

CORRELATION BETWEEN THE POLYPHENOL CONTENT AND ANTIOXIDANT EFFECT OF CYNARA SCOLYMUS L. MOTHER TINCTURE

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ABSTRACT. *Cynara scolymus* L. has been used for many years as natural remedy for liver and biliary tract diseases. Romanian spontaneous flora also includes *Cynara scolymus* L. The aim of this study was to evaluate the polyphenol content and antioxidant activity of *Cynara scolymus* L. mother tincture. Using thin layer chromatography (TLC) and spectrophotometric methods was demonstrated that *Cynara scolymus* L. mother tincture has a relative high concentration in polyphenols, such as caffeoylquinic acids and flavonoids which are responsible of the significant antioxidant activity and detoxifying effects. The antioxidant activity was evaluated using DPPH method.

Keywords: *Cynara scolymus* mother tincture, polyphenols, antioxidant activity, TLC, DPPH method

INTRODUCTION

Cynara scolymus L., having the popular name Artichoke or Globe artichoke, is a member of Compositae family, native from Mediterranean area. The commonly used parts of this plant are the dried basal leaves or the dried or fresh herb.

In popular medicine, the Artichoke leaves were always considered as vegetable remedy for general liver ailments, especially biliary dyspepsia. An improvement was empirically seen in minor liver insufficiencies such as post-prandial drowsiness, slow digestion, bitter mouth and bloating, especially after meals rich in fats and proteins. This is partly why the Artichoke and its extracts are commonly known as purifying or liver draining. Today, the most documented effects of Artichoke are the chologogic and lipid-reducing effects (Fintelmann, 1996; Fintelmann et al., 1996). The approved indications by Commission E for Artichoke are: liver and gallbladder complaints respectively loss of appetite.

These effects and indications are due by the presence in the Artichoke leaves and herb of the sesquiterpene bitter compounds, polyphenol acids, caffeic acids derivatives or dicaffeoylquinic acids, and flavonoids. The scientific data records for this plant a content of around 0,5 % of flavonoids, mainly rutoside (Hinou et al., 1989), but also luteolin-7O-rutoside and luteolin-7O-glucoside (Negro et al., 2012); chlorogenic, neochlorogenic, cryptochlorogenic acids and cynarine from caffeic acid derivatives class (Maros et al., 1965) respectively maximum 4 % of sesquiterpene lactones, like cynaropicrin, dehydrocynaropicrin, cynaratriol.

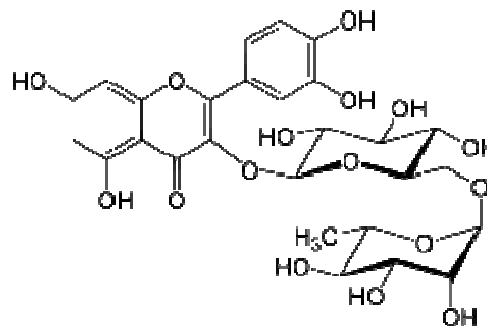


Fig. 1 Structure of rutoside



Fig. 2 Structure of cynarine

Reactive oxygen species (ROS) such as hydrogen peroxide (H₂O₂), superoxide radical, nitric oxide (NO•), singlet oxygen (1O₂) and hypochlorous acid (HOCl) react with biological molecules, causing cell and tissue injuries. The ROS are considered to contribute to a wide variety of degenerative processes and diseases such as atherosclerosis, Parkinson's disease, Alzheimer's dementia and reperfusion injury of brain or heart. Many studies have suggested that flavonoids exhibit biological activities, including antiallergenic, antiviral, anti-inflammatory, vessel dilating actions. These pharmacological effects are linked to the antioxidant properties of flavonoids. The

antioxidant activity was linked not only to the flavonoids, but generally to the polyphenols.

Studies demonstrate that the flavonoids from Artichoke leaves have xanthineoxidase inhibiting action (Sarawek et al., 2008). Other studies prove that the antioxidant activity of Artichoke is maintained also after cooking (Ferracane et al., 2008) or the antioxidant activity of different medicines containing this plant or extracts from this plant (Betancor-Fernandez et al., 2003).

