



## STUDY OF VOLOVATIC (*CARDUUS GLAUCINUS*) ACTIVE PRINCIPLES FOR POTENTIAL USE AS A MEDICINAL PLANT

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**Abstract:** The aim of this study is to establish the antioxidant capacity and phenolic content of one of the most popular medicinal plants in Bucovina area of Romania, named Volovatic or mountain Volovatic (*Carduus glaucinus*), a plant of the Asteraceae family, *Carduus* genus, *defloratus* *Carduus* species, *Carduus glaucinus* (*glaucus*) subspecies, less studied but widely used in the area of origin. It was determined Volovatic antioxidant potential, respectively polyphenol content and antioxidant activity, contents of metals and metalloids from the plant in order to assess a possible toxic effect.

**Keywords:** volovatic, polyphenol content, DPPH, heavy metals

### 1. Introduction

Medicinal plants constitute one of the main sources of dietary supplements, phytochemicals and pro-vitamins very important in maintaining good health. Most medicinal plants are used in the form of teas. The role of herbal tea in disease prevention and cure is due mostly to antioxidant properties of their constituents [1]. Although knowledge and literature about herbs are very vast, resources are not exhausted. In the mountainous area of Suceava County there are still plants used for therapeutic purposes by the native population, plants that are not at all or sporadically occurring references related to their therapeutic properties, respectively the content in active principles. In the Dorna area, Volovatic (*Carduus glaucinus*) flowers are used from ancestral herds to the preparation of liver tea [2, 3]. Tea is bitter, which confirms the existence of bitter substances that might be bitter or not. *Carduus glaucinus* is a perennial herbaceous plant in which renewal buds appear under the layer of dead leaves of

basal rosette [4]. Belongs to the genus *thistle*, although no spikes. Taxonomic classification and botanical characterization of the Volovatic is the following: Kingdom – *plantae*, Phylum – *spermatophyta*, Class - *dicotyledonatae*, Order -*asteridae/ compositae*, Family-*asteraceae*, Gender-*carduus*, Species - *carduus glaucinus/carduus defloratus*. The stem is simple, high 30-50 cm, up to 80 cm, without leaves and covered with bristles. The leaves are lanceolate to top undivided. They form a basal rosette, and if they are arranged on the stem, they alternate. The upper part of the leaves is milky; the bottom is covered with hairs [5]. The tubular flowers, purple, hermaphrodite form a chapel surrounded by sculptors. It blooms from June (July in the mountain area) until September. Flowers are pollinated by insects. The fruit is an achene clear ovoid with a white doll made of hairs [5].

Finally, in place of the flowers, the crook is made up of a tusk of white hairs like silver. Under each puff there is a nacelle, and inside a chicken of the plant. The wind

is blowing, the puffs are loosening, the sun lounges are stretched, and the baby is wrapped in the diapers [6].

These are an alpine species, which appear from the boreal to the subalpine. These love light, are not pretentious with regard to temperature, hears well the dryness and acidity of the mountain coast [7].

Although the sub-mountain hills and the coasts of the Dornelor Depression are full of blooming shrimps in the summer, Volovatic is a rare plant. It is mentioned in "The Flora and Vegetation of Moldova" as appearing in Suceava County and rarely in Neamt County [2]. It is not mentioned in the flora of other mountain areas (Retezat, Ciucaș, Bucegi) [8-10]. Other species of *carduus* (thistles) appear, such as: *c. candicans*, *c. kernerii*, *c. viridis*, *c. achantoides*, *c. personata*, în Bucegi Mountains [9]. Perhaps because Volovatic is a little widespread plant on the territory of Romania, and also quite rarely encountered in the world, there are few studies that remind it. Besides the fact that the inflorescences of this plant are used by the local population to prepare the tea for the regeneration and proper functioning of the liver, in the *Carduus genus* of which it is part, we find a number of representatives recognized for their sanogenic effects but which do not grow in the mountainous area of Suceava County: *Carduus marianus*, *Carduus benedicta*, *Carduus nutans*, *Silybum marianum*, *Armurariu* [11]. In view of the likely local ancestral use of volovace in combating indigestion/liver problems and the fact that many of its close relatives have demonstrated various beneficial actions on the liver, it is very necessary and challenging to research and find the active principles it contains as well their applicability to phytotherapy. Taking into account these premises, the antioxidant potential of the volovate, namely the content of polyphenols and the

antioxidant activity, as well as the mineral content, was determined. Particularly the presence of heavy metals and their content was compared with the maximum admissible limits.

