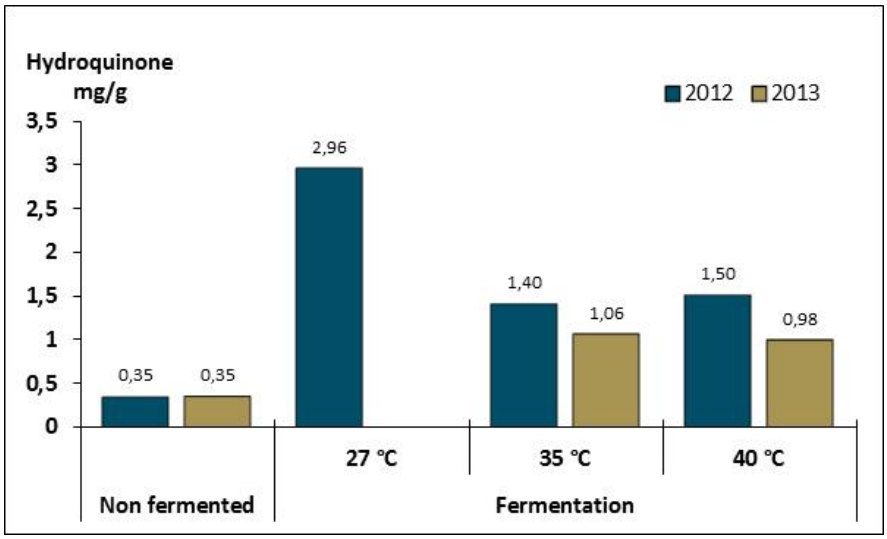

Figure 3. Effect of fermentation temperatures on the average hydroquinone content of three *Bergenia* accessions.

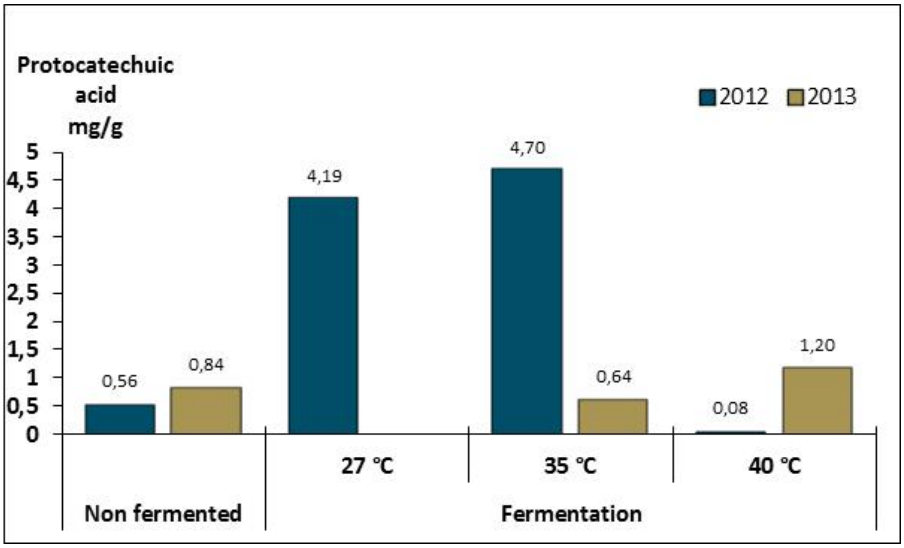

Figure 4. Effect of fermentation temperature on the average protocatechuic acid content of three *Bergenia* accessions.

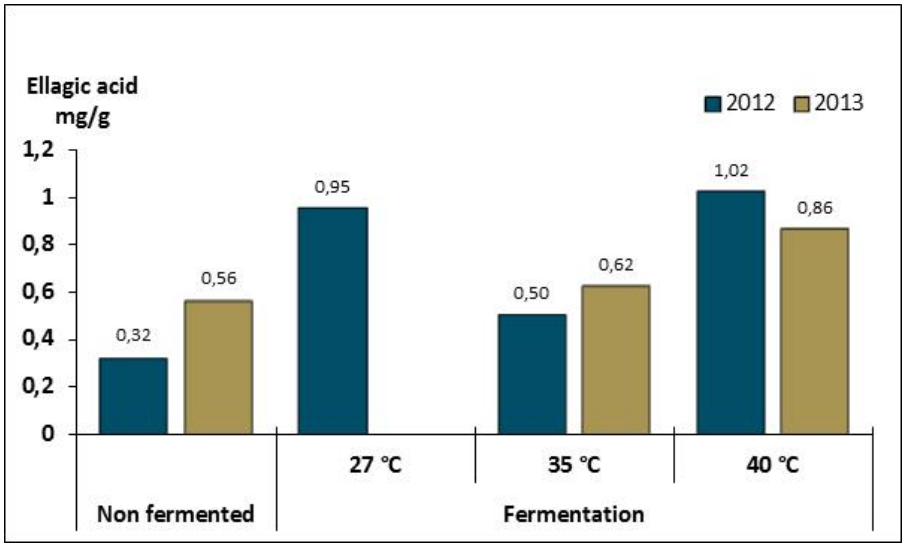

Figure 5. Effect of fermentation temperatures on the average ellagic acid content of three *Bergenia* accessions.

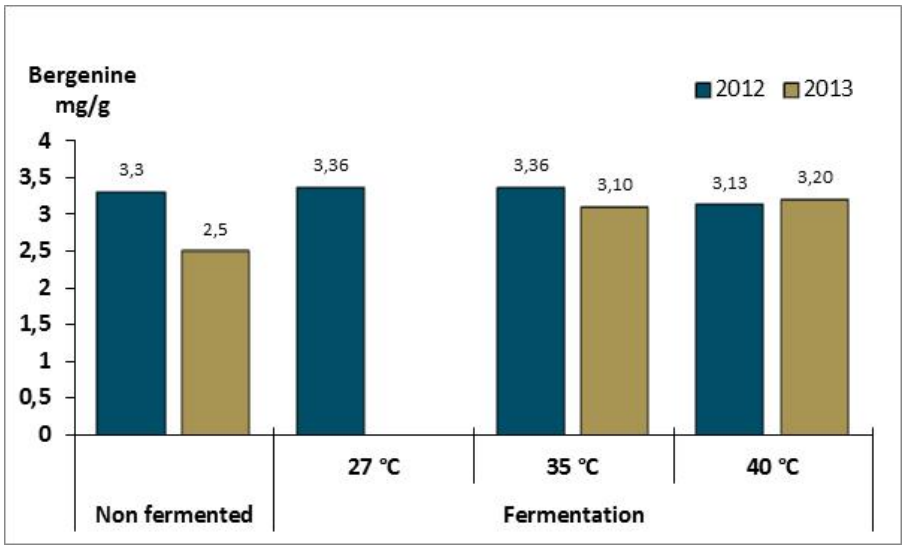

Figure 6. Effect of fermentation temperatures on the average bergenin content of three *Bergenia* accessions.

3.4.5. Post-fermentation procedures

A thin layer of fermented fresh plant material was put into drying boxes (15 x 25 x 3 cm in size) and dried at 40 °C for 12 hours.

The dry matter content of the fermented material ranged between 28.5 and 33.0 %. No difference was observed in the dry matter content fermented at 35 °C (mean 28.9 %, n= 9) and at 40°C (mean 29.1 %, n= 9). The ratio of fresh: dry material was 3.5:1 in average. From one kg of fresh fermented material we got 350 g of dry tea material in average. One kg of dry fermented material needs 2.8–3 kg of fresh crushed material.

The fermented dry plant material consisted of hard, small granulates and some plant parts, which were not crushed sufficiently. These were generally green in color. They were separated easily by using 3–5 mm screens. According to the fermentation in 2012, the average quantity of these plant parts was 1.3 % (0.7–2.7%) calculated on the dry weight.

The hard granulates were broken by crumbling/breaking over the 3 mm metal nets, but coffee mills are suitable as well. The final procedure is the equalization where dust is separated from the tea material. The equalized dry raw material can be stored in double package sacs (paper + plastic) at room temperature for 2 years.

3.4.6. Storage of the fermented material

For the determination how longer storing period effects on the quality of fermented material, we carried out a storage experiment. Fermented and non-fermented samples of two *Bergenia* accessions (*B. cordifolia* No.12, a *Bergenia* x hybrid "Piha") were stored at room temperature in double bags (inner bag of paper and outer bag of plastic). The length of the storage period was 23 months (nearly 2 years).

The chemical analyses were carried out at the same laboratory soon after the fermentation in 2012 and after 2 years in October/November 2014.

On the base of the obtained data we can state, that after a 2-year storage period changes in the content of studied compounds in non-fermented and fermented samples were generally low, ranging between 1–35 % (Table 2). Significant increase (33–80 %) was observed only in the protocatechuic acid content in the fermented samples at 35 °C. We may conclude that the stability of studied compounds in the fermented and non-fermented leaves of *Bergenia* species is quite good and during 2-year storage no detrimental changes occurred.

Table 2. Content (mg/g) of studied compounds of *Bergenia* species after a 2-year storage period.

Accession	Sample	Processing	Arbutin		Gallic acid		Hydroquinone		Protocatechuic acid		Bergenine		Ellagic acid	
	code	in 2012	2012	2014	2012	2014	2012	2014	2012	2014	2012	2014	2012	2014
Bergenia	1	Non-fermented, 40 °C	174.1±6.5	125.9±2.5	5.7±0.1	6,0±0.5	<0.35	<0.35	<0.08	<0.08	3.6±0.1	2.8±0.1	0.28±0.01	0,30±0.01
cordifolia	3	Fermented at 35° C	107.6±0.6	105.6±7.2	94,0±1.6	95.5±4.7	1.2±0.1	1.1±0.1	6,0±0.2	11,0±0.1	4.7±0.3	5.1±0.2	0.65±0.02	0.69±0.15
(No.12)	4	Fermented at 40° C	106.2±0.8	106.3±5.8	128.1±1.7	128.9±5.5	1.3±0.1	1.2±0.1	<0.08	<0.08	4.3±0.1	4.6±0.2	1.3±0.2	1.1±0.2
		mean of fermented	**106,9**	**106**	**111,1**	**112,2**	**1,25**	**1,15**	**3,04**	**5,54**	**4,5**	**4,85**	**0,97**	**0,91**
Bergenia	9	Non-fermented, 40 °C	150.7±0.7	133.5±0.6	13.4±0.2	13.1±0.1	<0.35	<0.35	1.8±0.1	1.4±0.1	3.9±0.1	3.4±0.1	0.28±0.01	0.18±0.01
Hybrid	11	Fermented at 35° C	91,0±0.1	71.1±0.7	81.1±0.5	80.1±0.6	1.2±0.1	0.9±0.1	8.1±0.2	10.8±0.1	4.4±0.3	4,0±0.1	0.51±0.06	0,50±0.03
cv."Piha"	12	Fermented at 40° C	109.1±0.7	104±0.4	45.6±0.3	47.6±0.7	0.8±0.1	0.7±0.1	<0,08	<0,08	3.9±0.1	4.1±0.2	0.78±0.07	0.8±0.1
		mean of fermented	**100,1**	**87,6**	**63,4**	**63,9**	**1,0**	**0,8**	**4,09**	**5,44**	**4,2**	**4,2**	**0,64**	**0,65**

3.5. Conclusion

On the base of results above we may state that the fermentation process elaborated for *Epilobium angustifolium* tea production can be used for the leaf yield of *Bergenia* species as well.

The optimum fermentation temperature seemed to be 40 °C (35–42 °C) and the fermentation period 3–3.5 days. The fermentation process ends when the green plant mass turns to deep tobacco

brown color and it has a nice, specific smell. Compared to the non-fermented leaves the fermentation process decreased significantly (by 20–60 %) the arbutin content, but the content of nearly all other compounds increased. The fermentation process can be carried out in farm level, without high cost investments. The critical phase is the suitable crushing machinery for the withered leaves. The practical details are presented in the technological recommendation (Appendix 1).

Picture 1. Mechanical harvest of *Bergenia* leaf yield. Photo Bertalan Galambosi.

Picture 2. Difficulties with mincing of leaves. Photo Bertalan Galambosi.

Picture 3. Succesful mixing of leaves. Photo Bertalan Galambosi.

Picture 4. Fermented minced and mixed *Bergenia* leaves. Photo Bertalan Galambosi.

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Appendix 1. Recommendation for the fermentation process of Bergenia leaf yield

Resource of the leaf yield: *Bergenia* leaves used for fermentation should consist mainly *Bergenia crassifolia* species. The raw material should contain high arbutin content of dry leaf (10–13%). Other species, like *B. cordifolia* or *Bergenia* hybrids can be used as well, if their arbutin content is high. Such accessions exist in medicinal plant gene collection of Natural Resources Institute Finland (Luke).

Harvest: The leaf yield should be harvested in autumn by hand or by machinery. Several hay harvesters, like Haldrup or Wintersteiger are suitable for machine harvesting. The harvest can be carried out during dry weather.

Quality of raw material: Raw plant material should contain *Bergenia sp.* leaves and stems, no buds and flowers. Leaf yield has to be free from impurities (dust, soil, dirt) and other contaminants such as fungal, insect and other animal contaminations. Leaf mass should be protected from rain and dust during transportation.

Withering: Harvested fresh leaf yield should be withered for 10–14 hours at room temperature. The harvest can be carried out in afternoon and the withering is done during the night. The leaves should be spread in 1–5 cm layers on the surface of clean floor (concrete or wood, not plastic, due to the moisture condensation). During withering the leaves of bad quality and other impurities should be taken away.

Slicing: Withered leaves have to be sliced for 1–2 cm plant parts. Suitable machinery for slicing is e.g. old style forage cutter, or modern cutter, like Wintersteiger.

Mincing/mixing: The sliced leaves have to be minced or mixed for destroying/crushing the cells of leaves. The crushing of leaves is necessary for promoting the enzyme activity of plant mass. A kitchen size cutting machine (Kenwood Gourmet 1600) machinery results a suitable small particles (0.1–1 mm). For commercial production an industrial size mixers should be found and tested.

Packing into boxes: Minced/mixed green plant mass should be put into plastic boxes with capacity of 2 to 10 kg plant material per box. The boxes have to be hygienic and easily cleaned and washed. The filled plastic boxes should be covered with the piece of paper, but not hermetically. Covering the surface with paper saves surface from drying but doesn't prevent the air circulation in the plant mass.

