ERAF EIROPAS REĢIONĀLĀS ATTĪSTĪBAS FONDS
IEGULDĪJUMS TAVĀ NĀKOTNĒ
EIROPAS SAVIENĪBA

"Promotion of International Cooperation Activities of Riga Stradiņš University in Science and Technologies", agreement No. 2010/0200/2DP/2.1.1.2.0/10/APIA/VIAA/006

RĪGAS STRADIŅA UNIVERSITĀTE

Perspectives of collaboration between «Altai companies» (Russia) and Riga Stradins University (Latvia)

Assist. Prof. Jelena Krasilnikova

LATVIA

Area – 64,6 t km²

Forest-covered area ~ 50 %

- **Conifers 56,4%**
- **Broadleaves 43,6%**

  Grey Alder (*Alnus incana*) 6,8%


RĪGAS STRADIŅA UNIVERSITĀTE

Algorythm of interaction

- Manufacturing: extraction, composition
- Bank of data
- Experiences
- Experimental results
  - Documentation
  - Certification
  - Legalization

- **Riga Stradins University:**
  - **Experimental series**
  - **Preclinical trials**
  - **Optimal choice of formulation**
    - **Patentaion (EU(Latvia))**
    - **Publication**

- **Promotion** *(etnopharmacology)*

Conferences («Drug discovery», «Polyphenols applications»)

LIWC TTO

LIAA

Latvian market

European market

Ministry of Economics

- **mission**
- **cooperation**
- **private companies**
- **research centres**

Actuality

- FR diseases
- Polyphenols = P vit (1998)
- Health Program (Finland, Denmark, Norway)
- Knownledge of polyphenol metabolism in Human
- Economical forms
- Domestic row

Metabolic food

- **Polyphenols, plants extracts, plants oil = Integral part of it.**
  - **Detoxication**
  - **Correction**
  - **Addition**

Strategy:

Vision

The Rīga Stradiņš University as a modern, prestigious university acknowledged in Europe and throughout the world in the fields of healthcare and social sciences, with the human being at its centre of attention.

Mission

The Rīga Stradiņš University's mission is to train highly qualified experts in the fields of health care and social sciences, so that they can serve the society of Latvia, the European Union, as well as the world. So that the knowledge, skills and attitudes gained by the student form a stable foundation for lifelong learning and are in line with humane traditions and the requirements of European Union. In developing research activities in accordance with the RSU's profile, the University is laying foundations to gain the status of a research university.

Strategic priorities (1)

Education. To enhance the student experience by continually improving the quality of academic programmes, thus adding value to the RSU degree for graduates and promoting the export of knowledge.

Research. To increase research opportunities for scientists and students at the RSU, in order to acquire applicable and exportable knowledge, as well as to make substantial contributions to the society.

Strategic priorities (2)

International activity. To increase the RSU's international cooperation and activity in attracting students in academic, research, and economic development areas.

Resource management. To ensure the RSU's financial stability by respecting people, taking care of property, economising on financial resources and increasing income, in order to ensure sound performance of the University.

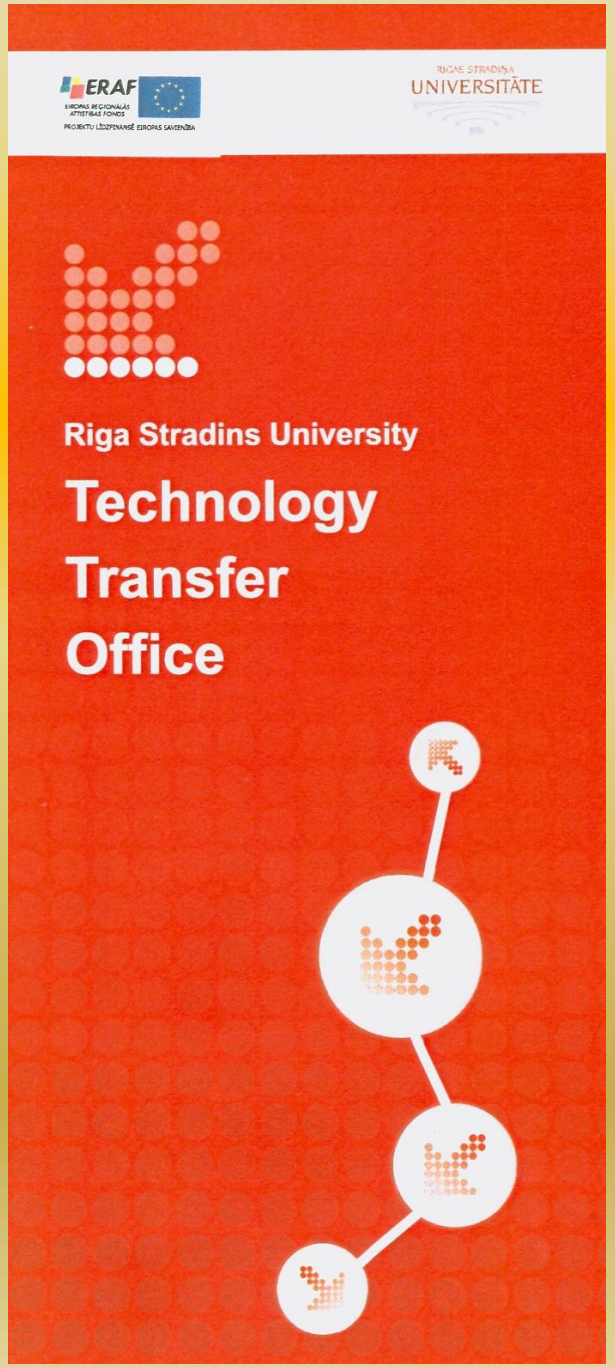
Benefit to the community. To enrich community, economic and family life through research and innovation, social inclusion and responsibility, entrepreneurship, and integration.

Management of quality (1)

In 2002 the Rīga Stradiņš University was the first establishment of higher education in Latvia to be acknowledged compliant to the ISO 9001 International Standard for Quality Management Systems. This certificate was issued by the "Bureau Veritas Quality International", the world's leading certification body. From that moment the RSU's quality management system is continually evaluated by internal audit and certified organizations.

