LATVIJAS VALSTS KOKSNES ĶĪMIJAS INSTITŪTS

LATVIJAS BIOZINĀTNU UN TEHNOLOĢIJU UNIVERSITĀTE

Sea buckthorn lignocelulosic biomass processing possibilities in Latvia

Waste-free rational use of fruit trees and shrubs lignocellulosic biomass

Mg. chem., LBTU PhD student Anna Andersone

BioRural Knowledge-exchange Workshops:
Advancing the European Rural Bioeconomy.
March 12-14, 2024

Why to use the wood of fruit trees?

- Wood from fruit trees in the world is still used in much smaller quantities than non-fruit trees wood. But the amount of fruit tree plantations is significant; if the total area of forests in the EU is estimated as 135 million ha - 2020 (EU27), the area of fruit trees in Europe occupies a total of 11.3 million ha.

Fruit trees need forced and planned pruning, which results in a large amount of wood waste. Garden trees without shaping and care give low yields.

- Theoretical calculation of wood waste EU estimates that up to 25 million tonnes of wood are produced in the form of branches each year as a result of pruning fruit trees.

References:
https://forest.eea.europa.eu/topics/forest-basic-data/key-facts
doi: 10.3390/biom12020238
Aliaño-González, M.J., Gabaston, J., Ortiz-Somovilla, V., Cantos-Villar, E., 2022. Wood Waste from Fruit Trees: Biomolecules and Their Applications in Agri-Food Industry. Biomolecules 12, 238. https://doi.org/10.3390/biom12020238

Sea Buckthorn (SBT) in the World and in Latvia

- **According to the 2023 report, SBT** (*Hippophae rhamnoides* L.) **grows in 52 countries with a total area of 3 million ha.** In Latvia, sea buckthorn is **the second most common fruit tree species after apple**. There are about **400 ha of SBT** orchards in Latvia, and about 250 ha of them are in the age of harvesting. The average yield is 4 tons/ha.
- **The global SBT berries export market** is valued at USD 2 billion. The top exporter in 2020 was Canada, with USD 0.417 billion and a yearly increase of 13.9%. On an industrial scale, SBT is cultivated in China, Canada, Finland, Russia, Germany, Latvia, Romania, Estonia, etc. The overall market of SBT products is ~17 times bigger than just for berries and is constantly growing.
- Industrial harvesting is mostly done by **cutting off the whole branch**. **The SBT tree should be almost completely cut back every 4 years.**
- There are **almost no applications for the green part of SBT, which comprises up to 20% of the harvested mass - on average per year – around 2.5-3.5 tons per ha.**

The aim of the study and its relevance for the Rural Economy

- Great competition in the market of SBT berries calls for the processing of **all plant parts** (fruits, branches, leaves and even roots), formed as residues.
- SBT lignocellulosic biomass contains **a variety of valuable secondary metabolites**, which plant synthesizes to protect itself from pests and diseases. Valuable secondary metabolites include **proanthocyanidins and serotonin**, with multifaceted chemical and biological activity, making them promising for use in health care, pharmaceuticals, and agriculture.
- Based on the necessity to develop flexible no-residue production schemes, the lignocellulosic biomass **residue after the separation** of biologically active compounds could be **used as a fodder additive or as a soil additive**, returning to the natural cycle the organic part taken from it, which is necessary for the beneficial functioning of the soil-biotic complex.
- The study aimed to propose **wasteless innovative scheme** providing usage of **all unique pool of biologically active compounds extracted from non-fruit trees**, on the example of SBT (*Hippophae rhamnoides* L.) biomass.
- **The proposed scheme could be valuable as a basis for creation of the biorefinery scheme for almost any fruit-tree.**
- **This new approach - complex and rational use of the whole fruit tree can help to promote regional development: work during non-season periods, new jobs in creating and selling side-products from lignocellulosic biomass.**

Sea buckthorn woody biomass waste material obtained after harvesting and from pruning

Evaluation of different sea buckthorn species

Evaluation of seasonal and yearly changes - characterisation, comparison

Summer - Autumn

Winter - Spring

Obtaining of extractives, with a focus on proanthocyanidins and serotonin content in the extract