Fermentation place: Suitable place for fermentation is chamber or room with correctly regulated and stable temperature. Suitable equipment is electric dryer. The temperature should be regulated between 25–40 °C and the temperature level should be easily controlled. The filled boxes should be put into the dryer at once and the door should not be opened frequently, not to disturb the fermentation process.

Fermentation temperature and sensory evaluation: The optimum fermentation temperature seems to be 40 °C (35–42 °C). The fermentation period at 35–40 °C lasts 3–3.5 days. The fermentation process needs to be ended, when the green plant mass turns to deep tobacco brown color and it has a nice, specific smell. The sensory observation is very important and needs a preliminary exercise!

Drying of fermented material: When the fermentation is ended, the moisture plant mass have to be dried. For quick drying the moisture plant material should be spread into drying boxes. It is advantageous if the bottom of boxes is made of 0.5 mm hole screen. The fermented plant mass is dried at 40°C during 10–12 hours.

Equalization and storing of dry fermented material: The dried plant material consists smaller and bigger hard particles/granulates and some non-fermented, bigger plant particles. For equalization the bigger plant parts and the possible dust should be separated by using 3–5 mm hole screen and dust screen. The equalized dry raw material is packed into a double sack (paper + plastic) and stored at room temperature.

Ratio of fresh and fermented dry yield: The dry matter content of dried fermented material is generally 29 %. From one kg of fresh fermented material can be obtained in average 350 g dry tea material. One kg of dry fermented material needs 2.8–3 kg of fresh crushed leaf material.

Quality control through the process: During the fermentation process the quality check points (chemical analyses) are proposed as follows: 1. The cultivated accession before starting cultivation: Focus should be on the high arbutin content of dry green leaves. 2. After the fermentation process: The chemical analyses should determinate the content of arbutin, gallic acid, hydroquinone, protocatechuic acid, bergenin and ellagic acid.

Storage of the fermented dry material: The samples can be stored for 2 years in room temperature in double sacs (inner bag of paper and outer bag of plastic).

Preconditions of the commercial fermentation in a farm-level processing unit

In the case of commercial quantities of fermented *Bergenia* leaf yield is needed, the process needs the following preconditions in a special farm:

1. Cultivation of *Bergenia* accessions with high arbutin content. Due to the stabile quality features of the three accessions studied, we propose accessions No 12 and Piha for further commercial propagation and industrial use. According to our experimental results, possible fresh leaf yield of *Bergenia* during the second and third year is 15–20 t/ha.
2. Processing building with official permission according to the hygienic standards of Finnish authorities. The building should allow sufficient space for withering, processing, drying and post-processing activities, with water washing and cleaning possibilities.
3. Sufficient machinery: field harvest machine, cutting machine, mincing/mixing machine, and drying/fermenting machinery: thermostats or other drying boxes regulated between the temperatures 30–50 °C.
4. Different size of plastic boxes for transport, fermentation and drying of *Bergenia* plant material. Storage place and sacks are necessary as well.
5. The farm has to organize the analytical expertise for quality controls, e.g. laboratory of the buyer company or independent laboratory service.

4. Clinical trial design for *Bergenia* samples, double blind placebo controlled crossover study

Nummela, A.[1], Joutsen, T.[2], Shikov, A.N.[3], Makarov, V.G. [3], Laajala, P.[4] and Galambosi, B.[5]

[1]Research Institute for Olympic Sports – KIHU, Rautpohjankatu 6, 40700 Jyväskylä, Finland

[2]Sports Lab / Sports Research and Development SRD Oy, Rautpohjankatu 6, 40700 Jyväskylä, Finland

[3]St.-Petersburg Institute of Pharmacy, 56, Bolshaja Porochovskaya, POBox 16, 195248, St-Petersburg, Russia

[4]Natural Resources Institute Finland (Luke), Kipinäntie 16, 88600 Sotkamo, Finland

[5]bertalan.galambosi@surffi.fi

Abstract

The study was planned to study whether acute or a 7-day *Bergenia* intake has any effects on endurance exercise capacity or muscle strength. The study included two experimental designs: to investigate the acute effects and the effect of 7-day use of *Bergenia*. The study was double blind placebo controlled crossover study. Thirty physically active men were divided into three groups who ingested one, two or four capsules containing either isomalt (placebo) or *Bergenia* extract (100 mg) plus isomalt. The order of the placebo and *Bergenia* tests was randomized so that half of each group had first placebo and then *Bergenia* tests and the other half had the opposite order. The endurance test was maximal incremental cycling test in which maximal power, maximal blood lactate, maximal heart rate, maximal ventilation, VO_{2max} as well as RPE and glucose concentration at exhaustion were determined. The strength tests were maximal isometric leg press and vertical counter-movement jump. The results of the present study showed that the *Bergenia* supplementation with the current doses of 100–400 mg does not have almost any acute or a 7-day usage effect on endurance and maximal force characteristics in physically active men. *Bergenia* supplementation did not cause any negative effects on the present subjects either. In the previous studies it was shown that supplementation of animals with the extract from fermented leaves of *Bergenia* enhanced the maximum swimming capacity of mice. We may make a hypothesis that probably other dose ranges and prolongation of supplementation time would be more correct for future human experiments.

Keywords: *Bergenia* supplementation, endurance, strength

4.1. Aim of the study

The aim of the study was to study the acute effect of *Bergenia* intake on endurance exercise capacity and muscle strength of physically active men (Study 1). Another aim of the study was to study the 7 day use of *Bergenia* intake on endurance exercise capacity and muscle strength (Study 2).

4.2. Material and methods

4.2.1. *Bergenia* food supplement and placebo

The *Bergenia* species were cultivated in the MTT Mikkeli experimental field. The fermentation procedures are described elsewhere in this publication (Galambosi et al. 2016). For the sport experiment the fermented green leaves were originated from the 2012 autumn fermentation.

Fermented *Bergenia* leaves were dried, crushed and extracted with hot water. Water extract was concentrated under vacuo and liophilized. Hard gelatin capsules were filled with isomalt (placebo) or mixture of isomalt with 100 mg of Bergenia extract.

4.2.2. Experiment design and subjects

The study included two experimental designs. In the first study, the acute effect of *Bergenia* intake on endurance performance, maximal oxygen uptake and muscle strength was compared to placebo in double blind controlled crossover design (Study 1). In the second study, the effect of 7 day use of *Bergenia* on endurance performance, maximal oxygen uptake and muscle strength was compared to placebo in double blind controlled crossover design (Study 2).

In the experimental design, the experiments in the first study were the pre experiments for the second study. Therefore, all subjects were measured four times with one week interval and after the first and third experiments were the 7 day use of *Bergenia* or placebo. The 7 day washout period was separated the second and third experiments. The subjects in the present study were healthy physically active men at the age of 18 to 45 years. Exclusion criteria were consistent intake of any medication, any medical conditions that might contraindicate for endurance and/or strength exercise testing, intake of any nutritional supplement two weeks before and during the study. Subjects were also asked to abstain from any medication during the period of study and avoid changes in their diet or level of physical activity. The subjects signed a written informed consent and they were randomly assigned to one of the six experimental groups (Table 1).

Table 1. Six experimental groups in the studies 1 and 2.

Group	Subjects (number)	Test sample 1-2	Test sample 3-4	Dose
Group A	5	Bergenia	Placebo	100 mg
Group B	5	Placebo	Bergenia	100 mg
Group C	5	Bergenia	Placebo	200 mg
Group D	5	Placebo	Bergenia	200 mg
Group E	5	Bergenia	Placebo	400 mg
Group F	5	Placebo	Bergenia	400 mg

All 30 subjects completed the measurements in the Study 1, but because of the illness of the subjects nine of them could not do one of the post measurements and therefore all of their data was excluded from the final results in Study 2.

4.2.3. Measurements

From the evening (10:00 PM) before the experiments until the end of each session, the subjects were asked to refrain from alcohol and caffeine containing beverages. However, they were, in each phase of the study, not compelled to be fasted at commencement of the experimental tests. The subjects came to the laboratory between 8:00 AM and 2:00 PM about one hour before the exercise tests. Upon arrival, they were weighed and they ingested one, two or four capsules containing either isomalt (Placebo) or 100 mg of a *Bergenia* extract plus isomalt (*Bergenia*) according to their experimental group (Table 1). They were seated in a semi-supine position in a comfortable chair for a 40 min period to obtain a full intestinal absorption. Before exercise tests a standard warm-up of 5 min was performed including 5 min cycling on an ergometer at the power of 1.5 x body weight (W) and 5 min squats (3 x 6) and vertical squat jumps (3 x 5). Thereafter, maximal isometric muscle strength, counter-movement jump and maximal endurance performance were assessed.

Maximal isometric knee-extension force was measured on an isometric leg press. Subjects performed three maximal voluntary isometric knee-extensions (3 s), interspersed by 1-min rest intervals, at a knee-angle of 100 degrees. Maximal isometric force (N) was measured as the highest 2 s period from the force-time curve. The highest of the three attempts was recorded as maximum force.

The other strength test was a vertical **counter-movement jump** (CMJ) on a force plate (AMTI AccuPower Force Platform, Advanced Medical Technology Inc, USA). The starting position was standing relaxed with legs apart and hands on the hips. In CMJ the subjects were squatting down quickly to 90 degree knee angle and jump immediately up as high as possible. Force-time curve was recorded to a computer. The height of the jump was calculated from the force impulse. Each subject performed three jumps with 30 s interval and the best jump was selected to final result.

The endurance test was **maximal incremental cycling test on a cycle ergometer** (Monark LC7, Monark, Sweden). In the maximal cycling test, the initial power was 60 W for one minute. Thereafter, the power was increased 20 W every minute until volitional exhaustion or to the point where the subject was not able to keep the cadence of 70 rpm. In the maximal cycling test ventilation, oxygen uptake (VO_2) and carbon dioxide production (VCO_2) were measured breath by breath (Oxycon Mobile, Viasys, Germany) and heart rate was measured as RR intervals (Suunto t6, Suunto, Finland) during the whole test. Furthermore, a 20 µl fingertip blood sample was taken before the test at 8 min and 2 min after the exhaustion for the determination of lactate and glucose concentration (Biosen, EKF Diagnostic GmbH, Germany). The rating of perceived exertion (RPE) was asked at 8 min and immediately after the exhaustion with the scale of 0–10.

From the maximal cycling test the exhaustion time (min), maximal power (W), maximal blood lactate (mmol/l), maximal heart rate (bpm), maximal 30 s ventilation (l/min), maximal 30 s VO_2 (ml/kg/min and l/min), maximal 30 s VCO_2 (l/min), RPE and glucose concentration (mmol/l) at exhaustion were determined. Furthermore, the respective values at 200 W (8 min) were determined to the final results. In the Study 2, the training as well as the symptoms and any feelings of discomfort were recorded daily during the 7 day experimental periods.

4.2.4. Statistical analysis

Standard statistical methods were used to calculate means and standard deviations for different groups. The acute effect as well as the effect of 7 day use of *Bergenia* supplementation (*Bergenia* vs Placebo) was tested with TTEST (Study 1) and ANOVA (Study 2) (SPSS for Windows, SPSS Inc., USA). Furthermore, the effect of dose was evaluated by TTEST (Study 1) and ANOVA (Study 2).

4.3. Results

The results show that there were almost no acute effects of *Bergenia* supplementation on measured variables (Table 2). The only statistical differences between *Bergenia* and placebo samples can be observed with the dose of 400 mg. There was a significant difference in RPE and VO_{2max} values in the bicycle ergometer test. There was also a significant difference in maximal ventilation when pooled data of all dose sizes were used.

The results of the Study 2 are presented in Tables 3–6 (in the end of the article). When pooled data of all sample sizes were used there were no significant differences between one week *Bergenia* and placebo supplementation (Table 3).

When all sample sizes were analyzed separately no significant differences were observed (Tables 4–6).

The only significant difference was observed in maximal heart rate and VO_{2max} (l/min and ml/kg/min) when the dose of 100 mg was analysed (Table 4). The VO_{2max} was increased more in placebo than in *Bergenia* supplemetation in the dose of 100 mg.

The only significant difference was observed in peak power output and VO_{2max} when the dose of 200 mg was analysed (Table 5). In *Bergenia* supplementation both peak power output and VO_{2max} were increased in *Bergenia* supplementation. The effect on peak power output was the opposite in *Bergenia* supplementation when the dose of 400 mg was used (Table 6).

4.4. Conclusion

Based on the results of Study 1 and 2 (Tables 2–6) it can be concluded that Bergenia supplementation does not have almost any effect on endurance and maximal force characteristics in physically active men. Only a few significant differences were observed in acute and one week Bergenia supplementation but the effects were partially contradictory to each other and therefore the results cannot confirm any effect of Bergenia supplementation using doses between 100–400 mg per day.

Bergenia supplementation did not cause any negative effects on the present subjects.

It was confirmed in current study that *Bergenia* extract doesn't affect to the body weight. Similar results were observed previously in mice (Shikov et al., 2010) and in rats with high calorie diet (Shikov et al., 2012) after treatment with *Bergenia crassifolia* extract over the 7 days.

However, the results of current study regarding the effect of *Bergenia* extract in physical exercise situation are in contrast with previous studies. *Bergenia* extract was evaluated for radical scavenging activity. Extract was proven an effective antioxidant which efficacy exceeded the efficacy of ascorbic acid (Pozharitskaya et al., 2007). Antioxidants play important positive role in exercise situation. It was shown that supplementation of animals with the extract from fermented leaves of *Bergenia* enhanced the maximum swimming capacity of mice without change of the body weight by increasing glucose utilization and decreasing lactate level compared to the control group (Shikov et al., 2010). The metabolism of animals and human are different and it is very difficult to translate results of animal studies to human. We may make a hypothesis that probably other dose ranges and prolongation of supplementation time will be more correct for future human experiments.

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Table 2. The acute effect of Bergenia and placebo samples. Values are averages ± SD.

