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UNIVERSITY OF AGRONOMIC SCIENCES AND VETERINARY MEDICINE OF BUCHAREST

Research Center for Studies of Food Quality and Agricultural Products

Scientific and technical report

for the project

„Natural phenolic systems for dietary lipid protection obtained from shrubs of the genus Vaccinium and their bioaccessibility during the gastrointestinal digestion" - BIOXVACCINI –

Phase II. Chemical and functional characterisation of Vaccinium functional food products, and their antimicrobial activity and lipid oxidation protection

Period covered: 15.12.2018-31.12.2019

In Phase II of the project, the phenolic composition and profile of the powders and extracts obtained in Phase I was assessed by UPLC/MS analyses. Analysis of procyanidins (determination of the degree of polymerization and flavanol unit constitution) by HPLC following thioacidolysis was applied directly on crude materials (powders). The *Vaccinium* products with the most complex phenolic composition were selected for the evaluation of the antimicrobial activity and lipid oxidation protection.

Phenolic composition of bilberry and blueberry extracts revealed the presence of caffeyl and *p*-coumaroyl derivatives, quercetin glycosides and (-) - epicatechin in the leaves, while in stems flavanol oligomers were also identified. For lingonberry, analyses by UPLC/MS showed the predominant presence of monomers and oligomers of catechin and epicatechin and quercetin glycosides in all the morphological parts. Arbutin was identified as major compound in lingonberry samples. His concentration was 8.37 mg / g SU in leaves and 0.63 mg / g SU in stems.

The antifungal activity of 50% aqueous ethanol extracts from leaves and stems on two food degradation fungi, *Aspergillus niger* and *Penicillium expansum*, was studied. The evaluation of the inhibition of lipid oxidation was performed by autoxidazing methyl linoleate (MeLo). Antibacterial tests were performed using *Bacillus subtilis* bacteria usually tested for food products both of animal and non-animal. For antifungal, antibacterial and antioxidant activities were used hydroethanolic (50%) extracts of samples.

In lingonberry, thioacidolysis revealed low degrees of polymerization (2.5 in stems and 3.1 in leaves) and (-)-epicatechin as the main flavan-3-ol unit. Procyanidins contain (+)-catechin and (-)-epicatechin as both extension and terminal units. In leaves, the flavanol monomers were detected as (+)-catechin and (-)-epicatechin, the former being highly preponderant. By contrast, almost similar amounts of flavanol monomers were quantified in stems. In bilberry and blueberry varieties, mDP ranging from 3.1 to 7.6 suggests the presence of small-size oligomers. Epicatechin was the only constituting unit of flavanol oligomers and the rest as extension units.

The lingonberry extracts showed a stronger antifungal activity against *Penicillium expansum*, compared to *Aspergillus niger*. The highest inhibition rate was obtained for leaves extracts (~14% on *Aspergillus niger* and ~36% on *Penicillium expansum*), for both fungi. The results obtained for the bacterial activity showed that the samples showed a area of inhibition around the discs impregnated with 50 and 70 μL extract. Regarding the antioxidant activity of MeLo, the stems and leaves extracts of bilberry showed a high inhibition compared to lingonberry extracts. The stems extracts showed a higher antioxidant activity that the leaf extracts.

Results from this study indicate that leaves and stems of different wild and cultivated *Vaccinium* species are suitable for valorization as sources of natural phenolic antioxidans as well as to be valuable raw material for the manufacture of herbal supplements.

The results obtained during the Phase II of the BIOXVACCINI project were disseminated by participating in **4 international conferences** and in **1 scientific publication***:*

International conferences:

1. **Oana-Crina Bujor,** Mona Elena Popa, Presentation of project *"BIOXVACCINI - „Natural phenolic systems for dietary lipid protection obtained from shrubs of the genus Vaccinium and their bioaccessibility during the gastrointestinal digestion",* Colloque Francophone "Aliments Fonctionnels et Produits Écologiques", 18 decembre 2018, Universitatea de Stiințe Agricole și Medicină Veterinară « Ion Ionescu de la Brad » din Iași, Iași, România.

2. **Oana-Crina Bujor**, Mona Elena Popa, *Phenolic profile and content of aerial parts of lingonberry (Vaccinium Vitis-idaea L.)*, "4th International Conference on Natural Products Utilization: from Plants to Pharmacy Shelf (ICNPU-2019)", 29.05.2019 – 01.06.2019, Albena, Bulgaria (poster).

3. **Oana-Crina Bujor**, Elisabeta Elena Popa, Mona Elena Popa, *Procyanidins characterization and antifungal activity of lingonberry leaves and stems*, "13th World Congress on Polyphenols Applications: Malta Polyphenols 2019", 30.09.2019 – 01.10.2019, Valletta, Malta (poster).

4. **Oana – Crina Bujor**, Ioana Oprică, Adrian Asănică, Liliana Bădulescu, Mona Elena Popa, *Phenolic content and antioxidant activity of leaves and stems of selected Vaccinium species, 15th International Symposium „Young people and agriculture research”,* USAMV Timișoara, Facultatea de Horticultură și Silvicultură Timișoara, 28 - 29 noiembrie 2019, Timișoara, România (poster).

Scientific publication

1. Oana-Crina Bujor, Corneliu Tanase, Mona Elena Popa, 2019. Phenolic antioxidants in aerial parts of wild Vaccinium species: towards pharmaceutical and biological properties, Antioxidants, 8, 649, pp. 1-13, doi:10.3390/antiox8120649; IF: 4,520.

References

1. Heinonen I. Marina, Lehtonen Pekka J., Hopia Anu I., *Antioxidant activity of berry and fruit wines and liquors*, J. Agric. Food Chem. 1998, 46, 25-31.
2. Kähkönen Marja P., Heinonen I. Marina, *Antioxidant activity of anthocyanins and their aglycons*, J. Agric. Food Chem. 2003, 51, 628-633.
3. Oana-Crina Bujor, Christian Giniès, Valentin I. Popa, Claire Dufour, 2018. Phenolic compounds and antioxidant ac tivity of lingonberry (*Vaccinium vitis-idaea* L.) leaf, stem and fruit at different harvest periods, Food Chemistry, 252, 356-365.
4. Oana-Crina Bujor, Carine Le Bourvellec C., Irina Volf, Valentin I. Popa, Claire Dufour, 2016. Seasonal variations of the phenolic constituents in bilberry (*Vaccinium myrtillus* L.) leaves, stems and fruits and their antioxidant activity, Food Chemistry, 213, 58-68.
5. Oana-Crina Bujor, Iulia Adina Talmaciu, Irina Volf, Valentin I. Popa, 2015. Biorefining to recover aromatic compounds with biological properties, Tappi Journal, 14(3), 187-193.
6. Fishwick, M. J.; Swoboda, P. A. T. *Measurement of oxidation of polyunsaturated fatty acids by spectophotometric assay of conjugated derivatives*. J. Sci. Food Agric. 1977, 28, 387-393.