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PHYTOCHEMICAL ANALYSIS OF SOME MEDICINAL PLANT PRODUCTS WITH ANTIOXIDANT POTENTIAL

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Abstract: The medicinal species *Cynara scolymus*, *Cichorium intybus* and *Taraxacum officinale* are known for their therapeutic properties, such as hepatoprotective, eupeptic, hypocholesterolemic, cholagogue, hypoglycemic, diuretic etc. The purpose of this comparative study was to determine the polyphenols content and the antioxidant potential of some natural products from these medicinal plants obtained from cultivated and spontaneous flora, but also purchased from commercial companies. The polyphenols contents (total polyphenols, flavonoids and caffeic acid derivatives) were spectrophotometrically determined. The samples from the spontaneous and cultivated flora revealed the highest polyphenolic contents: *C. scolymus* from culture (26.30 mg/g); spontaneous species *C.intybus* (26.01 mg/g) and *T. officinale* (32.56 mg/g). The antioxidant activity carried out with the DPPH· radical scavenging method and FRAP assay indicated that *C. scolymus* and *T. officinale* showed antiradical action in line with the polyphenol content. Medicinal plants products purchased from commercial companies had low concentrations of polyphenols, which led to low antioxidant activity or even no antioxidant potential. The large quantitative chemical differences between the studied samples highlights the need for controlling the quality conditions for the vegetable raw material, regarding the harvesting, drying, sorting, purification, preservation and storage.

***Keywords*:** antioxidant potential, *Cichorium intybus*, *Cynara scolymus*, *Taraxacum officinale*, polyphenolic compounds.

Introduction

Cynara scolymus L. (artichoke), *Cichorium intybus* L. (chicory) and *Taraxacum officinale* (L) Weber ex F.H. Wigg. (dandelion) are medicinal plants belonging to *Asteraceae* family. These vegetable products are used in

preparations for various diseases, such as hepato-biliary, digestive, diabetes etc., due to their therapeutic properties: hepatoprotective, eupeptic, hypocholesterolemic, cholagogue, hypoglycemic, diutretic etc. These species contains similar active compounds, such as polyphenols (flavonoids, phenolic acids), sesquiterpene lactones, with beneficial effects especially on the liver, often based on an antioxidant mechanism of action. The preparations containing these plant extracts represent important phytotherapeutic alternatives, as their safety and efficacy have been demonstrated over the time (Milala *et al*., 2009; Shad *et al*., 2013; Abbas *et al*., 2015; Fratianni *et al*., 2014; Milek *et al*., 2019).

The aim of this study was the comparative chemical and biological analysis of some plant raw materials harvested from *C. scolymus* (A), *C. intybus* (C) and *T. officinale* (D) species obtained from the cultivated and spontaneous flora of Romania, and also purchased from commercial companies. In order to obtain quality phytopreparations, it is necessary to use vegetable ingredients in accordance with the norms allowed in the Pharmacopoeia.

Materials and methods

Plant material

The vegetal material was represented by twelve samples (Table 1). Nine natural products were purchased from Romanian commercial companies, in the form of medicinal teas (t), as follows: three artichoke teas (At_1, At_2, At_3), three chicory teas (Ct_1, Ct_2, Ct_3) and three dandelion teas (Dt_1, Dt_2, Dt_3). The leaves of artichoke (Ac) were harvested from the cultivated fields of UASVM Cluj-Napoca. The aerial parts of chicory (Cs) and dandelion (Ds) were collected from the spontaneous flora (Cluj County, 2019).

Preparation of extracts

The plant materials of *C. scolymus*, *C. intybus* and *T. officinale* were grinded to fine powder (300 µm) after air drying at room temperature. The materials were extracted at 60°C (on a water bath) with 70% ethanol for 30 min. The supernatant was recovered after centrifugation at 4500 rpm for 15 min (Toiu *et al*., 2011; Benedec *et al*., 2014).

