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 (caffeic, chlorogenic, ferulic, cichoric, cinaric, rosemarinic), flavone derivatives (rutin, quercetin, kaempherol, 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, adition 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 caffeoylcinnamic acids, dicaffeoylcinnamic 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-ulcerogenic, hepatoprotective, is mentionated [27, 28].

Known for their antioxidant, antiinflammatory, antidiarrheal 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 sylimarin in the 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 cromathographic (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(R).

Quantitative determination of sylimarin 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=methanol [40].

Total polyphenols content and flavone derivatives were determined by UV-Vis spectrophotometry using a *JASCO 530* Spectrophotometer. The Folin-Ciocalteu reaction was applied to determine total polyphenols. The method implied the extraction of powders with aqueous methyl alcohol (40%) at the boiling temperature. The results were expressed in caffeic or chlorogenic equivalents [1, 35]. The flavone derivatives content was determined by the reaction with $AlCl_3$ and total content was expressed in rutin [1, 35].

An UV-Vis method described in European Pharmacopoeia Ed. 7 was used to determine rosmarinic acid [40].

The mineral contents of the powders and plants extracts were determined by AEA-spectrometry using an absorbtion and emission spectrometer AVANTA PM – air acetylene – flame equipped with computer and cathodic lamp for every element. The samples have been mineralized by HNO_3, H_2O_2, and HCl [21], using an under-pressure Berghof-MWS-Microwave digestion device.

Results and discussions

Table 1 presents the results obtained in the determination of polyphenol and flavone derivatives by UV-Vis spectrophotometry, expressed as caffeic/chlorogenic equivalents and rutin equivalents, for the powders and extracts of analyzed plants.

It is obvious that in almost all plants, the value of total polyphenols derivatives expressed in caffeic /chlorogenic acids equivalents and total flavone derivatives in rutin equivalents for the powders are greater than that of the plants extracts.

This phenomenon may be explained through the following equations:

$$m_1 = \frac{M_1 c_1}{100} (1);\ m_2 = \frac{M_2 c_2}{100} (2);\ \frac{M_2 c_2}{100} = \eta \frac{M_1 c_1}{100} (3);\ c_2 = \eta \frac{M_1}{M_2} c_1 (4);\ \eta < 1 (5);$$

$$M_1 < M_2 \Rightarrow \frac{M_1}{M_2} \ll 1 (6);\ \Rightarrow c_2 \ll c_1 (7)$$

M_1 = plant mass;
M_2 = extract mass;
m_1 = plant active ingredient mass;
m_2 = extract active ingredient mass;
c_1 = mass concentration of plant active ingredient;
c_2 = mass concentration of extract active ingredient;
η = extraction yield;

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

Extraction process is characterized both by extraction ratio (M_1:M_2), which is less that 1, and by extraction yield, which is also less that 1.

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

No	Plant	Powder / Extract	Total polyphenol [%]		Total flavone derivatives [%], in rutin equivalent
			In caffeic acid equivalent	In chlorogenic acid equivalent	
1.	Artichoke leaves	powder	2.18	4.40	2.00
		extract	0.58	1.17	0.58
2.	Rosemary leaves	powder	2.90	5.80	3.12
		extract	2.35	4.74	2.33
3.	Dandelion leaves	powder	1.55	3.13	2.33
		extract	0.16	0.32	0.19
4.	French tamarisk leaves	powder	2.90	5.80	1.90
		extract	0.37	0.75	0.26
5.	Burdock roots	powder	0.90	1.81	1.40
		extract	0.40	0.80	0.80

