

Stage III/2009

To be carried out between 01.07.2008 and 17.01.2009

Stage value:

- budget: 305,000 lei, of which:

- Coordinator – 155,000 lei
- PARTNER 1 – 100,000 lei
- PARTNER 2 – 50,000 lei

Co-funding: 38,750 lei

Objective of Stage

To design the technology to obtain standardised extractive fractions

3rd Stage activities

- The *in vitro* screening of the antioxidant action of the extractive fractions; the study of the effects at vascular level;
- Designing the extractive technology of the bioactive phytocomplexes at stage lab ;
- The physical and chemical characterization of the extractive fractions and standardization of the most promising potential from a biological viewpoint
- Designing the methods to characterise the extractive fractions; wide dissemination i.e. communication and publication the results;

Abstract

Bearing in mind the involvement of the oxygen reactive species (ORS) in the onset and development of cardiovascular conditions, Bearing in mind the involvement of the oxygen reactive species (ORS) in the onset and development of cardiovascular conditions, the **free radical scavenger ability** of the extracts obtained was tested as well as **their ability to inactivate some ORS**, namely the hydrogen peroxide (H₂O₂), the superoxide anion radical anion (O₂⁻), and the hydroxyl radical (HO[·]).

✓ **The free radical scavenger activity** was assessed by measuring the neutralising activity of the diphenyl-picrylhydrazyl (DPPH) radical, i.e. its being transformed into its reduced form (diphenyl-picrylhydrazine) by plant extracts under analysis.

The most active extracts from among those analyses proved to be the gross extract obtained from *Cynarae folium* and the purified extract obtained from *Sorbi aucupariae fructus*, which, at a 20mg/ml concentration, neutralised some 85.99% and 85.90%, respectively, of the DPPH radical.

The gross extract obtained from *Sorbi aucupariae fructus* neutralised some 36.64% of the DPPH radicals.

A 1.69 mg/ml quercetol-concentration neutralised 100% of the DPPH radical.

✓ **Scavenger activity with respect to the superoxide anion radical** was determined by assessing the analysed extracts capacity to inhibit the reduction reaction of nitroblue tetrazolium (a reaction where superoxide anions radicals are involved as well) in the reduced phenasin metosulphate/nicotinamid-adenin-dinucleotid system.

From among the extracts analysed, the most active proved to be the purified extract obtained from *Sorbi aucupariae fructus*, which, at 20 mg/ml concentration, neutralised 91.84% of the superoxide anion radicals.

The extract obtained from *Allii ursini herba* proved to be inactive.

A 1 mg/ml catechin concentration neutralised 90.29% of the superoxide anion radicals.

- **Scavenger activity with respect to hydroxyl radical** was determined by assessing the capacity of the analysed extracts to inhibit the reaction of forming the hydroxyl radicals within the Fe^{2+} /phosphate buffer system.

The extract analysis revealed two extracts that proved to be active, i.e. the one obtained from *Cynarae folium* and the purified extract of *Sorbi aucupariae fructus*, which, at a 20 mg/ml concentration, neutralised 49.56% and 69.83%, respectively, the hydroxyl radicals.

The extract obtained from *Avena sativa* L. caryopses, the one obtained from the aerial parts of *Allium ursinum* L., and the extract obtained from *Sorbus aucuparia* L. fruits were inactive.

A 5 mg/ml-concentration catechin neutralised 79.65% of hydroxyl radicals.

- **Scavenger activity with respect to hydrogen peroxide** was determined by assessing the capacity of the analysed extracts to break down the hydrogen peroxide, a process of reducing its absorption to 230 nm.

From among the extracts under analysis, the most active proved to be the purified extract of *Sorbi aucupariae fructus*, which are, at a 20 mg/ml-concentration, broke down 76.96% of the hydrogen peroxide.

A 5 mg/ml catechin concentration broke down 73.15% of the hydrogen peroxide.

The endothelial function is a major prognostic marker in assessing patient facing a cardiovascular risk or suffering from such conditions.

The effects of the extracts on arterial vascular response, i.e. conductivity and resistance, were tested in rats.

Relevant effects were obtained only for the purified extract of rowan fruits. Its administration in 15-minute incubation determined a significant inhibition of phenyl phrenic contraction. On the other hands, its administration in the contraction tableau determined an insignificant transitory myorelaxing effect (1-5%).

In order to select and standardise the extractive fractions having the maximum bioactive potential, the vegetal products under study, i.e. *Avena sativa*, *Allium ursinum*, *Cynara scolymus*, and *Sorbus aucuparia*, were extracted at lab stage in hydroalcoholic solvents of various degrees and/or water.

The extractive fractions obtained were characterised from a qualitatively phytochemical (HPTLC, HPLC) and quantitative (spectrophotometric and HPLC) points of view.

Each of the species analysed displayed the existence of a polyphenol and flavonoid fraction with antioxidant properties, which can be used in the composition of plant preparations formulae that in turn can be used in the treatment of some cardiovascular conditions.

The results obtained were disseminated in the abstract presented in the appendix.

CONCLUSIONS

We can therefore appreciate that of all the prepared extracts, the **purified extract obtained from *Sorbi aucupariae fructus*** proved to be **the most active antioxidant**. In a 20 mg/ml concentration, extract 5 neutralised 85.90% of the DPPH radical and inactivated a series of oxygen reactive species involved in the onset and development of many cardiovascular conditions; it neutralised 91.84% of the superoxide anion radical, 69.83% of hydroxyl radical, and 76.96% of hydrogen peroxide.

Furthermore, the antioxidant activity of the **extract obtained from *Cynarae folium*** is also remarkable. A similar concentration, i.e. 20 mg/ml, of this extract neutralised 85.99% DPPH radical and inactivated significant proportions of superoxide anion and hydroxyl radicals (i.e. up to 60.88% and 49.56%, respectively). Moreover, this extract broke down 41.25% of oxygenated water.

Tests run on the antioxidant effects and of the effects at the vascular level revealed the fact that the **purified extract of *Sorbi aucupariae fructus*** develops significant antioxidant and vasorelaxing effects.

A significant antioxidant action was also displayed by the **extract of *Cynarae folium***.