Management of quality (2)

In 2009 the quality management system was recertified in accordance with the ISO 9001:2008 standard. Once more, this is to prove that the RSU takes care of the quality of its education and that it has serious plans to establish itself in the environment of European higher education. The reputation of Rīga Stradiņš University is of high importance, since its ambition is to engage the brightest of young minds and to attract the best of experienced professors.



We offer

- Information on opportunities to cooperate with Riga Stradins University:
  - custom research projects,
  - laboratory services, analyses and expert assessments,
  - consultations from scientists and researchers.
- Information on technologies, methods and solutions developed by Riga Stradins University scientists in medicine and related fields.

We consult and support

- **We consult on how to prepare applications for registration of national and international patents, trademarks, and designs**
- **We provide financial support in registration of the intellectual and industrial property rights in the name of Riga Stradins University**
- **We adivise on the possibilities to commercialise specific research results**
- **We prepare technology commercialisation proposals.**

We organize

- **Workshops for coopration between entrepreneurs and scientists**
- **Creative discussions or so-called brainstorming sessions for scientists and entrepreneurs**
- **Participation, and financial support for participation in:**
  - **International fairs**
  - **Brokerage events**
  - **Direct visits and missions**
- **Exhibition and forum visits abroad**

BUREAU VERITAS
Certification

Sertifikāts

Izsniegts

Rīgas Stradiņa universitāte

Dzirciema iela 16, Rīga, LV-1007, LATVIJA

**Bureau Veritas Certification apliecina,
ka augstāk minētās organizācijas pārvaldības sistēma ir auditēta un atzīta kā atbilstoša zemāk norādītā pārvaldības sistēmas standarta prasībām**

STANDARTS

ISO 9001:2008

DARBĪBAS SFĒRA

AUGSTĀKĀ IZGLĪTĪBA, MŪŽIZGLĪTĪBA, ZINĀŠANU UN PRASMJU NOVĒRTĒŠANA, PĒTNIECĪBAS REZULTĀTU NOVĒRTĒŠANA, IZGLĪTĪBAS, AUGSTĀKĀS PROFESIONĀLĀS IZGLĪTĪBAS, AKADĒMISKO GRĀDU IEGUVI APLIECINOŠU DOKUMENTU IZSNIEGŠANA, PĒTNIECĪBA.

Sākotnējais apstiprināšanas datums: 2002. gada 13. decembris

Sertifikāta derīguma termiņš ir: 2014. gada 13. decembris,
organizācijai nodrošinot atbilstošu nepārtrauktu pārvaldības sistēmas darbību.

Lai pārliecinātos par šī sertifikāta derīgumu, lūdzu, zvaniet +371 67323246
Papildus informāciju par sertifikātā norādīto darbības sfēru un pārvaldības sistēmas standarta prasību pieņemšanu var saņemt Bureau Veritas Latvia SIA.

Datums : 2011. gada 9. novembris

Sertifikāta numurs : DNKFRC94686A

UKAS MANAGEMENT SYSTEMS
008

BUREAU VERITAS

Atbildīgais birojs: Bureau Veritas Latvia SIA, Duntes iela 17a, Rīga, LV-1005, Latvija
Izdevēj birojs: Bureau Veritas Certification Denmark A/S, Oldenborggade 1 B, 7000 Fredericia, Denmark

Latvijas Republikas Veselības ministrija

Sertifikāts

Nr. L-198-A

Rīgā 2007. gada 12.janvārī

Veselības statistikas un medicīnas tehnoloģiju valsts aģentūra ar šo apliecina, ka

RĪGAS STRADIŅA UNIVERSITĀTES

reģistrētas Latvijas Republikas Nodokļu maksātāju reģistrā nr. 90000013771, ārstniecības iestāžu reģistrā ar nr. 0100-19100

bioķīmijas laboratorijas

Dzirciema ielā 16, Rīgā

tehniskā kompetence ir novērtēta un atzīta par atbilstošu "Vispārējām prasībām testēšanas laboratoriju darbībai" un tā ir kompetenta veikt testēšanu šādās sfērās:

hematoloģija, bioķīmija.

Sertifikāts derīgs līdz 2010. gada 12.janvārim.
Reģistrācijas nr. 165/L 385-A
Pielikums ir sertifikāta sastāvdaļa un sastāv no 1 lapas.

Veselības statistikas un medicīnas tehnoloģiju valsts aģentūras direktors E.Lavendelis

VESELĪBAS STATISTIKAS UN MEDICĪNAS TEHNOLOĢIJU VALSTS AĢENTŪRA

Klīniski diagnostisko laboratoriju kompetences novērtēšanas komisijas priekšsēdētājs I.Trofimovs

COST Action CM0804 (Chemical Biology with Natural Products) of the European Commission, Brussels, Belgium

COST is an intergovernmental framework for European Cooperation in Science and Technology, allowing the coordination of nationally-funded research on a European level.

“The main objective of the action is to advance the use of natural products as tools for chemical biology. Applying modern techniques and advancing them, natural products will prove to be instrumental in discovering target proteins and biological pathways that are of relevance to diseases. This in turn, should facilitate and speed up subsequent drug discovery efforts in the pharmaceutical industry.“

RĪGAS STRADIŅA UNIVERSITĀTE

COST CM0804
Workshop on
"Natural Products as Drugs and Leads to Drugs"

koksnes ķīmijas institūts
institute of wood chemistry

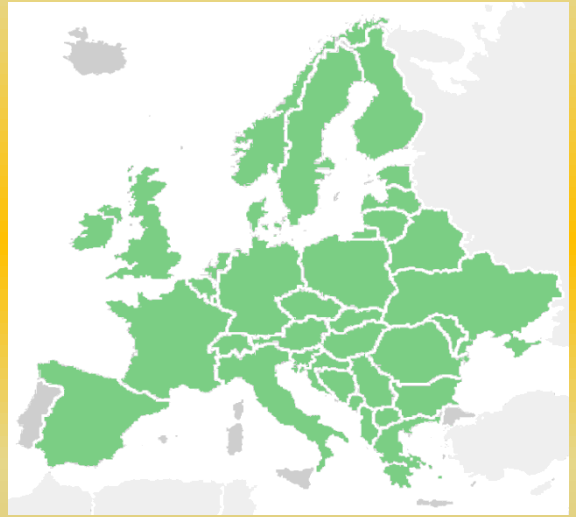
Diarylheptanoids and lignins: able and effective anti-aging natural compounds

