

**UNIVERSITY OF MEDICINE AND PHARMACY
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PhD THESIS ABSTRACT

***CHEMICAL STUDIES ON PLANTS OF CIRSIUM
ARVENSE SPECIES***

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The current context of research in the field of pharmacy is experiencing a shift towards natural medicine and thus to vegetal products with curative properties. The volume of scientific information in the field of phytopharmacy has doubled in the last decade, along with the improvement of the equipment and analytical methods necessary for such determination of the active principles of plants. This ultra-fast development of methods of investigation is sustained by extensive research which seek new resources yet uninvestigated.

Although traditional medicine uses the full therapeutic potential of native plants that are extremely common in the flora of our country, they are less valued at therapeutical level in the pharmaceutical industry.

These species, if it is found to be rich in secondary metabolites and are widespread in indigenous flora can provide cheap alternatives resources.

The proposed species, *Cirsium arvense* is a plant highly known in our country, on which studies are extremely poor.

It is known, however, that it can be used for the treatment of varicose veins and hemorrhoids in ethnomedicine.

As there are no studies on its chemical composition, it is necessary to conduct such a study covering a methodology as detailed as possible. Another argument that was the basis for choosing this theme was its spread in our country and the extremely high availability of this plant.

In this paper an interdisciplinary study was conducted following the determination of the identity and qualitative parameters for *Cirsium arvense* species, which as was said earlier is a native species commonly found in the wild flora of our country and completely uninvestigated until now.

Our study also focused on the chemical characterization of this species, and assessment of certain pharmaceutical activities that it may have.

The material is structured into two parts: a theoretical part consisting of two chapters and original contribution composed of six chapters.

PART ONE - CURRENT STATE OF KNOWLEDGE

1. The first chapter presents some general considerations concerning the *Cirsium arvense* species: botanical description, spread and ethnomedicinal uses.

2. The second chapter is dedicated to the theoretical aspects of the main methods of analysis of the active principles and the chemical composition of this species.

ORIGINAL CONTRIBUTIONS

CHAPTER 3. MACRO AND MICROSCOPIC ANALYSIS OF VEGETATIVE ORGANS FROM *CIRSIUM ARVENSE* SPECIES

In the third chapter a macroscopic and microscopic study of vegetative organs from *Cirsium Arvense* species was carried out, in order to establish the identity characteristics of the investigated species.

The proposed species, *Cirsium arvense* is a plant highly known in our country, on which studies are extremely reduced. Therefore must be settled certain morphological features that can be seen with the naked eye.

By the macroscopic analysis were described elements related to shape, appearance, size and color, and the features related to odor and characteristic taste.

Were studied the following parts: leaves, stems and roots at different times of the year when the active substance content is highest.

Since there are no studies on its the chemical composition, it was necessary to conduct such a study covering a detailed methodology.

For microscopic analysis a Bel Photonics BIO3 microscope was used with video camera Model MSE-100 and Bel MicroImage Analyser soft.

Microscopic analysis of the vegetative organs revealed structural details characteristic for this species corresponding to the existing data till present, and offers new details indicating structural differences between the species of the same genus.

CHAPTER 4. PRELIMINARY CHEMICAL ANALYSIS OF VEGETATIVE ORGANS FROM *CIRSIUM ARVENSE* SPECIES

In this chapter, several analyzes were performed to reveal the preliminary phytochemical composition of this species.

Thus in the first chapter was aimed determine the humidity and the ash for each vegetative organ of this species, observing that the percentages fits within limits stipulated by Pharmacopoeia (10-13% water content and less than 5% raw ash).

Subsection 4.2 includes evaluation by infrared spectroscopy of the main functional groups in the extracts.

In section 4.3. metal concentrations in the samples from *Cirsium arvense* were determined by atomic absorption spectrometry with flame and graphite furnace. The elements Ca, Cr, Ni, Fe, Mn, Zn and Pb were found to be present in various plant organs in varying proportions. Relative standard deviation for all measured metal concentrations was below 10%. The results are consistent with data reported in the literature.

In section 4.4. active principles were extracted by Soxhlet continuous extraction method using two solvents with different polarities: ethyl ether and alcohol. This extraction method was found to be effective to obtain the best extraction efficiency.

Leaf species had the highest percentage of alcohol-extractable compounds (12.9%) followed by root species (10.06%). Regarding the compounds extractable in the ether solution also the leaves are richer in such active ingredients (7.54%), followed by the roots species (4.96%).

In section 4.5 qualitative chemical analysis was performed for the leaves, stems and roots of this species, which revealed the presence of a lot of active principles such as phytosterols, terpenoids, flavonoids and tannins catechesis.

