RADICAL SCAVENGING CAPACITY OF SEEDS AND LEAVES ETHANOL EXTRACTS OF CYNARA SCOLYMUS L. – A COMPARATIVE STUDY

E. Georgieva¹, Y. Karamalakova¹, G. Nikolova¹, B. Grigorov¹, D. Pavlov², V. Gadjeva¹, A. Zheleva¹
¹Trakia University, Medical Faculty, Department Chemistry and Biochemistry, Stara Zagora, Bulgaria
²Trakia University, Department Agricultural Faculty, Stara Zagora, Bulgaria
Coresspondence to: Ekaterina Georgieva
E-mail: ekaterina.DGeorgieva@gmail.com

ABSTRACT
Artichoke with scientific name Cynara scolymus L. is an herbal medicinal plant widely used in traditional European medicine. This medicinal plant possesses many properties, including hepatoprotective ability and antioxidant effect. It has been reported that seeds and leaves of the artichoke contain different constituents such as caffeoylquinic acid derivatives, mono- and di- caffeoylquinic acids, bitter sesquiterpene such as cynatriol and cynaropicrin and flavonoids glycosides like apigenin, luteolin cynaroside, scolimoside, quercitine and others. The aim of the present study was by an Electron Paramagnetic Resonance (EPR) method and visible spectrophotometry to evaluate and compare the radical scavenging capacity towards 1,1-diphenyl-2-picrylhydrazyl (DPPH) of ethanol extracts prepared from Cynara scolymus L. seeds and leaves. By both methods we have demonstrated that at any studied concentration of the seeds extract the percent of the scavenged DPPH radicals was considerably higher than that calculated for the leaves extract.

Keywords: Cynara Scolymus L., DPPH scavenging capacity, spectrophotometry method, EPR spectroscopy, antioxidant activity

Introduction
Antioxidants have been widely used as food additives to avoid food degradation, and they play an important role in preventing many lifestyle-related diseases and ageing, being closely related to the formation of reactive oxygen species (ROS) and to lipid peroxidation (19). Consequently, during recent years, there has been much interest in the antioxidant activity of naturally occurring substances (22). A great number of aromatic, spicy, medicinal and other plants contain chemical compounds exhibiting antioxidant properties. Numerous studies were carried out on some of these plants, e.g. rosemary, sage, oregano, which resulted in a development of natural antioxidant formulations for food, cosmetic and other applications (3, 13, 23, 28). However, scientific information on antioxidant properties of various plants, particularly those that are less widely used in culinary and medicine is still rather scarce.

Cynara scolymus L., widely cultivated in the Mediterranean regions and adjoining of central Europe also known as globe artichoke is a perennial, frost sensitive, thistle-like plant with edible flower buds belongs to the family Compositae (12, 16). Artichoke is found to contain high amounts of flavone glycosides (8, 11), volatile oils (11), bitter sesquiterpene principles (10), phytosterol, tannins and sugars (8). An in vitro study determined that artichokes inhibit cholesterol biosynthesis by indirectly modulating and inhibiting HMG-CoA reductase, the key enzyme in the biosynthetic pathway for cholesterol synthesis. The cynaroside and particularly the aglycone, luteolin, were mainly responsible for HMG-CoA reductase inhibition (4, 7, 9, 10). The flavonoid of artichoke, luteolin, demonstrated anti-oxidant properties (5, 7, 24). Chemical and biological diversity of aromatic and medicinal plants depending on such factors, as cultivation area, climatic conditions, vegetation phase, genetic modifications and others is an important impetus to study flora present in different growing sites, countries and geographical zones (2, 15, 18). In most cases the use is validated by large amounts of literature data that refer to a single plant or extract (17).

The fact that pharmaceutical effects of Cynara scolymus L. have been attributed in part to its ability to scavenge free radicals (7) led us, using EPR spectroscopy and spectrophotometric methods to investigate and compare DPPH radical scavenging capacity of seeds and leaves ethanol extracts from Cynara scolymus L. grown in Bulgarian climatic conditions.

Material and Methods
Plant materials and chemicals
Leaves and seeds of Cynara scolymus L. were provided by Agricultural Faculty, Trakia University, Stara Zagora, Bulgaria. DPPH and ethanol (98%) were purchased by Sigma Chemical Co, St. Louis, USA. All other chemicals used in this study were analytical grade.

Extracts preparation
100 mg of Cynara scolymus L. seeds or leaves in powdered form were stirred in 10 ml of EtOH
EPR spectra of ethanol extracts isolated from seeds and leaves of *Cynara scolymus* L.

Ethanol extracts isolated from *Cynara scolymus* L. seeds and leaves were studied by direct EPR spectroscopy. EPR measurements were performed on an X-band EPR spectrometer Bruker, Germany, equipped with standard Resonator. The EPR spectra of both extracts were recorded at room temperature at the following EPR settings: center field, 3513.5 G; microwave power, 0.643 mW; modulation amplitude, 10 G; sweep width, 200 G; receiver gain, 2 x 10^3; time constant, 1310.72 ms; 1 scan per sample. Spectral processing was performed using Bruker WIN-EPR and Simfonia software.