The antioxidant activity may be determined using different free radicals. One of the methods use DPPH or 2,2-diphenyl-1-picrylhydrazyl which as radical is violet and in reduced form is yellow. The reduction is made with antioxidants and the color variation is used to evaluate the antioxidant concentration needed to reduce a certain amount of radicals. Spectrophotometric measurements can determine the color variation (Sanchez-Moreno, 2002).

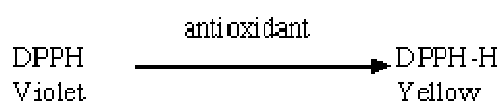


Fig. 3 DPPH method scheme

The objective of this study was to evaluate the polyphenol content and antioxidant activity of an indigenous *Cynara scolymus L.* mother tincture using chromatographic and spectral methods.

MATERIALS AND METHODS

Preparation of mother tincture

The *Cynara scolymus L.* mother tincture was prepared from herba harvested from Mures County. The fresh herba was chopped and mixed with ethanol 90% vol., according to the methods described by European Pharmacopoeia, French Pharmacopoeia and German Homeopathic Pharmacopoeia. 1 kg of chopped plant was mixed with 1,59 kg ethanol 90% vol. This mixture was mixed for 10 days periodically and than it was pressed. Five days later the extract was filtered.

TLC method

Polyphenols were determined using TLC method described by European Pharmacopoeia, German Homeopathic Pharmacopoeia and French Pharmacopoeia.

Stationary phase: plate with SilG F254 (Merck, Germania) 200 x 130 mm.

Mobile phase: ethyl acetate (Merck Germany) - formic acid (Merck Germany) - purified water (80:10:10, v/v).

Sample: *Cynara scolymus L.* mother tincture.

Standards: Caffeic acid (Merck, Germania) 1 mg/ml in methanol, Chlorogenic acid (Fluka, Germania) 1 mg/ml in methanol, Rutoside (Merck, Germania) 1 mg/ml in methanol and hyperoside (Merck, Germania) 1 mg/ml in methanol.

There were applied 30 microliter from sample and 10 microliter from each standard.

Visualisation: in ultraviolet light at 254 nm. Than the plate is sprayed with 1 % diphenylborate-ethanolamine, methanolic solution (Neu reagent) and 5% polyethylenglycol 400, ethanolic solution (PEG reagent). After 30 minutes the polyphenols can be observed in fluorescence at 365 nm.

From the chromatograms have been evaluated the Rf values.

Spectrophotometric determination of total flavonoids and polyphenols

The total flavonoids and polyphenols were evaluated using spectral methods according to Romanian Pharmacopoeia, using a Cintra 101, GBC Australia spectrophotometer.

Conditions for determination of total flavonoids:

Sample: to 1 ml *Cynara scolymus L.* mother tincture is added 5 ml of 100 g/l sodium acetate and 3 ml 25 g/l aluminium chloride, then is brought to 25 ml with methanol.

Sample blank: to 1 ml *Cynara scolymus L.* mother tincture is added 8 ml purified water and is brought to 25 ml with methanol.

Standard: to 1, 2, 3 and 4 ml of 0,101 mg/ml rutoside is added 5 ml of 100 g/l sodium acetate and 3 ml 25 g/l aluminium chloride, then is brought to 25 ml with methanol.

Standard blank: 8 ml purified water is brought to 25 ml with methanol.

The sample and the standard solutions are measured at 430 nm after 15 minutes of rest.

From results obtained with standard solutions was build a calibration curve in rutoside used to determine the *Cynara scolymus L.* mother tincture total flavonoids content.

Conditions for determination of total polyphenols:

Sample: to 0,1 ml *Cynara scolymus L.* mother tincture is added 0,5 ml of phosphotungstenic reagent, then is brought to 25 ml with 15 % sodium carbonate.

