BIOLOGIC ACTIVE COMPOUNDS IN NATURAL FOOD SUPPLEMENTS WITH HYPOLIPEMIANT PROPERTIES

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Abstract: Hof. Lipomin and Colerd, natural, original, food supplements were pharmacologically tested and well known for their hypolipemiant properties. They contain garlic (Allium sativum), leek (Allium porrum), juices and extracts, flax (Linum usitatissimum) seed -oil, rosemary (Rosmarinus officinalis) leaves extract as well as a propolis extract. The efficiency of the products is due to the synergism of the numerous biological active compounds present in their composition. The object of this work is to carry on a study aiming to determine the main active biologic compounds, using actual methods: Gas- Chromatography-mass spectrometry, High Performance Liquid Chromatography, UV-VIS Spectrophometry, and Absorption- Emission Atomic Spectrometry.

Key words: AEAS, GC-MS, HPLC, natural hypolipemiant food supplements, plant extracts, UV-VIS 3.

1. Introduction

As a result of our previous work [1], [14] concerning the natural treatment of cardiovascular diseases, two original vegetal food supplements having the generic name „Hof. Lipomin” and „Colerd” [2], [3] were developed. The pharmacologic studies of these products, emphasized a remarkable hypolipemiant, hypocholesterolemiant activity with animals having TRITON WR1339 induced hyperlipemia.

For the accuracy and results of analytical and pharmacological studies, the two patents Hof. Lipomin and Colerd received the highest prize (the Gold medal) at the Salon International Des Inventions Geneve, from the 12th of April, 2013 for Hof. Lipomin and Colerd and at the Salon International Des Inventions Cluj- Napoca, in 2008.

Obtained by the association of garlic (Allium sativum), leek (Allium porrum) juices and extracts, flax (Linum usitatissimum) seed oil, rosemary (Rosmarinus officinalis) leaves extract and propolis extract, the new products are able to decrease the total serum lipids, total cholesterol, LDL cholesterol and serum triglycerides, and having a cardio-protective effect. The plant choice was based on their known pharmacologic properties [4], [5], [6], [7], [8], [9].

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The pharmacologic properties are due to the composition of the used plants rich in various antioxidants, polyphenol and flavone derivatives, polyunsaturated fatty acids glycerides, sulphur compounds redox enzymes, and minerals.

The object of this study is to characterize the profile of antioxidants free radical scavengers in Hof. Lipomin and Colerd in order to explain the cardioprotective antiscorosclerotic hypolipemiant and hypocholesterolemiant activity.

2. Material and Methods

The juices of garlic and leek were obtained starting from fresh bulbs and sheaths, by pressing. The rosemary and propolis extract were prepared by usual standardized procedures (extraction with 70% ethanol), Propolis was supplied from our own crop (CC. ST 314) and the flaxseeds oil from Brad Society. The new products, Hof. Lipomin and Colerd, were prepared using the original formula which makes the object of two Romanian patents [2], [3].

Solvents reactive grade, reagents from (Fluka Germany) were used, and reference substances: chlorogenic galic, rosmarinic, p-cumaric, caffeic acids, epicatethin, vitexin, isovitexin, quercetin, kamferol, Allicin alliiin (Sigma Aldrich) were available.

The total polyphenol content was determined by UV-VIS spectrophotometry using a JASCO 530 spectrophotometer using a sodium wolframate reaction and reading the absorbency at 660 nm expressed in chlorogenic or caffeic acid equivalents [11]. Flavone derivatives were determined by their reaction with AlCl₃, reading their absorbency at 430 nm and expressing the total in rutin [11].

HPLC methods were developed starting from the literature data [12], [13].

The determinations were performed on a DIONEX HPLC system equipped with a Diode Array Detector 340. The extracts were injected directly into the HPLC system.

For the polyphenol and flavone derivatives: Column PP C18, 200x4.6 mm, 10µm, Gradient working, Mobile phases: A 0.01N phosphonic acid, B methanol ambient temperature Flow rate 1ml/min, wavelength 280 nm.

For the sulphur compounds in the garlic and leek juices the method mentioned in the European Pharmacopoea [15] was used.

The CFG-MS, method used for the determination of Polyunsaturated Fatty acids esters was presented in our previous work [1].