Jelena Krasilnikova, MD, PhD,
Riga Stradin University, Latvia
G. Telysheva, T. Dizhbite, O. Bikovens, J. Ponomarenko

Latvian State Wood Chemistry Institute

Grey alder as a promising feedstock

Distribution in **Europe**

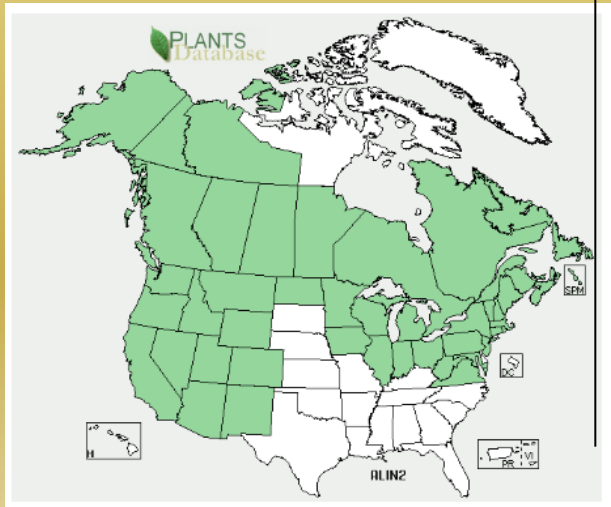


http://luirig.altervista.org/schedeit/ae/alnus_incana.htm

Grey alder covers large areas in almost all European countries and a lot of countries of other continents.

Significant advantage of the **grey alder** over other fast growing trees is **more efficient development on poor soils** that is connected with ability of the grey alder to bind atmospheric nitrogen thus enriching soil and improving it's fertility.

Distribution in **USA**



http://nerpn.org/Alnus_incana

Harvesting of **grey alder** for **bioenergy production** is the most beneficial at the age of 14 – 17 years, before beginning of reduction of annual increment..

Extension of fields of **grey alder usage** increases the feedstock for bark processing. Possibility of biologically active polyphenolics and other valuable chemicals obtaining from the bark can give **added value** make it's processing **more beneficial**.

Motivation for bark biorefinery

- Low cost of bark as fuel (it's calorific value is 17 MJ/kg)
- High price of products used for biomedical application and ability to obtain them from bark constituents.
- Modern policy of pharmacy and medicine in finding novel phyto-derived biological active compounds as a basic line for novel drug discovery and synthesis.

Orientation of Society on the development of bio-based economy

Scientific Background

Majority of the age-related pathologies: atherosclerosis, metabolic syndrome, sugar diabetes, neurodegenerative disorders (Parkinson, Alzheimer diseases) associate from one side, with metabolic disorders, where disbalance of lipids spectrum is prevalent and from the other side, with abnormal cellular response to oxidants and low tolerance of human antioxidant defence systems to them.

Natural products from plants were historically and remain today an undiminished source of new pharmaceuticals.

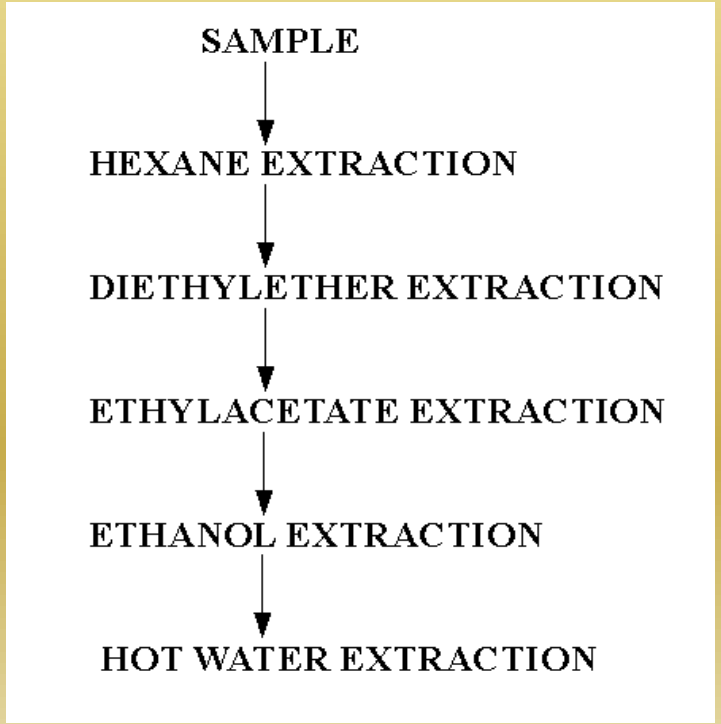
Our previous epidemiological data of RSU project "Biological Factors of aging process and life quality in the Latvian population" have illustrated that antioxidant (AO) deficiency and dysbalance in the human organism are in direct relation with aging process and disorders of metabolism of lipids and carbohydrates in the cells, membranes, blood.

One of the aim for Healthy Program is finding and adaptation of the efficient and economically accessible natural local AO for daily usage.