Evaluation of sequential two-stages extraction

1st stage - separation of non-polar and semipolar compounds. Innovative extraction by freon, comparison with traditional extraction by hexane

2nd stage – obtaining of hydrophilic compounds by water and ethanol-water extraction, after separation of lipophilic and semi-polar compounds

Evaluation of one-stage extraction of hydrophilic compounds

Separation of hydrophilic compounds

characterization and testing of obtained extracts

Extracts and purified group of compounds (proanthocyanidins and serotonin)

Anti-microbial properties

Anti-inflammatory properties

Antioxidative properties in lipid systems

Influence on digestive enzymes (amylase, lipase)

Solid remainders after extraction

Testing of animal digestibility and gas production

Modification and testing for plant growth and development

Application possibilities

Medicinal and cosmetics topical formulations ingredients

Food additives and medication ingredients

Animal feed additives

Plant growth stimulators and soil additives

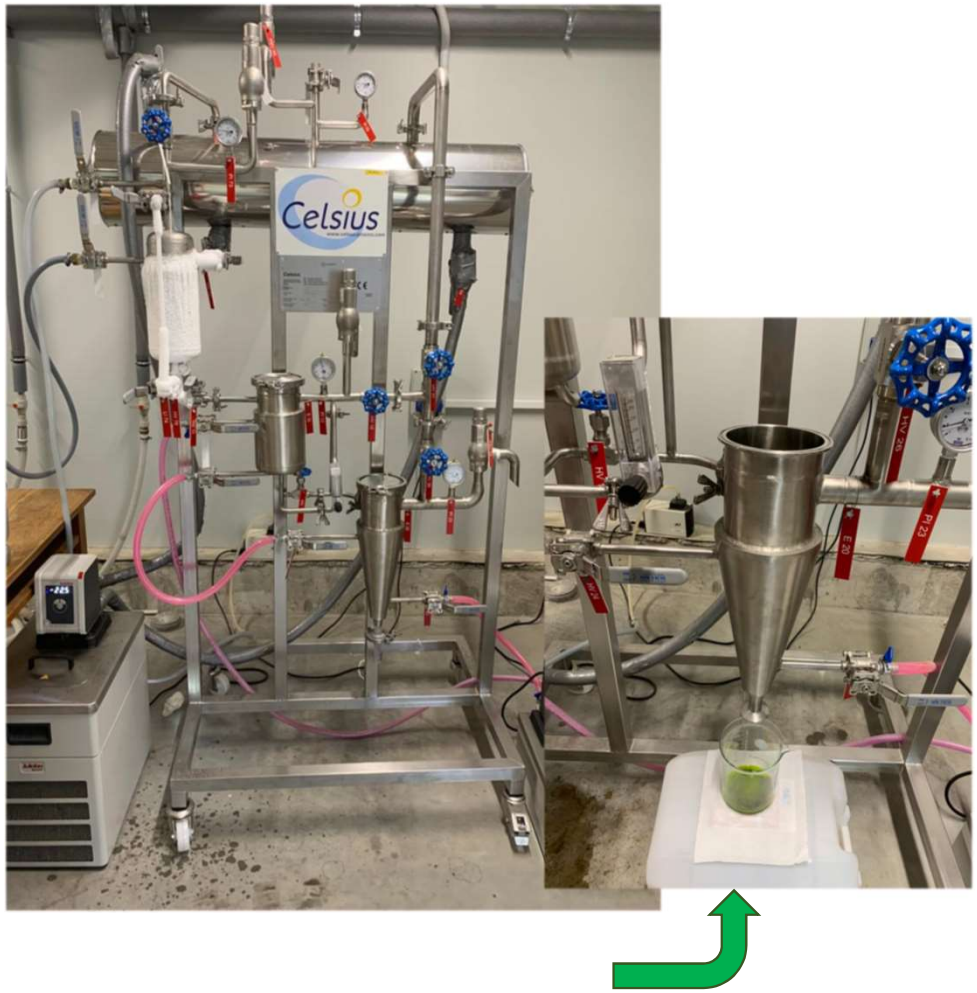
Characteristics of SBT branches of different seasons and growth ages

- **Biomass of branches collected in autumn is the most suitable raw material for the production of both serotonin and PAC.**
- **The highest content of serotonin and PAC was determined in the composition of SBT bark, which allows obtaining 26 g of dry extract with 14% serotonin and 53% PAC content from 100 g of bark, but peeling sea buckthorn branches is not an economically reasonable solution.** Thus the most suitable raw material from the analyzed samples is **unpeeled 1 and 2 years old branches with a serotonin content in the extract of 13.4 %/DM**.

