Polyphenols and Minerals, Antioxidants in the Plants Used in the Natural Treatment of Hepatobiliary Disorders

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The seeds of milk thistle (Silybum marianum), the leaves of rosemary (Rosmarinus officinalis), artichoke (Cynara scolymus), dandelion (Taraxacum officinale), and french tamarisk (Tamarix gallica) and burdock (Arctium lappa) roots are plants well known for their hepatobiliary protective properties. The present paper aims at realizing a comparative analysis concerning the polyphenol derivatives and minerals contents from the powders of the above mentioned plants and from their extracts. Actual methods, such as HPLC, UV-Vis Spectrophotometry, and AEA-Spectrometry were used. The results of this research confirm the higher contents of polyphenols in powders than in the plant extracts. Numerous polyphenolcarboxylic acids (cafeic, chlorogenic, fenolic, cichoric, cinarin, rosemarinic), flavone derivatives (rutin, quercetin, kaempferol, apigenin, luteolin) and flavolignans (silybin A, silybin B and derivatives) in the milk thistle seeds were identified and quantified thru HPLC methods. Various minerals were determined in the powders and the extracts of the plants. The data resulted in this analysis can be used to manufacture new food supplements as natural remedy in the treatment of hepatobiliary disorders.

Keywords: HPLC, UV-Vis, AEA-Spectrometry, polyphenols, minerals, plants

This research represents a continuation of our previous interest concerning natural polyphenols and minerals as antioxidants and free radical scavengers [1-3]. Considered as an important cause of several actual and grave disorders in connection to the oxidative stress, the problem of the dangerous surplus of the free radicals is an interesting one for both the patients, and the physicians [4, 5].

Working as antioxidants, the polyphenol derivatives scavenge free radicals by complex reaction mechanisms which imply substitution, addition and electron transfer reactions [1]. In this way, the devastating effect of free radicals which are able to damage the cell membrane, subcellular organelles, enzymes, proteins and nucleic acids is annihilated. The hepatic cells are very susceptible to the oxidative stress and to the assault of free radicals. For that reason a natural nontoxic remedy like phytopolyphenols are vital for liver disorders treatment. A large variety of plants, such as seeds of milk thistle (Silybum marianum), the leaves of rosemary (Rosmarinus officinalis), artichoke (Cynara scolymus), dandelion (Taraxacum officinale), french tamarisk (Tamarix gallica) and burdock roots (Arctium lappa) are well known for their hepatoprotective properties mostly due to the presence of polyphenol derivatives.

A valuable recent research [6] summarizes the phytotherapeutic properties of milk thistle seeds. The research underlines the antioxidant [7], hepatoprotective [8], anti-inflammatory [9], immunomodulatory [10], neurocardioprotective [11], antitumor [12], and antiviral properties [12] among several others [11, 13].

According to a recent paper, a series of polyphenol and flavone derivatives flavolignans are identified: silybin A, silybin B, isosylibin A, isosylibin B, silycristin and silydianin in the milk thistle seeds. All these constituents account for the phytotherapeutic properties [14]. The profile of polyphenols and phenolic acids of artichoke (Cynara scolymus) is the subject of certain recent articles [16-18]. Using HPLC-DAO-MS caffeoylchinnamic acids, dicafeoylchinnamic acids, apigenin, luteolin and their glycosides were identified in accordance to their antioxidant, antiviral, hepatoprotective, immunomodulatory, anticancerogenic activities [17, 18].

The roots of burdock (Arctium lappa), a popular plant, are used in hypertension, atherosclerosis, hepatitis, geriatric diseases due to their antioxidant activity of polyphenolic constituents [19-23]. The rosemary (Rosmarinus officinalis) shoots is the subject of recent research [24-27]. A great number of activities, antioxidant, antibacterial, diuretic, anti-ulcercogenic, hepatoprotective, is mentionated [27, 28].

Known for their antioxidant, antiinflammatory, antiarthritic properties, the leaves of french tamarisk (Tamarix gallica) were studied in connection to polyphenolic compounds, identified by HPLC [29, 30]. Dandelion leaves are known for their antioxidant, diuretic, hepatoprotective properties [31, 32].