MATERIALS

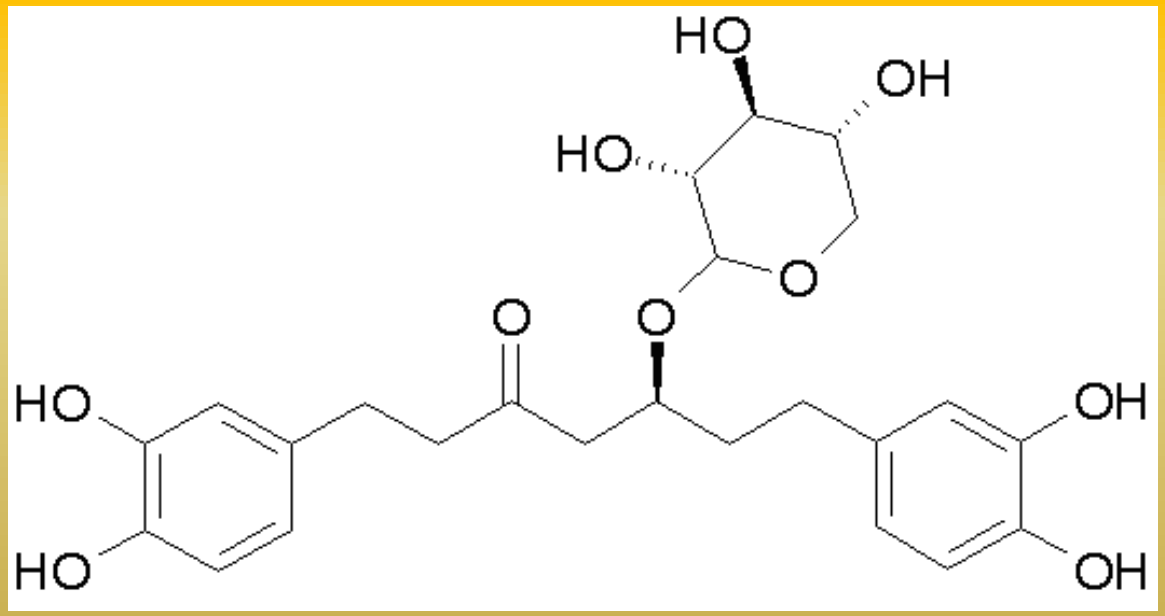
The bark of grey alder (Alnus incana) were collected in South-West part of Latvia.

Extraction Scheme



Freeze-dried and powdered bark was sequentially extracted in Sohxlet apparatus with solvents with increasing polarity.

For some *Alnus* species, oregonin, open chain diarylheptanoid, is found to be the major factor of the bark extracts bioactivity.



Oregonin: 1,7-bis-(3,4-dihydroxyphenyl)-heptan-3-one-5-O-β-D-xylopyronoside

Latvian deciduous tree species used for investigation

Grey Alder (*Alnus incana*), black alder (*Alnus glutinosa*), European aspen (*Populus tremola*), ash tree (*Fraxinus excelsior*) covered, respectively, 6.8; 2,6; 3,4 and 0,7% of Latvian forest area. The composition of their bark extractives was not yet studied systematically. The data about content of lipophilic and hydrophilic compounds was obtained using sequentially extraction with hexane and ethanol.

			grey alder	aspen	ash tree
• Hexane extraction	→ Lipophylic extraction	→ Yield, % of o.d. bark	8,1	6,6	1,7
• Ethanol extraction	→ Hydrophylic extraction	→ Yield, % of o.d. bark	36,8	22,5	28,5

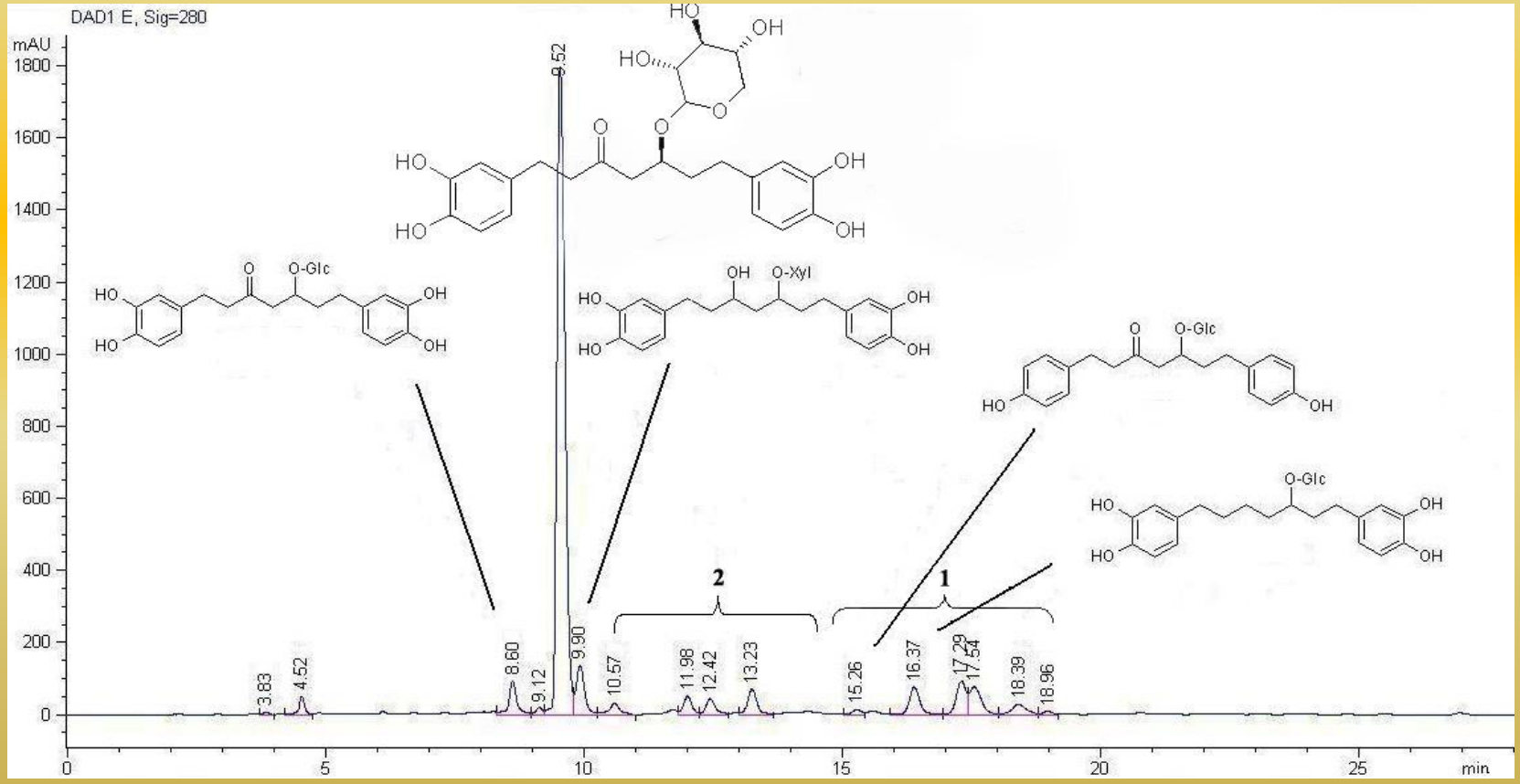
The grey alder bark differs from bark of other investigated species by the highest yield of hydrophilic extract and 4,5 times higher yield of lypophilic extracts.

