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BIOLOGICAL ASPECTS, COMPOSITION AND POTENTIAL APPLICATION OF *CYNARA SCOLYMUS* L.

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Abstract
Nature represents an inexhaustible resource for human people's health and care. In the last couple of years, medicinal and aromatic plants (MAPs) became one of the main research fields in Romania due to their therapeutic properties. In this study, Cynara scolymus L. plant was selected, due to its usage as an alternative to synthetic drugs, considering costs and side effects. Furthermore, this plant leaves are a reach source of compounds with powerful benefits in liver protection, cholesterol inhibition, cardiovascular and kidney diseases, indigestion, rheumatism, with antioxidant and antibacterial properties. The present study consisted of chlorogenic acid identification by HPLC, total polyphenol and flavonoids content determination and antioxidant activity evaluation. Due to its wide spread, according to our preliminary study, this plant was harvested from Romanian territory and its extract was processed in optimal conditions. In this study, flavones and polyphenolic compounds were found to be abundant in Cynara scolymus L., particularly the chlorogenic acid.

Keywords: antioxidant activity, Cynara scolymus L., flavonoids, total polypheonol.

1. INTRODUCTION

Cynara scolymus L. is a herbaceous, perennial, allogamous, melliferous plant, part of *Asteraceae* family, original from Mediterranean area (especially Southern Spain, Italy, France and Northern Morocco). This plant has medicinal benefits and as medicinal plant is cultivated in the Southern Romania (Ilfov, Ialomita, Teleorman, Giurgiu, Olt and Dolj Counties). For medical purposes are used the leaves of this medicinal plant. Leaves contributes to the achievement of high quality raw material and the development of plant for maximum production (Păun, 1995; Pârvu, 2006). *Cynara scolymus* L. leaves contain polyphenols, fenolic acid, caffeic acid, mono and dicaffeoylquinic derivatives acids, cynarin (1,3-di-O acid-caffeoylquinic) and chlorogenic acid, biologically active compounds as flavonoids (flavone glycoside, luteolin, apigenin and quercetin), volatile oils (sesqiterpene caryofilene and β-selinene, eugenol, phenylacetaldehyde and decanal), phytosterols (taraxasterol and β-taraxasterol), tannins, acids, glycolic, glyceric, sugars, inulin, enzymes such as peroxidase, cinaropicrină (bitter principle) and other related sesquiterpene lactones, vitamins (B1,

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B2, B3, B5, B6, B9, C, E, K) and minerals (Brand, 1990; Hammounda et al., 1993; Chevallier, 2001; Zhu et al., 2004; Fratianni et al., 2007; Lattanzio et al., 2009; Shen et al., 2010; Lutz et al., 2011; Pandino et al., 2011; EMA/HMPC/150209/2009, 2012; Gouveia et al., 2012; Mutalib et al., 2012;). Inflorescence receptacle contains crude protein, lipids, un-nitrate extractive substances.
Bioactive compounds in leaves of *Cynara scolymus* L., has many beneficial effects on health, having therapeutic applications in human and veterinary medicine. The pharmacological properties of this plant are improving digestive functions (Walker et al., 2001; Bundy et al., 2004), improving hepatic (Omata et al., 2007; Mehmetçik et al., 2008; Miccadei et al., 2008) and bile functions (Kirchhoff et al., 1994), balancing cholesterol and glucose levels (Englisch et al., 2000; Qiang et al., 2012), impressive antioxidant activity of leaves, flowers and seeds and antibacterial activity on *Staphylococcus aureus* and *Escherichia coli* (Zhu et al., 2004; Falleh et al., 2008). Futhermore, *Cynara scolymus* L. regenerates liver cells, increases appetite, strengthens the liver's antitoxic function, lowers blood cholesterol levels, increases urinary flow, it works favorably in hepatic steatosis and treating various forms of jaundice (Pârvu, 2006).
This medicinal plant is also recommended in coronary hepatitis, cirrhosis, congestive and hepatic failure, corneal nephritis, enteritis, angina pectoris, atherosclerosis, hemorrhoids, asthenia, overuse, gout, rheumatism, intoxication (Chevallier, 2001; Pârvu, 2006).
The present study consisted of identification of the importans biologically active compounds from *Cynara scolymus* L. using nowadays methods.