Having an important contribution in determination of antioxidant activity, the minerals are present in all studied plants. Some of them as Cu, Fe, Ni, Mn, Zn are implied in the structure of redox-enzymes like superoxide dismutase [33, 34]. They all ensure a well balance of the organism functions.

Taking into account the mentioned data, the object of this research is to realize a comparative study concerning the contents of polyphenol derivatives and minerals in the powders and extract of these plants, aiming to formulate new natural remedy in the treatment of hepatobiliary disorders.

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 Experimental parts

Materials and methods

Locally cultivated rosemary, artichoke, dandelion, french tamarisk leaves, burdock roots and milk thistle seeds were used for extraction.

Reference substances: High-purity standards (95%) chlorogenic acid, gallic acid, p-cumaric acid, caffeic acid, ferulic acid, rosmarinic acid, quercetin, luteolin, cynarin, apigenin, silybin A and silybin B obtained from SIGMA-ALDRICH and PhytoLab.

Reagents: High-purity methanol, ethanol, acetonitrile.

gradient grade for liquid chromatography were obtained from Merck (Germany).

The leaves of rosemary, dandelion, artichoke and french tamarisk, burdock roots and milk thistle seeds were evenly dried and ground in a mill. Plant extracts were obtained by maceration for 7 days at room temperature. A solvent extraction with ethyl alcohol aqueous solution of 40% concentration in a variable ratio of powders weight: solvent volume was applied.

Two HPLC methods to determine polyphenols and flavone derivatives, based on the literature data [25, 36 - 38] were performed and validated.

The first HPLC method, qualitative, was based on comparisons of retention times and UV spectra database stored in the computer. The UV spectra in our database was obtained from standards injection of gallic acids, chlorogenic acid, caffeic acid, p-cumaric acid, ferulic acid, tannins, epicatechin, rutin, quercetin, luteolin, kaempferol, and apigenin. This method was qualitatively validated by testing sensitivity (resolution between peaks: ferulic acid – p-cumaric acid 3.5), recovery (83%) and precision (RDS <2.5%) [39].

The second HPLC method used for quantitative determination of sylimar in the milk thistle seeds and extracts was validated by testing for sensitivity, linearity, precision and recovery with silybin A used as standard.

The reversed-phase high performance liquid chromatographic (HPLC) analyses were carried out on DIONEX system equipped with a Diode Array detector (200-600 nm) and a gradient performing pump (P580).

Flavone and flavonoids separation from artichoke, french tamarisk, dandelion and burdock extracts was performed in a 200 mm long and ∅ =4.6 mm C18 column, at 35°C temperature, mobile phase A=0.5% V/V phosphoric acid and mobile phase B=acetonitrile.

Quantitative determination of sylimar in milk thistle seeds and extract was validated by testing for sensitivity, linearity, precision and recovery with silybin A used as standard.

The reversed-phase high performance liquid chromatographic (HPLC) analyses were carried out on DIONEX system equipped with a Diode Array detector (200-600 nm) and a gradient performing pump (P580). Flavone and flavonoids separation from artichoke, french tamarisk, dandelion and burdock extracts was performed in a 200 mm long and ∅ =4.6 mm C18 column, at 35°C temperature, mobile phase A=0.5% V/V phosphoric acid and mobile phase B=acetonitrile.

Quantitative determination of sylimar in milk thistle seeds and extract were carried out in the same equipment of 200 mm length and ∅=4 mm RP-8 column, at 35°C temperature, mobile phase A=0.01 M phosphoric acid and mobile phase B=acetonitrile.

The active ingredient amounts found in plant and extract are described by (1) and (2).

Following partial mass balance applied to active ingredient (3) it is obvious that mass concentration of extract active ingredient is less that mass concentration in plant active ingredient (7). These observations are found also in the literature data [41].