Latvian deciduous tree species used for investigation

Sequential extraction of bark with solvents of increasing polarity has shown that hydrophylic extracts are rich in polyphenolics which are concentrated mainly in ethylacetate and ethanol fraction.

		The yield, % of o.d. bark			The yield of total phenolics, %of o.d. fraction	
		Alder	**Aspen**	**Ash**	**Alder bark**	**Aspen bark**
• Hexane →	Lypophilic extractives	8,1	6,6	1,7	-	-
• Diethylether →	Low molecular phenols	3,5	4,2	2,9	0,48	0,31
• Ethylacetate →	Flavanoids, polyphenols, monoglycosides	**12,9**	6,2	10,8	0,44	0,12
• Ethanol →	Tannins, polyglycosides	**17,4**	5,5	14,7	0,14	0,04
• Hot water →	Proteins, polysaccharides, water soluble salts	13,9	11,5	5,2	0,13	0,03
• Bark extracted residue						

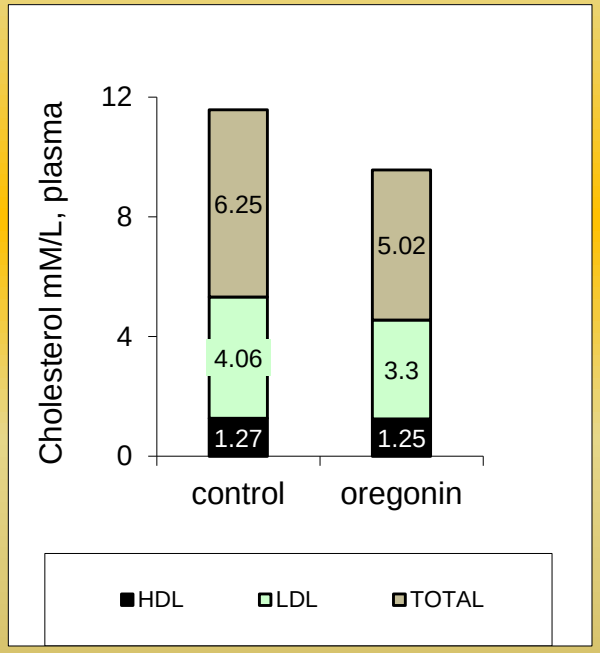
Composition of EtAc fraction of grey alder bark extract

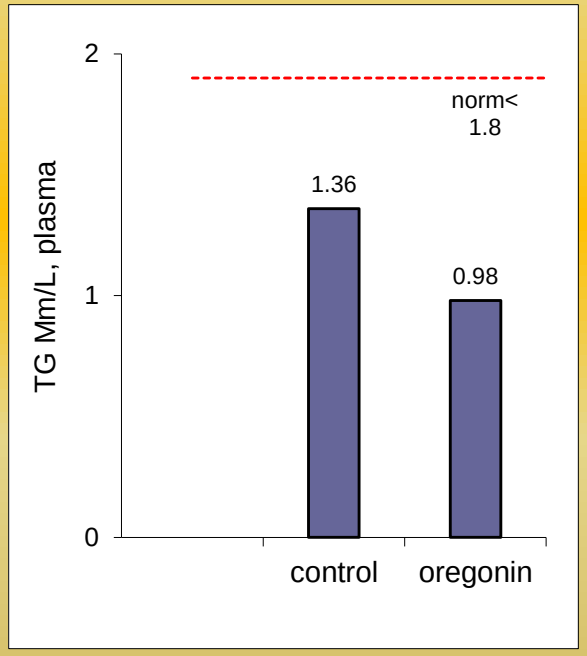


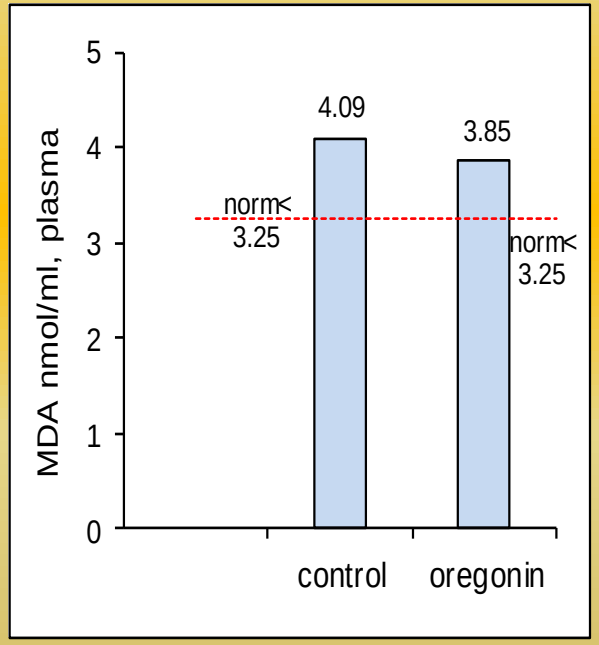
The main part, more than 80% of EtAc fraction (or ~ 7% from o.d. bark) is identified as linear diarylheptanoids (DAH) (the data of FTIS, Pyr-GS/MS, HPLC UV/MS and NMR). More than 90% of these DAH is presented by 1,7-bis-(3,4-dihydroxyphenyl)-heptan-3-one-5-O-D-xylopyronoside or oregonin. Radical scavenging activity of DAH fraction 1 was a little higher, whereas of DAH fraction 2 lower than that for oregonin: IC_{50} values in the DPPH* test were, respectively, 3,0 ± 0,1; 8,8 ± 0,3 and 4,5 ± 0,3 mg/L.

