

## THE FOURTH STAGE /2009

Execution: 18.01.2009 – 15.12.2009

### Stage value -

<b>budget :</b>	<b>105,295 lei</b> , of which:
▪ Coordinator:	79,075 lei
▪ Partner:	26,220 lei
Co-funding:	19,770 lei

### Objective of stage

**To design the association formulae in order to obtain the standardized extracts**

### Activities

➤ To design the associations variants in order to obtain the standardized extracts; standardizing the association extracts;

### Summary

At this stage, the phytochemical assessment of some plant extracts that can be used to their composition and standardisation became mandatory. In order to obtain the extracts, it is necessary to assess the bioproductivity of a natural population sampled from the spontaneous flora, less anthropic areas or conventional cultures. From among all the natural populations assessed until this stage, we concluded that *Allium ursinum* is the most widely spread in the Baisa area, Botosani County, as compared to the populations in other areas such as Strunga, Iasi County, or Nemtisor, Neamt County.

The plant matter made of leaves, flowers, bulbs, and roots underwent successive extractions with absolute methanol in order to obtain **dewatered methanolic extract** as well as methanolic extract 70% to capture hydrosoluble fractions. The HPLC analysis allows for highlighting the fact that in common extracts from fresh matter, the compound quantities detected belonging to the kaempferol group are more reduced than those obtained from dry matter. The **dewatered methanolic extract** is richer in active principles than the 70% one, the extraction allowing for a larger quantity of active principles to be solubilised. In the dry matter, at dry flower level the larger quantity of flavonoids was dosed (i.e. 967.58 mg / 100g vegetal matter), followed by the one at leaf level (i.e. 228.5 mg / 100g vegetal matter); **all in all, fresh vegetal matter has small quantities of flavonoids as compared to the dry one**. This is a significant aspect as it can be used as an argument to support the idea that drying plant matter is an optimal

solution to preserve raw material in order to be processed throughout the year and not only during the maximum plant vegetation season.

The analysis of the extracts obtained from two populations of *Sorbus aucuparia* revealed:

From among flavonoids, rutosidea and apigenin were identified in compliance with the samples. Apigenin is present only in the methanolic extracts obtained from leaves whereas rutosidea was obtained from leaves and fruits. Polyphenols are in greater number and in larger quantities. Caffeic acid and chlorogenic acid are found in leaves extracts. The fruits extracts lack caffeic acid but contain ferulic and chlorogenic acid.

The extracts obtained as a result of previously conducted experimental activities as well as the phytochemical analysis of the extractive fractions created the basis for the design of some models to extract the active complexes that can be standardised. Four selective extraction models were suggested for the industrial valuation of artichoke leaves, of oat grains and aerial part, of rowan fruits and wild garlic aerial part.

The selective extracts were characterised from a physical and chemical point of view and standardising intervals were suggested for the dominant/representative biologically active principle.

Based on the phytochemical composition, four extract association formulae were designed, which will be used in the composition of some nutritive supplements effective as antioxidants, in lowering cholesterol, lipids, blood pressure, protecting the heart, and venotonic, designed to maintain a healthy cardiovascular system.

The association formulae were standardised according to the active principle dominant and representative from a pharmacological point of view.

Technological diagrams were designed to obtain the four association extracts during the pilot stage.

## **Conclusions**

From among all the natural populations assessed until this stage, we concluded that *Allium ursinum* is the most widely spread in the Baisa area, Botosani County, as compared to the populations in other areas such as Strunga, Iasi County, or Neamt County.

The plant matter made of leaves, flowers, bulbs, and roots underwent successive extractions with absolute methanol in order to obtain **dewatered methanolic extract** as

well as methanolic extract 70% to capture hydrosoluble fractions. The HPLC analysis allows us to highlight the fact that in common extracts from fresh matter, the compound quantities detected belonging to the kaempferol group are more reduced than those obtained from dry matter.

The **dewatered methanolic extract** is richer in active principles than the 70% one, the extraction allowing for a larger quantity of active principles to be solubilised. In the dry matter, at dry flower level the larger quantity of flavonoids was dosed (i.e. 967.58 mg / 100g vegetal matter), followed by the one at leaf level (i.e. 228.5 mg / 100g vegetal matter); all in all, fresh vegetal matter has small quantities of flavonoids as compared to the dry one. This is a significant aspect as it can be used as an argument to support the idea that drying plant matter is an optimal solution to preserve raw material in order to be processed throughout the year and not only during the maximum plant vegetation season.

The analysis of the extracts obtained from two populations of *Sorbus aucuparia* revealed:

- From among flavonoids, rutosidea and apigenin were identified in compliance with the samples.
- Apigenin is present only in the methanolic extracts obtained from leaves whereas rutosidea was obtained from leaves and fruits.
- Polyphenols are in greater number and in larger quantities.
- Caffeic acid and chlorogenic acid are found in leaves extracts.
- The fruits extracts lack caffeic acid but contain ferulic and chlorogenic acid.

The plant species under study were processed by means of selective extractions. The standardised extracts have the following characteristics:

**1.1. Artichoke hydro alcoholic extract (leaves)**, standardised in polyphenol-carboxylic acids acid, expressed in caffeic acid min. 1,0 %, containing *chlorogenic acid*, *cynarin*, *apigenol*, *apigenol-7-glucoside*, *luteolin*, *luteolin-7-glucoside*, *stigmasterol*.

**.2. Artichoke aqueous extract (leaves)**, standardised in polyholsides, min 20%

Both extracts are antioxidant, lower cholesterol and fight against atherogenesis.

**2.1. Oat grains extract**, standardised in polyphenols, expressed in galic acid, min. 12.0 %

**2.2 Oat hydro alcoholic extract (aerial part)**, standardised in flavones expressed in *rutoside*, min 3%, containing amino acids, polyphenol-carboxylic acids and flavonosides (*apigenol* and *rutosidea*) anti-oxidising active principles.

**3. Rowan fruit extract**, standardised in polyphenol-carboxylic acids, expressed in chlorogenic acid, min. 4.0 %, containing *caffeic*, *chlorogenic*, and *ferulic acid*, *rutosidea* and *apigenol-7-glucoside*, *ursolic acid*, highly antioxidant active principles.

**4. Wild garlic extract (aerial part)**, standardised in polyphenols, expressed in gallic acid, min 3%, containing *ferulic acid*, *ursolic acid* as well as *sulphur compounds*, active principles responsible for the platelet anti-aggregation.

The extracts thus obtained formed the basis of new association that can be used in producing nutritive supplements that target the cardiovascular system health.