## 2. Materials and methods

### Materials

Harvested plants were used from the commune of Coșna, Suceava County, from two neighboring areas, located at 47,378558 latitude and 25,171808 long, stereo GPS coordinates 70 x = 513111,239m, y = 653287,138 m respectively 47,375816 and 25,175646 long, stereo coordinates 70: x = 513349,851 m, y = 6256978,629m and at about 860m altitude.

For the determinations, aerial parts of plants were harvested during the flowering and naturally dried, shade, and fresh roots, autumn harvested at the end of the vegetation period.

Reagents. All reagents were of analytical grade and were purchased from Sigma Aldrich (Germany).

### Methods

The moisture (W) was determined by weighing at the analytical balance of samples from all parts of the plant, dried in the oven until the mass remained constant using ZRD-A5055 oven (Zhicheng, China).

$$W\% = (m_{\text{sample}} - m_{\text{dried sample}}) * 100 / m_{\text{sample}}$$

where:  $m_{\text{sample}}$  - sample mass before drying  
 $m_{\text{dried sample}}$  - sample mass after drying

**The ash** was obtained by calcining at 800°C the samples from all parts of the plant using a Nabertherm LE 2/11/R6 Muffle Furnace.

**Determination of antioxidant capacity** was achieved by the DPPH (2,2-diphenyl-1-picrylhydrazyl) method. The methanolic solution of DPPH has a maximum absorption at the wavelength of 517 nm due to the unpaired electron. In the presence of an antioxidant, the electron is mated to form the discoloured DPPH-H form, the discoloration being directly proportional to the number of electrons captured. The determinations were carried out for the aqueous extract: 5 g sample to 200 ml water, 30 min boiled extract and alcohol extract: 1 g sample to 50 ml ethanol obtained in an ultrasonic bath at 40°C and 25 KHz frequency from the aerial parts of the plant: leaves, flowers, stem. The samples of 0.5 ml were mixed with 2.5 ml of DPPH solution,  $6 \times 10^{-5}$  M. The absorbance was measured with 3600 Shimadzu UV-VIS-NIR Spectrophotometer. The percent inhibition of free radicals, calculated by comparison with DPPH, was calculated using the formula:

$$I\% = A_0 - A_p / A_0 \times 100 \quad (1)$$

where:  $A_0$  = the absorbance of the reference substance,

$A_p$  = absorbance of the analysed sample.

**Determination of the polyphenol content** was achieved by the Folin-Ciocalteu method.

As aromatic chemical compounds as several hydroxyl groups inserted on the aromatic ring, the polyphenols can be oxidized by the Folin Ciocalteu (Fc) reagent, with a blue colour formation, with a maximum absorption of 750 nm. The Folin - Ciocalteu Index is specific only for phenolic compounds with reducing properties. Total phenolic content was determined with Folin Ciocalteu method, using gallic acid as a standard. The results were expressed as g of gallic acid equivalents (GAE)/L on a dry weight basis [16].

For analysis 2 mL of extract, 1 mL of Folin Ciocalteu reagent and 8 mL of 7.5%  $\text{Na}_2\text{CO}_3$  were mixed for 5 min and stored in the dark for 30 min then it was measured the absorbance at 750 nm [17].

**Determination of the mineral content** in different samples was performed using a mass spectrometer with inductively coupled plasma (ICP-MS Agilent Technologies 7500 Series) Digestion of the samples was performed with concentrated nitric acid and hydrochloric acid, using double deionized water obtained with a Water purification system Thermofisher. Concentration (C) of heavy metals in samples obtained is expressed in  $\mu\text{g/g}$  sample and is calculated from the formula [2]:

$$C = a \cdot \frac{V}{m} \quad (2)$$

where: a - concentration value measured, [ppb];

V - volume of acid that dissolved sample [ml]

m - mass of mineralized sample [g].

### 3. Results and discussion

The ash content shows (Table 1) the mineral richness of the plant, and its determination was used for their elemental analysis.