Biomedical properties of grey alder polyphenolics *in vitro*

Cholesterol, Triglyceride (TG), Malondialdehyde (MDA)





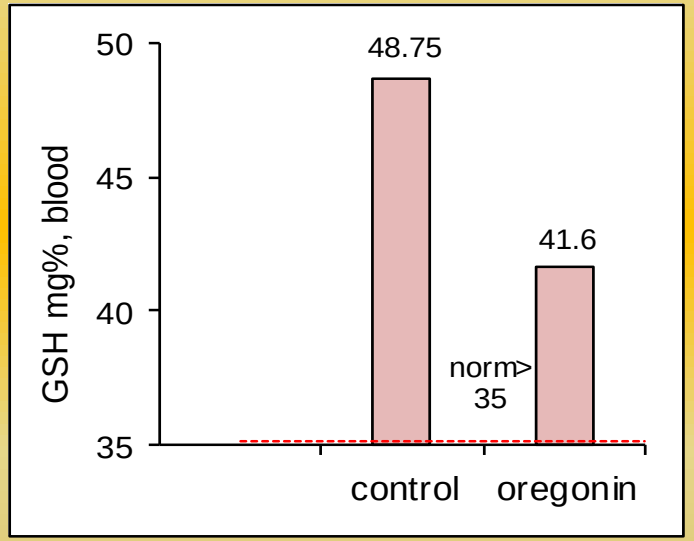


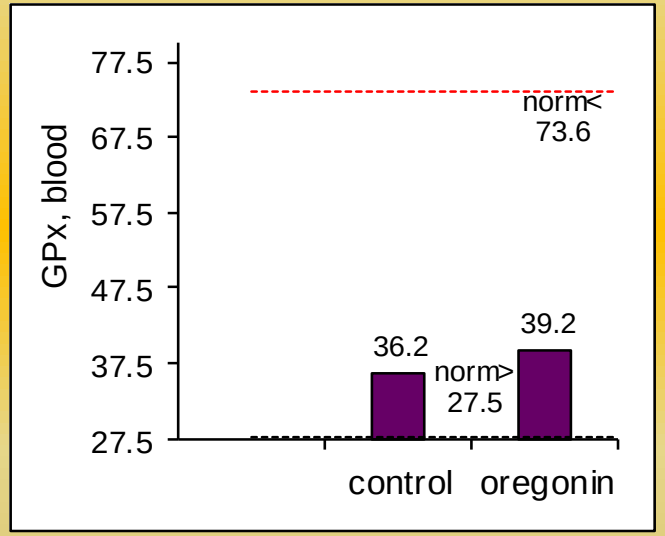
For the initial blood samples, imbalance in the cholesterol pool was observed. As a result of the 30 min incubation with **oregonin**, content of **Total** and **LDL – Chol decreased by ~20%** and **TG** level – **by ~40%**.

Oregonin reduced to some extent **peroxidation of Lipids** that followed from the changes in **MDA** content in blood plasma.

Biomedical properties of grey alder polyphenolics *in vitro*

Glutathione, Superoxide Dismutase (SOD), Glutathionperoxidase (GPx)





Glutathione content is often used as actual parameter for determination of various degenerative, age-related pathologies. In our experiment it was shown that **oregonin decreased Glutathione content in the blood by ~15%**.

The level of SOD, GPx and catalase was in norm both before and after incubation of blood samples with oregonin.

The data show that diarylheptanoids obtained from *Alnus Incana* bark could be considered as a part of integral means for prevention of the free radicals and aging related pathologies.

Biomedical properties of grey alder phenolics in experiments *in vivo*

Results of the 2 months experiments *in vivo* with rats in conditions of induced hypercholesterolemia have confirmed conclusions made from *in vitro* study

Design of the experiment

The experiment with 21 white rats (Vistar line, weight of 200g) has been realized in the vivarium at the Riga Stradin University

I step: the blood samples have been taken from all health rats for biochemical analysis

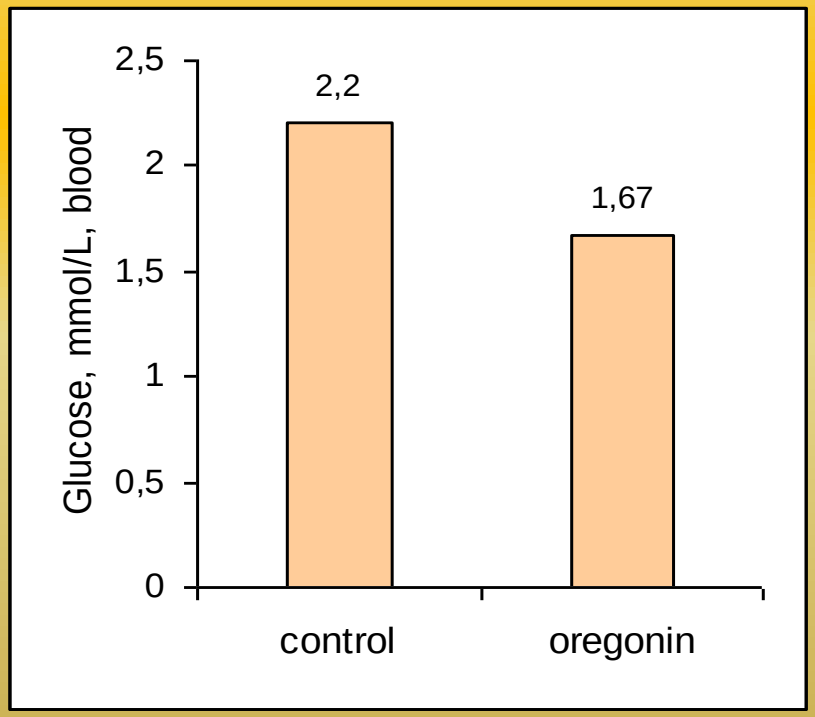
II step (duration 30 days): all rats got the increased cholesterol content (1.3 g per day that was twice amount on the norm). The cholesterol solution was introduced *per oral.* The blood samples have been taken on the 30th day.

III step (duration 30 days): The control group was fed with a standard physiological ration. The experimental group got oregonin (0.2 mg for every 100 g of animal weight) in 0.9% NaCl solution. The blood samples have been taken on the 30th day.

