ACTIVE PRINCIPLES WITH POSITIVE EFFECTS ON LIPID METABOLISM

Diana GROPOȘILĂ-CONSTANTINESCU, Ovidiu POPA, Narcisa BĂBEANU, Gabriela MĂRGĂRIT, Andreea ÎNSURĂȚELU

University of Agronomic Sciences and Veterinary Medicine - Bucharest, Faculty of Biotechnologies 59 Marasti, 011464, Bucharest, Romania Phone: +40 (21) 318 22 66, Fax: +40 (21) 318 28 88 E-mail: dikiconst@yahoo.com, ovid_popa@yahoo.com, bnarcisa@yahoo.com, gabriela.margarit@biotehnologii.usamv.ro, andreea.insuratelu@yahoo.com

Corresponding author e-mail: dikiconst@yahoo.com

Abstract

Today attention is given increasingly higher to plants, considering that they represent an inexhaustible source of raw materials that regenerates every year. Plants can be used as raw materials in the preparation of drugs or in industrial extraction of active principles. The advantage of phytotherapy is that plant based drugs have a positive impact not only on a single organ or a specific system, but on the whole body. Experimental research focused on the following plant materials: sea buckthorn (Hippophae rhamnoides), hawthorn leaves and flowers (Crataegus oxyacantha), garlic bulbs (Allium sativum) and maple buds (Acer campestre). They were tested in order to characterize, identify and determine the content of active compounds. The analysis of the extracts revealed a high content of lipase in sea buckthorn, 6,3 U/g, as well as in garlic, 4,9 U/g, while the buds maple had a very low lipolytic activity, only 0,4 U/g. The highest content of flavones was determined in leaves and flowers of hawthorn and sea buckthorn. Reducing compounds were determined in large quantities in sea buckthorn, while the other materials tested showed a 50% lower content. The highest content of polyhydroxyphenols was determined in hawthorn and sea buckthorn. Sea buckthorn was also noted by a very high content of vitamin C, 483 mg/100 g. The results obtained in these experiments allowed the selection of plant products rich in many active principles involved in the regulation of lipid metabolism.

Keywords: phytotherapy, active principle, lipases, lipid metabolism.

INTRODUCTION

Currently phytotherapy has become an important science, practiced by competent doctors. The effects of medicinal plants on the human body was demonstrated by numerous studies of organic chemistry, phytochemistry, pharmacology, pharmacognosy, biochemistry and microbiology (Grigore, 2005).

Though phytotherapy is based on the curative effects of certain biological compounds from plants or other plant part, this ancient science of healing follows the classical rules, exploiting the plant material without selectively extract certain substances or principles from plant tissues, as do other sciences as pharmaceutical or aromatherapy (Grigore and Grigore, 2008).

For this reason we can say that phytotherapy remains the cleanest and most natural of sciences. (Parvu, 2002) The active principles from medicinal plants are synthesized in the plant cells. In order to properly exploit them, these natural substances must be found in various phytotherapeutic forms (Grigore, 2005).

The main purpose of our research was to obtain active principles with positive effect on lipid metabolism.

The main objectives were: a) selection of plant materials with high content of active principles involved in the regulation of lipid metabolism; b) pharmacognostical characterization of the selected plant products; c) qualitative and quantitative analysis of the active compounds found in the plant extracts.

MATERIALS AND METHODS

Experiments focused on the following plant materials: sea buckthorn (*Hippophae rhamnoides*), hawthorn leaves and flowers (*Crataegus oxyacantha*), garlic bulbs (*Allium sativum*) and maple buds (*Acer campestre*).

The selected materials were tested in order to characterize, identify and determine the content of active compounds.

The pharmacognostical analysis consisted of a set of qualitative and quantitative methods carried out to determine the identity, purity and quality of the plant product. Thus, several determinations have been performed: macroscopic examination, determination of purity, determination of humidity and imbibition factor (Gird et al., 2008).

Extraction of the biologically active substances from plant materials was performed by maceration using alcoholic and hydroalcoholic solutions (Dragota, 2005; Ionescu and Savapol, 1997).

Qualitative determinations of the active principles in the plant products aimed mainly to identify flavones, phytosterols and reducing compounds.

Quantitative determinations aimed: lipolytic activity, flavones, phytosterols, reducing sugars and ascorbic acid.

