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Abstract of the PhD thesis

Evaluation of some phytochemical compounds on metabolic alterations caused by hyperglycemia

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Hyperglycemia is a serious condition, which in time leads to a large number of abnormalities in the metabolic pathway, causing serious and life-threatening complications. Researches for natural compounds have become a great interest in recent years, proving that a number of phytochemicals not only lower the blood glucose, but have a number of beneficial effects on the complications caused by long-term hyperglycemia. *Vaccinium* species are well known for their high polyphenolic content. Traditional medicine in Romania uses the leaves from *Vaccinium myrtillus* as a complementary treatment in diabetes, but this use is mostly based on empirical data. *Vaccinium corymbosum* is a relative new species, introduced in cultures in Romania for the production of fruits. The leaves are at this moment a waste product.

The present thesis is structured in two chapters. The first chapter contains a brief description of the physiological mechanisms involved in diabetes and its complications, the pharmacological treatment and the most recent discovered plants and phytochemicals proved to have beneficial effects in the management of metabolic syndrome.

In the second chapter, eleven experiments that were taken during this thesis are described. The four herbal drugs analyzed were the leaves and fruits collected from *Vaccinium myrtillus* and *Vaccinium corymbosum*. The samples from *Vaccinium myrtillus* were collected from a natural habitat in Mureș County, Romania. The samples from *Vaccinium corymbosum* were collected from a culture near Trei sate village, Mureș County, Romania.

In the first three studies qualitative determinations were performed using chromatographic methods: TLC, CC, HPLC-UV, LC-MS/MS. Using these methods main differences between the herbal drugs used in this study and other reported data were highlighted. In the fourth and fifth study, quantitative determinations were assessed: total polyphenols, tannins, flavonoids and proanthocyanidins content were determined for the leaves extracts and total polyphenols and total monomeric anthocyanins were determined for the fruits extracts. Optimizations of the extractions methods were also performed, in order to obtain extracts with optimal composition and safe for *in vivo* studies. In the next study, 15 elements were determined using an ICP method. No toxic concentrations of heavy metals were found in the herbal drugs. Antioxidant capacity was determined using DPPH and ABTS free radical scavenging methods. The leaves from both species

have a high antioxidant capacity. The fruits collected from *Vaccinium myrtillus* have a significantly higher antioxidant capacity than the fruits collected from *Vaccinium corymbosum*.

The eighth study was an *in vivo* evaluation of four types of extracts on female rats with diabetes induced by streptozotocin (STZ). Extracts tested were: VML - *Vaccinium myrtillus* leaves extract, VMLF - *Vaccinium myrtillus* leaves and fruits extract, VCL - *Vaccinium corymbosum* leaves extract, and VCLF - *Vaccinium corymbosum* leaves and fruits extract. Three control groups were included in this study: normal control, diabetic control and positive control. Body weight and glucose level variations were measured for eight weeks. After the sacrifice, blood was collected for serum analysis (insulin, glucose, malondialdehyde, triglycerides, total cholesterol, aspartate aminotransferase, alanin aminotransferase, creatinine) and organs (eyes, kidneys, liver) for histopathological analysis. The results showed that *Vaccinium myrtillus* leaf extracts have the potential to lower blood glucose, to improve serum insulin and to ameliorate diabetic complications induced by STZ like the development of cataract and hepatic alterations. The association of fruit and leaves extract from *Vaccinium myrtillus* had a lower outcome, but this combination was able to prevent the development of cataract. *Vaccinium corymbosum* leaves extract did not have any remarkable effects during this study, but the combination of fruit and leaves extract from this species had a low to moderate effect in preventing or ameliorate diabetes complications.

The ninth study was an *ex vivo* experiment made on *Gallus gallus* lens, where the extracts effects against opacification induced by hyper osmosis were assessed. *Vaccinium myrtillus* leaves extract showed a remarkable anti-cataractogenic effect.

The last but one experiment represented the determination of the capacity of inhibition on alpha-glucosidase. Both extracts from leaves had a high alpha-glucosidase inhibition capacity. *Vaccinium myrtillus* fruits also have a high capacity of inhibition on alpha-glucosidase.

The last study assessed the antibacterial effect of the extracts on six bacterial stains: *Staphylococcus aureus* ATCC 25923, MRSA 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* 27853. Extracts obtained from *Vaccinium myrtillus* showed a higher antibacterial activity compared with the extracts obtained from *Vaccinium corymbosum*.

Phytochemical analysis through high and sensible methods, evaluation of the therapeutic potential on an experimental diabetic model, *ex vivo* and *in vitro* studies on the complications caused by sustained hyperglycemia in time, are the main characteristics that offer a degree of originality to this thesis and opens new avenues for future research.