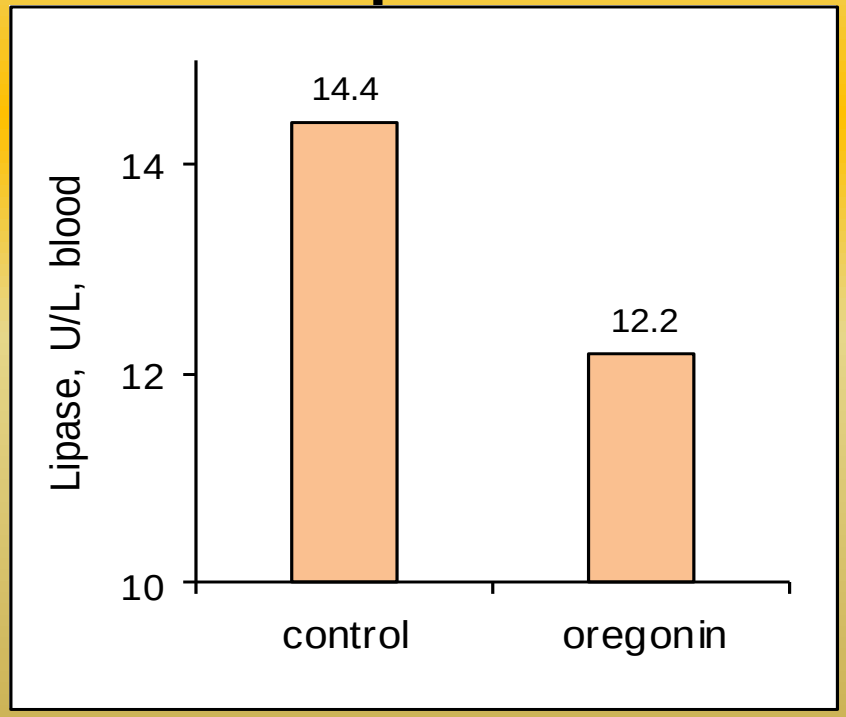
The microscopic morphological investigations of the animal tissues were carried out after the end of the III step of the experiment. The tissue samples for microscopy were prepared from rat kidneys, liver and heart.

Biomedical properties of grey alder phenolics in experiments *in vivo*

Glucose



Lipase

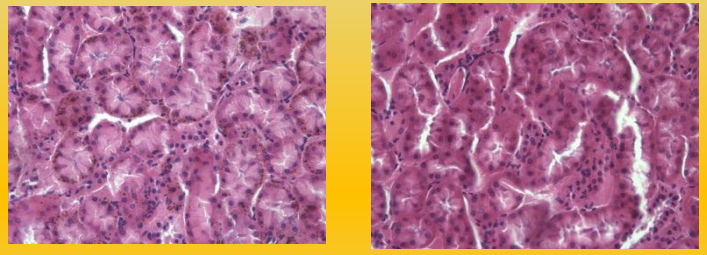


Results of this experiment have confirmed the ability of **Oregonin** to **normalize Protein**, **Lipid** and **Carbohydrate metabolism**.

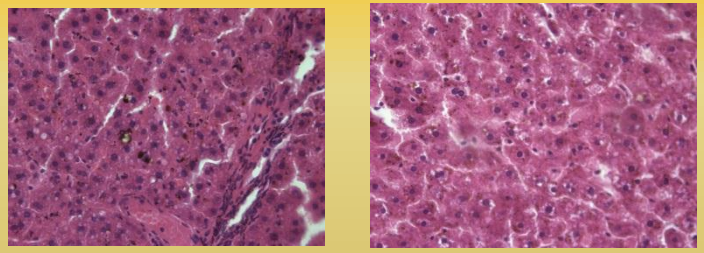
Biomedical properties of grey alder phenolics in experiments *in vivo*

Morphology of kidney and liver tissues

Control **Oregonin**



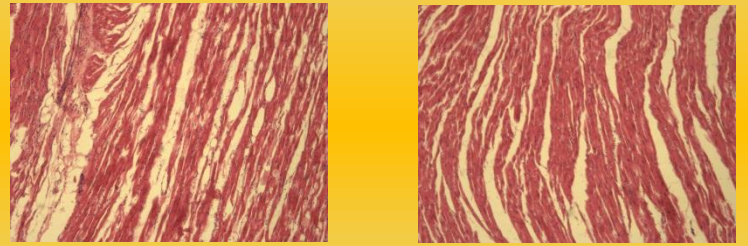
Kidney

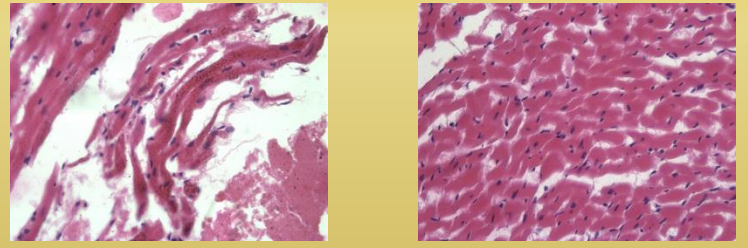


Liver

Morphology of heart tissues (myocardium and endocardium)

Control **Oregonin**

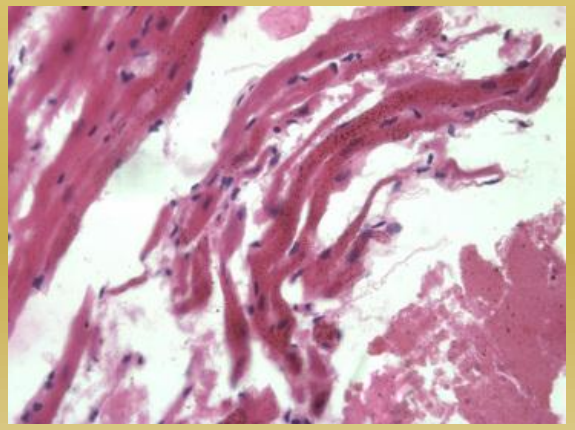




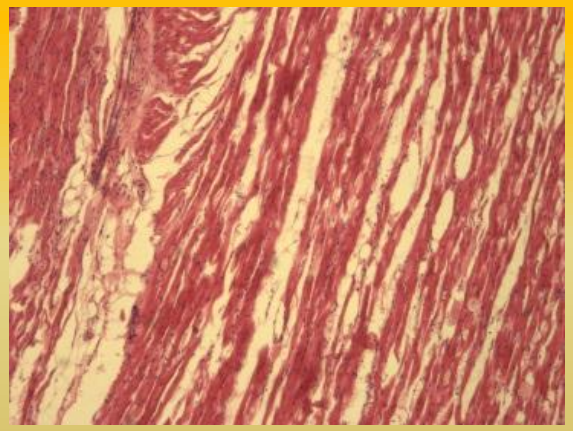
It has been shown that application of oregonin provided high level of regeneration of kidney, liver, myocardium and vessel walls.