**Table 1.**  
Water and ash content the various anatomical parts of the medicinal plant *Carduus glaucinus*

Samples	Moisture %	Ash %
Dried leaves	6.25	8.54
Dried flowers	6.05	3.85
Dried strain	6.38	2.24
Fresh root	30.58	5.24

It is noticeable that the highest mineral content was found in the leaves, followed by root, flowers and plant stem.

**Table 2.**

Metals and metalloids content of the various anatomical parts of the medicinal plant *Carduus glaucinus*, in µg/g.

Tea 1 (Leaves)		Tea 2 (Flowers)
Li	0.366	9.129
Na	30.853	656.689
Mg	32.361	675.819
Al	0	13.035
Ca	305.613	2789.826
Ti	59.375	553.567
V	0.065	3.958
Cr	1.649	39.300
Mn	1.928	55.729
Fe II	0.116	3.545
Fe III	0	0.907
Co	0	1.219
Ni	0.142	10.130
Cu	0.100	9.102
Zn	0.014	1.687
Ga	0	0.104
As	0.072	0.350
Se	0.083	1.101
Sr	0	136.398
Mo	57.546	519.011
Pd	0	0.013
Ag	0	0.014
Cd	0.630	0.735
In	4.019	0.527
Sn	0	6.117
Sb	0.105	0.193
Te	0	2.065
Cs	0.278	4.432
Ba	0.270	4.360
Pt	0	0
Au	0	0.657
Hg	0.059	0.056
Tl	0.010	0.144
Pb	0	0
U	0	0

Tea 3 (Strain)		Tea 4 (Root)
Li	5.097	2.982
Na	290.811	302.707
Mg	185.585	247.688
Al	294.393	1019.286
Ca	2021.654	2749.519
Ti	360.831	822.616
V	1.831	13.717
Cr	19.751	2.744
Mn	25.737	200.609
Fe II	1.979	8.144
Fe III	0.417	1.782
Co	0.571	1.697
Ni	4.769	8.384
Cu	4.572	6.818
Zn	1.095	1.493
Ga	0.081	0.248
As	0.387	0.492
Se	1.015	4.179
Sr	157.211	4674.264
Mo	597.403	4542.413
Pd	0.010	0.039
Ag	7.393	0.019
Cd	0.763	0.010
In	0.786	0.276
Sn	7.394	3.884
Sb	0.178	0.143
Te	0.428	1.338
Cs	9.218	10.729
Ba	8.839	13.805
Pt	0	0
Au	0	0
Hg	0.032	0.046
Tl	0.083	0.121
Pb	0	0
U	37.234	0

Table 2 shows the high Mg and Ca content of the plant flowers, followed by those of the root and then of the stem, the smallest values being in the leaves. Fe<sup>2+</sup>, Se and Zn are high in flowers and roots. In terms of heavy metals, Pb is absent in the entire plant, and Hg and Cd to be well below the maximum limit of 10mg/kg for children. [18].

From the analysis of the results it can be concluded that, from the point of view of the elemental composition, the flowers are

most suitable for use in the form of tea. Cadmium, arsenic, mercury and antimony were within the safety limits and lead was below the detection limits.

**Table 3.**  
**Antioxidant capacity of the various anatomical parts of the medicinal plant *Carduus glaucinus* (I %)**

Samples	Aqueous extract	Alcoholic extract
Leaves	61.348	2.033
Flowers	89.947	20.444
Strain	78.652	30.497

It can be seen that for all parts of the plant, the aqueous extract is much richer in antioxidants, the biggest difference being observed in the case of the leaves.

**Table 4.**  
**Polyphenol content of the various anatomical parts of the medicinal plant *Carduus glaucinus***

Samples	Aqueous extract	Alcoholic extract
Dried leaves	8.526 g/L	0.865 g/L
Dried flowers	9.107 g/L	1.009 g/L
Dried strain	11.116 g/L	0.755 g/L

In accordance with the previous result, the concentration of polyphenols is much higher in the aqueous extract than in the alcohol, being the highest in the strain. In the alcoholic extract the highest amount of polyphenols is found in the flowers.

#### 4. Conclusion

Volovatic (*Carduus glaucinus*) is a rich source of antioxidants and phenolic compounds and can protect the human body from the action of free radicals. The determined metals and metalloids show

that the traditional medicinal plant is safe to use. Along with the other plants of the *Cardus* species, tea from *Carduus glaucinus* can be used for the regeneration and proper functioning of the liver especially in the treatment and control of liver disease.

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