

## THERAPEUTICAL AND FREE RADICAL SCAVENGING PROPERTIES OF *CYNARA SCOLYMUS* L. LEAVE EXTRACTS

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### Abstract

*Cynara scolymus* L. is a perennial herbaceous plant of the Asteraceae family and is one of the most common and important medicinal plants used of the Mediterranean ethnopharmacy. In folk medicine *Cynara scolymus* L. leaf extracts have been widely used as astringent, blood cleanser, cardiogenic, detoxifier, digestive stimulant, diuretic and hypocholesterolemic because was proved to inhibit cholesterol biosynthesis and LDL oxidation. In the present study we investigated the antioxidant characteristics, of most chemical important constituents of extracts from leaves of *Cynara scolymus* L. (phenolic and flavonoid compounds, amino acids,) as well as of selective extracts obtained after technological processes (filtration, concentration, separation, precipitation). Antioxidant properties was studied using the chemiluminescence technique and DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The total flavonoids and polyphenols content was spectrophotometrically determined according to Romanian Pharmacopoea (FR). In addition the viability of cells were detected by MTS - assay that emphasize significant stimulation of the growth of mouse fibroblast 3T3 in a dose-dependent manner.

**Key words:** chemiluminescence, *Cynara scolymus*, pharmacological test.

### INTRODUCTION

*Cynara scolymus* L. is a plant herbaceous perennial belonging to family Asteraceae of Mediterranean origin, North Africa, Canary isles and Southern Europe.

It is well adapted to xerothermic conditions of Southern Europe (Moglia, 2008) (Raccuia, Cavallaro, & Melilli, 2004), (Gominho, 2001), (Bianco, 2005;).

A complex of biologically active compounds is responsible for its therapeutic properties. The principles important constituent of *Cynara scolymus* L. leaves include three principal chemical compounds classes: phenolic acids, flavonoids and sesquiterpene lactones. (Nasser, 2012)

Phenolic acids are represented the combinations of caffeic acid (Figure 1a) and quinic acid (Figure 1b) as well as by two important compounds cynarin (Figure 1c) and chlorogenic acid (Figure 2). Flavonoids compounds are represented mainly by glycoside of apigenin (apigenin-7-O- glycoside) and luteolin

(Figure 3) including luteoline-7- glycoside (cynaroside) and luteoline-7- rutinoside (sculomoside). Sesquiterpene lactones represented by the cynaropicrin (Figure 4), dehydrocynaropicrin, cynaratriol (Figure 5).

(Brand, 1999), (Brand Z. N., 1990) (Nasser, 2012)

In addition, a total of 15 amino acids were found in *Cynara scolymus* L. leaves (aspartic acid, threonine, serine, glutamic acid, glycine, alanine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine (Orlovskaya, 2007) ).

Leaves of *Cynara scolymus* L. have been used for hepatoprotection and as a choleric and diuretic for its lipid-lowering, hepato stimulating, and appetite-stimulating actions. Different pharmacological researches about *Cynara scolymus* L. have demonstrated their health-protective potential, especially their anticarcinogenic, hypocholesterolemic, anti-HIV activities, bile-expelling, lipid-lowering effects and antimicrobial agent. (Xianfeng Zhu, 2004)

(Kraft, 1997), (Kirchhoff R. et.al, 1994), (Gebhardt, 1997), (Gebhardt R. , 1998). (McDougall, et al., 1998).

Should be emphasized the fact that, artichoke is a potential good source of antioxidant activity because it contains large amounts of caffeic