	All doses (n = 30)			Dose 100 mg (n = 10)			Dose 200 mg (n = 10)			Dose 400 mg (n = 10)		
	Bergenia	Placebo	P-value	Bergenia	Placebo	P-value	Bergenia	Placebo	P-value	Bergenia	Placebo	P-value
Body mass (kg)	79.7 ± 11.1	79.4 ± 11.2	0.079	82.3 ± 10.6	82.2 ± 10.4	0.608	78.2 ± 12.9	78.1 ± 13.3	0.698	78.5 ± 10.3	78.0 ± 10.1	0.037
Isometric force (kg)	306 ± 66	300 ± 66	0.115	327 ± 86	323 ± 85	0.605	311 ± 52	298 ± 50	0.114	281 ± 52	278 ± 54	0.689
CMJ (cm)	33.1 ± 6.6	33.4 ± 6.1	0.559	34.2 ± 6.5	34.5 ± 6.1	0.601	34.9 ± 6.8	34.9 ± 6.6	0.997	30.4 ± 6.1	30.7 ± 5.3	0.691
B-La rest (mmol/l)	0.9 ± 0.3	1.0 ± 0.4	0.178	1.2 ± 0.3	1.3 ± 0.5	0.406	0.8 ± 0.2	0.8 ± 0.4	0.749	0.9 ± 0.3	1.0 ± 0.2	0.273
B-Glu rest (mmol/l)	4.5 ± 0.5	4.5 ± 0.4	0.600	4.4 ± 0.5	4.6 ± 0.3	0.262	4.7 ± 0.5	4.4 ± 0.4	0.057	4.5 ± 0.4	4.5 ± 0.5	1.000
Bicycle ergometer test max												
Time max (min)	14.62 ± 1.72	14.60 ± 1.67	0.959	14.31 ± 1.95	14.24 ± 1.90	0.581	15.48 ± 1.86	15.57 ± 1.80	0.368	14.02 ± 0.91	13.99 ± 0.77	0.837
Power max (W)	332 ± 34	332 ± 33	0.930	326 ± 39	325 ± 38	0.540	350 ± 37	351 ± 36	0.387	320 ± 18	320 ± 16	0.852
RPE max (0-10)	9.1 ± 1.1	9.3 ± 0.9	0.277	9.7 ± 0.9	9.5 ± 1.3	0.177	8.5 ± 1.1	8.7 ± 0.5	0.555	9.1 ± 0.8	9.7 ± 0.5	0.030
HR max (bpm)	177 ± 7	178 ± 7	0.487	176 ± 8	178 ± 9	0.098	180 ± 8	181 ± 6	0.295	177 ± 6	176 ± 6	0.506
VO_{2max} (l/min)	3.96 ± 0.38	3.90 ± 0.36	0.130	3.88 ± 0.50	3.82 ± 0.41	0.245	4.05 ± 0.35	4.11 ± 0.38	0.255	3.96 ± 0.24	3.78 ± 0.17	0.021
VO_{2max} (ml/kg/min)	50.3 ± 6.3	49.8 ± 6.4	0.249	47.3 ± 3.9	46.7 ± 3.7	0.299	52.6 ± 7.4	53.5 ± 7.7	0.158	50.1 ± 6.6	49.1 ± 5.8	0.043
VCO_{2max} (l/min)	4.52 ± 0.42	4.50 ± 0.46	0.381	4.46 ± 0.52	4.43 ± 0.49	0.595	4.74 ± 0.36	4.80 ± 0.42	0.609	4.37 ± 0.27	4.26 ± 0.32	0.333
RER max	1.14 ± 0.05	1.15 ± 0.05	0.423	1.15 ± 0.06	1.16 ± 0.04	0.553	1.17 ± 0.04	1.17 ± 0.04	0.811	1.10 ± 0.04	1.13 ± 0.07	0.442
VE max (l/min)	132 ± 22	137 ± 21	0.050	133 ± 24	136 ± 28	0.285	134 ± 18	140 ± 17	0.258	128 ± 25	134 ± 19	0.286
TV max (l)	3.22 ± 0.30	3.19 ± 0.30	0.573	3.29 ± 0.31	3.25 ± 0.29	0.287	3.21 ± 0.23	3.15 ± 0.28	0.078	3.14 ± 0.38	3.15 ± 0.35	0.195
RF max (bpm)	50.6 ± 8.6	52.5 ± 8.7	0.146	49.6 ± 9.6	51.5 ± 10.7	0.112	51.3 ± 6.9	54.0 ± 6.7	0.162	50.8 ± 10.1	52.0 ± 8.9	0.728
B-La max (mmol/l)	11.5 ± 2.3	12.0 ± 2.2	0.166	11.5 ± 1.3	11.9 ± 1.7	0.138	13.1 ± 1.8	13.1 ± 1.9	0.913	9.8 ± 2.3	11.2 ± 2.7	0.276
B-Glu max (mmol/l)	5.7 ± 0.7	5.8 ± 0.7	0.508	5.9 ± 0.9	5.8 ± 0.8	0.543	5.7 ± 0.5	6.0 ± 0.9	0.158	5.5 ± 0.6	5.5 ± 0.5	0.871
Bicycle ergometer test 200 W												
RPE (0-10)	3.4 ± 1.4	3.3 ± 1.2	0.318	4.2 ± 1.3	3.8 ± 1.1	0.182	2.5 ± 1.1	2.5 ± 1.1	0.725	3.5 ± 1.2	3.5 ± 1.1	1.000
HR (bpm)	143 ± 9	143 ± 8	0.825	142 ± 9	145 ± 10	0.199	143 ± 9	144 ± 6	0.941	143 ± 10	141 ± 6	0.333
VO_2 (l/min)	2.72 ± 0.16	2.73 ± 0.15	0.952	2.71 ± 0.22	2.72 ± 0.23	0.874	2.73 ± 0.13	2.68 ± 0.07	0.306	2.74 ± 0.13	2.78 ± 0.08	0.372
VO_2 (ml/kg/min)	34.7 ± 4.2	34.8 ± 4.2	0.688	33.1 ± 2.3	33.3 ± 3.1	0.753	35.6 ± 5.3	35.1 ± 4.8	0.355	35.3 ± 4.4	36.1 ± 4.4	0.173
VCO_2 (l/min)	2.45 ± 0.14	2.46 ± 0.15	0.595	2.44 ± 0.17	2.44 ± 0.15	0.993	2.46 ± 0.14	2.43 ± 0.16	0.681	2.45 ± 0.12	2.53 ± 0.14	0.068
RER	0.90 ± 0.05	0.91 ± 0.05	0.554	0.90 ± 0.06	0.90 ± 0.04	0.807	0.90 ± 0.04	0.90 ± 0.05	0.828	0.90 ± 0.06	0.91 ± 0.06	0.279
VE (l/min)	52.6 ± 6.9	52.9 ± 5.9	0.771	54.6 ± 8.9	52.3 ± 6.9	0.203	50.0 ± 5.1	50.5 ± 5.3	0.785	53.2 ± 6.0	55.8 ± 4.8	0.011
TV (l)	2.51 ± 0.31	2.55 ± 0.29	0.376	2.62 ± 0.24	2.64 ± 0.22	0.853	2.47 ± 0.33	2.48 ± 0.24	0.927	2.44 ± 0.35	2.53 ± 0.39	0.112
RF (bpm)	26.0 ± 4.3	25.7 ± 3.8	0.283	25.5 ± 4.7	24.3 ± 3.5	0.301	25.3 ± 3.9	24.9 ± 3.0	0.678	27.3 ± 4.5	27.9 ± 4.1	0.885
B-La (mmol/l)	2.2 ± 0.9	2.3 ± 1.0	0.316	2.4 ± 0.8	2.6 ± 0.9	0.480	2.1 ± 1.1	2.0 ± 1.1	0.459	2.0 ± 0.7	2.4 ± 0.9	0.182
B-Glu (mmol/l)	4.5 ± 0.4	4.4 ± 0.3	0.443	4.5 ± 0.3	4.6 ± 0.3	0.409	4.6 ± 0.6	4.3 ± 0.4	0.306	4.4 ± 0.4	4.4 ± 0.5	0.616

Abbreviations: CMJ = counter movement jump, B-La = blood lactate; B-Glu = blood glucose; RPE = rated perceived exertion; HR = heart rate; VO_2 = oxygen uptake; VCO_2 = carbondioxide production; RER = respiratory exchange ratio; VE = ventilation; TV = tidal volume; RF = respiratory frequency

Table 3. The effect of one week *Bergenia* supplementation. Values are averages ± SD.

	All doses (n = 21)				
	Bergenia		Placebo		
	Pre	Post	Pre	Post	P-value
Body mass (kg)	78.2 ± 10.7	78.2 ± 10.8	78.0 ± 10.9	78.2 ± 10.7	0.234
Isometric force (kg)	305 ± 54	304 ± 52	296 ± 52	299 ± 59	0.482
CMJ (cm)	33.1 ± 6.2	33.3 ± 5.8	33.6 ± 5.6	33.4 ± 5.9	0.462
B-La rest (mmol/l)	0.9 ± 0.3	1.0 ± 0.3	1.1 ± 0.5	0.9 ± 0.4	0.053
B-Glu rest (mmol/l)	4.5 ± 0.5	4.5 ± 0.3	4.4 ± 0.4	4.4 ± 0.5	0.928
Bicycle ergometer test max					
Time max (min)	14.58 ± 1.76	14.64 ± 1.82	14.57 ± 1.70	14.68 ± 1.66	0.822
Power max (W)	332 ± 35	333 ± 36	331 ± 34	334 ± 33	0.815
RPE max (0-10)	9.0 ± 1.0	9.0 ± 1.2	9.2 ± 1.0	9.2 ± 1.1	0.392
HR max (bpm)	178 ± 7	178 ± 8	178 ± 8	179 ± 6	0.285
VO_{2max} (l/min)	3.94 ± 0.36	3.92 ± 0.36	3.90 ± 0.37	3.90 ± 0.35	0.754
VO_{2max} (ml/kg/min)	51.0 ± 6.8	50.4 ± 6.4	50.6 ± 6.6	50.5 ± 5.9	0.413
VCO_{2max} (l/min)	4.50 ± 0.41	4.51 ± 0.44	4.48 ± 0.47	4.56 ± 0.37	0.436
RER max	1.14 ± 0.05	1.15 ± 0.04	1.15 ± 0.05	1.17 ± 0.04	0.470
VE max (l/min)	129 ± 20	131 ± 21	135 ± 18	135 ± 17	0.197
TV max (l)	3.12 ± 0.30	3.07 ± 0.32	3.09 ± 0.29	3.05 ± 0.29	0.728
RF max (bpm)	51.1 ± 8.1	52.5 ± 9.1	53.4 ± 8.4	53.8 ± 7.7	0.582
B-La max (mmol/l)	11.5 ± 2.4	11.9 ± 2.2	12.2 ± 2.4	12.3 ± 1.8	0.852
B-Glu max (mmol/l)	5.5 ± 0.6	5.5 ± 0.6	5.5 ± 0.5	5.6 ± 0.5	0.607
Bicycle ergometer test 200 W					
RPE (0-10)	3.4 ± 1.4	3.7 ± 1.4	3.2 ± 1.2	3.4 ± 1.3	0.973
HR (bpm)	143 ± 9	142 ± 11	143 ± 8	141 ± 9	0.483
VO_2 (l/min)	2.70 ± 0.14	2.68 ± 0.12	2.72 ± 0.15	2.68 ± 0.13	0.948
VO_2 (ml/kg/min)	35.1 ± 4.7	34.9 ± 4.6	35.4 ± 4.4	34.5 ± 4.2	0.822
VCO_2 (l/min)	2.43 ± 0.14	2.42 ± 0.16	2.44 ± 0.17	2.42 ± 0.16	0.917
RER	0.90 ± 0.05	0.90 ± 0.04	0.90 ± 0.05	0.90 ± 0.4	0.771
VE (l/min)	51.4 ± 5.3	52.1 ± 6.0	52.8 ± 5.5	52.0 ± 6.2	0.339
TV (l)	2.44 ± 0.30	2.48 ± 0.32	2.48 ± 0.27	2.46 ± 0.26	0.638
RF (bpm)	26.2 ± 3.9	26.0 ± 4.1	26.0 ± 3.5	25.8 ± 4.4	1.000
B-La (mmol/l)	2.3 ± 0.9	2.2 ± 0.7	2.4 ± 1.0	2.1 ± 0.8	0.478
B-Glu (mmol/l)	4.5 ± 0.5	4.3 ± 0.5	4.4 ± 0.4	4.4 ± 0.3	0.579

Abbreviations: CMJ = counter movement jump, B-La = blood lactate; B-Glu = blood glucose; RPE = rated perceived exertion; HR = heart rate; VO_2 = oxygen uptake; VCO_2 = carbondioxide production; RER = respiratory exchange ratio; VE = ventilation; TV = tidal volume; RF = respiratory frequency

Table 4. The effect of one week daily 100 mg *Bergenia* supplementation. Values are averages ± SD.

