

THE FIFTH STAGE /2010

Execution: 16.12.2009 – 15.09.2010

Stage value -

budget : 197,150 lei, of which:		
▪ Coordinator		142,150 lei
▪ Partner		55,000 lei
	Co-funding	52,795 lei

Objective of stage

To develop the experimental variants and draft the documentation thereof.

Activities

- ✓ *Designing and experimenting the association formulae and conditioning methods (solutions, extracts, granules, tablets)*
- ✓ *Developing and characterising – from a physical, chemical, and microbiological point of view – the standardised extracts of the plant preparations*
- ✓ *Drafting and compiling the technical documentation of the plant preparations development; Disseminating the information; developing informative materials*

SUMMARY

During this stage, we carried on the experiments in order to design technologies to extract the determining active principles from the association formulae of the four plant products under study. The experiments conducted to determine the extracting technologies relied on the best results obtained in the experiments at the laboratory stage related to the extraction of the phyto complexes from the unit plants under study.

Based on the results obtained during the previous stage, at the laboratory stage, we designed and optimised five highly productive extracting technologies suitable both for the extractible product (i.e. dry substance) and for the active principle specific to each plant matter. In order to optimise the in-progress extracting experiments, we focused on the variation of the total extractible content total (dry substance) in the extractive solutions according to the extraction time.

We aimed at obtaining dry association extracts, standardised in the active principles with an outstanding therapeutic value, which would allow their use as such or in mixtures, and the conditioning formulae and forms were decided upon. The plant extracts obtained were characterised from a qualitative and quantitative point of view. The qualitative analysis aimed at highlighting the main bioactive compound classes (flavonoid compounds, polyphenolic compounds, polysaccharide compounds, anthocyanins, and sulphur organic compounds).

The quantitative analysis was conducted by using various methods such as UV VIS spectrophotometry, e.g. the two UV VIS spectrophotometers, Cary 50 and Cintra 101.

The qualitative and quantitative characteristics determined laid the basis for drafting the Product Technical sheets.

The microbiological characteristics were determined by means of the existing specific equipment (e.g. autoclave, incubator, colony counter) and of the latest generation culture media, as provided by the harmonised European Pharmacopoeia (USP+EP+JP), 6th edition, chapters 2.6.12 (for the total number of viable aerobe germs) and 2.6.13 (for some Enterobacteria, *Pseudomonas* and *Staphylococcus aureus*), respectively, and the international standard NF ISO 16649-2 (for *E. coli*).

Starting from the associating extract studies developed in the previous stage more associating formulae were developed in various conditioning forms from all the extracts obtained.

For all conditioning forms, i.e. tablets, capsules, and glycerinated solutions, production technologies were developed, qualitative and quantitative characterisation methods were determined as well as physical, chemical, and microbiological parameters.

To characterise from the physical and chemical parameters, the methods decided for dry extracts were used and adjusted to these conditioning forms.

The microbiological assessment was especially careful both in terms of characterising the end products and in monitoring the production process.

The research team compiled the technical files of the development of CARDIOFIT product range. Such files contain the composition of the product, technical specifications for the intermediate product (standardised extract), the end-product (i.e. tablets, capsules, or glycerinated solution), and the process control, the physical and chemical methods to control the technological flow, the technological instructions, and the flow chart.

At micro-pilot level, the following pre-dosed products were produced:

ANGHICORD – tablets from artichoke leaves and rowan fruits, to be used in fighting the free radical effects in the organism and lowering cholesterol.

FIBRAVEN – tablets based on artichoke leaves and oat grains recommended as adjuvant in high cholesterol and lipid levels.

URSITON – capsules from wild garlic leaves and rowan fruits, effective in lowering lipids and cholesterol, platelet anti-aggregating and blood pressure, and cardio-protective.

AVENOX – capsule from artichoke leaves, wild garlic leaves and oat grass, likely to be effective as neuroprotector, vasoprotector, cardioprotector, venotonic, inducing calmness, and reducing blood pressure.

TONOHERB – **glycerinate** from artichoke leaves, green oat aerial part and rowan fruits, with highly antioxidant effects, liver protective, venotonic, and vessel protective.

The scientific results obtained until this stage of the project development were communicated as two posters in “*The 6th Conference on medicinal and Aromatic Plants of Southeast European Countries*”, 18-22 April 2010, Antalya, Turkey, and as abstracts of communications were published in **Pharmacognosy Magazine April-June 2010/Volume 6/Issue 22 (Suppl); Medknow Publications and Media Plv. Ltd., Mumbai, India.; ISSN: 0973-1296**, as follows:

The Effect of Solvent Extraction on the Extractability of Some Bioactive Substances and Antioxidant Activity of Some Medicinal Herbs Indicated for Cardiovascular Protection Note I: *Cynara Scolymus L.* Aerial Part

Authors: R. Mihailescu, E. Iacob, G. Mitroi, R. Verdes, C. Bedreag, V. Ionescu, L. Cioclu, A. Macavei

The Effect of Solvent Extraction on the Extractability of Some Bioactive Substances and Antioxidant Activity of Some Medicinal Herbs Indicated for Cardiovascular Protection Note II: *Fructus of Sorbus Aucuparia L.*

Authors: C. Bedreag, R. Mihailescu, E. Iacob, M. Gabriela, R. Verdes, C. Tebrencu, E. Ionescu, M. Chiriac

The results of the research materialised as **4 extracts unitary, fluid, 4 associating extracts, dry**, and **5 end products** (2 capsules, 2 tablets, 1 glycerinate) will be presented during our participation to **Salonul cercetarii – 2010** (Bucharest - October 2010).