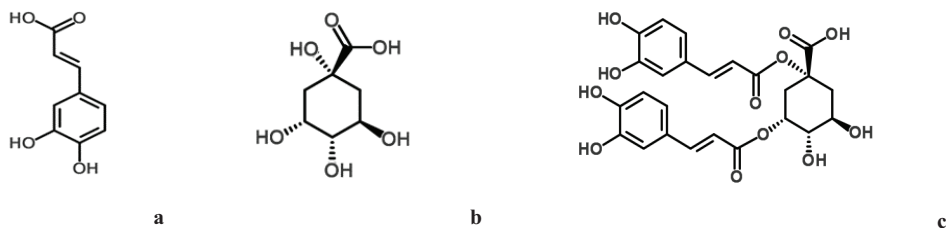


Figure 1. Chemical structure a) caffeic acid b) quinic acid c) cynarin

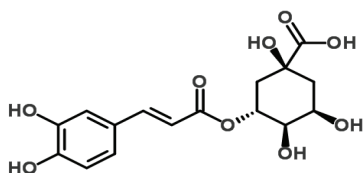


Figure 2 Chemical structure of chlorogenic acid

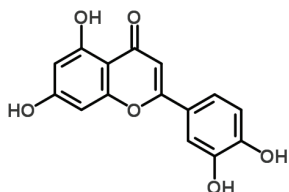


Figure 3. Chemical structure of luteolin

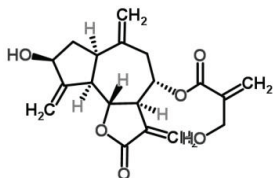


Figure 4. Chemical structure of cynaropicrin

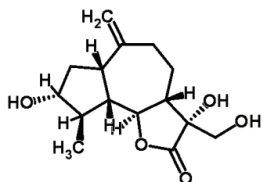


Figure 5. Chemical structure of cynaratriol.

acids. Caffeic acid derivatives are the main phenolic compounds in *Cynara scolymus* L., with a wide range of caffeoylquinic acid derivatives with chlorogenic acid (5-O-

caffeoylquinic acid) as the most important of these derivatives. (Liorach, 2002), (Chen & Ho, 1997) (Tomás-Barberan, Ferreres, & Gil, 2000) (Lattanzio, Cardinali, di Venere, Linsalata, & Palmieri, 1994).

In the present study, we show that *Cynara scolymus* L. extracts possesses the ability to scavenge free radicals and reduce oxidative stress. Phytochemical studies of *Cynara scolymus* L. extracts revealed that several phenolic compounds greatly contribute to the antioxidant activities of this plant. (Sheng-Yang Wang, 2003), (Simonetti, Gardana, & Pietta, 2001).

## MATERIALS AND METHODS

**Plant materials.** The *Cynara scolymus* L. leaves are commercial samples, obtained from FARES: S.C. Romania.

**Chemicals.** Aluminium chloride, Sodium acetate, Folin-Ciocalteu phenol reagent, Arnou reagent, ethanol, methanol, acetic acid, rutin, quercetin, chlorogenic acid, caffeic acid, glutamic acid, glycine, methionine, leucine, tyrosine, histidine, were purchased from Sigma-Aldrich and ultrapure water (Millipore water system).

**Reagents for antioxidant activity/** radical scavenging activity determination : luminol. 5-amino-2,3-dihydrophthalazine-1,4-dione-H<sub>2</sub>O<sub>2</sub> in buffer TRIS-HCl, at pH 8.6 (chemiluminescence methods) and DPPH ( $\alpha,\alpha$ -Diphenyl- $\beta$ -Picrylhydrazyl).

**Reagents for in vitro pharmacological tests:** Dulbecco's Modified Essential Media (DMEM), Fetal Calf Serum (FCS) and antibiotics (penicilin and streptomycin) were purchased

from Sigma-Aldrich (St. Louis, MO). Indicators-MTS 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) was procured from Promega GmbH.

**Cell cultures:** 3T3 fibroblasts obtained from ATCC (LGC Standards, Germany) were cultured in DMEM supplemented with 10% FCS and 1% antibiotics (10,000 units/ml penicillin and 10,000 µg/ml streptomycin in 0.85% saline).

**Equipments.** Soxhlet extraction system, *Spectrophotometer* UV-Vis, Jasco, Japan V-570 for DPPH method and quantitative determination of flavonoids, polyphenols, polyphenol-carboxylic acids and total hydroxycinnamic derivatives.

Digital Rotary Evaporator RE100-Pro LCD (Dragon Laboratory Instruments Limited) Chemiluminometer (Sirius Luminometer Berthelot - GmbH Germany): for *antioxidant activity* measurements by chemiluminescence technique (CL).