EPR spectroscopy method for determination of DPPH radical scavenging capacity

Determination of DPPH radical scavenging capacity of the *Cynara scolymus* L. seeds and leaves extracts was based on the decay of DPPH signal after addition the tasted samples to the stock ethanol solution of DPPH (80 µM). Briefly, to 250 µl stock ethanol solution of DPPH were added correspondingly 5, 10 or 20 µl of leaves or seeds ethanol extract of *Cynara scolymus* L. Every sample was incubated for 10 min and a quartz tube was filled with the sample and placed in the EPR cavity. Control sample for each tested concentration of the extracts was prepared by adding 5, 10 or 20 µl of ethanol to 250 µl ethanol solution of DPPH. The percent of DPPH radicals scavenged by the studied extract concentration was calculated according to the following equation:

\[
\text{Scavenged DPPH radical (\%)} = \left(\frac{I_0 - I_S}{I_0}\right) \times 100\% \quad (1)
\]

where \(I_0\) was integral intensity of the DPPH signal of the control sample and \(I\) was integral intensity after addition of the studied extract concentration to the control sample. All EPR measurements were performed at room temperature and carried out in triplicate. Spectral processing was performed using Bruker WIN-EPR and Simfonia software. EPR spectrum of the DPPH radical in the studied and control samples was recorded at the following EPR settings: center field, 3516.0 G; microwave power, 0.643 mW; modulation amplitude, 5 G; sweep width, 200 G; receiver gain, 5.02 x 10^3; time constant, 163.84 ms; 1 average percent of the scavenged DPPH radicals (calculated from three independent measurements) as a function from the amount of the added extract (expressed in µl). This approach has been implemented in a computer program KORELIA-Dynamics for calculation of IC\(_{50}\) and EC\(_{50}\) parameters of *Cynara scolymus* L. seeds and leaves extracts (25, 26).

**Statistical analysis**

Statistical analysis was performed with Statistica 6.1, StaaSoft, Inc. and results were expressed as means ± standard error (SE). Statistical significance was determined by the Student’s t-test. A value of \(p<0.05\) was considered statistical significant.

**Results and Discussion**

EPR spectra registered in the seeds and leaves ethanol extracts of *Cynara scolymus* L. are presented in Fig. 1a, Fig. 1b.

As is seen the EPR spectra registered in both studied extracts exhibited EPR singlet signals with g values 2.00375 for the seeds and 2.00223 for the leaves extract correspondingly. Having in mind the shape of the EPR spectra, their g values (16, 17, 18) and the presence of cynarine, chlorogenic acid and other polyphenols in both extracts (3, 7, 10) we consider that the registered EPR singlet signals were due to presence of semiquinone radical structures. The difference between the two EPR spectra might be explained by the presence of different constituents in both studied extracts.

The DPPH test is easy to perform, reliable and reproducible, for this reason has been widely used in the analysis of antioxidant activities, both in EPR spectroscopy and in the techniques, which measure the optical absorption intensity at 517 nm. The fact that the DPPH EPR spectrum has a relatively...
simple signal due only to the free radical is an advantage in relation to the optical spectroscopy. In the latter, besides the appearance of absorptions due to the presence of other compounds in the extract, there is also an increment at 320 nm, corresponding to the increase in DPPH-H concentration due to DPPH reduction to the respective hydrazine (27, 20). Results obtained for the DPPH radical scavenging capacity of the studied extracts by the EPR spectroscopy technique are presented on Fig. 2.

**Fig. 1.** EPR spectra of ethanol extracts isolated from *Cynara scolymus* L. leaves (A) and seeds (B)

**Fig. 2.** Percent of scavenged DPPH radicals by leaves and seeds ethanol extracts of *Cynara scolymus* L. and controls determined by EPR method

After addition the corresponding tested extract concentration no changes in the shape of the DPPH spectrum was found but relative intensity of the spectrum was decreased. As is seen, for any studied concentration the percent of the scavenged DPPH radicals by the seeds extract was considerably higher than that calculated for leaves extract (Fig. 2). Results obtained by the spectrophotometry method are presented on Fig. 3 and as is shown they positively correlate with those established by the EPR method.

The most important parameter for characterizing and comparing the antioxidant activity of the naturally isolated extracts is determination of EC<sub>50</sub> (IC<sub>50</sub>) value. That is the amount (concentration) of crude extract required for 50% reduction in the initial concentration of DPPH.
radical (1, 21). As, the value of this parameter is lower as the extract antioxidant activity is higher (14).

![Graph showing percent of scavenged DPPH radicals by leaves and seeds ethanol extracts of Cynara scolymus L. and controls determined by spectrophotometry method.](image)

The results from calculation of EC$_{50}$ and IC$_{50}$ by both methods are shown on Fig. 4 and Fig. 5.

The values of EC$_{50}$ determined spectrophotometrically ranging from 3.115 µl for Cynara seeds extract to 24.828 µl for the Cynara leaves extracts. The same parameter (IC$_{50}$) determined by the EPR method was 4.4352 µl for seeds extract and 25.619 µl for leaves extract correspondingly. It is evident, that Cynara seeds extract exhibits better radical scavenging capacity towards DPPH in comparison with that of Cynara leaves extract.

![Graph showing determination of EC$_{50}$ by spectrophotometry from leaves extract (A) and seeds extract (B).](image)

![Graph showing determination of IC$_{50}$ by EPR spectroscopy from leaves extract (A) and seeds extract (B).](image)

**Conclusions**

By the present preliminary study we have demonstrated that the percent of DPPH radical scavenging capacity of Cynara seeds extract determined either by EPR spectroscopy or by spectrophotometry method was higher than that calculated for the leaves extract. The better DPPH radical scavenging capacity of the Cynara seeds extract was confirmed once again by calculation.
and comparing the values of the IC\textsubscript{50} (EC\textsubscript{50}) parameters of both studied extracts.

These our finding being contrary to the results of other authors, we could explain by Bulgarian climatic conditions and soils in which the \textit{Cynara scolymus} has been cultivated.

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REFERENCES