2. MATERIALS AND METHODS

Plant extracts

Cynara scolymus L. plant material used was cultivated in Romania and the leaves necessary for analysis were harvested at maturity when a dark-green coloration appears. Plant extracts used in the analysis methods were obtained by different procedures. All the reagents and standards were analytic grade, purchased from Merck KGaA (Germany) and were used without any purification. The bidistilled water used in the experiments was obtained in the laboratory.
Plant extract (test solution) used for identification of the chlorogenic acid from *Cynarae folium* by HPLC was obtained by taking 1g of dried crushed leaves mixed with 50mL methanol and heated to reflux in a water bath at 70°C for 1 hour; the solution was centrifuged and the supernatant was transferred into a 200 mL Erlenmeyer flask. In the end, the procedure was repeated and the extract was diluted to 200 mL with bidistilled water.
Plant extract (test solution) used for identification of the total polyphenols content expressed as chlorogenic acid equivalents was obtained by taking 10g of dried crushed leaves mixed with 100 mL ethanol solution 50% (v/v) and heated to boiling in a water bath to reflux for 30 minutes. The hot solution was filtered through cotton in a flask and, after cooling, made up to 100 mL by washing the residue with the same solvent. A 5 mL solution was diluted to 50 mL in a volumetric flask with ethanol 50% (v/v).
Plant extract (test solution) used for identification of the flavones expressed in rutin was obtained by taking 5 g of dried crushed leaves mixed with 100 mL ethanol 50% (v/v) and heated to boiling in a water bath to reflux for 30 minutes. The hot solution was filtered through cotton in a flask and, after cooling, made up to 100 mL by washing the residue with the solvent.
Plant extract used for antioxidant activity was obtained by taking 2 g of dried crushed leaves mixed with 40 mL bidistilled water at 87°C for 2 hours for the water extract. For the hydroalcoholic extract was mixed 2 g of dried crushed leaves with 20 mL of water and 20 mL of ethanol at 85°C

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for 2 hours. For alcoholic extract was mixed 2 g of dried crushed leaves with 40 mL of ethanol at 70°C for 2 hours.

Identification of the chlorogenic acid in *Cynarae folium* by HPLC analysis

The method used for identify the chlorogenic acid in *Cynarae folium* by HPLC analysis performed by a system equipped with a column with 0.25 m length and 4.6 mm diameter (European Pharmacopoeia 8.0, 2014). The stationary phase was octadecylsilyl silica gel for chromatography (5 µm), at 40°C temperature, mobile phase A was phosphoric acid and water (0.5:99.5) (v/v), mobile phase B was acetonitrile. The reference solution was obtained by dissolving 5 mg of chlorogenic acid in 50 mL methanol and transferring 5 mL of solution resulted into an Erlenmeyer flask, before adding 5 mL of methanol and diluting to 20 mL with water. The flow rate was 0.9 mL/minute; injection 20 µL and spectrophotometric detection set at 330 nm.

Chlorogenic acid content was calculated using the formula:

$$Chlorogenic\ acid\ content\ (\%) = \frac{A_1 \cdot m_2 \cdot p}{A_2 \cdot m_1} \cdot \frac{100}{100 - U} \quad (1)$$

where:

A_1 is peak area determined by chlorogenic acid in the chromatogram obtained with the test solution;

A_2 is peak area determined by chlorogenic acid in the chromatogram obtained with the reference solution;

m_1 is the sample mass to be analyzed (g);

m_2 is chlorogenic acid mass of the reference solution (g);

p is purity of the chlorogenic acid standard used;

U is the loss on drying.

Determination of the total polyphenols content expressed in chlorogenic acid equivalents from *Cynarae folium*

This determination was performed by spectrophotometric method (Ionescu et al., 2014), using UV-VIS spectrophotometer and measuring the absorbance at 660 nm. The reagents used were ethanol solution 50% (v/v), sodium tungstate, sodium carbonate solution 200 g/L, caffeic acid, water and phosphoric acid. Sodium phosphotungstate solution consists of 10g sodium tungstate mixed with 10 mL phosphoric acid and 75 mL water. The mixture was boiled to reflux, for 2 hours, cooled and diluted at 100 mL with water. Compensating solution consists of 1.25 mL of sodium phosphotungstate solution diluted 25 mL with water.