CHAPTER 5. ANALYSIS OF SECONDARY METABOLITES FROM *CIRSIMUM ARVENSE* SPECIES BY GC-MS AND HPLC

Chapter 5 presents the analysis of secondary metabolites from *Cirsium arvense* species by modern coupled analytical techniques (gas chromatography coupled with mass spectrometry, high performance liquid chromatography coupled with UV-VIS detector).

Analysis by GC-MS (section 5.1) shown in the chemical profile of the investigated species the presence of three phytosterols with highly significant curative properties such as lowering blood cholesterol, prevention of heart diseases, prevention of the formation of free radicals in the body.

By HPLC analysis (section 5.2) were found in this species twelve polyphenols: three polyphenolic acids derived from hydroxycinnamic acid, eight flavonoids and one compound belonging to the class of catechins tannins (catechins). All present compounds are secondary metabolites extremely studied for their outstanding curative properties for human health.

The proposed method proved to be selective, the peaks of all analytes were well separated without significant interference caused by endogenous compounds. Furthermore, the time of analysis is quite low, up to 30 minutes, resulting in the simultaneous separation of the thirteen distinct compounds in this range.

CHAPTER 6. DETERMINATION OF TOTAL FLAVONOIDS, TOTAL POLYPHENOLS AND OXIDANT SUBSTANCES BY SPECTROPHOTOMETRIC METHODS

In the study performed in the chapter six, the research was focused on determination of total flavonoids, total polyphenols and oxidant substances by spectrophotometric methods from the investigated species.

Of the three samples, the leaves have the highest content polyphenolic (42.07 mg/100 mL alcohol 30%, 52.83 mg/100 mL alcohol 50% and 47.35 mg/100 mL alcohol 70%)

and at the same time the highest flavonoids content (34.22 mg/100 mL in alcohol 50% and 32.36 mg/100 mL in 70% alcohol).

After the leaves of the species, the highest polyphenolic content and flavonoids is in *Cirsium arvense* roots, followed by stems.

It can be concluded that for medicinal purposes of tinctures from this species, 50-70% ethanol can be used to obtain a tincture rich in active ingredients, especially in antioxidants (polyphenols and flavonoids).

From the Hydroethanolic mixtures of obtained at different concentrations (70°C, 50°C, 30°C) it was found that the ethanol 50°C extract is the richest in polyphenols, while the ethanol 70°C extract proved to be the richest in flavonoids.

CHAPTER 7. ANALYSIS OF PHYTOTOXICITY OF CIRSIUM ARVENSE SPECIES BY TRITICUM ASSAY

For phytotoxicity analysis of the extracts obtained from *Cirsium arvense*, Triticum test was elected. The Extracts showed at low concentrations a stimulating effect particularly on the primary root of the seeds treated with 0.25-0.5% extract concentration. In this case the elongation of the root was exceeding that of the seeds treated with distilled water from the blank. Thus, the 0.25% extract from the bark of the *Cirsium arvense* species stimulated by 18% the main root growth whereas the 0.25% extract from the stems stimulated by 13% the main root growth. At higher concentrations, of 1-2%, the effect is the opposite, the greater inhibition of 87% was given by the 2% extract from the leaves of the species.

CHAPTER 8. PREPARATION AND CHARACTERIZATION OF CIRSIUM ARVENSE TINCTURES

In the last chapter were evaluated tinctures obtained from this species, which were organoleptic characterized, the refractive index was determined, relative density and the total content of heavy metals also were determined. According to the results, tinctures are within the limits provided by pharmacopoeia.

In the thesis are included a number of **original contributions**:

- analysis of macro and microscopic anatomy of the species *Cirsium arvense* spontaneous flora of our country;

- was carried out the inorganic profile of this species, determining the level of Mo, Pb, Zn, Ni, Ca, Cr and Fe by atomic absorption spectrometry with graphite furnace;

- by GC-MS and HPLC were determined a number of secondary metabolites from the following classes: phytosterols, polyphenols and catechins.

Cirsium arvense species deserve special attention from researchers due to its abundance in the wild flora and as shown by the analyzes in this thesis, due to the diversity of secondary metabolites contained in this species, it may be an unlimited source of compounds that might subsequently be used in the pharmaceutical industry.

Future research perspectives:

Deoarece la ora actuală materia primă este furnizată de materialul vegetal recoltat din flora spontană, în cazul utilizării controlate a materialului vegetal, ar trebui să se treacă la cultura convențională pe care se vor face studii de specialitate, în acest caz potențialul biosintetic fiind influențat de condițiile pedoclimatice din arealul de prelevare a plantelor.

Since the raw material is currently provided by the plant material harvested from spontaneous flora, in the case of controlled use of the vegetal material it should proceed with conventional culture on which will be made studies, in this case the biosynthetic potential being influenced by the climatic conditions of the area of plants collecting.

PhD thesis contains eight chapters, 55 figures and 17 tables.

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