**Qualitative analysis** (LC-ESI-MS *analysis*). Qualitative information was gathered using a mass spectrometer instrument LCMS Shimadzu 2010 EV, consisting of a single quadrupole analyzer and a diode array detector, operated in the negative electrospray mode and by direct infusion of sample. Ion source parameters were as follows: capillary voltage 1.5kV, interface voltage 2.5kV, source temperature 120°C, desolvation temperature 250°C, nitrogen gas flow 1.5l/min and the sample injection flow rate of 10µl/min. The acquisition was made both in a total ion scan mode (from 50 to 600 m/z) and by single ion monitoring (of ions of interest: 179, 353, 431, 447, 515 m/z). Sample solutions were filtered with a 0.45-µm (pore size) disposable syringe filter (Sigma-Aldrich)

**Quantitative analysis:** The quantitative determination of the total flavonoids content, total polyphenols content, polyphenolcarboxylic acids and the specific physical-chemical indicators were done according to the FR X (Romanian Pharmacopoea, 1993), (Ciulei, Istudor, Palade, Albulescu, & Gard, 1995)

### Antioxidant activity

**CL method.** The antioxidant activity (AA%) of samples (CN1, CN2, CN3, CN4, CN5) has been determined and compared with that of

pure standards: Rutin, quercetin, chlorogenic acid, caffeic acid, glutamic acid, glycine, alanine, methionine, leucine, tyrosine, histidine were purchased from Sigma-Aldrich.

Chemiluminescence method (CL), was applied using luminol - H<sub>2</sub>O<sub>2</sub> as generator system, in tampon TRIS-HCl, pH= 8.4 by using Sirius Luminometer Berthelot - GmbH Germany. The antioxidant activity of samples was calculated by using the relation (Iftimie N., 2004)

$$AA \% = \frac{I_0 - I}{I_0} \cdot 100$$

where: I<sub>0</sub>=the maximum CL for standard at t=5 s; I= the maximum CL for sample at t=5 s.

### DPPH Radical Scavenging Activity

The free radical scavenging activity (SR%) was quantitatively tested using 2, 2'-diphenyl-1-picrylhydrazyl according to the modified method of Brand-Williams *et al.* (Brand-Williams W, 1995). A DPPH solution (80 µM) was freshly prepared in 95% methanol. A volume of 250 µl of this solution was allowed to react with 35 µl sample and the absorbance was measured at 515 nm. The radical scavenging activity (SR%) was calculated as follows:

$$SR\% = 100 \left( 1 - \frac{Abs_{sample} - Abs_{blank}}{Abs_{control}} \right)$$

The DPPH• assay was repeated three times.

## RESULTS AND DISCUSSIONS

### Obtaining of vegetal selective extracts from *Cynara scolymus* L. leaves

The vegetal selective extracts (CN1, CN2, CN3, CN4, CN5) were obtained by a succession of technological stages, consisting in the first stage in the solid-liquid extraction in a Soxhlet installation. Following the extraction procedure (3 cycles of extraction), the vegetal material used was removed, and the obtained filtrates were brought together (crude extract CN1) were processed by vacuum concentration (Digital Rotary Evaporator RE100-Pro LCD, water bath at constant temperature) until obtaining a residue which, passed through successive precipitations with polar and non-polar solvents, centrifugation, filtering at low pressure and purification. The various fractions extractive (CN2, CN3, CN4, CN5) of non-

hygroscopic, fine powdery samples have been obtained by variation of the operational parameters: crushing degree of the plant, the solvent used for, plant-solvent ratio, extraction time, temperature, type of concentrating, precipitating and purifying.

Qualitative analysis

For crude extract of *Cynara scolymus* L. obtained by Soxhlet method (CN1), before carrying out the processing, was assessed qualitatively analysis by identifying representative chemical compounds. The direct injection negative ion mode MS spectrum of crude extract of *Cynara scolymus* L leaves showed the presence of only two pseudomolecular peaks with 191 m/z and 447 m/z (Figure 6). On the basis of their m/z these peaks could be easily ascribed to caffeoylquinic acids that were previously identified in many plants, especially in *Cynara scolymus* L. Also the molecular ion, m/z = 191 is found in fingerprint in the following compounds: quinic acid, 1-O-caffeoylquinic acid, 5-O-caffeoylquinic acid (chlorogenic acid), 3,5-di-O- caffeoylquinic acid, 1,5-di-O-caffeoylquinic acid, belonging to the chemical composition of *Cynara scolymus* L leaves. (Schutz, 2004). In mass spectra (Scan mode TIC- total ion current) were identified at m/z 353 (chlorogenic acid), m/z 515(cynarin) value of the deprotonated molecular ion. The structures of peaks with [M-H]<sup>-</sup> at m/z= 133, m/z= 215, m/z= 285, m/z= 327, m/z= 379, m/z= 425, m/z=489 can be attributed to molecular fragments generated from isomers of caffeic acid, quinic acid, dicaffeoylquinic acid etc, and the other compounds. (Kapusta, et al., 2013)