CONCLUSIONS

The results of the research during this stage are as follows:

1. the development of **4 extracting technologies** at pilot stage for the **4 plant material** under study;
2. **five associating plant extracts** were obtained - *four dry* and *one fluid*, standardized in their active principles determinant for the use (as such or in mixtures) in the production of nutritive supplements:
 - **associating dry extract**, from **artichoke leaves** and **rowan fruits**, standardised in *polyphenolcarboxylic acids*, expressed in **acid cafeic**;
 - **associating dry extract**, from **artichoke leaves** and **oat grains**, standardised in *polysaccharides*;
 - **associating dry extract**, from **wild garlic aerial part** and **rowan fruits**, standardised in *polyphenols*, expressed in **galic acid**;
 - **associating dry extract**, from **wild garlic leaves**, **artichoke leaves** and **oat aerial part**, standardised in *flavonosides*, expressed in **rutoside**;
 - **associating fluid extract**, din **oat grass**, **artichoke leaves** and **rowan fruits**, standardised in *flavonosides*, expressed in **rutoside**;
3. the development of *three conditions forms were decided*: **tablets**, **capsules**, **glycerinate** and the technologies to obtain them;
4. the determination of the qualitative, quantitative, and microbiological characterisation parameters and methods for the dry extracts and the end products obtained;
5. the development of five Technical Sheets for the dry standardised extracts;
6. the development of the Technical Specifications for the five products conditioned as tablets, capsules, and glycerinate, obtained from the five associating extracts, standardised, belonging to the “CARDIOFIT” range, products intended for the prophylaxis of cardiovascular⁴ conditions;
7. the compilation of five technical files for the **CARDIOFIT** range products.

By conditioning the studied plant products as selective extracts and associating extracts, five end products were developed; they are pre-dosed as tablets, capsules, and glycerinated solution, as detailed below:

ANGHICORD – tablets – is a product based on associating extract obtained from processing **artichoke leaves** (*Cynarae folium*) and **rowan fruits** (*Sorbi fructus*) with *hydroalcoholic solvent*. Characterised by a complex composition, rich in *polyphenolcarboxylic acids* and *flavonoides*, this association has **outstanding antioxidising effects, anti-atherogenic, and platelet anti-aggregating effects**, contributing to **increasing blood vessel elasticity** and as **venous system tonic**. Owing to the highly antioxidant character of phenolic fractions, this association can be used to **fight against free radicals in the body.**

FIBRAVEN – tablets – is a products based on associating extract obtained from processing by water extraction of **artichoke leaves** (*Cynarae folium*) and **oat grains** (*Avenae fructus*). This association characterised by its *fibre* input (beta-glucans, fructosans) and *polyholosides* **lowers cholesterol and lipids**, and is recommended as an adjuvant in **high cholesterol** and **high lipid levels.**

URSITON – capsules – is a product based on associating extract obtained by processing **wild garlic aerial parts** (*Allium ursini herba*) and **rowan fruits** (*Sorbi fructus*). Its composition contains *sulphur organic compounds* (S-methyl-L-cysteine sulfoxide, allicin, ajoene, allyl disulfide), flavonoids and polyphenols, and is likely to be effective in **lowering lipids, cholesterol, platelet anti-aggregating, lowering blood pressure**, and as **protecting the heart**.

AVENOX – capsules – is a product based on associating extract obtained by hydroalcoholic solvent extraction from **artichoke leaves** (*Cynarae folium*), **wild garlic aerial part** (*Allium ursini herba*) and **oat aerial part** (*Avenae herba*). The composition contains *sulphur organic compounds* (S-methyl-L-cysteine sulfoxide, allicin, ajoene, allyl disulfide), *flavonoids* and *polyphenols*, and is likely to be **effective as neuroprotector, vasoprotector, cardioprotector, venotonic, inducing calmness, and lowering blood pressure.**

TONOHERB – glycerinated extract obtained from **artichoke leaves** (*Cynarae folium*), **oat aerial part** (*Avenae herba*) and **rowan fruits** (*Sorbi fructus*). It is characterised by a high content of *flavonoid and polyphenol compounds, tannins, proanthocyanins, macro- and microelements* with high **antioxidising effects, liver protecting and venotonic effects.**