Chemicals and Apparatus

Gallic acid, DPPH· were acquired from Roth (Karlsruhe, Germany); caffeic acid, rutin, were purchased from Sigma (St. Louis, MO, USA).

Sodium molybdate dihydrate, sodium nitrite, sodium hydroxide, sodium carbonate were purchased from Sigma-Aldrich (Steinheim, Germany). Hydrochloric acid, aluminum chloride, sodium acetate, ethanol and Folin-Ciocalteu reagent were purchased from Merck (Darmstadt, Germany). All spectrophotometric data were acquired using a Jasco V-530 UV-vis spectrophotometer (Jasco International Co., Ltd., Tokyo, Japan).

Table 1

The studied samples

Medicinal plants/ common name	**Origin**	**Plant organs**	**Abbreviation**
Cynara scolymus/ Artichoke (A)	commercial company	leaves	At_1
	commercial company	leaves	At_2
	commercial company	leaves	At_3
	cultivated flora	leaves	Ac
Cichorium intybus/ Chicory (C)	commercial company	aerial parts	Ct_1
	commercial company	aerial parts	Ct_2
	commercial company	aerial parts	Ct_3
	spontaneous flora	aerial parts	Cs
Taraxacum officinale/ Dandelion (D)	commercial company	aerial parts	Dt_1
	commercial company	aerial parts	Dt_2
	commercial company	aerial parts	Dt_3
	spontaneous flora	aerial parts	Ds

Determination of phenol contents (total polyphenols, flavonoids, and caffeic acid derivatives)

Determination of total polyphenols content

Total polyphenols content (TPC) was determined using by slightly modified Folin-Ciocalteu procedure. Each hydro-alcoholic extract of plant products was mixed with Folin- Ciocâlteu reagent and sodium carbonate solution. Absorbance was measured at 760 nm, after 30 min. Gallic acid was used as standard for the preparation of a calibration curve ($R^2 = 0.999$) and

the results were expressed as mg of gallic acid equivalent (GAE)/g dry plant material (Eur. Ph., 2014; Benedec *et al*., 2016).

Determination of flavonoids content

Total flavonoids content (TFC) was determined by a spectrophotometric assay based on flavonoid-aluminum chloride ($AlCl_3$) complexation. The absorbance of the solution was measured at 430 nm. Rutin was used as a standard for the preparation of a calibration curve (R^2 = 0.999). The results were expressed as mg of rutin equivalents per gram of dry plant material (mg RE/g) (FR, 1993; Benedec *et al*., 2014).

Determination of caffeic acid derivatives content

Spectrophotometric method was used to determine the caffeic acid derivatives content (CADC) using hydrochloric acid (0.5 N), Arnow's reagent and sodium hydroxide solution (1 N). Absorbance was measured at 500 nm and the results were expressed as caffeic acid equivalent (mg CAE/g), using an equation derived from the calibration curve of caffeic acid (R^2 = 0.994) (FR, 1993; Benedec *et al*., 2014).

Antioxidant Assessment

The antioxidant capacity of the extracts was determined by two *in vitro* methods, testing the behaviour of the samples towards radicals generated *in vitro*: DPPH bleaching assay and the ferric reducing antioxidant power assay.

DPPH• radical scavenging assay

The antioxidant potential of the extracts was quantified using the stable DPPH radical (2,2-diphenyl-1-picrylhydrazyl) method. Ascorbic acid was used as a positive control and absorbance was measured at 517 nm. The percent DPPH scavenging ability was calculated as:

$$DPPH\ scavenging\ ability = \frac{A_{control} - A_{extract}}{A_{control}} \times 100, \text{ where:}$$

$A_{control}$ is the absorbance of DPPH radical and methanol (containing all reagents except the sample) and $A_{extract}$ is the absorbance of the mixture of DPPH radical and sample extract. The antioxidant activity was expressed as IC_{50} (μg/mL), the concentration of vegetal material required to cause a 50% DPPH inhibition (Simirgiotis, 2013; Benedec *et al*., 2014; Andriamadio *et al*., 2015).