Antioxidant activity

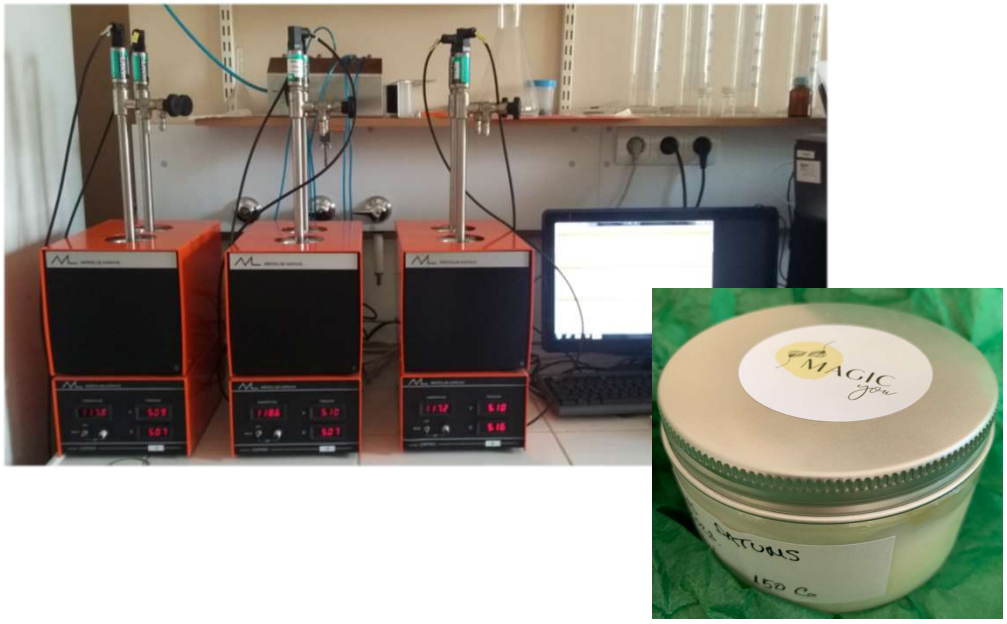
As the content of PAC in the extract increases, the radical deactivation activity in the ABTS+● and DPPH• tests increases. Among the extracts studied, **50% EtOH extracts showed the highest antioxidant activity compared to aqueous extracts**. In comparison with the synthetic antioxidant Trolox as a reference, which is a water-soluble derivative of vitamin E, the **PACs showed higher antioxidant activity.**

Innovative freon extraction



Nectacel (Celisus, France) extractor for liquiefied gases was used for the preliminar extraction of lipophilic compounds

- **Influence of the SBT extracts on the oxidation time of lipid-based system (LBS, Oxipress method) was tested for** application of hydrophilic and lipophilic extracts in creams



Antimicrobial activity of the SBT extracts, serotonin-rich fraction and PACs

Samples	PACs content in extract, %	*E. coli*	*P. aeruginosa*	*S. aureus*	*B. cereus*	*S. pyogenes*	*C. acnes*
		MIC/MBC, mg/mL					
Water extract	26.4	0.39/0.39	0.39/3.13	0.39/0.78	0.78/>50	0.20/0.20	0.78/0.78
50% EtOH extract	40.6	0.20/0.20	0.39/0.78	0.20/0.39	0.39/50	0.20/0.20	0.39/0.39
Purified PACs	92.1	0.04/0.04	0.08/0.16	0.08/0.16	0.63/1.25	0.10/0.10	0.39/0.39
Serotonin-rich extract (26.2%/ODE)	0	0.78/0.78	0.78/0.78	0.39/0.78	0.78/6.25	0.10/0.20	0.39/0.39
Serotonin standard (purity ≥98.0%)						0.10/0.20	0.39/0.78