	Dose 100 mg (n = 6)				
	Bergenia		Placebo		
	Pre	Post	Pre	Post	P-value
Body mass (kg)	79.6 ± 8.0	79.9 ± 8.3	79.3 ± 8.1	79.9 ± 8.0	0.521
Isometric force (kg)	316 ± 51	313 ± 51	305 ± 42	308 ± 48	0.377
CMJ (cm)	33.4 ± 6.3	34.0 ± 6.3	34.6 ± 5.6	33.7 ± 5.7	0.118
B-La rest (mmol/l)	1.2 ± 0.3	1.2 ± 0.4	1.4 ± 0.5	1.2 ± 0.5	0.404
B-Glu rest (mmol/l)	4.4 ± 0.6	4.5 ± 0.3	4.6 ± 0.3	4.4 ± 0.8	0.437
Bicycle ergometer test max					
Time max (min)	14.02 ± 2.16	14.17 ± 2.34	14.00 ± 2.18	14.26 ± 2.24	0.682
Power max (W)	321 ± 43	323 ± 47	320 ± 44	325 ± 45	0.547
RPE max (0-10)	9.5 ± 1.2	9.2 ± 1.6	9.3 ± 1.6	9.4 ± 1.4	0.374
HR max (bpm)	175 ± 8	173 ± 7	177 ± 9	178 ± 7	0.018
VO_{2max} (l/min)	3.75 ± 0.49	3.77 ± 0.48	3.69 ± 0.43	3.82 ± 0.43	0.025
VO_{2max} (ml/kg/min)	47.1 ± 4.1	47.2 ± 3.2	46.5 ± 3.4	47.8 ± 2.1	0.069
VCO_{2max} (l/min)	4.31 ± 0.47	4.30 ± 0.47	4.24 ± 0.48	4.48 ± 0.42	0.085
RER max	1.15 ± 0.05	1.14 ± 0.04	1.15 ± 0.03	1.18 ± 0.04	0.433
VE max (l/min)	132 ± 18	132 ± 21	134 ± 19	141 ± 18	0.737
TV max (l)	3.12 ± 0.26	3.01 ± 0.25	3.11 ± 0.27	3.00 ± 0.28	0.586
RF max (bpm)	51.8 ± 8.5	53.2 ± 9.2	53.0 ± 9.8	56.7 ± 7.4	0.910
B-La max (mmol/l)	11.5 ± 1.0	11.6 ± 0.9	12.0 ± 1.7	12.3 ± 1.3	0.872
B-Glu max (mmol/l)	5.5 ± 0.5	5.2 ± 0.6	5.3 ± 0.5	5.5 ± 0.5	0.065
Bicycle ergometer test 200 W					
RPE (0-10)	4.5 ± 1.2	4.3 ± 1.3	3.8 ± 1.1	3.8 ± 0.9	0.765
HR (bpm)	140 ± 10	139 ± 11	146 ± 12	141 ± 15	0.078
VO_2 (l/min)	2.62 ± 0.11	2.63 ± 0.11	2.65 ± 0.24	2.63 ± 0.12	0.693
VO_2 (ml/kg/min)	33.0 ± 2.4	33.2 ± 3.2	33.6 ± 3.4	33.2 ± 3.6	0.617
VCO_2 (l/min)	2.37 ± 0.14	2.41 ± 0.14	2.41 ± 0.19	2.41 ± 0.15	0.729
RER	0.91 ± 0.06	0.92 ± 0.06	0.91 ± 0.04	0.92 ± 0.5	0.996
VE (l/min)	53.2 ± 4.9	54.8 ± 4.4	53.2 ± 4.9	52.8 ± 3.87	0.275
TV (l)	2.53 ± 0.26	2.60 ± 0.39	2.56 ± 0.23	2.49 ± 0.31	0.671
RF (bpm)	25.8 ± 4.0	26.7 ± 3.9	25.3 ± 3.0	26.0 ± 4.4	0.930
B-La (mmol/l)	2.5 ± 0.7	2.4 ± 0.6	2.8 ± 0.7	2.5 ± 1.0	0.739
B-Glu (mmol/l)	4.5 ± 0.3	4.6 ± 0.3	4.7 ± 0.3	4.6 ± 0.3	0.355

Abbreviations: CMJ = counter movement jump, B-La = blood lactate; B-Glu = blood glucose; RPE = rated perceived exertion; HR = heart rate; VO_2 = oxygen uptake; VCO_2 = carbondioxide production; RER = respiratory exchange ratio; VE = ventilation; TV = tidal volume; RF = respiratory frequency

Table 5. The effect of one week daily 200 mg *Bergenia* supplementation. Values are averages ± SD.

	Dose 200 mg (n = 7)				
	Bergenia		Placebo		
	Pre	Post	Pre	Post	P-value
Body mass (kg)	78.2 ± 15.7	77.9 ± 15.7	78.3 ± 16.2	78.2 ± 15.7	0.436
Isometric force (kg)	312 ± 61	299 ± 58	298 ± 61	299 ± 63	0.103
CMJ (cm)	34.6 ± 6.7	33.9 ± 6.5	34.6 ± 6.4	34.4 ± 6.5	0.460
B-La rest (mmol/l)	0.8 ± 0.2	0.8 ± 0.3	0.8 ± 0.5	0.8 ± 0.2	0.695
B-Glu rest (mmol/l)	4.7 ± 0.5	4.5 ± 0.3	4.4 ± 0.5	4.4 ± 0.5	0.472
Bicycle ergometer test max					
Time max (min)	15.41 ± 1.94	15.69 ± 1.92	15.58 ± 1.83	15.45 ± 1.84	0.030
Power max (W)	348 ± 39	354 ± 38	352 ± 37	349 ± 37	0.028
RPE max (0-10)	8.4 ± 1.0	9.1 ± 0.7	8.7 ± 0.5	8.6 ± 1.0	0.224
HR max (bpm)	182 ± 6	184 ± 6	182 ± 6	182 ± 8	0.153
VO_{2max} (l/min)	4.04 ± 0.34	4.07 ± 0.38	4.15 ± 0.37	4.07 ± 0.41	0.008
VO_{2max} (ml/kg/min)	52.9 ± 8.6	53.6 ± 8.8	54.4 ± 8.8	53.3 ± 8.4	0.007
VCO_{2max} (l/min)	4.78 ± 0.36	4.76 ± 0.48	4.84 ± 0.44	4.76 ± 0.43	0.318
RER max	1.18 ± 0.05	1.17 ± 0.03	1.17 ± 0.04	1.17 ± 0.03	0.419
VE max (l/min)	131 ± 16	137 ± 18	139 ± 17	135 ± 17	0.122
TV max (l)	3.11 ± 0.18	3.09 ± 0.27	3.04 ± 0.22	3.11 ± 0.25	0.234
RF max (bpm)	51.6 ± 6.1	54.4 ± 8.7	55.6 ± 5.3	52.6 ± 6.9	0.137
B-La max (mmol/l)	13.3 ± 1.7	13.6 ± 2.1	13.3 ± 2.0	13.3 ± 1.9	0.623
B-Glu max (mmol/l)	5.7 ± 0.5	6.0 ± 0.5	5.8 ± 0.5	5.8 ± 0.7	0.291
Bicycle ergometer test 200 W					
RPE (0-10)	2.5 ± 1.3	2.9 ± 1.5	2.5 ± 1.3	2.7 ± 1.5	0.425
HR (bpm)	144 ± 8	143 ± 11	144 ± 7	141 ± 5	0.814
VO_2 (l/min)	2.73 ± 0.14	2.66 ± 0.12	2.69 ± 0.08	2.70 ± 0.15	0.226
VO_2 (ml/kg/min)	36.0 ± 6.5	35.2 ± 6.1	35.4 ± 5.8	35.3 ± 5.1	0.389
VCO_2 (l/min)	2.46 ± 0.16	2.36 ± 0.16	2.38 ± 0.15	2.40 ± 0.16	0.183
RER	0.90 ± 0.04	0.89 ± 0.04	0.89 ± 0.04	0.89 ± 0.04	0.319
VE (l/min)	49.3 ± 5.5	48.1 ± 6.3	49.9 ± 6.3	48.7 ± 6.5	1.000
TV (l)	2.43 ± 0.38	2.44 ± 0.28	2.43 ± 0.22	2.47 ± 0.25	0.860
RF (bpm)	25.4 ± 4.4	24.3 ± 4.1	25.0 ± 3.1	23.7 ± 3.5	0.920
B-La (mmol/l)	2.3 ± 1.3	2.1 ± 0.9	2.0 ± 1.3	2.1 ± 0.8	0.274
B-Glu (mmol/l)	4.6 ± 0.7	4.5 ± 0.3	4.2 ± 0.3	4.3 ± 0.4	0.603

Abbreviations: CMJ = counter movement jump, B-La = blood lactate; B-Glu = blood glucose; RPE = rated perceived exertion; HR = heart rate; VO_2 = oxygen uptake; VCO_2 = carbondioxide production; RER = respiratory exchange ratio; VE = ventilation; TV = tidal volume; RF = respiratory frequency

Table 6. The effect of one week daily 400 mg *Bergenia* supplementation. Values are averages ± SD.

	Dose 400 mg (n = 8)				
	Bergenia		Placebo		
	Pre	Post	Pre	Post	P-value
Body mass (kg)	77.3 ± 8.2	77.2 ± 8.3	76.6 ± 7.8	76.8 ± 8.0	0.586
Isometric force (kg)	290 ± 54	302 ± 53	288 ± 56	293 ± 70	0.484
CMJ (cm)	31.6 ± 6.3	32.3 ± 5.5	32.0 ± 5.1	32.3 ± 6.2	0.762
B-La rest (mmol/l)	0.9 ± 0.3	0.9 ± 0.2	1.0 ± 0.2	0.7 ± 0.2	0.041
B-Glu rest (mmol/l)	4.4 ± 0.4	4.4 ± 0.4	4.4 ± 0.5	4.4 ± 0.3	0.703
Bicycle ergometer test max					
Time max (min)	14.23 ± 0.94	14.07 ± 0.86	14.12 ± 0.70	14.34 ± 0.73	0.019
Power max (W)	324 ± 19	322 ± 17	323 ± 14	327 ± 15	0.030
RPE max (0-10)	9.1 ± 0.7	8.9 ± 1.3	9.6 ± 0.5	9.5 ± 0.8	1.000
HR max (bpm)	177 ± 4	175 ± 5	175 ± 7	177 ± 5	0.024
VO_{2max} (l/min)	4.01 ± 0.22	3.90 ± 0.16	3.83 ± 0.15	3.81 ± 0.16	0.666
VO_{2max} (ml/kg/min)	52.3 ± 6.3	50.0 ± 4.8	50.4 ± 4.7	50.0 ± 4.71	0.995
VCO_{2max} (l/min)	4.43 ± 0.28	4.43 ± 0.26	4.34 ± 0.32	4.43 ± 0.22	0.903
RER max	1.11 ± 0.03	1.13 ± 0.04	1.13 ± 0.06	1.16 ± 0.05	0.959
VE max (l/min)	125 ± 26	126 ± 24	132 ± 20	132 ± 17	0.938
TV max (l)	3.12 ± 0.44	3.09 ± 0.44	3.11 ± 0.38	3.05 ± 0.36	0.040
RF max (bpm)	50.0 ± 10.4	50.3 ± 10.1	51.8 ± 9.9	52.8 ± 8.8	0.097
B-La max (mmol/l)	9.8 ± 2.6	10.8 ± 2.3	11.4 ± 2.9	11.5 ± 1.7	0.451
B-Glu max (mmol/l)	5.5 ± 0.7	5.3 ± 0.6	5.5 ± 0.5	5.4 ± 0.4	0.261
Bicycle ergometer test 200 W					
RPE (0-10)	3.4 ± 1.2	3.9 ± 1.3	3.4 ± 1.0	3.8 ± 1.3	0.893
HR (bpm)	145 ± 9	143 ± 12	142 ± 7	140 ± 7	0.796
VO_2 (l/min)	2.74 ± 0.14	2.74 ± 0.13	2.79 ± 0.09	2.70 ± 0.11	0.520
VO_2 (ml/kg/min)	35.8 ± 4.4	35.8 ± 4.3	36.7 ± 3.8	34.7 ± 4.1	0.577
VCO_2 (l/min)	2.44 ± 0.14	2.49 ± 0.16	2.52 ± 0.16	2.44 ± 0.19	0.333
RER	0.89 ± 0.06	0.91 ± 0.04	0.91 ± 0.06	0.90 ± 0.05	0.562
VE (l/min)	52.0 ± 5.5	53.5 ± 5.7	55.0 ± 4.6	54.6 ± 6.9	0.367
TV (l)	2.38 ± 0.27	2.44 ± 0.33	2.47 ± 0.34	2.42 ± 0.26	0.573
RF (bpm)	27.3 ± 3.7	27.1 ± 4.3	27.4 ± 4.1	27.6 ± 4.8	0.851
B-La (mmol/l)	2.0 ± 0.8	2.1 ± 0.7	2.4 ± 1.0	1.9 ± 0.6	0.123
B-Glu (mmol/l)	4.4 ± 0.4	4.3 ± 0.4	4.4 ± 0.5	4.4 ± 0.3	0.556

Abbreviations: CMJ = counter movement jump, B-La = blood lactate; B-Glu = blood glucose; RPE = rated perceived exertion; HR = heart rate; VO_2 = oxygen uptake; VCO_2 = carbondioxide production; RER = respiratory exchange ratio; VE = ventilation; TV = tidal volume; RF = respiratory frequency

5. Chokeberry (X *Sorbaronia mitschurinii*) for health

Marnila, P.[1], Shikov, A.N.[2], Laine A. [1], Kaseva J.[1], Hellström J.[1], Pihlava J-M.[1], Pozharitskaya O.N. [2], Kauppinen S.[3], Makarov V.G. [2], Makarova M.N. [2], Mattila P.[1], Galambosi, B.

[1]Natural Resources Institute Finland (Luke), Alimentum, Humppilantie 7, 31600 Jokioinen, Finland

[2]St.-Petersburg Institute of Pharmacy, 56, Bolshaja Porochovskaya, POBox 16, 195248, St-Petersburg, Russia

[3]Natural Resources Institute Finland (Luke), Lönnrotinkatu 3, 50100 Mikkeli, Finland

Abstract

High consumption of berries and fruits as whole (not as high sugar juices) have in numerous clinical studies consistently shown to have an anti-obesity effect. There are several mechanisms behind. A part of them are attributed to phytochemicals including phenolic compounds. Obesity is associated with increased risk for widespread diseases, including hypertension, metabolic syndrome, type II diabetes, atherosclerosis and arthritis, disorders with increasing incidence. Chokeberries are exceptionally rich source of phenolic compounds. The content of total phenolics is so high that no other common edible fruit or berry can surpass it. The beneficial effects of chokeberry juice on lipid metabolism are well documented. Chokeberry is considered to be beneficial food for diabetes patients. An interesting new area is the effects of berries and fruits on central nervous system. Hypertension is an important risk factor for cardiovascular disease which is the most common cause of morbidity and mortality in Europe, usually due to atherosclerosis. Chokeberry phenolic extracts have in clinical trials been shown to reduce blood pressure in hypertensive patients. In Specicrop project we studied effects of different dosages of Finnish chokeberry (*X Sorbaronia mitschurinii, var. Viking*) juice on blood pressure of the spontaneously hypertensive rats. We also measured the inflammatory cytokines in rat blood in order to see the potential beneficial effects of the chokeberry juice on chronic inflammation which is typical in hypertension. The study provided encouraging data.

Key words: *Aronia mitschurinii*, phenolic compounds, polyphenols, hypertension, vascular disease, diabetes mellitus, inflammation, metabolic health

5.1. Study of effects of chokeberry juice in Specicrop project

Hypertension is an important risk factor for cardiovascular disease which is the most common cause of morbidity and mortality in Europe, usually due to atherosclerosis. There is strong evidence that chronic inflammation and abnormal immune responses are involved in development and pathogenesis of hypertension, cardiovascular disease, type 2 diabetes and older age memory diseases (Dinh 2014). Food compounds having anti-hypertensive or anti-inflammatory properties are in general considered to have beneficial effects with regard to above mentioned diseases. Growing evidence obtained from test animal model studies and from studies with humans suggests that berry phenolics and polysaccharides have beneficial effects on cardiovascular health.