Table 1
TOTAL POLYPHENOL AND FLAVONE DERIVATIVES IN THE PLANT POWDERS AND EXTRACTS

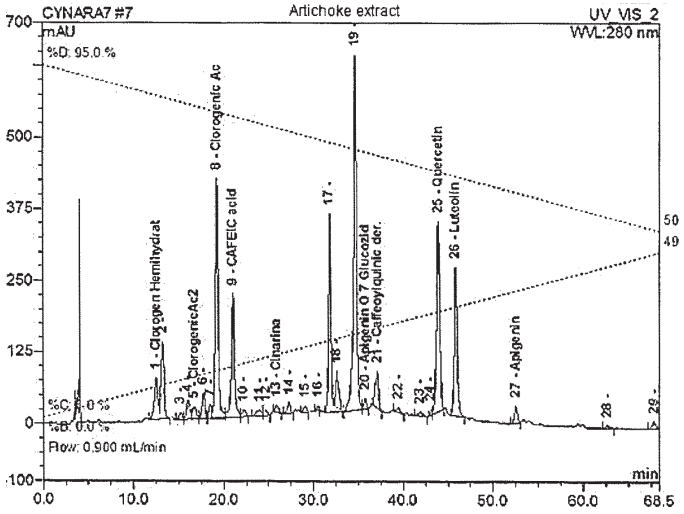

Fig.1. HPLC chromatogram for artichoke extract

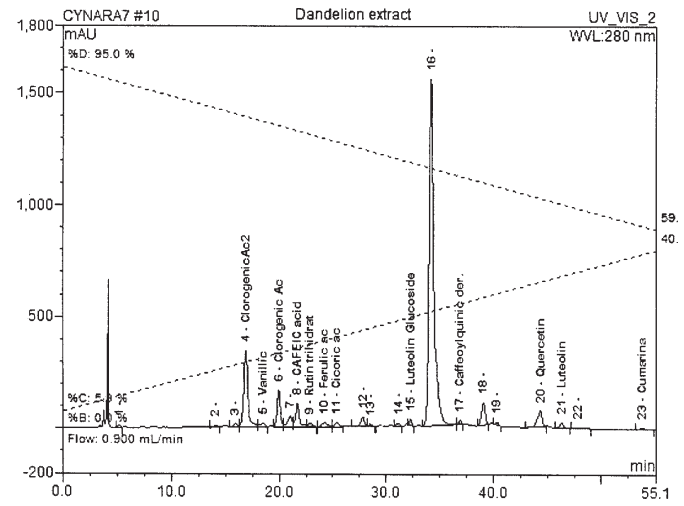

Fig.2. HPLC chromatogram for dandelion extract

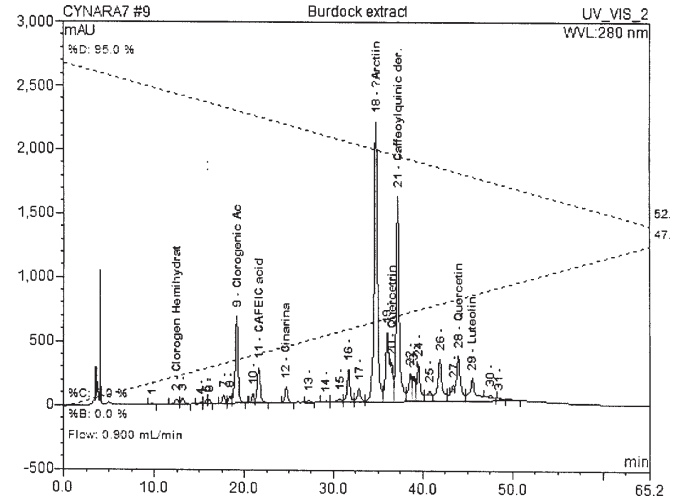

Fig.3. HPLC chromatogram for burdock extract

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.

