

Objective of stage II/2008

Qualitative and quantitative phytochemical investigations into the species *Allium ursinum* and *Sorbus aucuparia*

2nd Stage Activities

Activity 2.1 Investigation into the chemical composition of the species *Sorbus aucuparia* and *Allium ursinum*

Activity 2.2 Laboratory study to determine the optimal conditions to extract polysaccharides, sulphur and polyphenols compounds.

Activity 2.3 Obtaining extractive fractions at the lab stage.

Activity 2.4. Physical, chemical, microbiological, and phytobiological (cytogenesis, cytotoxicity) characterisation of extractive fractions; dissemination of results

Abstract

In this study, research focuses on a higher valuation of *Allium ursinum*, namely the synthetic potential of antioxidant compound – e.g. polyphenols and flavones – is assessed. In order to characterise the plant raw matter from a morphobiochemical viewpoint, a number of natural populations of *Allium ursinum* were assessed with a view to selecting those that correspond to several parameters such as bioproductivity, biomass + biochemical composition.

Currently, raw matter is supplied from the spontaneous flora. In future, conventional culture should be considered (in fields) so that natural pools are not exhausted and to allow for the control of the biosynthetic potential of the plant matter used in processing. Nonetheless, sampling the matter as bulbs or in-bloom plant may make vegetal multiplication impossible (naturally) of the species *Allium ursinum*. Investigating natural populations is also necessary because of the fact that the synthetic potential can be influenced by soil and climate conditions in the area plants were sampled. Hence, the processing personnel cannot have the same raw material throughout a longer time, with the same biochemical features. The study we the study we undertook aimed at assessing the morphological and biochemical populations of *Allium ursinum* plants, potentially usable in obtaining plant preparations that can administered in cardiovascular conditions. Finding the best extractive methods is a goal in preserving antioxidant principles mainly as processing fresh raw matter is essential.

In order to determine the optimal conditions to extract antioxidant active principles, i.e. flavonoid derivatives, polyphenol compounds, sulphur compounds, from the two plant products under study, such principles were extracted with various polarity solvents: absolute ethanol and hydroethanolic mixtures with various concentrations (e.g. 70°, 50°, 30°). For *Allii ursini herba* extracts, the determinations focused on the content of flavonoid, polyphenols, and sulphur compounds whereas for *Sorbi aucupariae fructus* extracts, the flavonoid and polyphenol content was determined.

The quantitative chemical study performed highlighted the following:

- for *Allii ursini herba* product, extracts in absolute ethanol and ethanol 70° contain over 8 mg flavonoids per 100 ml whereas the extracts obtained with ethanol 50° and ethanol 30° the flavonoid content is of 3.9 and 2.25 mg, respectively, per 100 ml extract; the extract in ethanol 50° proved to be the richest in polyphenols (25.22 mg polyphenols per 100 ml extract). Mention should be made that in the other

extracts (obtained with absolute ethanol, ethanol 70° and ethanol 30°), the content in polyphenols is similarly high varying between 21.17 and 23.62 mg per 100 ml. For sulphur compounds, the election solvent proved to be ethanol 70° (11.33 mg sulphur ions per 100 ml extract).

- for *Sorbi aucupariae fructus*, the election solvent for flavonoids is represented by absolute ethanol (4.55 mg flavonoids per 100 ml extract) whereas hydroethanolic extracts obtained with ethanol 70° , 50° , and 30° , the flavonoid content does not exceed 1.7 mg per 100 ml extract; extracts in ethanol 30° and ethanol 50° proved to be richer in polyphenols (20.18 and 18.13 mg polyphenols, respectively, per 100 ml extract) than extracts obtained with ethanol 70° and absolute ethanol (11.34 and 8,93 mg, respectively, per 100 ml extract).

To conclude, we can appreciate that in case of *Allii ursini herba* product, to obtain an extract richer in antioxidant principles (e.g. flavonoids, polyphenols, and sulphur compounds) the use of ethanol 70° as an extraction solvent is recommended.

For *Sorbi aucupariae fructus*, absolute ethanol is the election solvent for flavonoids whereas ethanol 30° extracts the highest percentage of polyphenols.

The results obtained were disseminated in the abstract presented in the appendix, for the paper which will be presented at...

CONCLUSIONS

As based on the qualitative and quantitative phytochemical investigation of the species *Allium ursinum* and *Sorbus aucuparia* the authors reached the following conclusions: the morphobiochemical analysis was conducted on two populations of wild garlic sampled from different areas (i.e. Iasi and Botosani) on plants undergoing various development stages.

Three natural populations of rowan, sampled in pools that belong to the counties of Suceava, Neamt, and Covasna, were investigated.

After conducting the biometrical measurements, the matter was divided in two batches, one was processed on fresh matter, and the other after the matter underwent a natural drying process.

In order to identify the active principles major groups, successive extractions were conducted with various polarity solvents, e.g. dichloromethane, ethanol, water.

- the qualitative chemical analysis of the extracts obtained from vegetal organs of the species under study led to identifying the major active principles groups: coumarin, flavonosides, amino acids, reducing compounds, and carbohydrates.