Ferric reducing antioxidant power (FRAP) assay

The antioxidant capacity of the extracts was estimated by the spectrophotometrically ferric reducing antioxidant power assay. This method uses the reduction of the ferric (Fe^{+3}) to the ferrous ion (Fe^{+2}) in a complex formed with iron, of the radical TPTZ (2,4,6-tripyridyl-s-triazine). Trolox was used as reference and absorbance was measured at 593 nm (Benedec *et al*., 2017). Results are expressed as mM Trolox equivalents/g plant material, using a calibration curve ($R^2 = 0.989$) constructed with 10-40 mg/L Trolox standard (Benzie and Strain, 1996; Thaipong *et al*., 2006; Olah *et al*., 2016; Benedec *et al*., 2017).

Statistical analysis

The samples were analysed in triplicate; the average and the relative SD were calculated using the Excel software package.

Results and discussion

The total polyphenol contents expressed in gallic acid equivalents, the flavonoid contents expressed in rutin equivalents and the phenolic acid contents expressed in caffeic acid equivalents are shown in Table 2.

The richest samples in TPC were the vegetal products obtained from culture (*Cynarae folium* - Ac, 26.30 mg GAE/g), and also from the spontaneous flora of Cluj (*Cichorii herba* - Cs, 26.01 mg GAE/g and *Taraxaci herba* - Ds, 36.56 mg GAE/g). The lowest contents in total polyphenols were recorded in commercial samples, with values between 12.99 and 25.66 mg GAE/g. The levels of polyphenols in the three medicinal species were in the following order: *T. officinale* > *C. scolymus* > *C. intybus*, the richest in total phenols being dandelion from spontaneous flora (Ds). Thus, our results obtained on dandelion sample are superior to other reports in the literature (Sengul *et al*., 2009; Milek *et al*., 2019). The comparison of the results obtained for TPC of *C. intybus* and *C. scolymus* species with those obtained by other researchers is limited by the different expression of the polyphenols contents and other types of extracts (Milala *et al*., 2009; Shad *et al*., 2013; Abbas *et al*., 2015).

The concentrations of flavonoids determined in our samples had values in the range of 3.05-8.41 mg RE/g dry vegetal product, except for the sample representing the leaves harvested from *C. scolymus* (Ac) which was the richest in these active principles (25.78 mg RE/g). *C. intybus* samples showed low concentrations in flavonoids, similar to the concentration of total polyphenols. Some previous researches on chicory hydroalcoholic

extracts have shown similar values (Abbas *et al*., 2015). For *T. officinale*, lower values compared to those determined by us were reported (Popescu *et al*., 2010).

In connection with the analysis of phenolic acids, the highest amount of caffeic acid derivatives was found in *T. officinale* samples (10.91-18.57 mg CAE/g), with a maximum value for a commercial sample (18.57 mg CAE/g). High concentrations were also determined in *C. scolymus* harvested from crops (9.03 mg CAE/g), but the commercial samples showed the lowest amounts of these compounds (1.22-4.54 mg CAE/ g). Mean values (4.31-13.00 mg CAE/g) were recorded for chicory samples. Regarding others researches on the studied species, similar values of active principles were reported for *C. scolymus* and higher concentrations were found in *T. officinale* (Popescu *et al*., 2010; Fratianni *et al*., 2014). The variations in the active principles content may be due to the different development conditions of the plants, the moment of harvesting and the conservation of the vegetal material before processing.