Sample blank: 0,1 ml *Cynara scolymus L.* mother tincture is brought to 25 ml with 15 % sodium carbonate.

Standard: to 0,1; 0,2; 0,3 and 0,4 ml of 1 mg/ml caffeic acid is added 0,5 ml of phosphotungstenic reagent, then is brought to 25 ml with 15 % sodium carbonate.

Standard blank: 0,1 ml of 1 mg/ml caffeic acid is brought to 25 ml with 15 % sodium carbonate.

The sample and the standard solutions are measured at 715 nm after 2 minutes of rest.

From results obtained with standard solutions was build a calibration curve in caffeic acid used to determine the *Cynara scolymus L.* mother tincture total polyphenols content.

DPPH method for evaluate the antioxidant activity

The method used in order to evaluate the antioxidant activity of *Cynara scolymus* L. mother tincture was DPPH spectrophotometric method, in visible light, at 517 nm (Burits et al., 2000). The spectrophotometric determinations were performed using a Cintra 101, GBC Australia spectrophotometer.

Sample: 0,5 ml *Cynara scolymus* L. mother tincture was diluted to 10ml with methanol. Then 0,2; 0,4; 0,6; 0,8 and 1,0 ml from diluted solution were brought to 10 ml with methanol. 5 ml from every solution was mixed with 5 ml 0,25 mM DPPH solution. The solutions were maintained 30 minutes at 40°C.

Reference sample: 5 ml 0,25 mM DPPH solution was mixed with 5 ml methanol. The mixture was maintained 30 minutes at 40°C.

Blank sample: methanol.

Standards: 0,4; 0,8; 1,2; 1,6; 2,0; 2,4; 2,8; 3,2, 3,6 and 4,0 ml from a 1,024 mg/ml BHT (butyl-hydroxy-toluol) methanolic solution was diluted to 10 ml with methanol. 5 ml from every solution was mixed with 5 ml 0,25 mM DPPH solution. The solutions were maintained 30 minutes at 40°C.

The sample and standard solutions were evaluated at 517 nm. For every solution was determined the inhibition percentage using the following formula: $I\% = [(A_{reference} - A_{sample}) / A_{reference}] \times 100$.

From results obtained with samples and standards were used to build curves to determine the 50 % inhibition index IC50.

RESULTS AND DISCUSSIONS

In figure 4 can be observed the TLC chromatograms of *Cynara scolymus* L. mother tincture and the used phenolic acid respectively flavonoids standards.

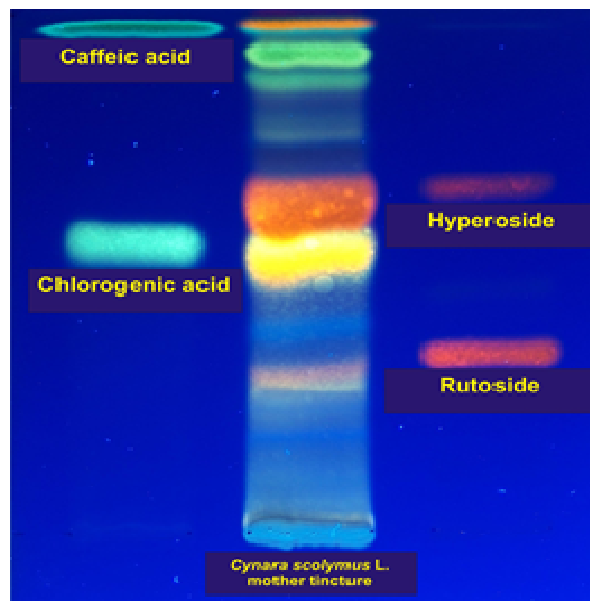


Fig. 4 TLC chromatogram in fluorescence

In the given TLC determination conditions the flavonoids appear in yellow to orange color and the caffeic acid derivatives from green to blue color. According to position and the color of the bands can be identify in the *Cynara scolymus* L. mother tincture the rutoside, the hyperoside and probably the caffeic acid. The other green bands from the sample chromatogram could be caffeic acid derivatives and the yellow band can be a flavonoid.