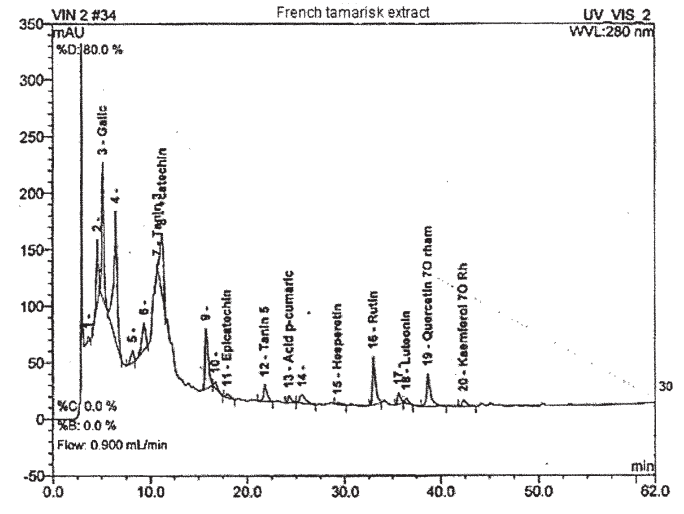

Fig.4. HPLC chromatogram for french tamarisk extract

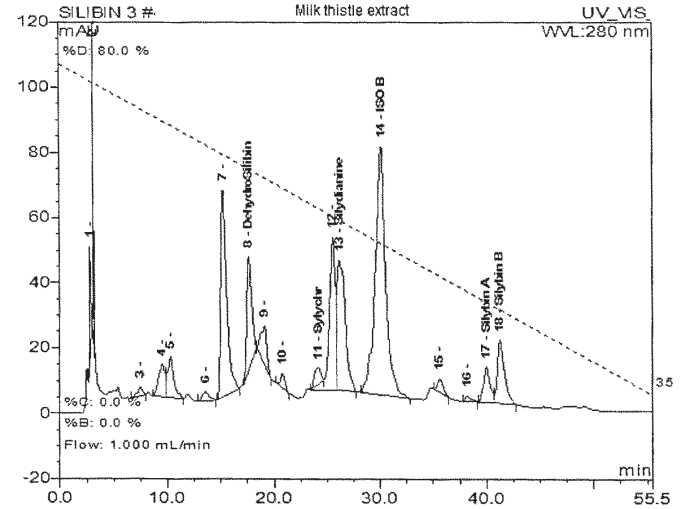

Fig.5. HPLC chromatogram for milk thistle extract

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, quercetrin, 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.

No	Plant	Powder Extract	Mineral content [mg %]			
			Ca	Mg	Na	K
1.	Artichoke leaves	powder	1600	700	1900	5000
		extract	40	80	200	300
2.	Rosemary leaves	powder	300	550	140	840
		extract	6	80	30	900
3.	Dandelion leaves	powder	400	450	160	3000
		extract	7	25	15	300
4.	French tamarisk leaves	powder	1000	530	600	400
		extract	16	70	450	200
5.	Burdock roots	powder	600	560	320	2000
		extract	4	15	30	500
6.	Milk thistle seeds	powder	120	340	40	800
		extract	9	80	30	500

Table 2
Ca, Mg, Na AND K CONTENTS IN POWDERS AND EXTRACTS OF ANALYZED PLANTS

No	Plant	Powder Extract	Mineral content [mg %]			
			Mn	Fe	Zn	Cu
1.	Artichoke leaves	powder	6	30	8	3
		extract	0.15	0.7	0.2	0.1
2.	Rosemary leaves	powder	3.2	25	5.8	0.8
		extract	0.4	0.2	0.8	0.06
3.	Dandelion leaves	powder	40	470	7	0.3
		extract	0.2	0.5	0.3	0.2
4.	French tamarisk leaves	powder	1.7	15	5.5	0.4
		extract	0.2	0.05	0.9	0.3
5.	Burdock roots	powder	7	450	5	0.7
		extract	0.07	0.4	0.2	0.3
6.	Milk thistle seeds	powder	5.2	30	7.8	2.8
		extract	0.1	0.1	0.5	0.1

Table 3
Mn, Fe, Zn AND Cu CONTENTS IN POWDERS AND EXTRACTS OF ANALYZED PLANTS

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 poliphenols 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.

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