Earlier, we reported that chokeberry juice and phenolic fraction of chokeberry (X *Sorbaronia mitschurinii,* var. 'Viking') had blood pressure-lowering effects in spontaneously hypertensive rats (Hellström et al. 2010). The effects were clear but the study period was only 10 days. This rat model is a well known experimental model to study hypertension and it has been also proposed as a model of insulin resistance. These rats typically show abdominal obesity, hypertension and high levels of triacylglycerols in blood (Artiñano and Castro 2009).

In Specicrop project we studied effects of different dosages and longer term administration of the chokeberry (*X Sorbaronia mitschurinii, var.* Viking) juice on blood pressure of the spontaneously hypertensive rats. We also measured the inflammatory cytokines in rat blood in order to see the

potential beneficial effects of the chokeberry juice on chronic inflammation. The study provided encouraging data and due to good results the research group decided to offer the study to be published first in an international scientific journal. In order not to endanger the publication process or to violate issues concerning novelty of results and copyrights we can not yet present more specific information on the results. We hope that the article is soon available for the readers. Instead of our results in Specicrop project we present here a brief insight to the main findings and some selected studies on the effects of chokeberry and chokeberry phenolics on the risk factors of common widespread diseases.

5.2. Chokeberry and risk of widespread diseases

Obesity is a major public health problem in industrialized countries and it is associated with increased risk for obesity related inflammatory diseases, including metabolic syndrome, type II diabetes, atherosclerosis and arthritis, disorders with increasing incidence. Novel approaches based on nutritional and diet changes are needed. Although berries and fruits contain large amounts of simple sugars which are well known to induce obesity, epidemiological research has consistently shown that most types of berries and fruits have anti-obesity effects. All the mechanisms of this effect are not fully known but the evidence is strong (Sharma et al. 2016). Dietary phenolic compounds are shown in numerous clinical studies to support metabolic health and to act in a number of ways which include inhibition of inflammatory cytokine secretion, inhibition of leukocyte activation and reduced nitric oxide production and oxidative stress.

Chokeberries are exceptionally rich source of phenolic compounds and the reported contents have varied from 3.4 up to 7.8% of dry matter (Kulling & Rawel, 2008). Actually, the content of total phenolics is so high that no other common edible fruit or berry can surpass it. Proanthocyanidins (condensed tannins) is the most abundant phenolic group followed by anthocyanins and caffeoyl-quinic acids. Proanthocyanidins are essentially B-type procyanidins and highly polymerized. The astringent, dry puckering mouthfeel of the berry is caused by proanthocyanidins precipitating the saliva proteins. Four major anthocyanins in chokeberry are all based on cyanidin. They give the dark blue color of the berry. Chlorogenic and neochlorogenic acids are the dominating caffeoyl-quinic acids. While the contents of phenolics are remarkably high in fresh berries they, anyhow, can be significantly reduced in processed products. Especially anthocyanins are known to be sensitive for processing and storage conditions (Hellström et al., 2013). Moderate heat treatments during processing and cold storage conditions are hence recommended to minimize the loss of phenolics.

Chokeberry phenolic extracts have in clinical trials been shown to reduce blood pressure. For instance in test subjects who suffered hypertension and metabolic syndrome the use of chokeberry phenolic extract (3 x 100 mg/day) for two months decreased systolic and diastolic blood pressures in average by 11 mmHg and 5 mmHg, respectively (Broncel et al. 2010), and in a later study by 10.5 and 6.3, respectively (Sikora et al. 2014). In our earlier study with the spontaneously hypertensive rat model (Hellström et al 2010) administering lyophilized chokeberry juice and extract for ten days (50mg/kg/day) decreased the mean systolic blood pressure by 20 ±8 and 15 ± 7 mm Hg, respectively. Corresponding mean decreases in the diastolic blood pressures were 23 ± 6 and 13 ± 2 mmHg, respectively. Consumption of juice seems to result in similar reduction in blood pressure than phenolic extract. The mechanism is partly inhibion of angiotensin 1 convertase enzyme and partly other pleiotrophic effects of berry juice (Hellström et al. 2010, Sikora et al. 2014).

Blood platelet function is an important specific factor in cardiovascular health. Bioactive compounds including phenolic acids in plant foods are known to modulate platelet activation. The effect of chokeberry extract was tested in a clinical trial in patients with metabolic syndrome (Sikora et al 2012). Administering 100 mg of the extract three times per day for one or two months resulted in favourable changes to overall potential for plasma clotting as well as in lipid profiles (Sikora et al. 2012).

The beneficial effects of chokeberry juice on lipid metabolism are well documented. In several clinical trials consumption of chokeberry juice and its extract are reported to exert considerable beneficial effects on total and LDL cholesterol and triglyseride levels, on fatty acid composition as well as on oxidative stress and inflammatory markers in blood (Naruszewicz et al. 2007, Broncel et al. 2010, Duchnowicz et al. 2012, Sikora et al. 2014, Kardum et al. 2014, 2015). Distorted lipid metabolism is commonly treated with statins. Berries can be used together with statins as supportive means to prevent ischemic heart disease. For instance, in clinical trial with 44 elderly patients who had suffered myocardial infarction, and had received simvastatin therapy at least for 6 months administering of chokeberry extract (3 x 85mg/day) for 6 weeks lowered the LDL –levels in serum as well as markers of chronic inflammation and reduced both systolic and diastolic blood pressures (Naruszewicz et al. 2007).

The beneficial effects of chokeberry on lipid metabolism may also protect liver. In recent animal model study on mice with high-fat diet induced non-alcoholic fatty liver disease mixing 0.5 or 1% of chokeberry powder to high fat diet for 8 weeks lowered serum triglyseride levels, decreased size of fat droplets in liver and corrected biochemical markers of fatty liver disease (Park et al. 2016).

Due to high polyphenol content chokeberry is considered to be beneficial food for diabetes patients. This is demonstrated in clinical study with subjects having type 2 diabetes mellitus (Simeonov et al. 2012). Ingestion of 200 ml sugar free chokeberry juice daily for 3 months lowered fasting glucose levels of subjects from in average 13.3 to 9.1 mmol/l. Recently, Kozuga et al (2015) identified a compound from chokeberry, cyanidin 3,5,-diglucoside, which act same way than a group of commonly used blood sugar lowering diabetes drugs (dipeptyl peptidase IV inhibitors). In subsequent study by Yamane et al. (2016) administering chokeberry juice to diabetic mice reduced their body weights, amount of white adipose tissue and blood glucose levels.

An interesting area is the effects of berries and fruits on central nervous system. Many polyphenols e.g. in cacao are known to improve brain blood circulation. In novel test animal studies administering chokeberry juice was reported to improve memory and learning in rats (Valcheva-Kuzmaseva 2014) and to inhibit memory impairment in a mouse model that mimics metabolic changes in Alzheimer's disease (Lee et al. 2016). It is not yet known, if these observations from animal models can be translated to human. We are looking forward to human studies on the effects of chokeberry on memory and learning. Anyway, what is good for circulation is good for brain.

5.3. Conclusions

Most of the beneficial health effects presented above can be achieved also by eating other berries. It is probable that the best health benefit can be obtained by eating regularly many different kinds of berries and fruits. However, chokeberry is exceptionally rich in phenolic compounds and has its own unique composition. Identification of these compounds and their mode of action will help to address the very real public health issue in providing nutritional solutions for improving health. The published scientific research demonstrates clearly that berries and fruits contain a diverse range of bioactive compounds that are able to favourably influence on our health and thus, they should be an integral part of our everyday food.

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6. Development of herb education in Begunitsky Agro-technological College

Galambosi, B.[1], Kirakosyan, G.M.[2], Dembickaya, E.[2], Lapshin, A.[2], Nemtshenko N.F.[2], Kauppinen, S.[3], Sorvari, J.[4]

[1]bertalan.galambosi@surffi.fi

[2]Begunitsky Agrotechnological College, 188423 Begunitsy, Volosovsky area, Leningrad region, Russia

[3]Natural Resources Institute Finland (Luke), Lönnrotinkatu 3, 50100 Mikkeli, Finland

[4]Euro-Rahoitus Oy, Nummitie 18 A, 01800 Klaukkala, Finland

Abstract

During the last decade the interest for domestic grown herb raw materials have increased among pharmaceutical companies in the Leningrad region, Russia. There are eight vocational agricultural colleges in the Leningrad region, and none of them teaches herb cultivation. There is a lack of cultivation expertise, also in herb cultivation education material. Due to the cross border cooperation with the support of EU Interreg and ENPI programs, during 1995–2014 several projects have resulted specialisation for herb education in the Begunitsky Agrotechnological College, in connections with Finnish institutes. Due to the similar northern climate conditions in regions of South-East Finland and North-West Russia, the cooperation focused on cold tolerant medicinal plants, and berry and fruit plants. In these projects we made efforts for transferring the experiences of organic cultivation obtained in small-scale herb farms in Finland into Russia. During the last 3-year project *"Special crop education for economic development in North-West Russia and South-East Finland SPECICROP*" we strengthened the preconditions of the professional education in the College by creating of demonstration herb garden with 30 species, renovation of the greenhouse and the herb-vegetable educational cabinet supplied by an infrared experimental dryer for herbs. For increasing the professional skills of teachers and entrepreneurs study trips were organized in family size herb farms in Finland, in fruit institutes and companies around Moscow, and in herb-processing companies in Altai. Regular seminars were organized in Begunitsky Agrotechnological College for young and adult students on organic herb and berry cultivation, on introduction of new herbs into cultivation. A practical seminar was arranged for demonstrating the advances and machinery of plastic mulch technology. A series of Power Point lectures on several specific subjects and growing technologies of the most important medicinal and berry cultures were prepared and provided for educational purposes. Update, modern herb-cultivation text books in Finnish and Russian are under finalization.

Keywords: Herb cultivation, South-East Finland, North-West Russia, black plastic mulch, herb, berry, demonstration garden

6.1. Lack of herb educational expertise in North West Russia

During the last years the interest for domestic grown herb raw material has increased among pharmaceutical companies in the Leningrad region. At the same time has become clear that there is a lack of cultivation expertise, also in education of herb cultivation. There are eight vocational agricultural colleges in the Leningrad region, and none of them teaches herb cultivation. The available technological information is from the technical books written in 1960–70 and needs updating.

Due to cross border cooperation with the support of EU Interreg and ENPI programs, during 2002–2014 several projects have resulted specialization for herb education in the Begunitsky Agrotechnological College, in connections with Finnish institutes (Table 1). The partners in this

cooperation have been MTT Agrifood Research Finland, ProAgria South Savo (coordinators) and South Savo Vocational College in Mikkeli, Finland, and the Begunitsy Agrotechnological College, V. Komarov Botanical Garden, St- Petersburg, St-Petersburg Institute of Pharmacy, St-Petersburg in Russia and several companies in the Leningrad region. The administration task has belonged to Euro-Rahoitus Oy, Klaukkala, Finland.

Table 1. Cooperative herb projects between Russia and Finland during 2002–2014.

Period	Project name	Program
2002-2004	European Nordic Herb Production (EPY)	Interreg
	Euroopan Pohjoinen Yrttituotanto (EPY)	
2005-2006	Development of new medicinal herb-based products in South-East Finland (YKE)	Interreg
	Yrttijalosteiden kehittäminen Kaakkois-Suomessa (YKE)	
2007-2008	New herbs for Leningrad region (New Herbs)	Interreg-TACIS
	Uutuusyrtit Lenigradin alueelle (New herbs)	
2010	Commercialisation of special crop production in the Leningrad region (Herbfruit)	Ministry of
	Erikoiskasvien tuotannon kaupallistaminen Leningradin alueella (Herbfruit)	Foreign Affairs
2012-2014	Special crop education for economic development in North-West Russia and South-East Finland (SPECICROP)	ENPI CBC
	Erikoiskasvien opetus Luoteis-Venäjän ja Kaakkois-Suomen taloudellisen kehityksen edistämiseksi (SPECICROP)	2007-2013

Since South-East Finland and North-West Russia represent similar northern climate conditions, the cooperation focused on the cultivation of those cold tolerant medicinal plants, in which marketing interest occurred (Table 2). Additionally in these projects we made effort for transferring the experience of organic cultivation obtained in small-scale herb farms in Finland to Russia, and made effort on the scientific basis to develop new herb-based products. The long-term cooperation made possible to plan and realize several long-term research activities.

6.2. Some achievements of the projects between 2002–2011

- We organized regularly herb seminars in Begunitsky Agrotechnological College of several subjects. Seminars gave special information for young and adult students and for the growers in this region.
- We developed the preconditions of education in the College. A herb demonstration garden was created including 30 species of herb and medicinal plants (Table 2).The quality of locally grown herb seeds was regularly determined and herb seeds were given for students and farmers (Picture 1).
- A reconstruction of the plastic house was carried out for modern nursery production (Picture 1).
- A modern drying machinery (Ferusa) was obtained with infrared drying technology
- The herb and vegetable teaching cabinet was reconstructed and supplied with modern equipment (demonstration samples and a microscope, picture 2).
- The demonstration garden has supplied research samples for cooperative research between Russia and Finland.
- Several market analyses have been carried out to have basic information for the most important herb species and needs of the industry in Leningrad region.
- In cooperation with St-Petersburg Botanical Garden and Vavilov Institute we carried out comparable experiences in Mikkeli both indoors and outdoors with 5 Russian dill and 10 Russian coriander varieties.
- In cooperation with Russian scientists we introduced several new medicinal plants into Finland, which are well known in the Russian official and traditional pharmacology. The species are: Bergenia (*Bergenia* sp.), trifid bur-marigold (*Bidens tripartita*), common melilot (*Melilotus officinalis*), fenugreek (*Trigonella foenum-graecum*), lucerne (*Medicago sativa),* marsh cudweed *(Gnaphalium uliginosum)*.