The R_f values for standards and the separated bands from sample are represented in Table 1. The intensity grades for fluorescence were marked as: +/- small, + observed, ++ normal intensity, +++ intensive, ++++ very intensive. Also according the R_f values can be identified the same 3 compounds: rutoside, hyperoside and caffeic acid.

The intensity of the bands suggests a relative high polyphenol content of the studied sample.

Table 1.

Name of the component	R _f	The R _f values	
		UV 254	Fluorescence
Rutoside	0,34	++++	Pink-orange
Chlorogenic acid	0,56	++++	Blue-green
Hyperoside	0,67	++++	Pink-orange
Caffeic acid	0,97	++++	Blue
<i>Cynara scolymus</i> L. mother tincture	0,19	Blue	Blue
	0,24	Blue	
	0,30	+/-	Pink-orange
	0,38	+/-	
	0,41		Blue
	0,48	Blue	
	0,54	++++	Yellow
	0,63	++++	
	0,66		Orange
	0,73	++	
	0,76		Green
0,80	++		
0,86	+++	Green	

0,92	++++	Green
0,97		Blue
0,98	++++	Orange

In figure 5 and 6 are presented the calibration curves for rutoside respectively for caffeic acid, used then for determine the total flavonoid and total polyphenol content of the studied mother tincture.

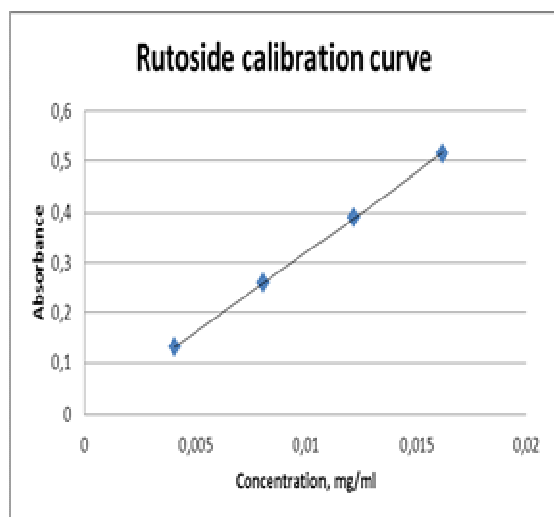


Fig. 5 Calibration curve in rutoside

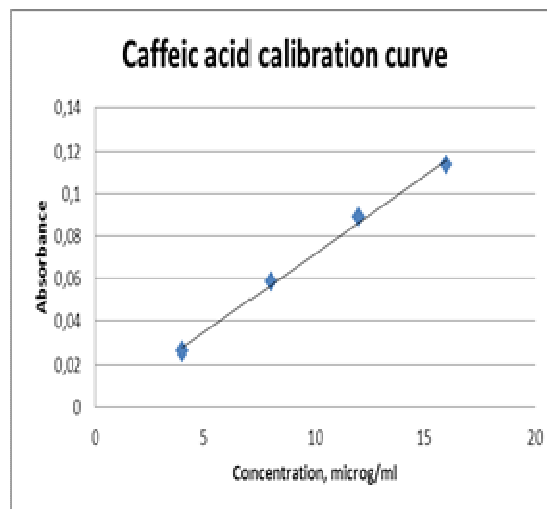


Fig. 6 Calibration curve in caffeic acid

The equation of the curves and the correlation index together with the determined contents in studied mother tinctures are presented in table 2.

Table 2. Spectral data

Calibration curve equation	The curve correlation index	Content
Rutoside: Absorbance = 31,77 x concentration + 0,0028	0,9999	Total flavonoid content of <i>Cynara scolymus</i> L. mother tincture: 0,13 mg/ml
Caffeic acid: Absorbance = 0,0074x concentration - 0,0019	0,9955	Total polyphenol content of <i>Cynara scolymus</i> L. mother tincture: 0,20 mg/ml

The results show a high concentration of polyphenols in *Cynara scolymus* L. mother tincture, data that can be correlated with the intensity of the bands separated by TLC.