The greatest value for the total polyphenols was determined for rosemary and french tamarisk and the

<table>
<thead>
<tr>
<th>No.</th>
<th>Plant</th>
<th>Powder</th>
<th>Total polyphenol [%]</th>
<th>Total flavone derivatives [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>In caffeic acid</td>
<td>In chlorogenic acid</td>
</tr>
<tr>
<td>1.</td>
<td>Artichoke leaves</td>
<td>powder</td>
<td>2.18</td>
<td>4.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>extract</td>
<td>0.58</td>
<td>1.17</td>
</tr>
<tr>
<td>2.</td>
<td>Rosemary leaves</td>
<td>powder</td>
<td>2.90</td>
<td>5.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>extract</td>
<td>2.35</td>
<td>4.74</td>
</tr>
<tr>
<td>3.</td>
<td>Dandelion leaves</td>
<td>powder</td>
<td>1.55</td>
<td>3.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>extract</td>
<td>0.16</td>
<td>0.32</td>
</tr>
<tr>
<td>4.</td>
<td>French tamarisk leaves</td>
<td>powder</td>
<td>2.90</td>
<td>5.80</td>
</tr>
<tr>
<td></td>
<td></td>
<td>extract</td>
<td>0.37</td>
<td>0.75</td>
</tr>
<tr>
<td>5.</td>
<td>Burdock roots</td>
<td>powder</td>
<td>0.90</td>
<td>1.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>extract</td>
<td>0.40</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Table 1

TOTAL POLYPHENOL AND FLAVONE DERIVATIVES IN THE PLANT POWDERS AND EXTRACTS
values vary as follows: rosemary > french tamarisk > artichoke > dandelion < burdock and for flavone derivatives: rosemary lives > dandelion > artichoke > french tamarisk.

Working according to European Pharmacopoeia [40], 3.4% rosmarinic acid was found in rosemary powders and 0.41% in rosemary extract.

Using the above described HPLC method, 1.94% silymarin expressed in silybin A was quantified in milk thistle powders and 0.33% in milk thistle extract.

The HPLC chromatograms, presented in figures 1 to 5, identified a series of natural compounds having antioxidant free scavenging properties.

Numerous polyphenol carboxylic acids, such as caffeic, chlorogenic, ferulic, cichoric, cinaric, and flavone derivatives, such as rutin, quercetin, quercetin, luteolin, apigenin were identified in all analyzed plants, while silybin A, silybin B, and derivatives were found in the milk thistle seeds.

The AEA spectrometry determinations of minerals in powders and extracts of analyzed plants are presented in tables 2 and 3.

For the analyzed plants, the mineral content in plant powders is bigger than the extract content they originate from.

Artichoke and french tamarisk powder are rich in Ca, artichoke, burdock, rosemary and french tamarisk are rich in Mg, artichoke and french tamarisk are rich in Na, and artichoke, dandelion and burdock are rich in K.

Among the analyzed plants, dandelion and burdock is distinguished by the highest content of minerals with antioxidant properties (Mn, Fe, Zn, Cu).

The biggest contents of Zn and Cu were found in the milk thistle seeds powder, followed by artichoke.

The richest powder in Fe is dandelion and burdock.
Conclusions

A comparative study was performed concerning the contents of polyphenols, flavone derivatives and minerals in powders and extract of milk thistle seeds, rosemary, artichoke, dandelion, French tamarisk leaves and burdock roots.

Determined by UV-Vis spectrophotometry and expressed in caffeic/chlorogenic acids equivalents, the content in polyphenol derivatives is greater in powders than in extracts.

This content varies as follows: for polyphenols rosemary > French tamarisk > artichoke > dandelion < burdock and for flavone derivatives: rosemary lives > dandelion > artichoke > French tamarisk. Using HPLC methods, numerous polyphenol carboxylic acids, such as caffeic, chlorogenic, ferulic, cichoric, cinaric were identified. Flavone derivatives, such as rutin, quercetin, quercetrin, luteolin, apigenin were identified in all analyzed plants.

Silybin A, silybin B, and their derivatives were found in milk thistle seeds.

Various minerals such as Na, K, Ca, Mg, Mn, Fe, Zn, Cu were found in plants' powders and extracts, and the mineral contents are bigger in plant powders than in the plant extracts.

The data resulted in this analysis can be used in manufacturing of new natural food supplements with hepatoprotective, choleretic and cholagogue properties.

References

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