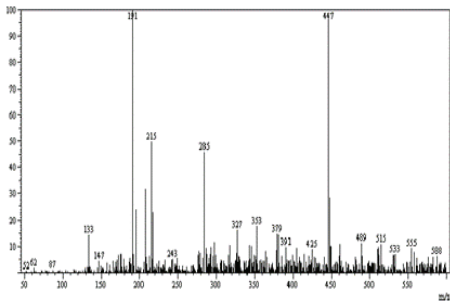


Figure 6. MS fingerprint for crude extract CN1 (SCAN mode by direct injection

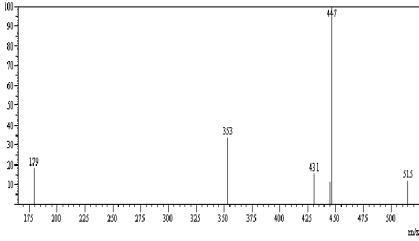


Figure 7. MS fingerprint for crude extract CN1 (SIM mode by direct injection)

Table 1. Chemical compounds identified in crude extract of *Cynara scolymus* L leaves – SIM mode

Compounds	Molecular formula	Molecular weight g·mol <sup>-1</sup>	[M-H] <sup>-</sup> m/z
Caffeic acid	C <sub>9</sub> H <sub>8</sub> O <sub>4</sub>	180.16	179
Chlorogenic acid	C <sub>16</sub> H <sub>18</sub> O <sub>9</sub>	354.31	353
Apigenin-7-O-glycoside	C <sub>21</sub> H <sub>20</sub> O <sub>10</sub>	432.38	431
Luteolin-7-O-glycoside	C <sub>21</sub> H <sub>20</sub> O <sub>11</sub>	448.38	447
Cynarine	C <sub>25</sub> H <sub>24</sub> O <sub>12</sub>	516.45	515

The figure 7 and table 1 show, the compounds identified by ESI-MS in the negative single ion monitoring (SIM).

Quantitative analysis:

Table 2. Physical-chemical characteristics from extract of *Cynara scolymus* L.

Samples	CN 1	CN2	CN3	CN4	CN5
Ash %	-	2.14	1.98	2.10	1.87.
Humidity %	-	3.95	3.74	3.21	4.11
Flavonoids, mass % (as rutin)	0.93	2.72	2.41	3.02	2.27
Polyphenols, mass % (as gallic acid)	1.07	3.41	3.56	7.11	4.29
Polyphenol carboxylic acids, mass % (as caffeic acid)	1.14	3.29	2.73	4.89	4.67

Table 2 shows the amount of flavonoids expressed in rutin, total polyphenols expressed in gallic acid and the polyphenol carboxylic acids expressed in caffeic acid for the samples analysed (CN1, CN2, CN3, CN4, CN5)

In the case of crude extract (CN1) is observed smaller amount of flavonoids, total polyphenols, polyphenol carboxylic acids. After application the specific processing, for crude extract it is found a noticeable increase, of the amount of biologically active chemical compounds (CN2, CN3, CN4, CN5).

The total phenolic content ranged in a wide range from 1.07%-7.11%. The flavonoids and polyphenol carboxylic acids contents, followed a similar pattern as the total phenolics, but the increase was not so great (table 2).

**Antioxidant activity**

The antioxidant activity evaluated by CL and the results of DPPH radical scavenging activities is shown in Figure 9. Both of these methods demonstrated similar values for samples analyzed, as well as, for biological chemical compounds responsible for the therapeutic action. It should be noted antioxidant activity values of *Cynara scolymus* L leaves crude extract (CN1) and high levels for selective extracts. Processing technologies, of crude extract (CN1) led to the enrichment of active principles (flavonoids, total polyphenols, polyphenol carboxylic acids), which generates a very high antioxidant activity between 61.9<sub>CL</sub>/60.2<sub>DPPH</sub> - 94.5<sub>CL</sub>/93.7<sub>DPPH</sub>. We can mention that the values AA%/SR% obtained

for selective extracts (CN2, CN3, CN4, CN5) are comparable to those of standards tested.