- From the year 2010 on we enlarged the object of cooperation to fruit and berry plants. We created contacts to Russian fruit institutes and companies.
- We started cooperation on studying the pharmacological research of special fruit plants, like sea buckthorn (*Hippophae rhamnoides*) and aronia (*Aronia mitschurinii*).
- The results of cooperative research activities were presented in different research forums, in Russian, Finnish and International seminars. All together 26 lectures and posters were presented (Appendix 1).
- During 2002–2011 we published numerous research and professional articles on our common research studies. All together 23 research papers were published (Appendix 2).

Table 2. Medicinal plants in the demonstration herb garden of Begunitsky Agrotechnological College.

Scientific name	Russian name	Finnish name	Germination %*
Achillea millefolium	Тысячелистник обыкновенный	siankärsämö	99
Althaea officinalis	Алтей лекарственный	rohtosalkoruusu	21
Arctium lappa	Лопух большой	takiainen	
Bergenia crassifolia	Бадан толстолистный	soikkovuorenkilpi	40-89
Bidens tripartita	Череда трехраздельная	tummarusokki	
Calendula officinalis	Ноготки лекарственные	kehäkukka	
Chelidonium majus	Чистотел большой	keltamo	
Dracocephalum moldavica	Змееголовник молдавский	tuoksuampiaisyrtti	
Echinacea purpurea	Эхинацея пурпурная	kaunopunahattu	76
Epilobium angustifolium	Кипрей узколистный	maitohorsma	
Gnaphalium uliginosum	Сушеница топяная	savijäkkärä	
Helichrysum arenarium	Бессмертник песчанный	hietaolkikukka	
Hypericum perforatum	Зверобой продырявленный	mäkikuisma	
Inula helenium	Девясил высокий	isohirvenjuuri	
Leonorus cardiaca	Пустырник сердечный	rohtonukula	72
Leuzea carthamoides	Стемаканта сафлоровидная (Левзея сафлоровидная)	maraljuuri	59
Matricaria chamomilla	ромашка аптечная	kamomillasaunio	89
Medicago sativa	Люцерна посевная	sinimailanen	
Melissa officinalis	Мелисса лекарственная	sitruunamelissa	
Origanum vulgare	душица обыкновенная	mäkimeirami	63-83
Plantago lanceolata	Подорожник ланцетолистный	heinäratamo	
Polemonium caeruleum	Синюха голубая	lehtosinilatva	83
Potentilla argentea	Лапчатка гусинная	hopeahanhikki	
Rhodiola rosea	Родиола розовая	pohjanruusujuuri	
Salvia officinalis	Шалфей лекарственный	rohtosalvia	
Serratula coronaria	Серпуха венценосная	rohtoliuskalääte	
Trifolium pratense	Клевер луговой	puna-apila	
Valeriana officinalis	Валериана лекарственная	rohtovirmajuuri	
			* during 2009-2010

6.3. Activities in the SPECICROP project

During 2012–2014 a 3-year project was carried out: *Special crop education for economic development in North-West Russia and South-East Finland SPECICROP*". The overall objective of project was to enhance the professional cultivation of special crops (herbs and medicinal plants, fruits and berries) and commercialize their production and processing through improved education, advisory activities and new research.

6.3.1. Development of special crop education

Begunitsky Agrotechnological College has added into its present "Landlady of the Manor" education program a new special subject: the cultivation of herbs, berries and fruits. The program was accredited in the year 2014 and is accepted as a part of their official education program. Altogether

43 students have graduated from this educational program during the project. College has engaged to continue the subject after the project: The education program has been signed for the year 2018 by now.

Based on our previous cooperation and experiences we made effort to achieve above mentioned long-term goal by developing the precondition of the education of herb and fruit cultivation in North-West Russia, being its centres in the Begunitsky Agrotechnological College and in South Savo Vocational College, Mikkeli. The focus was on increasing the professional skills of teachers and providing modern educational material and demonstration gardens for young and adult students.

6.3.2. Seminars

During the project period we organized several intensive courses (seminars) for all together 190 participants (76 students, 14 teachers, 18 researchers, 17 company representatives, 11 farmers, 4 NGO representatives, 27 home gardeners, 23 project organizers.)

- 2012, February 29 th –March 1st: Intensive course of herb and berry production was held in Begunitsy: 43 participants (Picture 3).
- 2012, May: Medicinal plant workshop was held in Begunitsy for five professional participants.
- 2013, May 30th: Practical seminar for establishing the perennial herb and fruit garden was held in Begunitsy: 32 participants (Picture 4).
- 2014, June 24th: Practical seminar of establishing the perennial herb field in Lembolovo as a part of international seminar called "Medicinal crop science and modern pharmaceutical industry" arranged by Saint-Petersburg Chemical Pharmaceutical Academy: 37 participants including 21 students of the Academy.
- 2014, September 30th: Final seminar of project results was held in Begunitsy with 22 Russian participants.
- 2014, October 29th: Final seminar of project results was held in Mikkeli for 51 Finnish participants.

6.3.3. Study trips

For increasing the professional skills of Russian and Finnish teachers, growers and advisers several study trips were organized with 61 participants.

- 2012, August 19th–22nd: Study trip for Russian teachers, entrepreneurs and project partners was arranged to Eastern Finland and Karkkila to have experience with the activities of the family size herb farms in Finland (Kauppinen et al. 2012, Picture 5).
- 2013, September 30 th–October 6 th :Study trip for Finnish teachers, researchers, company representatives, farmers and project organizers to study the fruit and berry production and breeding activities in Moscow, Tula and Orel in Russia (Sorvari et al. 2013)
- 2014, August 17th–24th: Study trip for Finnish herb specialists, teachers, researchers, project organizers, advisory and authority representatives to study the modern herb and berry production and processing in the Altai region, Russia (Sorvari et al. 2014, Picture 6).

Picture 1. Herb demonstration garden and new plastic houses in Begunitsky. Photo Bertalan Galambosi.

Picture 2. Refacilitated teaching cabinet in Begunitsky. Photo Bertalan Galambosi.

Picture 3. Seminars for students and teachers in Begunitsky. Photo Bertalan Galambosi.

Picture 4. Spreading of black plastic mulch during practical seminar in Begunitsky. Photo Bertalan Galambosi.

Picture 5. Russian entrepreneurs visiting *Echinacea* farm in South Savo, Finland in 2012. Photo Bertalan Galambosi.

Picture 6. Finnish herb specialists visited Central Siberian Botanical Garden in Novosibirsk, Russia in 2014. Photo Sanna Kauppinen.

6.3.4. Contribution to the technological development of special crops

Black plastic mulch technology

On the base of the experience of study trip in Finland 2012–visiting in practical farms – where herbs and berries are cultivated in black plastic mulch – there was a strong wish to introduce the black plastic cultivation method into the Begunitsky Agrotechnological College. Machinery and black plastic mulch was provided by the SPECICROP project. Also Saint-Petersburg Chemical Pharmaceutical Academy got interested in the technology and uses it in its demonstration farm in Lembolovo.

Herb, fruit and berry demonstration gardens

After spreading the plastic mulch, the herb demonstration garden was enlarged and the perennial herbs were transferred into the plastic mulch area. Additionally the fruit demonstration garden was formed in Begunitsky including 14 fruit and berry species. Similarly an organic apple garden was created in South Savo Vocational College in 2014, where 23 Russian apple cultivars will be tested for their disease resistance during the following years.

Root herb harvest machinery

Several important medicinal plant species are producing sufficient root yield under the cool Nordic climatic condition. For enhancing their adequate and effective harvest method, a single-row root harvester was obtained for Begunitsky Agrotechnological College for further root harvest experiments.

Technologies for production of fermented tea raw materials

Based on several previous experiments during the project we finalized the technological parameters of production of Ivan tea (*Epilobium angustifolium*) suitable for small scale enterprises (Galambosi et al. 2016a). Additionally we adapted the Ivan tea production methods for the production of Siberian tea (*Bergenia crassifolia*) and provided practical recommendations for producers (Galambosi et al. 2016b).

Fruit cultivar descriptions

A Finnish book was prepared with recommendations of 84 apple cultivars and 15 pear cultivars for Finnish climate for the use of fruit producers in Finland (Nissinen et al. 2016). Most of the cultivars originate from Russia, but also Belorussian, Ukrainian, Baltic and American cultivars are introduced.

6.3.5. Research and development activities in the project

During the project the research and development activities resulted all together 18 publications, including 6 scientific articles and 6 congress abstracts (Appendix 3.)

6.3.6. Modern educational material

One of the important final goals of Specicrop project was providing modern educational material for Russian and Finnish colleges. Series of Power Point lectures on several specific subjects and growing technologies of the most important medicinal and fruit plants and techniques were prepared in Russian and were provided to the teachers of Begunitsky Agrotechnological College. The PP-presentations can be found from the web site of College (http://gbouspobat.ru/soc-partner/world-sotrud/specicrop/).

Educational herb cultivation material by B. Galambosi.

1. Climate and soils in herb cultivation
2. Direct sowing and mechanical weed control in herb cultivation
3. Herb cultivation in potato ridge
4. Herb cultivation in mulched bed
5. Herb cultivation technology in black plastic mulched bed
6. Spreading technology of black plastic mulch

7. Harvest of seed herbs and flower herbs
8. Harvest of leafy herbs and root herbs
9. Harvest and post harvest processing of root herbs
10. Good Agriculture Practice for aromatic and medicinal plants
11. Agropharmaceutica (G. Kirakosyan)

Educational fruit and berry cultivation material by S. Kauppinen
1. Cultivation of strawberry
2. Cultivation of raspberry
3. Black currant (short)
4. Cultivation of sea buckthorn
5. Methodology of research of fruit and berry plants in Russia (N. Bezkina)
6. Medicinal use of berry and fruit plants (G. Kirakosian)

6.3.7. Herb growing manuals in Finnish and in Russian

One of the important goals of the project was to prepare an updated, modern herb cultivation handbook both in Finnish and in Russian.

A Finnish herb cultivation manual

The new Finnish cultivation handbook was prepared in accordance with Finnish requirements. The manuscript was prepared in close collaboration with four Finnish teachers who are teaching herb cultivation in vocational colleges in Finland.

Altogether 69 herbal plants were selected to include into a new cultivation handbook. The handbook summarized the latest agronomical research results and practical cultivation experiences. Additionally it contained several special aspects of the herb cultivation among Nordic climatic conditions. The handbook was published by the Finnish National Board of Education in November 2016 (Galambosi 2016, Appendix 4).

A Russian herb cultivation manual

Herb cultivation handbooks in Russian language have been published 20–25 years ago and there was a need for up to date herb growing manual. In the frame of the project, the Russian herb growing manual manuscript contains 22 important herbs, whose cultivation is possible in North-West Russia, in the Leningrad region (Appendix 4). The Russian manuscript is based on the Finnish version but is revised by Russian specialists to suit Russian conditions. This manuscript is freely delivered in the homepage of Begunitsky Agrotechnological College (http://gbouspobat.ru/soc-partner/world-sotrud/specicrop/). Furthermore there is an aim to publish it also in a paper version.

6.4. Summary

Based on the previous long-term cross border cooperation with Russian and Finnish research and educational institutes, a 3-year project was planned to improve the special crop education in the Begunitsky Agrotechnological College. The Specicrop project fulfilled the planned activities and gave an important impact for the new special educational subject in the College: the cultivation of herbs, berries and fruits. The precondition for the implementation of project goals was the active collaboration of specialist in the Russian and Finnish institutes and colleges involved in the project. The research and educational seminars, the demonstration gardens, the new technological improvements and diversified educational material has given a strong background for the development of the special crop production in North-West Russia.

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Appendix 1. Participation in different congresses with posters and lectures between 2003–2010

Abstracts of the Congresses

2003

Galambosi, B., Kaarlas, M., Moilanen, T.2003. **Implementing of Good Agricultural Practice (GAP) for medicinal and aromatic plants in Finland**. Phytopharm Congress 2003.

Galambosi, B., Sairanen, J., Domokos, J. 2003. **Effect of harvesting methods on the seed yield and oil quality of *Borago officinalis* and *Oenothera biennis* in South-East Finland**. Phytopharm Congress 2003.

Lampinen, P., Leskinen, M., Lehesvaara, M. 2003. **Microbiological quality of fresh and dried dill (*Anethum graveolens*) in Finland**. Phytopharm Congress 2003.

Makarov, V.G., Zenkevcih, I.G., Shikov,A.N., Pimenov, A.I., Pozharitskaya, O.N., Ivanova, S.A., Galambosi, B. 2003 **Comparative analyses of *Rhodiola rosea* of Scandinavian and Russian origin**. Phytopharm Congress 2003.

2004

Galambosi, B., Kaarlas, M. 2004. **Preconditions for elaboration of Dynaforce, a new *Rhodiola rosea* extract from field research to legislation**. Phytopharm Congress 2004.

Leskinen, M., Lampinen, P. 2004. **Microbiological quality of fresh and dried nettle (*Urtica dioica*) in Finland**. Phytopharm Congress 2004.

Sairanen, J., Sorvari, J. 2004 **Herbs in both sides of the border by European Nordic Herb Production (EPY)**. Developing project No: 17/3521/02 by Interreg III-A. Phytopharm Congress 2004.

Shikov, A.N., Makarov, V.G., Dragland, S., Vender, C., Volodin, V. L., Galambosi, B. :2004 **Comparative analyses of *Rhodiola rosea* of European and Russian origin**. Phytopharm Congress 2004.

2005

Holm, Y., Dergounova, E., Galambosi, B., Hiltunen, R. 2005. **Comparison of greenhouse and open field cultivated basil, parsley, dill and leaf coriander using hydrodistillation and SPME**. In: Jenö Bernáth, Éva Németh and Anita Kozak (eds.). Programme and book of abstracts: 36th international symposium on essential oils, 4–7 September, 2005 Budapest, Hungary. Budapest: Faculty of Horticultural Sciences. p. 172.

2006

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Galambosi, B., Galambosi, Zs., Hethelyi, B.E. 2006. **Evaluation of biomass potential and oil yield of *Myrica gale* L. for possible field cultivation.** pp. 415–421. Phytopharm Congress 2006.

Hellström, J., Mattila, P., Pihlanto, A., Ryhänen, E.-L., Kivijärvi, P. 2006. **ACE inhibitory activity of chokeberry (*Aronia melanocarpa*) polyphenols**. Pp.426–428. Phytopharm Congress 2006.