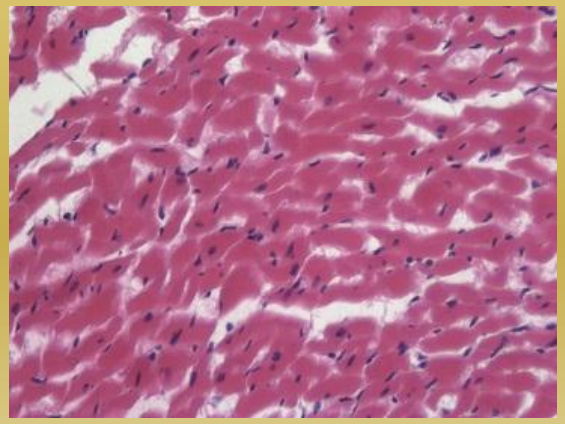
Morphology of heart tissues (myocardium and endocardium)

Control



Oregonin





Cardioprotection of oregonin in the chronic alcoholic intoxication

Just now the results were obtained from our colleagues from Grodno Medicine University (Belarus) showing that oregonin significantly decreased degradation of animals myocard cardiomiocites resulting from alcoholic intoxication: cells with significantly damaged myofibrillar apparatus practically were not observed.

Content of the Free Aminoacids and their derivatives in the HAI and using of the Oregonin (µM/g)

LIVER

Aminoacids	Control	HAI	HAI + Oregonin
Cis	46,7 ± 3,9	48,0 ±2,2	50,0 ±4,0
Glutamate	2285 ± 128	2801 ± 156*	2566 ± 272
Asp	90,1 ± 4,0	116,0 ±11,1*	130,0 ±7,0*
Ser	1198 ± 154	940 ±128	1097 ±119
Gly	3465 ± 163	3473 ± 225	4137 ±238*
β-Ala	237,3 ± 38,1	328,8 ±24,0*	339,4 ±22,2*
Ala	774,1 ± 62,4	1225 ± 122*	1292 ± 170*
Taurin	4862 ± 1681	3707 ±1121	2330 ±274

Content of the Free Aminoacids and their derivatives in the HAI and using of the Oregonin (µM/g) LIVER.

Aminoacids	Control	HAI	HAI + Oregonin
GABA	16,7 ± 2,0	16,1 ± 2,5	26,9 ± 6,8
Tyr	91,0 ± 8,5	132,1 ± 10,9*	126,9 ±11,0*
Met	46,1 ± 3,5	64,5 ± 5,4*	64,2 ± 3,6*
I-Ley	144,1 ± 7,4	166,6 ±14,7	162,2 ± 11,8
Phe	100,7 ± 5,3	126,4 ±8,6*	125,7 ±7,8*
Ley	196,2 ± 16,5	254,9 ±25,2*	276,8 ±19,5*
Pro-OH	18,5 ± 1,5	22,4 ± 1,7	29,1 ±3,3*
Ornitin	437,0 ± 31,4	426,8 ±38,8	507,0 ±23,5
Lys	645,3 ± 76,9	635,7 ±95,3	793,4 ±51,8
Pro	158,8 ± 9,7	212,0 ±14,1*	220,3 ± 15,8*

Oregonin (in vivo)

HAI

- **↑ activation of protein synthesis**
- **↑ depo of AA**
- **↑ proteinogenic AA**
- **↑ non-essential/essential AA**
- **↓ Taurin degradation**

↑ adaption to the intoxication

↑ metabolic response

High antioxidant properties clearly documented antimicrobial activity and positive influence on human metabolic processes make grey alder bark extractives prospective source for novel cosmetic products development, incl. special low-irritation cosmetics.

At present experimental batches of face cream and mask formulated by specialists of FIDE Ltd. (Riga, Latvia) using bark extracts and isolated diarilheptanoid oregonin are subjected to the tests before commercial realization.

Patents – LV (2009 – 2010)

- **Diarylheptanoids natural substances,**
  - **↓ TG in the blood**
  - **↑ Hb in the erythrocytes**
  - **↓ Lipase in the blood**
  - **↑ Glutathion in the blood**

Lignin

Among the objects of the present investigation there was ash tree *(Fraxinus excelsior)* lignin isolated from wood by the alkaline treatment similar to the industrial process for pulp (technical cellulose) production.

Phenylpropanoid polymer lignin is one of the main component of vascular plants in nature and main by-product of phytomass chemical processing in industry.

Lignin action on the Digestive enzymes

Reagents	n1	n2	n3	n4
Milk	4,0	4,0	4,0	4,0
Pancreatin solution (ml)	1,0	1,0	1,0	1,0
Bile (ml)	–	–	1,0	1,0
Lignin	–	1,0	–	1,0
0,1N NaOH quantity after incubation (38°C, 40min)	2,4	3,8	5,3	6,8

- Enzyme: Amylase (oral cavity)
- Amyloclastic force of Saliva
- $D_{38/30min}$ 640 → 5120 (activator)

Lignin

Lignin *(in vitro)*

- **Dg: MS (overweight type)**
  - **CHOL – 7,2µM/L → ↓ 34%**
  - **TG – 1,9µM/L → ↓ 0,94%**
  - **Glu – 6,5µM/L → ↓27%**

  - **GGT – 29,8u/L (N) → ↓ 10%**
  - **α–Amylase – 56,2 u/L(N) → ↓ 12%**
  - **Myoglobine – 32,2 ng/mL → ↓ 31,6ng/mL**

1ml of plasma, 50µL of Lignin (100mg/L);

45min incubation

Conclusion remarks

The results obtained provide new evidences of the antioxidant activity and positive effects on lipid, protein and carbohydrate metabolism of **oregonin** and **lignins** indicating possibility its usage as a part of integral means for:

- prevention of the free-radicals and aging-related diseases
- their pathogenetic correction
- metabolic food preparing
- health program design.

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Thank you for attention!