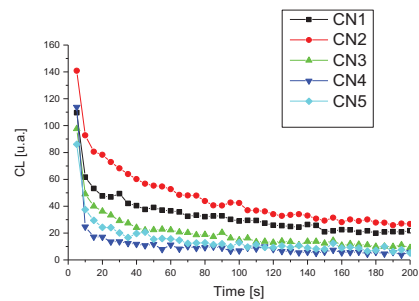


Figure 8. CL evolution in time of the samples CN1, CN2, CN3, CN4

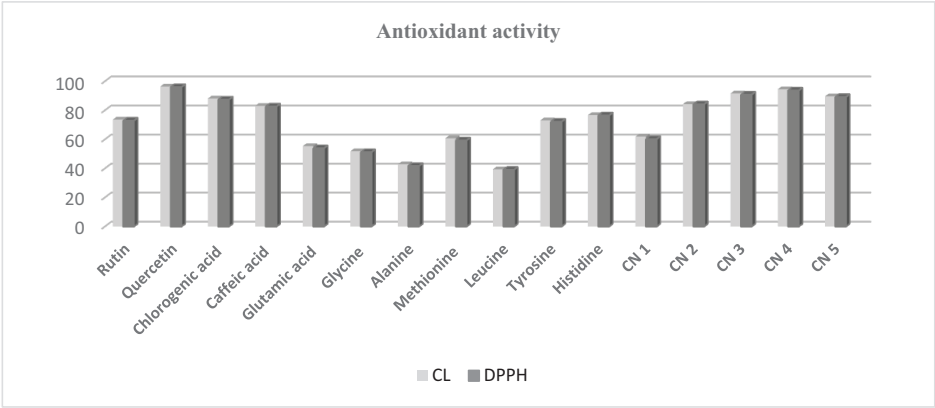


Figure 9. Evaluation of antioxidant activity by CL and DPPH method.

**Cell viability**

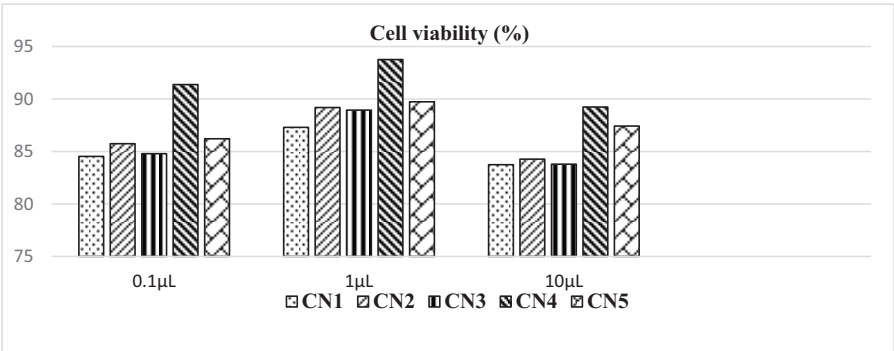


Figure 10. Evaluation of cell viability

The effect of crude extract (CN1) and of various selective extracts of *Cynara scolymus* L. leaves (CN2, CN3, CN4) on cell viability in 3T3 fibroblasts cells is shown in Figure 10.

The cell viability of 3T3 fibroblasts cells was increased with the treatment of various selective extracts. Tests performed with samples CN1, CN2, CN3, CN4, CN5 had a dose-dependent effect on cell viability. The percentage of cells viability at 24 h was between 84.53% - 91.37% in cells incubated.

## CONCLUSIONS

Qualitative and quantitative analysis, highlights chemical composition of the extracts of *Cynara scolymus* L. leaves. The value of antioxidant activity and phytochemical analysis results suggest that the design of biotechnology-processing of *Cynara scolymus* L. extract can lead to obtain selective extracts enriched in active principles. Antioxidant properties evaluated by the chemiluminescence technique and DPPH(2,2-diphenyl-1-picrylhydrazyl) method emphasized significant values. The total flavonoids and polyphenols content was spectrophotometrically determined according to Romanian Pharmacopoeia (FR). In addition the viability of cells were detected by MTS - assay have emphasized significant stimulation of the growth of mouse fibroblast 3T3 in a dose-dependent manner but were also emphasized of the antioxidant properties and amount of biological compounds.

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