The recipe for identification of total polyphenols contents consists in mixing 5 mL of test solution with 5 mL of sodium phosphotungstate solution, the solution resulted was shaked, filtered and was removed the first portions of the filtrate. In a volumetric flask was mixed 2.5 mL of this filtered solution with 22.5 mL sodium carbonate (200 g/L solution).

The total polyphenols contents was calculated using standard curve, determined in parallel and in the same conditions as the test solution, taking standard solutions of caffeic acid and the compensating solution. The percentage content of total polyphenols expressed as chlorogenic acid is calculated according to the formula:

$$Total\ polyphenols\ contents\ (\%) = \frac{C \cdot V \cdot 2.016}{m_p \cdot 100} \cdot \frac{100}{100 - U} \quad (2)$$

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where:
C is the concentration read on the calibration curve (μg/mL);
V is the volume of the quenched flask (mL);
m_p is the mass of sample to be analysed (g);
2.016 is the factor of transformation of caffeic acid into chlorogenic acid;
U is the loss on drying.

Determination of the total flavones expressed in rutin equivalents from *Cynarae folium*

The method used for identification of the flavones expressed as rutin equivalents from *Cynarae folium* is the spectrophotometric method based on $AlCl_3$ complexation using UV-VIS spectrophotometer (Constantinescu et al., 1979; Ionescu et al., 2014). The principle of the method consists in the formation of flavones having free OH groups at the ortho, para or peri positions, extracted into hydrophilic solvents, to treatment with aluminum (III) chloride, complexes (chelates) with intense yellow coloration, colorimetry.

The solution A was obtained by taking 5g dried material of *Cynarae folium* mixed with 100 mL methanol solution 50% (v/v) and was boiled on water bath to reflux for 30 minutes. The obtained solution was filtered through cotton into a 100 mL volumetric flask and after cooling, is made up to the mark by washing the residue with the same solvent. The compensating solution consist of 5 mL test solution diluted to 25 mL methanol.

The absorbance of the test solution is measured against the compensating solution after 20 minutes at a spectrophotometer at 430 nm in the 1 cm cuvette. If the extinction of the solution was greater than 0.3, then it was made the appropriate dilution.

The quantity of flavones was calculated by reference to the standard curve of the routine, as follows: was pipetted 1 mL, 2 mL and 3 mL of 0.01% rutin solution in methanol into three 25 mL volumetric flasks. Was added in each flask, 5 mL of sodium acetate solution (100 g/L) and 3 mL of aluminum chloride solution (25 g/L), shaking after each addition of the reagent; the solutions were completed with methanol and shaked vigorously. The solutions obtained were read from the spectrophotometer at 430 nm, in a 1 cm cuvette, relative to methanol.

The flavone content, expressed as rutin equivalents, was calculated using the formula:

$$Flavones\ content\ (\%) = \frac{C \cdot V_1}{m_p \cdot V_2} \cdot 10^{-4} \cdot F \cdot \frac{100}{100 - U} \quad (3)$$

where:
C is the concentration read on the calibration curve (μg/mL);
m_p is the mass of sample to be analysed (g);
V_1 is the volume of the used flask (mL);
V_2 is the volume of solution A used (mL);
F is the dilution factor;
U is the loss on drying.

Antioxidant activity determination

The antioxidant activity was performed using DPPH obtained by mixing 0.5 mL of the plant extract with 1mL of DPPH solution. After an incubation of 30 minutes, solutions were tested by reading the absorbance at 517 nm on the UV-VIS spectrophotometer.

The antioxidant activity was calculated using the formula:

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$$AA(\%) = \frac{A_{control} - A_{plant\ extract}}{A_{control}} \cdot 100 \quad (4)$$

where:
$A_{control}$ is the absorbance of the DPPH solution without sample;
$A_{plant\ extract}$ is the absorbance of the extract mixed with DPPH solution (Fierascu et al., 2014).

3. RESULTS AND DISCUSSIONS

Using the methods describes in the previous section, it was obtained the chromatogram shown in Figure 1 and it was determined a chlorogenic acid content based on the dry substance of 2.68%.