In figure 7 and 8 are presented the curves for BHT respectively *Cynara scolymus* L. mother tincture after the evaluation of antioxidant effect and determination of inhibition percentage.

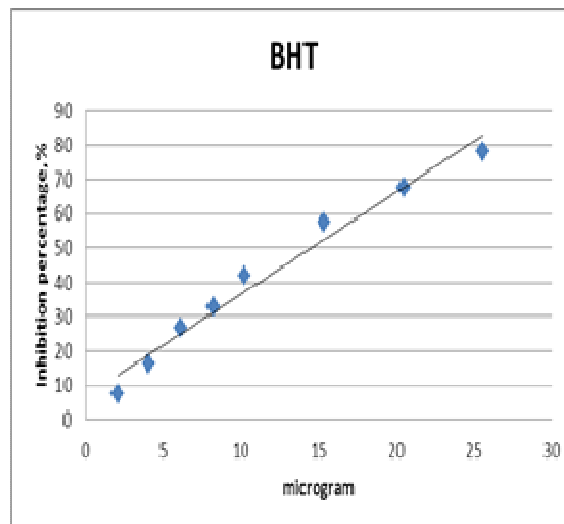


Fig. 7 Antioxidant effect evaluation for BHT

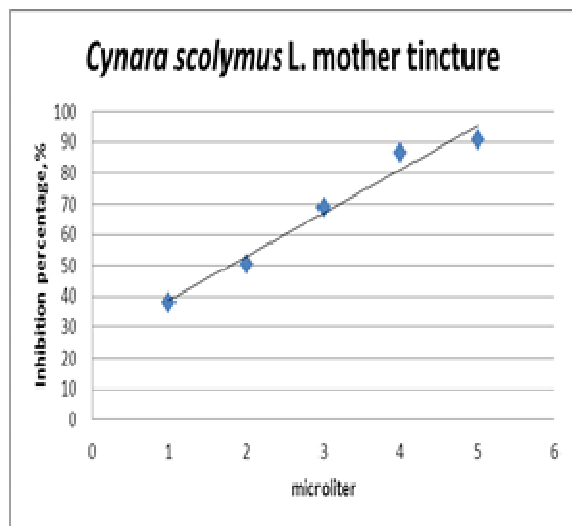


Fig. 8 Antioxidant effect evaluation of *Cynara scolymus* L. mother tincture

In table 3 are presented the equation and correlation index of the above presented curves and the calculated IC50 index for BHT and the studied mother tincture.

Table 3. Antioxidant effect evaluation

Curves equation	The curve correlation index	IC50
BHT: $I\% = 2,9778 \times \text{microgram} + 6,8099$	0,9739	BHT: 14,50 microgram
<i>Cynara scolymus</i> L. mother tincture: $I\% = 14,122 \times \text{microliter} + 24,514$	0,9723	<i>Cynara scolymus</i> L. mother tincture: 1,81 microliter

The results show that the *Cynara scolymus* L. mother tincture has a very good antioxidant effect, comparative with, even better as, the standard antioxidant BHT. These results can be correlated with the relative high polyphenol content and from these the high concentration of flavonoids. 65 % of the polyphenols from the studied extract are represented by flavonoids, these being the main polyphenols and mainly responsible by the *Cynara scolymus* L. mother tincture antioxidant effect.

CONCLUSIONS

This study demonstrates that the studied, indigenous *Cynara scolymus* L. mother tincture has semnificative concentration of polyphenols, mainly flavonoids, such as rutoside, hyperoside, but also caffeic acid derivates. This concentration lead to a significant antioxidant activity demonstrated by DPPH method.

The high polyphenol content correlated with the significant antioxidant activity can be the explanation for the beneficial effect of this product on liver and gallbladder.

From these results can concluded also that the *Cynara scolymus* cultivated in Mures county, Romania, has a good quality, high polyphenol and flavonoid content that can recommend it to be used in alimentary and pharmaceutical fields.

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