Purified PACs and 50% EtOH extract (with higher PACs content than in water extract), had the best antimicrobial activity against all bacteria, except for 50% EtOH extract against *B. cereus*. Serotonin-rich extract was less active than purified PACs against *B. cereus* bacteria. **The antibacterial activity of the serotonin-rich extract against *C. acnes* was even a little bit higher than that of pure serotonin**. Considering the complexity of serotonin purification, **the serotonin-rich extract, alone or together with purified PACs or 50% EtOH extract, can be used against *S. pyogenes* and *C. acnes* without further SBT-derived serotonin purification.**

Antimicrobial and anti-fungus activity of PAC from SBT and Aronia (AR)

MIC/MBC (mg/mL)	*E.coli*	*B. Cereus*	*C.albicans*	*P. aeruginosa*	*S. aureus*
PAC from SBT	0.20/0.20	0.39/50	0.20/>50	0.39/0.78	0.2/0.39
PAC from SBT	0.04/0.04	0.63/1.25	1.25/>2.5	0.08/0.16	0.08/0.16
PAC from AR	0.04/0.04	0.63/1.25	2.50/>5.0	0.08/0.16	0.08/0.16

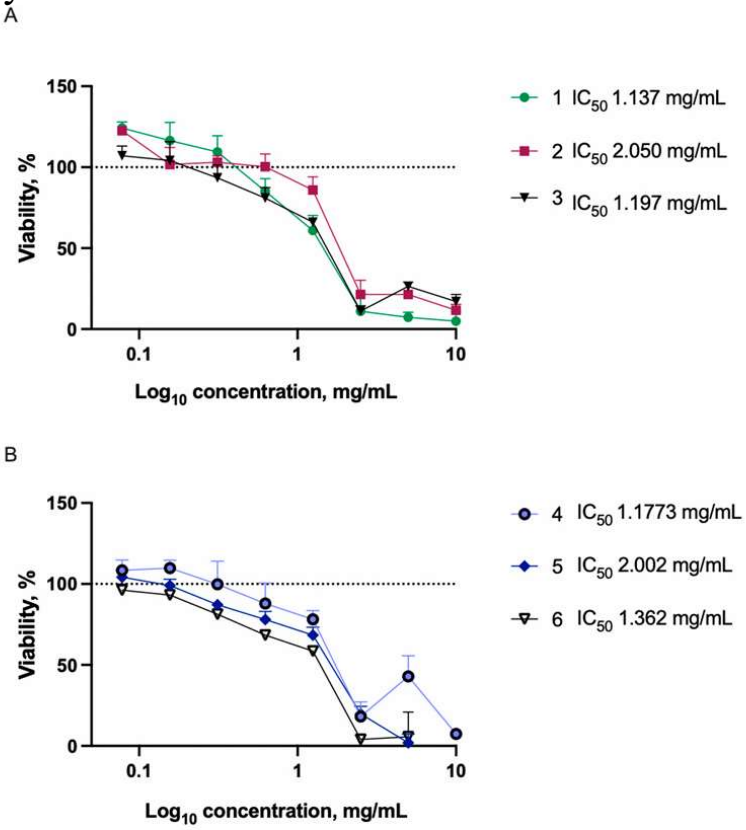
The results showed that fruit-trees samples have high antimicrobial/anti-fungal activity against both Gram-positive and Gram-negative bacteria.