Lipolytic activity was evaluated by a titrimetric method, based on the determination of fatty acids released into the reaction medium after the action of the enzyme on the substrate, olive oil. One unit of lipase activity is defined as the amount of enzyme that releases 1 umol of fatty acid (Groposilă-Constantinescu, 2010). Determination of flavones was performed by a spectrophotometric method, based on the determination of absorbance of the chelate complexes resulted from the reaction of flavones with aluminum chloride (Hooper et al., 2008). The amount of reducing sugars was determined by a widely used titrimetric method, known as Schoorl method (Jurcoane et al., 2010). The amount of ascorbic acid was determined by a titrimetric method, based on the property of vitamin C to reduce 2-6dichlorophenol-indophenol to a leucoderivate (Dumitru and Iordachescu, 1981).

RESULTS AND DISCUSSIONS

1. Pharmacognostical determinations

From the point of view of macroscopic characterization of plant materials, they have met all the quality requirements of their use for subsequent qualitative and quantitative analysis, as described in the Romanian Pharmacopoeia, Xth Edition (FR X, 1998). Determination of humidity and imbibition factor led to the following results:

| Table 1. | Humidity | and | imb | ibi | tion | factor | of | the | tested | |
|----------|----------|-----|-----|-----|------|--------|----|-----|--------|--|
| | | | | | | | | | | |

| plant materials | | | | |
|--------------------------|----------|------------|--|--|
| Plant material | Humidity | Imbibition | | |
| | (%) | factor (%) | | |
| Pulverized sea buckthorn | 1 | 10,3-10,9 | | |
| Pulverized hawthorn | 0,8 | 10,7-11,2 | | |
| leaves and flowers | | | | |
| Garlic bulbs | 93 | 2,8-2,9 | | |
| Maple buds | 82 | 2,1-2,3 | | |

Relatively high values obtained in case of pulverized products can be correlated with the presence of relatively large amounts of various mucilaginous substances.

2. Identification of active principles

Identification reactions of active principles revealed the targeted compounds in most of the materials tested.

Evaluation of the results obtained in this analysis is presented in the following tables:

Table 2. Identification of flavones

| Plant material | Type of reaction: | Intensity of | |
|-----------------|-------------------|--------------|--|
| | Shibata reaction/ | the reaction | |
| | NaOH reaction | | |
| Pulverized sea | orange/yellow | ++/+++ | |
| buckthorn | oralige/yenow | ++/+++ | |
| Pulverized | | | |
| hawthorn leaves | orange/yellow | +++ / +++ | |
| and flowers | | | |
| Garlic bulbs | - / - | - / - | |
| Maple buds | orange/yellow | + / + | |

Table 3. Identification of reducing compounds

| Plant material | Intensity of the | | |
|--------------------------------|------------------|--|--|
| | reaction | | |
| Pulverized sea buckthorn | +++ | | |
| Pulverized hawthorn leaves and | | | |
| flowers | | | |
| Garlic bulbs | ++ | | |
| Maple buds | - | | |

Table 4. Identification of phytosterols

| Plant material | Intensity of the | | |
|--------------------------------|------------------|--|--|
| | reaction | | |
| Pulverized sea buckthorn | +++ | | |
| Pulverized hawthorn leaves and | | | |
| flowers | $\tau \tau \tau$ | | |
| Garlic bulbs | ++ | | |
| Maple buds | + | | |

3. Quantitative determination of the active principles

Analyses conducted to determine the lipolytic activity of plant extracts showed a high lipolytic activity in sea buckthorn fruits, 6.3 U/g and garlic bulb extract, 4.9 U/g. Also, a high lipolytic activity was found in leaves and flowers of hawthorn, 3.2 U/g, while the maple buds extract was very low, only 0.4 U/g.

The results obtained for the determination of lipolytic activity in plant extracts are shown in the following figure:

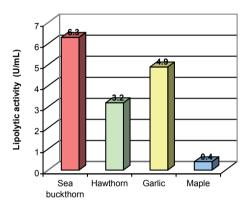


Figure 1.Lipolytic activity of plant materials

Thus, the results obtained for flavones content are shown in the figure below.