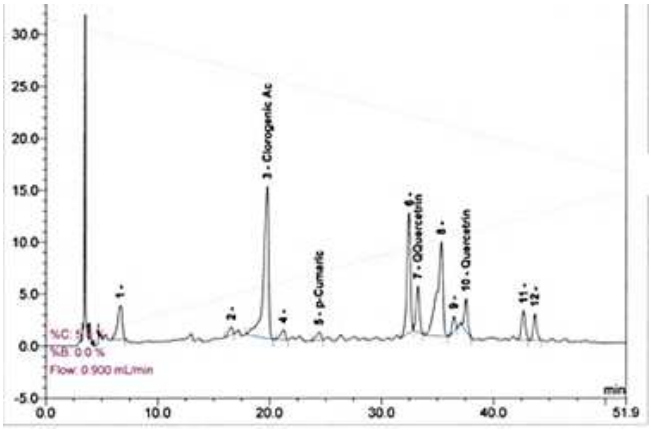

Figure 1. HPLC Chromatogram for Cynarae folium

Applying the method described earlier about identification of total polyphenols contents expressed in chlorogenic acid, it was obtained the percentage of 4.40% and in caffeic acid of 2.18%.
The flavones content expressed in rutin were identified using the standard curve of the routine (Figure 2) and it was calculated as being 2% using the formula (3).

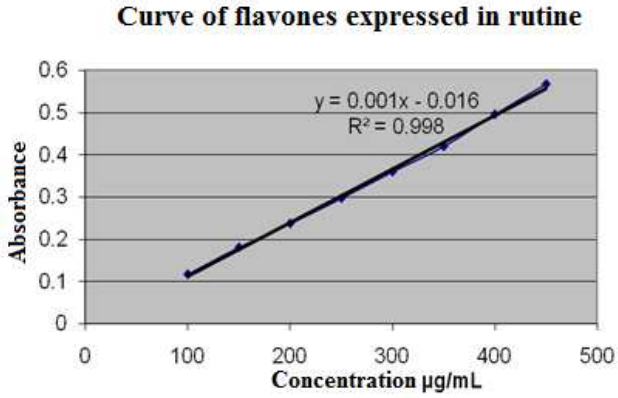

Figure 2. Ethalon curve

The results obtained after antioxidant activity test by DPPH calculated with formula (4) were 48.98% for aqueous extract, 52.64% for hydroalcoholic extract and 59.64% for alcoholic extract shown in Figure 3.

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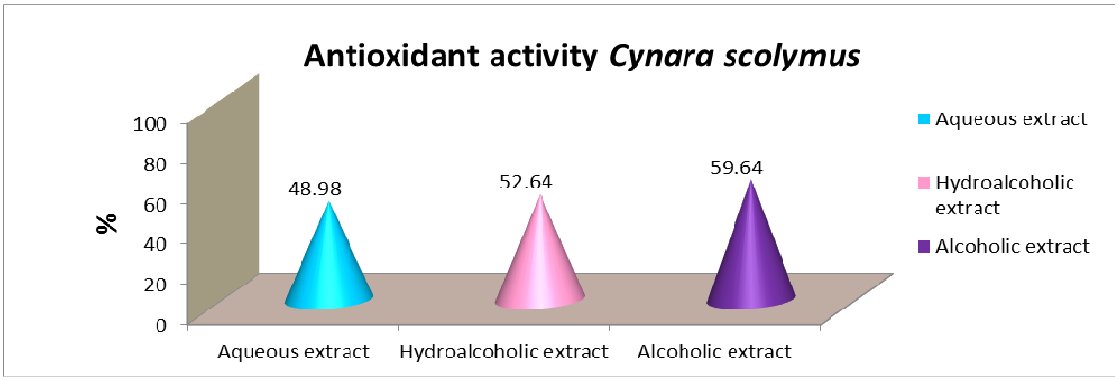

Figure 3. Antioxidant activity

4. CONCLUSIONS

The most important biologically active components including chlorogenic acid, polyphenols and flavones of *Cynara scolymus* L. were determined in significative concentration.

The study of *Cynara scolymus* L. extracts supports its use as an alternative to traditional medicine for liver and gallbladder diseases.

The alcoholic extract showed a relatively high antioxidant activity, supporting its use in various tinctures and supplements.

5. ACKNOWLEDGEMENTS

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