The mineral contents were determined by AEA spectrometry, using an AVANTA Spectometer equipped with a computer and lamps for every element. Mineralization was realized with chlorhydric and nitrous acids.

3. Results and Discussions

The contents in the main antioxidants free radical scavengers: polyphenol, flavone derivatives. The main antioxidant contents in Hof.Lipomin and Colerd are shown in Table 1.

<table>
<thead>
<tr>
<th>No.</th>
<th>Product</th>
<th>Polyphenol derivs in chlorogenic acid equiv, [mg/cpr]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Hof.Lipomin</td>
<td>2.6</td>
</tr>
<tr>
<td>2</td>
<td>Colerd</td>
<td>2.4</td>
</tr>
</tbody>
</table>
Polyphenol and flavone derivatives determined in Hof. Lipomin and Colerd are mainly due to the presence of rosemary and propolis extracts. The HPLC chromatogram for rosemary extract is presented in Figure 1 and for the propolis extract in Figure 2.

**Fig. 1. HPLC Chromatogram of the Rosemary Extract**

**Fig. 2. HPLC Chromatogram of the Propolis Extract**
Various polyphenolcarboxilic acids, p-cumaric, rosemarinic, caffeic and flavone derivatives were identified. Aliciin, alliin and other sulphur compounds were quantified in Hof.Lipomin and in Colerd (Figure 3 and Figure 4).

Fig. 3. HPLC Chromatogram for Hof.Lipomin

Fig. 4. HPLC Chromatogram for Colerd
Polyunsaturated fatty acids-glycerides were determined in Hof.Lipomin, by HPLC after derivatization to methylesters [1]. The content of the various fatty acids in total fatty acids in Hof.Lipomin are presented in Table 2.

The content of the main fatty acids in Hof.Lipomin

<table>
<thead>
<tr>
<th>No.</th>
<th>Fatty acid</th>
<th>Total fatty acids in Hof.Lipomin, [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Palmitic</td>
<td>5.66</td>
</tr>
<tr>
<td>2</td>
<td>Stearic</td>
<td>5.99</td>
</tr>
<tr>
<td>3</td>
<td>Oleic</td>
<td>6.03</td>
</tr>
<tr>
<td>4</td>
<td>Linoleic</td>
<td>16.50</td>
</tr>
<tr>
<td>5</td>
<td>Linoleic</td>
<td>4793</td>
</tr>
</tbody>
</table>

High percents of unsaturated and polyunsaturated fatty acids are present in Hof.Lipomin. The content of minerals for Hof.Lipomin and Colerd are presented in Table 3.

The contents of minerals in Hof. Lipomin and Colerd

<table>
<thead>
<tr>
<th>Product</th>
<th>Na</th>
<th>K</th>
<th>Ca</th>
<th>Mg</th>
<th>Fe</th>
<th>Mn</th>
<th>Zn</th>
<th>Cu</th>
<th>Pb</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hof.Lipomin</td>
<td>80</td>
<td>100</td>
<td>900</td>
<td>10</td>
<td>15</td>
<td>2.0</td>
<td>2.5</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Colerd</td>
<td>60</td>
<td>80</td>
<td>1200</td>
<td>11</td>
<td>17</td>
<td>1.0</td>
<td>3.3</td>
<td>0.7</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Besides Na, K, Mg, Ca, small quantities of Fe, Mn, Zn, Cu, minerals, involved in the redox enzymes structure, were detected in Hof.Lipomin and Colerd [10]. The performed study justifies the antisclerotic, hypcholesterolemiant, hypolipemiant activity determined in the case of animals, having induced hyperlipemia, in a recently published paper.

4. Conclusions

A study was performed using actual methods, HPLC, UV-VIS spectrometry, GC-MS, AEA spectrometry aiming to realize the antioxidant compounds profile for Hof.Lipomin and Colerd, new hypolipemiant natural food supplements. The total content of polyphenol and flavone derivatives established by UV-VIS Spectrophotometry and HPLC, the sulphur compounds, and polyunsaturated fatty acids glicerides determined by GC-MS, in addition to the minerals detected by AEA Spectrometry, account for the antioxidant hypolipidemic, hypcholesterolemiant, antisclerotic activity of the new products.

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References