Various types of vegetal extract were obtained from the leaves of wild garlic, e.g. methanolic, ethanolic, dichloromethanic and aqueous. The ethanolic ones recorded 70% concentration obtained via refluxing, infusion of the matter with hot ethanol, and then extraction at room temperature for 24 hours, 50% and 70% concentration tinctures (raw matter versus solvent ratio: 1:10, maceration for 14 days at room temperature. They served to dosing the polyphenols and flavonoids acids.

The assessment of the quantities obtained from the vegetal matter infused with hot ethanol, followed by 24 hours extraction at room temperature, ascertained that in the leaves sampled from both populations, flavonoids recorded high values, i.e. some 0.913 g % for the former, and 1.496 g % for the latter population, respectively. Thus, it is

confirmed that plant population in Botosani record higher values for this parameter, which correlates with the morphological properties reflected quantitatively (100 plants = 717.20 g).

It was also ascertained that the Botosani population recorded the highest polyphenols values (i.e. in leaves), 0.913 g% and 1.496 g% for flavonoids, whereas in bulbs, the quantity of gross polyholosides is of 4.36 g as compared to 4.53 g% in roots.

The rowan variants analysed revealed that these plants belong to the Dorna Arini (county of Suceava) population. They displayed high values, i.e. 0.741 g% polyphenols expressed in caffeic acid, 1.146 g% flavonoids expressed in rutoside and gross polyholoside of 1.83 g% (gravimetric dosage), as compared to the Vaduri – Neamt and Ojdula – Covasna population.

The quantitative determinations conducted with a view to determining the selective extractive methods led to the following results:

- for the *Allii ursini herba* product, the extracts in absolute ethanol and ethanol 70° contain over 8 mg flavonoids per 100 ml whereas the extracts obtained with ethanol 50° and ethanol 30° , the flavonoid content is of 3.29 and 2.25 mg per 100 ml extract, respectively.
- for *Sorbi aucupariae fructus*, the election solvent for flavonoids is represented by absolute ethanol (4.55 mg flavonoids per 100 ml extract) whereas in the hydroethanolic extracts obtained with ethanol de 70°, 50° and 30° the flavonoid content does not exceed 1.7 mg per 100 ml extract.

In the case of *Allii ursini herba* product, the extract in ethanol 50° proved to be the richest in polyphenols (25.22 mg polyphenols per 100 ml extract). Mentioned should be made that in the other extracts (obtained with absolute ethanol, ethanol 70° and ethanol 30°) the polyphenol content is similarly high varying between 21.17 and 23.62 mg per 100 ml.

For *Sorbi aucupariae fructus*, extracts in ethanol 30° and ethanol 50° proved to be richer in polyphenols (i.e. 20.18 and 18.13 mg polyphenols la 100 ml extract, respectively) than the extracts obtained with ethanol 70° and absolute ethanol (11.34 and 8.93 mg per 100 ml extract, respectively).

In terms of the sulphur compounds in *Allii ursini herba*, the election solvent proved to be ethanol 70° (11.33 mg sulphate ions per 100 ml extract).

To conclude, we can appreciate that, in the case of *Allii ursini herba* product, it is recommended to use ethanol 70° as extraction solvent in order to obtain an extract richer in antioxidant principles (e.g. flavonoids, polyphenols, and sulphur compounds).

For *Sorbi aucupariae fructus*, the absolute ethanol is the election solvent for flavonoids whereas ethanol 30° extracts the highest percentage of polyphenols.

Preliminary phytobiological testing of the *Allium ursinum* ethanolic extracts, obtained from leaves, bulbs, and roots, revealed the fact that do not have cytotoxic and cytogenetic action on the of cells of *Triticum aestivum* plantlets used as testing matter.

Description/presentation of various events carried out within the project

Participations to scientific events

The 5th Conference on Medicinal and Aromatic Plants of Southeast European Countries, BRNO,2-5.09.2008

Paper title: *Obtaining of phytoproducts for the cardiovascular diseases profilaxy*

Note I : Some investigations of the Allium ursinum chemical composition

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ABSTRACT:

It has been shown that Allium species may help to prevent cardiovascular diseases and aging specially when are associated with free radicals. Wild garlic, (*Allium ursinum*L.) [Fam. Alliaceae; Liliaceae s.l.], is a bulbous plant found in Europe and northern Asia, known for healthy cholesterol levels activity, antioxidant properties, and antifungal and antibacterial properties. The qualitative and quantitative chemical composition of leaves, flowers and roots of *Allium ursinum*, Romanian origin, were investigated. Some extracts of wild garlic

were investigated for flavonoids, polyphenolcarboxylic acids, aminoacids, micro-and macroelements, chlorophyll, carotens, sulfur-containing constituents, using Molecular Spectrophotometry UV-VIS, Atomic Absorption Spectrophotometry, High Performance Thin Layer Chromatography - densitometry, gas-cromatography.

Radical scavenging activity was tested against 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and OH radicals, on some water and ethanol extracts of wild garlic.

Keywords: *Allium ursinum*, cardiovascular prophylaxy, radical scavenging activity.