The low concentrations needed to inhibit the growth of specific microorganisms, especially *C. albicans* and *S. aureus*, along with the absence of cytotoxicity at low concentrations, indicate to the **potential of the tested PACs to be further developed for various antimicrobial/antigungal applications.**

Reference: Anna Andersone, Sarmite Janceva, Liga Lauberte, Ingus Skadins, Vizma Nikolajeva, Konstantins Logviss, Natalija Zaharova, Gints Rieksts, Galina Telysheva, A comparative analysis of the proanthocyanidins from fruit and non-fruit trees and shrubs of Northern Europe: Chemical characteristics and biological activity,
Sustainable Chemistry and Pharmacy, Volume 36, 2023, https://doi.org/10.1016/j.scp.2023.101266

Evaluation of cytotoxicity of the extracts

The cytotoxicity of the extracts was tested for the BALB/c3T3 cell line by the neutral red (NR) uptake assay.



Cytotoxicity of extracts in Balb/c 3T3 cell line.
Results expressed as a relative change compared to untreated control.

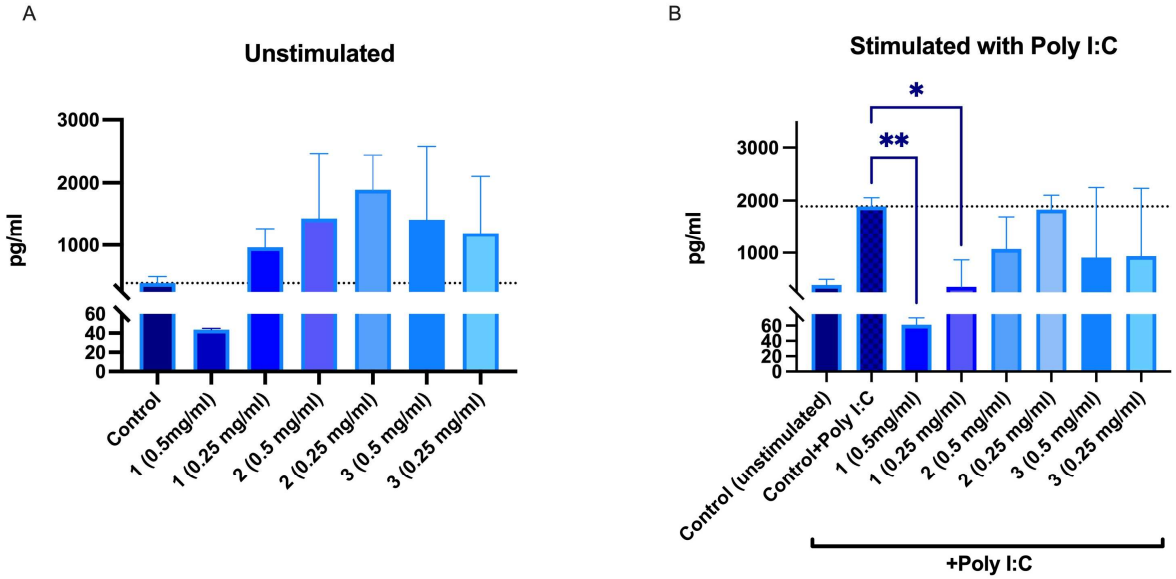
<u>In most cases, at concentrations similar to the MIC values observed in antimicrobial activity tests, no toxic effect was observed</u>. Water extracts were slightly more cytotoxic than ethanol extracts.
Cytotoxic concentrations of ethanol extracts ('Maria Bruvele', 'Tatiana') did not exceed the concentrations needed to inhibit the growth of the tested microorganisms.

(An extract at a specific concentration was considered to be cytotoxic if the cell viability was reduced by more than 20%).

Reference: Janceva, S.; Andersone, A.; Lauberte, L.; Bikovens, O.; Nikolajeva, V.; Jashina, L.; Zaharova, N.; Telysheva, G.; Senkovs, M.; Rieksts, G.; et al. Sea Buckthorn (Hippophae rhamnoides) Waste Biomass after Harvesting as a Source of Valuable Biologically Active Compounds with Nutraceutical and Antibacterial Potential. Plants 2022, 11, 642. https://doi.org/10.3390/ plants11050642

Anti-inflammatory activity of proanthocyanidins

PACs at concentration of 0.5 mg/mL reduced IL-8 secretion in unstimulated Peripheral blood mononuclear cells (PBMNCs) and **significantly reduced IL-8 and IL-6 secretion in** polyinosinic:polycytidylic acid (**poly I:C) stimulated PBMNCs**. PACs, 50% EtOH extract, and water extract reduced secretion of IL-8 in presence of Poly I:C.
Since experiment with poly I:C mimics inflammation related to viral infections, the results indicate **the ability of plant PACs and PACs containing extracts to reduce inflammation related to viral infections.**



Changes in IL-8 secretion from unstimulated (A) and Poly I:C stimulated (B) human peripheral blood mononuclear cells after 24h incubation with SBT samples: 1 – PACs, 2 - 50% EtOH extract, 3 – water extract.