Kosman, V.M., Melihkova, M.V., Galambosi, B., Ryzhenkov, V.E., Makarov, V.G. 2006. **Anticoagulant activity of some medicinal plants**. Pp. 463– 269. Phytopharm Congress 2006

Makarova, M.N., Tesakova S.V., Eschenko, A.Yu., Siivari, J., Galambosi B., Zenkevics, I.G. 2006. **Study of antiradical activity of *Bergenia* ssp. leave samples in vitro.** Pp. 488–493. Phytopharm Congress 2006.

Mattila, P., Hellström, J., Ryhänen, E.-L., Kivijärvi, P.2006: **Contents of polyphenols in chokeberries (*Aronia Medik)*** pp. 494–496. Phytopharm Congress 2006

2008

Galambosi, B., Galambosi, Zs., Siivari, J., Shikov, A., Pozharitsakaya, O., Ivanova, S., Tikhonov, V. 2008. **Ecophysiological studies for optimizing field growing methods of *Bergenia* sp**. In: The 12th international congress PHYTOPHARM 2008, Saint-Petersburg, Russia, 2–4 July 2008: abstracts book. Saint-Petersburg: Interregional Center Adaptogen. p. 40.

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2009

Galambosi, B., Galambosi, Zs., Kosman, V.M., Faustova, N.M., Shikov, A.N., Pozharitskaya, N., Makarov, V.M. 2009. **Leaf yield and quality of Hungarian *Medicago sativa* varieties grown in Finland**. In: Lippay János – Ormos Imre – Vas Károly scientific conference, 28–30 October 2009, Budapest: abstracts. Budapest: Corvinus University of Budapest. p. 97.

2010

Galambosi, B., Galambosi, Zs., Shikov, A. N., Pozharitskaya, O. N., Kosman, V. M., Ivanova, S. A., Makarov, V. G. 2010. **Biomass production of *Epilobium parviflorum* and *Epilobium hirsutum* in cultivation.** In: The 14th international congress Phytopharm 2010, Saint-Petersburg, Russia, 1–3 July 2010: abstracts book. Saint-Petersburg: Interregional Center "Adaptogen". p. 45–46.

Galambosi, B., Galambosi, Zs., Shikov, A. N., Pozharitskaya, O.N., Kosman, V. M., Ivanova, S. A., Makarov, V. G. 2010. **Challenges in elaboration of growing methods of *Gnaphalium uliginosum* L.**. In: The 14th international congress Phytopharm 2010, Saint-Petersburg, Russia, 1–3 July 2010: abstracts book. Saint-Petersburg: Interregional Center "Adaptogen". p. 47.

Galambosi, B., Galambosi, Zs., Shikov, A. N., Pozharitskaya, O. N., Kosman, V. M., Ivanova, S. A., Makarov, V. G., Kirakosyan, G. 2010. **Biomass accumulation of *Epilobium angustifolium* in natural and cultivated populations.** In: The 14th international congress Phytopharm 2010, Saint-Petersburg, Russia, 1–3 July 2010 : abstracts book. Saint-Petersburg: Interregional Center "Adaptogen". p. 42.

Galambosi, B., Shikov, A. N., Pozharitskaya, O. N., Ivanova, S. A., Makarov, V. G., Hethelyi, B. E. 2010. **Experiences with fermentation on *Epilobium angustifolium* shoots**. In: The 14th international congress Phytopharm 2010, Saint-Petersburg, Russia, 1–3 July 2010 : abstracts book. Saint-Petersburg: Interregional Center "Adaptogen". p. 43–44

Appendix 2. List of scientific publications between 2003–2012

2003

Makarov, V.G., Zenkevich, I.G., Shikov, A.N., Pimenov, A.I., Pozharitskaya, O.N. , Ivanova, S.A., Galambosi, B. 2003. **Comparative analyses of Rhodiola rosea of Scandinavian and Russian origin**. In: The 7th international congress Phytopharm 2003: Actual problems of creation of new medicinal preparations of natural origin, Proceedings of congress, St.-Petersburg-Pushkin, Russia July 3–5, 2003. p. 390–396.

2004

Kosman, V.M., Shikov, A.N., Makarov, V.G., Galambosi, B., Dragland, S., Vender, C. 2004. **Comparative analysis of European and Russian Rhodiola rosea samples.** In: Actual problems of creation of new medicinal preparations of natural origin: the 8th international congress Phytopharm 2004 Mikkeli, Finland June 21–23, 2004: Proceedings of congress. St.-Petersburg: VVM.co. p. 268–274.

2006

Galambosi, B., Uotila, L., Tkatchenko, K., Demchenko, D., Makarov, V., Shikov, A. 2006. **Yield and quality of Bidens tripartita L. effected by agronomical factors**. In: Actual problems of creation of new medicinal preparations of natural origin: the 10th international congress Phytopharm 2006, St.-Petersburg, June 27–30, 2006: Proceedings of congress. p. 410–415.

Kosman, V.M., Melihkova, M.V., Galambosi, B., Ryzhenkov, V.E., Makarov, V.G. 2006. **Anticoagulant activity of some medical plants**. In: Actual problems of creation of new medicinal preparations of natural origin: the 10th international congress Phytopharm 2006, St.-Petersburg, June 27–30, 2006: Proceedings of congress. p. 463–469.

Makarova, M.N., Tesakova, S.V., Eschenko, A.Yu., Siivari, J., Galambosi, B., Zenkevich, I.G. 2006. **Study of antiradical activity of *Bergenia* ssp. leave samples in vitro**. In: Actual problems of creation of new medicinal preparations of natural origin: the 10th international congress Phytopharm 2006, St.-Petersburg, June 27–30, 2006: Proceedings of congress. p. 488–493.

2007

Galambosi, B., Galambosi, Z., Kosman, V., Melikhova, M., Ryzenkov, V., Makarov, V. 2007. **Verenohennukseen soveltuvien rohdoskasvien viljelykokeet**. In: Pirjo Kivijärvi and Bertalan Galambosi (eds.). Uutuusrohdoskasvit sekä tyrni ja marja-aronia terveyden edistäjinä. Maa- ja elintarviketalous 105: s. 36–51. http://urn.fi/URN:ISBN:978-952-487-117-4

Galambosi, B., Galambosi, Z., Shikov, A., Tkachenko, K., Siivari, J. 2007. **Vuorenkilven viljelykokeet**. In: Pirjo Kivijärvi and Bertalan Galambosi (eds.). Uutuusrohdoskasvit sekä tyrni ja marja-aronia terveyden edistäjinä. Maa- ja elintarviketalous 105: s. 10–22. http://urn.fi/URN:ISBN:978-952-487-117-4

Galambosi, B., Tkachenko, K., Demchenko, D., Makarov, V., Shikov, A. 2007. **Tummarusokin viljelykokeet**. In: Pirjo Kivijärvi and Bertalan Galambosi (eds.). Uutuusrohdoskasvit sekä tyrni ja marja-aronia terveyden edistäjinä. Maa- ja elintarviketalous 105: s. 23–35. http://urn.fi/URN:ISBN:978-952-487-117-4

Ivanova, S.A., Pozharitskaya, O.N., Shikov. A.N., Makarov, V.G. 2007. **Study of free radical scavening activity of extracts of leaves of Bergenia by HPTLC-DPPH-method**. Planta Medica 73. DOI: 10.1055/s–2007–987233.

Kivijärvi, P., Galambosi, B. (eds.). 2007. **Uutuusrohdoskasvit sekä tyrni ja marja-aronia terveyden edistäjinä**. Maa- ja elintarviketalous 105: 96 s. http://urn.fi/URN:ISBN:978-952-487-117-4

Pozharitskaya, O. E., Ivanova, Sv. A., Shikov, A. N., Makarov, V. G., Galambosi, B. 2007. **Separation and evaluation of free radical-scavenging activity of phenol components of green, brown, and black leaves of Bergenia crassifolia by using HPTLC-DPPH method**. Journal of separation science 30: doi:10.1002/jssc.200700178

Shikov, A.N., Pozharitskaya, O.N., Dorman H.J.D., Makarov, V.G., Tikhonov, V.P., Hiltunen, R. 2007. **Chemical composition of extracts from green, brown and black leaves of Bergenia crassifolia L**. Planta Medica 73. DOI: 10.1055/s–2007– 987016

2008

Galambosi.B. 2008. **Vuorenkilpeä peltoviljelyyn** (Bergenia for field cultivation). Maaseudun Tiede. Liite 1/2008. s. 14.

Galambosi, B., Shikov, A.N., Ivanova, S.A., Pozharitskaya, O.N., Makarov, V.G. 2008. **Root yield and resveratrol content of *Fallopia japonica* grown in Finland**. Olaj Szappan Kozmetika 57, 4: 133–136.

2009

Galambosi, B., Tkachenko, G.K., Shikov, A. 2009. **Elaboration of growing methods of *Bidens tripartita* L. in Finland**. In: Index seminum 2009. Catalog de seminte. Note botanice XXXV: 35–40.

Kosman, V.M., Faustova, N.M., Pozharitskaya, O.N., Shikov, A.N., Galambosi, B., Makarov, V.G. 2009. **Phytochemical analysis of fruit of Russian sort *Hippophae rhamnoides*, cultivated in Finland and food products, containing them**. Voprosi Pitanija 78, 3: 38–42. (in Russian)

2010

Kosman, V. M., Pozharitskaya, O. N., Shikov, A. N., Faustova, N. M., Ivanova, S. A., Demchenko, D. V., Galambosi, B., Makarov, V. G., 2010. **Variability of biologically active compounds composition in *Bidens tripartia* (Asteraceae) herb cultivated in North-Western Russia and in Finland**. Rastitelnye Resurssi 1: 77–86. (in Russian)

Pozharitskaya, O.N., Shikov, A.N., Makarova, M.N., Kosman, V.M., Faustova, N.M., Tesakova, S.V., Makarov, V.G., Galambosi, B. 2010. **Anti-inflammatory activity of a HPLC-fingerprinted aqueous infusion of aerial part of *Bidens tripartita* L.** Phytomedicine 17, 6: 463–468. doi:10.1016/j.phymed.2009.08.001

Shikov, A. N., Kundracikova, M., Palama, T. L., Pozharotskaya, O. N., Kosman, V. M., Makarov, V. G., Galambosi, B., Kim, H. J., Jang, Y. P., Choi, Y. H., Verporte, R. 2010. **Phenolic constituents of *Gnaphalium uliginosum* L.** Phytochemistry Letters 3, 1: 45–47. doi:10.1016/j.phytol.2009.11.002

Shikov, A. N., Pozharitskaya, O. N., Makarova, M. N., Dorman, H.J.D., Makarov, V.G., Hiltunen, R., Galambosi, B., 2010**. Adaptogenic effect of black and fermented leaves of *Bergenia crassifolia* L. in mice**. Journal of Functional Foods 2, 1: 71–76. doi:10.1016/j.jff.2009.11.003

Shikov, A. N., Pozharitskaya, O. N., Ivanova, S. A., Makarov, V. G., Tikhonov, V. P., Galambosi, B. 2010. **Improved and validated HPTLC method for quantification of oenothein B and its use for analysis of *Epilobium angustifolium* L.**. Journal of Planar Chromatography 23, 1: 70–74. DOI:10.1556/JPC.23.2010.1.12

2011

Galambosi, B., Shikov, A. 2011. **Lehtiyrttien hiostuskokeet teollisuuden näkökulmasta**. in: Lavola, A., Julkunen-Tiitto, R., Saastamoinen, O. (eds.). Luonnontuotealan valtakunnallinen tutkimusseminaari, 5–10.2010. Joensuu. Publication of the University of Eastern Finland, Reports in Forestry and Natural Sciences, No.7:38–43. http://epublications.uef.fi/pub/urn_isbn_978-952-61-0643-4/urn_isbn_978-952-61-0643-4.pdf

2012

Kosman, V.M., Faustova, N.M., Shikov, A.N., Pozharitskaya, O.N., Makarov, V.G. , Galambosi, B. 2012. **Variation of flavonoid and coumarins content in *Trigonella foenum-graecum* cultivated in Finland**. in: " Fenolic compounds" – basic and applied aspects. Proceedings of VIII. Int. Symposium, 2–5 October, Moskova. Russian Academy of Sciences, pp. 210–215.

Appendix 3. Scientific papers, congress abstracts and popular articles in SPECICROP-project 2012–2014

Scientific publications

2012

Shikov, A.N., Pozharitskaya.O.N., Makarova, M.N., Kovaleva, M.A., Laakso, I., Dorman, J.J.D., Hiltunen, R., Makarov, V.G., Galambosi, B. 2012. **Effect of Bergenia crassifolia L. extracts on weight gain and feeding behavior of rats with high-caloric diet-induced obesity**. Phytomedicine 19, 1250–1255. http://dx.doi.org/10.1016/j.phymed.2012.09.019

2013

Kosman, V.M., Shikov, A.N., Pozharitsjaya, O.N., Makarov, V.G., Galambosi, B., Kauppinen, S. 2013. **Variation of chemical composition of Epilobium angustifolium during fermentation**. Planta Medica, 79–PJ42. http://dx.doi.org/10.1055/s-0033-1352246

2014

Chernetsova, E.S., Shikov, A.N., Crawford, E.A., Grashorn, S. Laakso, I., Pozharitskaya, O.N., Makarov, V,G., Hiltunen, R., Galambosi, B., Morlocka, G.E. 2014. **Characterization of volatile and semivolatile compounds in green and fermented leaves of Bergenia crassifolia L. by gas chromatography-mass spectrometry and ID-CUBE direct analysis in real time-high resolution mass spectrometry**. Eur. J. Mass Spectrom. 20, 199–205. http://www.ncbi.nlm.nih.gov/pubmed/24895781

Salminen, J-P., Shikov, N.A., Karonen, M., Pozharitskaya, O. N., Kim, J., Makarov, V.G., Hiltunen, R., Galambosi, B. 2014. **Rapid profiling of phenolic compounds of green and fermented *Bergenia crassifolia* L. leaves by UPLC- DAD-QqQ-MS and HPLC-DAD-ESI-QTOF-MS**. Natural Product Research 28 (19): 1530–3. DOI: 10.1080/14786419.2014.923999. http://www.ncbi.nlm.nih.gov/pubmed/24896228

Shikov, A.N., Pozharitskaya. O.N., Makarova, M.N., Makarov, V.G., Wagner, H. 2014. ***Bergenia crassifolia* (L.) Fritsch – Pharmacology and phytochemistry**. Phytomedicine 21 (12): 1534–1542. http://www.ncbi.nlm.nih.gov/pubmed/25442262

Abstracts of congress lectures and posters

2012

Кауппинен С., Логре́н Й. Увеличение объемов выращивания специальных культур как средство диверсификации производства и создания предприятий малого и среднего агробизнеса. (**Enhancing special crop cultivation diversifies production and gives living to small and medium size agricultural enterprises**.) Ресурсный потенциал растениеводства — основа обеспе-чения продовольственной безопасности: Труды Между-народной заочной научно-практической конференции (10 декабря 2012 г.). — Петрозаводск: Изд-во ПетрГУ, 2012. — 196 с. ISBN 978-5-8021-1578-7. http://petrsu.ru/Chairs/Agronomy/resourse_pr.pdf

2013

Kosman, V.M., Shikov, A.N., Pozharitskaya, O.N., Makarov, V.G., Galambosi, B., Kauppinen, S. 2013. **Variation of chemical composition of *Epilobium angustifolium* during fermentation**. Poster on 61st International Congress and Annual Meeting of the Society Medicinal Plant and Natural Product Research (GA) in Muenster, Germany, on 1–5 September 2013.
http://jukuri.luke.fi/handle/10024/481892

2014

Galambosi, B., Galambosi, Zs., Kauppinen, S., Kosman, V.M., Shikov, A.N., Pozharitskaya, O.N., Makarov, V.G. 2014. **Fermentation experiments with green leaves of *Bergenia* sp**. In: Abstract book of the 18th Int. Congress Phytopharm2014. St-Petersburg, Russia 3–5 July 2014. Reviews of clinical pharmacology and drug therapy. TOM 12/2014/Supplement, p. 20.