Table 2

The content of polyphenols (mg/g dry plant material) in the studied samples

No. crt.	Samples	TPC (mg GAE/g)	TFC (mg RE/g)	CADC (mg CAE/g)
***Cynara scolymus*/artichoke**				
1.	At_1	17.50± 0.45	6.00±0.09	1.22±0.03
2.	At_2	19.99±1.01	5.01±0.08	1.53±0.07
3.	At_3	20.18±0.82	6.45±0.12	4.54±0.16
4.	Ac	**26.30**±1.40	**25.82**±0.95	**9.03**±0.47
***Cichorium intybus*/chicory**				
5.	Ct_1	17.45±0.76	3.11±0.08	4.31±0.24
6.	Ct_2	12.99±0.24	3.23±0.06	3.96±0.04
7.	Ct_3	22.90±1.42	4.16±0.07	6.13±0.47
8.	Cs	**26.01**±1.01	**5.84**±0.16	**13.00**±1.21
***Taraxacum officinale*/dandelion**				
9.	Dt_1	26.77±0.23	5.41±0.15	**18.57**±1.21
10.	Dt_2	26.97±0.81	5.88±0.12	12.72±0.73
11.	Dt_3	25.56±0.44	**8.42**±0.30	11.68±0.65
12.	Ds	**36.56**±0.94	3.05±0.05	10.91±0.76

Notes: Each value is the mean ± SD of three independent measurements

Regarding the antioxidant activity performed with the DPPH radical assay (Table 3), the lowest IC_{50} value (IC_{50}=59.32 µg/mL) was obtained for cultivated artichoke, *C. scolymus* (Ac) which demonstrates a good

antioxidant activity. *T. officinale* (Ds) from the spontaneous flora showed a modest antioxidant activity (IC_{50} = 132.55 µg/mL).

Table 3

Evaluation of the antioxidant activity

No. crt.	Samples	DPPH IC_{50} (µg Trolox/mL)	FRAP (µM Trolox/100 mL)
***Cynara scolymus*/artichoke**			
1.	At_1	>200	330.71±8.29
2.	At_2	>200	333.61±6.39
3.	At_3	>200	502.49±7.51
4.	Ac	**59.32**±2.68	**2023.24±13.75**
***Cichorium intybus*/chicory**			
5.	Ct_1	>200	506.64±3.36
6.	Ct_2	>200	466.80±4.15
7.	Ct_3	>200	632.78±7.22
8.	Cs	>200	**1191.70±8.30**
***Taraxacum officinale*/dandelion**			
9.	Dt_1	160.71±4.29	1046.06±10.94
10.	Dt_2	>200	909.13±8.87
11.	Dt_3	>200	795.85±4.15
12.	Ds	132.55±4.45	**1162.66**±9.34
13.	Ascorbic acid	15,09±0.31	
13.	Trolox	11.20±0.20	

Notes: Each value is the mean ± SD of three independent measurements.

The antioxidant capacity of the extracts evaluated by FRAP method was better for the plants obtained by spontaneous or cultivated flora (*C. scolymus* Ac > *C. intybus* Cs > *T. officinale* Ds), than for commercial samples. The obtained results were in accordance with those obtained by other authors (Milala *et al*., 2009; Fratianni *et al*., 2014).

The determination of *in vitro* antioxidant activity, both by the FRAP and DPPH radical method, confirmed the superior activity of the cultivated species *C. scolymus* (Ac), in accordance with the high content of phenolic compounds.

Conclusions

In the present study, the polyphenols contents as well as the antioxidant potential were determined for three medicinal plants commonly used for their therapeutic properties: *C. scolymus*, *C. intybus* and *T. officinale*. The studied plant materials were obtained from cultivated flora, spontaneous flora, as well as from commercial companies. The comparative phytochemical analysis showed quantitative differences between the studied samples. Large amounts of polyphenolic compounds (TPC, flavonoids, and caffeic acid derivatives) have been determined in vegetable products from spontaneous and cultivated flora, which developed a good antioxidant activity, especially for *C. scolymus*. The commercial samples showed low concentrations of polyphenolic compounds and a poor antioxidant potential. These quantitative differences could be due to the quality of plant materials (determined by pedo-climatic factors, harvesting, drying, preservation etc.) that influence the chemical composition of the raw material and their biological properties. That is why the quality of the vegetal material is very important in order to obtain extracts suitable for medicinal use.

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