None of the extracts at a concentration of 0.5 mg/mL **induced hemolysis after 1 h or 8 h incubation, indicating the high biocompatibility and safety of the extracts.**

Reference: Andersone, A.; Janceva, S.; Lauberte, L.; Ramata-Stunda, A.; Nikolajeva, V.; Zaharova, N.; Rieksts, G.; Telysheva, G. Anti-Inflammatory, Anti-Bacterial, and Anti-Fungal Activity of Oligomeric Proanthocyanidins and Extracts Obtained from Lignocellulosic Agricultural Waste. *Molecules* **2023**, *28*, 863. https://doi.org/10.3390/molecules28020863

Determination of the *in vitro* gas production and digestibility of SBT biomass

According to *in vitro* test data, the extract showed the greatest digestibility after the separation of CT. The leaves have a much higher digestibility than stem.

- It can be seen that the digestibility of the leaves is a bit less than of MB residual fraction after CT separation, but at the same time, the gas emissions are much less for leaves.
- **Therefore, for the reduction of GHG emissions, the SBT leaves should always be in the composition of the SBT-based animal feed. The leaves can be combined with other types of biomass.**

Feed pellets: A - MB/LV: MB/ST/MT (1:1, w/w) + 5% TM/R; B - MB/LV; C – MB/ST/MT.

Reference: Andersone, A.; Janceva, S.; Lauberte, L.; Zaharova, N.; Chervenkov, M.; Jurkjane, V.; Jashina, L.; Rieksts, G.; Telysheva, G. Granulated Animal Feed and Fuel Based on Sea Buckthorn Agro-Waste Biomass for Sustainable Berry Production. *Sustainability* **2023**, *15*, 11152. https://doi.org/10.3390/su151411152

Sea buckthorn biomass biorefinery scheme

Sea buckthorn

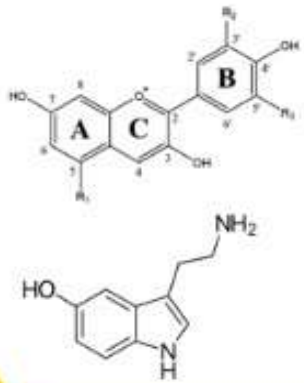
- Berry
- By-product of harvesting - branches and leaves
- Wood from winter/spring pruning – twigs
- Plantations grown for amelioration and soil erosion control – annual pruning wood – twigs, leaves

Berry – for food

Oil, pomaces

Lignocellulosic biomass

Extracts, proanthocyanidins Serotonin



Active ingredients for multifunctional products:

- Anti-inflammatory
- Vitamines-containing
- Nutritional
- Antioxidants for lipids-containing systems
- Antibacterial agents
- Fungicides
- Digestive enzyme regulators

for food additives, cosmetic and medical formulas

Residue after extraction

Granulated animal feed additive

Plant growth stimulator

Conclusions

- SBT **lignocellulosic biomass**, which is formed as a by-product as a result of berry harvesting and as a waste result of shrub trimming, is a new valuable raw material for the production of **proanthocyanidins and serotonin.**
- Proven high antimicrobial activity against *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Bacillus cereus* and *Candida albicans*, with the possibility of replacing antibiotics in the fight against resistant microbes (obtaining antimicrobial preparations).
- In the range of antimicrobial activity of extracts and PACs, cytotoxic concentrations did not exceed the concentration required to kill pathogenic microorganisms. Thus, extracts and PACs may have been used in food additives.