Galambosi, B., Galambosi, Zs., Kauppinen, S., Kosman, V.M., Shikov, A.N., Pozharitskaya, O.N., Makarov, V.G. 2014. **Optimization the technological parameters in the fermentation of *Epilobium angustifolium* shoot yield**. In: Abstract book of the 18th Int. Congress Phytopharm2014. St-Petersburg, Russia 3–5 July 2014. Reviews of clinical pharmacology and drug therapy. TOM 12/2014/Supplement, pp. 22–23.

Galambosi, B., Galambosi, Zs., Kirakosyan, G.M., Alexandrova, O.A., Morozova, M.A., Trusov, S.A., Enikeev, A.H. 2014. **Evaluation of *Rhodiola rosea* gene collection from different geographical origin**. In: Posters of the 18th Int. Congress Phytopharm2014. St-Petersburg, Russia 3–5 July 2014.

Galambosi, B., Galambosi, Zs., Kirakosian, G.M., Alexandrova,O.A., Morozova, M.A., Trusov, S.A., Enikeev, A.H.2014. **Experiments for improving root harvest of *Rhodiola rosea***. In: Abstract book of the 18th Int. Congress Phytopharm2014. St-Petersburg, Russia 3–5 July 2014. Reviews of clinical pharmacology and drug therapy. TOM 12/2014/Supplement, p. 23–24.

Galambosi, B., Galambosi, Zs., Shikov, AN., Pozharitsakaya, ON., Kosman,VM., Makarov, VG. 2014. **Field production and fermentation of *Bergenia* sp. leafe yields**. In: Abstract book of the 18th Int. Congress Phytopharm2014. St-Petersburg, Russia 3–5 July 2014. Reviews of clinical pharmacology and drug therapy. TOM 12/2014/Supplement. P. 22.

Galambosi, B., Kauppinen, S., Kirakosyan, G.M., Dembickaya, E., Lapshin, A., Sorvari, J. 2014. **Development of herb education program in Begunitsky Agrotechnological College**. In: Abstract book of the 18th Int. Congress Phytopharm2014. St-Petersburg, Russia 3–5 July 2014. Reviews of clinical pharmacology and drug therapy. TOM 12/2014/Supplement, p.21.

Shikov, A.N., Pozharitskaya. O.N., Makarov, V.G. 2014. ***Bergenia crassifolia* (L.) Fritsch – A versatile adaptogen**. In: Abstract book of the 18th Int. Congress Phytopharm2014. St-Petersburg, Russia 3–5 July 2014. Reviews of clinical pharmacology and drug therapy. TOM 12/2014/Supplement, p. 60.
https://uu.diva-portal.org/smash/get/diva2:790982/FULLTEXT01.pdf

2015

Kosman, V.M., Pozharitskaya, O.N., Shikov, A.N., Galambosi, B., Makarov, V.G. 2015. **Effect of fermentation on concentration of phenolics in leafs of *Bergenia* species cultivated in Finland**. ФЕНОЛЬНЫЕ СОЕДИНЕНИЯ: ФУНДАМЕНТАЛЬНЫЕ И ПРИКЛАДНЫЕ АСПЕКТЫ. СБОРНИК і МАТЕРИАЛОВ IX. МЕЖДУНАРОДНОГО СИМПОЗИУМА. Москва , 20–25 апреля 2015. pp.753–757

Popular seminar presentations and articles

2012

Galambosi, B. 2012. **Cultivation of aromatic and medicinal plants in Finland**. Presentation on FAO Round Table on Medicinal and Aromatic Plants (MAP) workshop in Budapest on 2–5 April 2012.

Galambosi, B., Galambosiné Sz. Zs. 2012. **How the Russian tea is produced?** (Miből lesz az orosz tea?) Kertészet és Szőlészet. 2012/42. szám) 2012.10.18.,(Horsma tee, in Hungarian)

Galambosi, B., Galambosine Szebeni, Zs. 2012. **Life-cycle of *Bergenia*** (Börlevél életpálya). Kerteszet es Szöleszet 2012/44. (Bergenia tee, in Hungarian)

Logren, J., Kauppinen, S. 2012. **Uudet hankkeet**. Maaseudun Tiede 69 3(22.10.2012): 12. https://issuu.com/mttelo/docs/mtiede_3_2012?backgroundColor

2013

Galambosi, B. 2013. **Venäläisten yrttitietoa 1: Tummarusokin uusi ulottuvuus**. Pähkylä 3/2013. 9–12. (in Finnish).

Galambosi, B. 2013. **Venäläisten yrttitietoa 2: Vuorenkilpi: Koristekasvi? Kosmetiikkakasvi? Lääkekasvi?** Pähkylä 3/2013. 12–18.(in Finnish)

Galambosi, B. 2013. **Mustaa muovia esiteltiin venäläisille**. Puutarha & Kauppa 13:30. (in Finnish)

Galambosi, B., Kauppinen, S. 2013. **Uusi yrttiviljelykirja on tulossa – kaksikielinen aktiivisuus Specicrop – hankkeessa**. Valtakunnalliset luonnontuotepäivät. 09.10.2013. Mikkeli, Anttolanhovi. Luentomateriaalit. (in Finnish)

Kauppinen, S. 2013. **Ruvenkestävät omenalajikkeet luomuun**. Tutkittua tietoa luomusta -luentosarja 29.10.2013. Finnish Organic Research Institute.

http://luomuinstituutti.fi/wp-content/uploads/sites/2/2013/10/Ruvenkest%C3%A4v%C3%A4t-omenalajikkeet-luomuun-Sanna-Kauppinen-29.10.13.pdf

2014

Kauppinen, S. 2014. **Yrtit ja rohdoskasvit Siperiassa – perinteillä saadaan kulutusta myös korkean arvonlisän tuotteille**. Luonnontuotepäivät 24.–25.9.2014, Kokkola. Suomen luontoyrittäjyysverkosto ry. http://urn.fi/URN:NBN:fi-fe2014101745261

Galambosi. B.. 2014. **Siperia yllättää. Matkakertomus Specicrop-hankkeen Altajin opintomatkasta** Mikkelin Puutarhayhdistys – luento 10.11.2014.

Appendix 4. The content of Finnish and Russian herb cultivation book

Cultivation of herbs and medicinal plants under Nordic climatic conditions suitable for North-West Russia and South-East Finland

Galambosi, B., Kirakosyan, G., Shikov, A., Pozharitskaja, O., Makarov, V. G.

General chapters

1. Overview on herb cultivation in Europe (South-Europe, Central-Europe, Scandinavia, Baltic, Russia)
2. Herb cultivation in Finland
   2.1 Production models in cultivation of herbs and medicinal plants
   2.2 Features of herb and medicinal cultivation in Finland
   2.3 Strategies of successful small scale enterprises
3. Special questions of herb cultivation in the North
   3.1 Narrow plant spectrum
   3.2 Contents of the aromatic compounds of herbs in the North
   3.3 Cleanness of raw material (heavy metals, microbiology)
   3.4 Production costs of Nordic cultivation
4. The suitability of herb species to the Northern climatic features
5. Well adapted herb varieties to Nordic climate, studied in Finland
6. Seed production possibilities between 60°–65° Nordic latitudes (Finnish results and experiences)

Plant list of Finnish herb cultivation book

1.	Amerikanginsengjuuri	*Panax quinquefolius*
2.	Apilat (puna-ja valkoapila)	*Trifolium pratense, T. repens*
3.	Aaprottimaruna	*Artemisia abrotanum*
4.	Basilika	*Ocimum basilicum*
5.	Etelänarnikki	*Arnica montana*
6.	Euroopanalppitähti	*Leontopodium alpinum*
7.	Hapro	*Oxyria dygina*
8.	Humala	*Humulus lupulus*
9.	Iisoppi	*Hyssopus officinalis*
10.	Kamomillasaunio	*Matricaria recutita*
11.	Kangasajuruoho	*Thymus serpyllum*
12.	Kaunopunahattu	*Echinacea purpurea*
13.	Kehäkukka	*Calendula officinalis*
14.	Keltakatkero	*Gentiana lutea*

15. Keltasinappi, sareptansinappi	*Sinapis alba, Brassica juncea*
16. Kesäkynteli	*Satureja hortensis*
17. Keto-orvokki	*Viola tricolor*
18. Kevätesikko	*Primula vulgaris*
19. Kihokki	*Drosera rotundifolia*
20. Koiruoho, mali	*Artemisia absinthium*
21. Korianteri	*Coriandrum sativum*
22. Kultapiisku	*Solidago virgaurea*
23. Kumina	*Carum carvi*
24. Laventeli	*Lavandula officinalis*
25. Lipstikka	*Levisticum officinalis*
26. Maurinkiiltomalva	*Malva sylvestris ssp. Mauritiana*
27. Maustefenkoli	*Foeniculum vulgare*
28. Maustekirveli	*Anthriscus cerefolium*
29. Maustemeirami	*Origanum majorana*
30. Mäkikuisma	*Hypericum perforatum*
31. Mäkimeirami	*Origanum vulgare*
32. Niittyhumala	*Prunella vulgaris*
33. Niittymaarianheinä	*Hierochloe hirta*
34. Nokkonen	*Urtica dioica*
35. Nukkahorsma	*Epilobium parviflorum*
36. Oopiuminunikko	*Papaver somniferum*
37. Persilja	*Petroselinum hortense*
38. Piparminttu ja muut mintut	*Mentha piperita, Mentha sp*.
39. Pohjanruusujuuri	*Rhodiola rosea*
40. Poimulehdet	*Alchemilla sp*.
41. Rakuuna	*Artemisia dracunculus*
42. Ratamot (piha- ja heinäratamo)	*Plantago major, P. lanceolata*
43. Rohtomaraljuuri	*Leuzea carthamoides*
44. Rohtonukula	*Leonorus cardiaca*
45. Rohtopaju	*Salix*
46. Rohtopurasruoho	*Borago officinalis*
47. Rohtosamettikukka	*Tagetes lucida*
48. Rohtosinilatva	*Polemonium caeruleum*
49. Rohtotulikukka	*Verbascum phlomoides*
50. Rohtovirmajuuri	*Valeriana officinalis*
51. Rosmariini	*Rosmarinus officinalis*
52. Ruohosipuli	*Allium schoenophrasum*
53. Ryytisalvia	*Salvia officinalis*
54. Saksankirveli	*Myrrhis odorata*
55. Siankärsämö	*Achillea millefolium*
56. Sitruunamelissa	*Melissa officinalis*
57. Suvikynteli	*Satureja biflora*
58. Takiaiset	*Arctium sp.*
59. Tilli	*Anethum graveolens*

60.	Timjami	*Thymus vulgaris*
61.	Tummarusokki	*Bidens tripartita*
62.	Tuoksuampiaisyrtti	*Dracocephalum moldavica*
63.	Tuoksusimake	*Anthoxanthum odoratum*
64.	Veripeippi	*Perilla frutescens*
65.	Voikukka	*Taraxacum officinalis*
66.	Vuorenkilvet	*Bergenia sp*.
67.	Väinönputki	*Angelica archangelica*
68.	Yrtti-iiso	*Agastache foeniculum*
69.	Tyrni	*Hippophaë rhamnoides*

Plant list of Russian herb cultivation book

1.	*Achillea millefolium*	Тысячелистник обыкновенный
2.	*Bergenia crassifolia*	Бадан толстолистный
3.	*Bidens tripartita*	Череда трехраздельная
4.	*Calendula officinalis*	Ноготки лекарственные
5.	*Dracocephalum moldavica*	Змееголовник молдавский
6.	*Echinacea purpurea*	Эхинацея пурпурная
7.	*Hypericum perforatum*	Зверобой продырявленный
8.	*Hippophaë rhamnoides*	Облепиха крушиновидная
9.	*Leonorus cardiaca*	Пустырник сердечный
10.	*Leuzea carthamoides*	Стемаканта сафлоровидная (Левзея сафлоровидная)
11.	*Matricaria chamomilla*	ромашка аптечная
12.	*Melissa officinalis*	Мелисса лекарственная
13.	*Origanum vulgare*	душица обыкновенная
14.	*Panax quinquefolius*	женьшень американский
15.	*Plantago lanceolata*	Подорожник ланцетолистный
16.	*Polemonium caeruleum*	Синюха голубая
17.	*Rhodiola rosea*	Родиола розовая
18.	*Salvia officinalis*	Шалфей лекарственный
19.	*Serratula coronata*	Серпуха венценосная
20.	*Trifolium pratense*	Клевер красный
21.	*Urtica dioica*	Крапива
22.	*Valeriana officinalis*	Валериана лекарственная

Natural Resources Institute Finland
Latokartanonkaari 9
FI-00790 Helsinki, Finland
tel. +358 29 532 6000