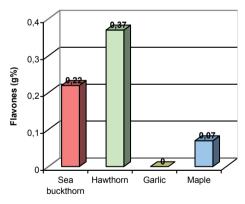


Figure 2. Flavones content of plant materials

The highest values of flavones content was obtained in the leaves and flowers of hawthorn. A fairly large content was also obtained in case of sea buckthorn fruit. A lower content was observed in maple buds. In case of garlic bulbs analysis confirmed the preliminary identification of these compounds in which the results of flavones content was zero.

For the quantitative determination of polyhydroxyphenols, a spectrophotometric method was used, in which was determined the izonitrozoderivates formed from the phenols in the alkaline media, in the presence of nitrous acid.

The content of polyhydroxyphenols can be seen in the following figure.

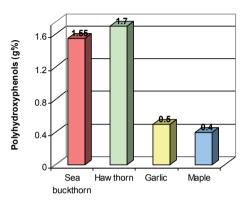


Figure 3. Polyhydroxyphenols content of plant materials

From the results shown in Figure 4, it can be observed that the highest values of reducing sugar content was obtained in case of sea buckthorn fruit. Also, a relatively high content of reducing sugar was obtained in garlic and hawthorn. A very low content was observed in maple buds.

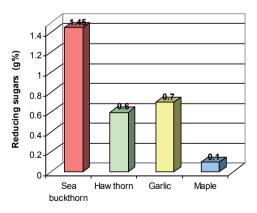


Figure 4. Reducing sugars content of plant materials

The results showed that, as mentioned in the literature, the highest content of vitamin C was

obtained for sea buckthorn fruit, reaching a maximum of 483 mg/100 g dry product.

In case of the leaves and flowers of hawthorn, the amount of vitamin C was 23.4 mg/100 g dry product and for garlic bulbs, 17.3 mg/100 g fresh product.

Maple buds did not presented in their composition viatmina C.

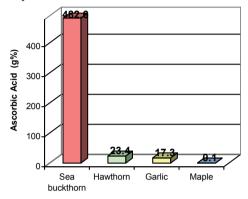


Figure 5. Ascorbic acid content of plant materials

CONCLUSIONS

Of all the plant materials tested, sea buckthorn fruit have the highest content of flavones, lipases, ascorbic acid and reducing sugars.

Corroborating the results obtained in the qualitative and quantitative analyzes of the plant materials tested, we selected, in order to obtain a complex bioproduct, the sea buckthorn fruit powder, the powder of hawthorn leaves and flowers and the extract of garlic bulbs, rich in many active principles involved in regulation of lipid metabolism.

REFERENCES

Dragota D., 2005, *Tehnologii noi de extracție și izolare a unor substanțe active din flora României*, Ed. Chiminform Data, București

Dumitru I. F., Iordachescu D., 1981, *Biochimie practica-proteine si enzime*, Ed. Medicala, Bucuresti

Farmacopeea Română X, 1998, Ed. Medicală, București Gird C.E., Dutu L.E., Popescu M.L., Pavel M., Iordache A.T., 2008, *Bazele teoretice si practice ale analizei farmacognostice*, vol. I, Ed. Curtea Veche, Bucuresti

Grigore G., 2005, *Plante medicinale, ceaiuri si recomandări fitoterapeutice*, Ed. All, București

Grigore G., Grigore C., 2008, *Ghidul plantelor medicinale si al bolilor tratate cu plante*, Ed. All, Bucuresti, 75-80

Gropoșilă-Constantinescu Diana-Gabriela, 2010, *Teză* de doctorat: Studii privind obținerea unor biopreparate cu activitate lipolitică din surse microbiene și plante medicinale indigene, Bucuresti

Hooper L., Kroon P., Rimm E., Cohn J., Harvey I., Le Cornu K., Ryder J., Hall W., Cassidy A., 2008, *Flavonoids: flavonoid rich-foods and cardiovascular risk, a meta-analysis of randomized cpntroled trails*, The American Journal of Clinical Nutrition

Ionescu Stoian P., Savapol E., 1997, *Extracte farmaceutice vegetale*, Ed. Medicală, București

Jurcoane Ștefana, Gropoșilă-Constantinescu Diana, Diguță Camelia-Filofteia, 2010, *Biotehnologie Generală-Îndrumător de lucrări practice*, Ed. Univ. Buc. "ARS DOCENDI"

Parvu C., 2002, Enciclopedia plantelor, Plante din flora României vol. 1, Ed. Tehnic, București.