Conclusions

- **PAC have high anti-inflammatory activity.**
- SBT biomass extracts can **protect lipid-containing systems from oxidation**, and have **high antimicrobial activity**. Thus they can be used as antioxidants and antibacterial agents **in medical and cosmetic cream formulations**.
- Znalyzes in the digestive system of small ruminants showed the possibility of using SBT biomass and extracts for the production of **animal feed additives**.
- A soil additive obtained from the post-extraction residues of biomass **promoted the growth and development of plants**.
- **Waste-free sea buckthorn biorefinery scheme was developed**.

Publications

- Andersone, A.; Janceva, S.; Lauberte, L.; Skadins, I.; Nikolajeva, V.; Logviss, K.; Zaharova, N.; Rieksts, G.; Telysheva, G. A comparative analysis of the proanthocyanidins from fruit and non-fruit trees and shrubs of Northern Europe: Chemical characteristics and biological activity. *Sustainable Chemistry and Pharmacy*, Volume 36, 2023, 101266. https://doi.org/10.1016/j.scp.2023.101266
- Andersone, A.; Janceva, S.; Lauberte, L.; Zaharova, N.; Chervenkov, M.; Jurkjane, V.; Jashina, L.; Rieksts, G.; Telysheva, G. Granulated Animal Feed and Fuel Based on Sea Buckthorn Agro-Waste Biomass for Sustainable Berry Production. *Sustainability* **2023**, *15*, 11152. https://doi.org/10.3390/su151411152.
- Andersone, A.; Janceva, S.; Lauberte, L.; Ramata-Stunda, A.; Nikolajeva, V.; Zaharova, N.; Rieksts, G.; Telysheva, G. Anti-Inflammatory, Anti-Bacterial, and Anti-Fungal Activity of Oligomeric Proanthocyanidins and Extracts Obtained from Lignocellulosic Agricultural Waste. *Molecules* **2023**, *28*, 863. https://doi.org/10.3390/molecules28020863.
- Andersone, A.; Janceva, S.; Lauberte, L.; Krasilnikova, J.; Zaharova, N.; Nikolajeva, V.; Rieksts, G.; Telysheva, G. Lignocellulosic Waste Compounds for Pancreatic Lipase Inhibition: Preliminary Extraction by Freon, Obtaining of Proanthocyanidins and Testing on Lipase Activity. *Metabolites* **2023**, *13*, 922. https://doi.org/10.3390/metabo13080922
- Janceva, S**.;** Andersone, A.; Lauberte, L.; Zaharova, N.; Krasilnikova, J.; Rieksts, G. A Comparative Assessment of Sea Buckthorn (Hippophae rhamnoides L.) Pruning Waste as a Potential Source of Serotonin. Bioresources. **2023.** *DOI: 10.15376/biores.19.1. (pieņemts publicēšanai).*
- Janceva, S.; Andersone, A.; Lauberte, L.; Bikovens, O.; Nikolajeva, V.; Jashina, L.; Zaharova, N.; Telysheva, G.; Senkovs, M.; Rieksts, G.; et al. Sea Buckthorn (*Hippophae rhamnoides*) Waste Biomass after Harvesting as a Source of Valuable Biologically Active Compounds with Nutraceutical and Antibacterial Potential. *Plants* **2022**, *11*, 642. https://doi.org/10.3390/plants11050642.
- Andersone, A.; Janceva, S.; Zaharova, N.; Svarta, A.; Telysheva, G. Lignin and lignocellulose-based organomineral complex for organic agriculture. Proceedings of 23rd SGEM International Multidisciplinary Scientific GeoConference **2023**. Volume 23, 247-256. DOI:10.5593/sgem2023/3.1/s13.30.

Thank you for your kind attention!

Acknowledgments:

ERDF project nr. Nr.1.1.1.1/19/A/146 “Biorefinery processing of sea buckthorn non-fruit biomass using innovative techniques and comprehensive analytical investigation, for obtaining prospective for Latvian bioeconomy high value-added products, including serotonin”

Researcher Orcid ID - 0000-0002-7177-7752

Phone number: +371 29104319
E-mail: anna.andersone@kki.lv
aandersone@gmail.com