Biologically active compounds with therapeutic benefits from *Prunella vulgaris* L.
Analytical and pharmacological approaches

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Prunella vulgaris L. is a plant known in oriental medicine as a species used since ancient times and is officially recorded in the latest edition of the European Pharmacopoeia with a number of scientifically proven beneficial effects. The multiple pharmacotherapeutic actions due to the complexity and diversity of the active principles (phenolic acids, flavonoids, pentacyclic triterpene compounds, sterols, polysaccharides) and the lack of data on the *Prunella vulgaris* L. species from the spontaneous flora were the benchmarks taken into account when choosing the topic of the research.

Taking into account the variability of morphological characteristics and phytochemical composition of the species in different parts of the globe, in my personal research I chose the study of *Prunella vulgaris* L. in Mureș County to validate or disprove the properties of the plant compared to existing data in the literature.

Research on the *Prunella vulgaris* L. species focused on 3 different directions:

- Morphological and botanical studies
- Phytochemical studies
- *In vitro* studies to determine the antioxidant, antimicrobial and antineoplastic potential on cell lines; *in vivo* studies on animal models of induced ulcer and diabetes.

The first study aimed at analyzing the macroscopic and microscopic characteristics of the spiciform inflorescence of the *Prunella vulgaris* L. species, taking into account its resemblance to other species of the *Lamiaceae* family. The research results of the histological analysis confirm the few and general data in the literature, but also bring new information on macroscopic and microscopic features with practical relevance in the botanical identification of the plant product and the prevention of impurity/ falsification.

The second study focused on the qualitative and quantitative analysis of the plant product used (spiciform inflorescence, leaves, nucules) and also a detailed and complete analysis of the *Prunellae spica* plant product. A preliminary qualitative analysis was performed using CSS with standard substances: rosmarinic acid, caffeic acid, betulinic acid, rutoside, quercetin resulting in the fact that the main component of *Prunellae spica* extract is rosmarinic acid. A more complex qualitative and quantitative analysis was performed with a high performance HPLC liquid chromatographic method with two types of detection (UV and MS) - the method described by Vlase et al. Qualitative analysis aimed at evaluating the chemical composition of plant organs and the extraction yield according to the solvent used (aqueous, hydroalcoholic) the purpose being the selection of a plant organ/ extract with the richest concentration of active principles to standardize doses administered in animal models.

The two modes of detection (UV, MS) led for the most part to the same results in terms of qualitative and quantitative composition, except:

- ♦ from the hydroalcoholic extract of spiciform inflorescence the identification of caftaric, caffeic, chlorogenic acid could be achieved only by HPLC/ MS/ MS
- ♦ from the hydroalcoholic extract from the leaves, caffeic and chlorogenic acid were identified only by HPLC/ MS/ MS.

All qualitative / quantitative analyses were corroborated with the optimal harvesting periods. The results indicated that rosmarinic acid is the major compound in the hydroalcoholic extract (spiciform leaves and inflorescences), whereas the richest extract in active principles is from the spiciform inflorescences harvested in July. Regarding the pentacyclic triterpene compounds, ursolic acid being isomer with oleanolic acid, the sum of the two compounds expressed in ursolic acid was quantified. The studied plant extracts contained betulinic acid, but not betulin.

The focus of the last study was to determine the pharmacological action of the *Prunella vulgaris* L. species. *In vitro* studies aimed to evaluate the antimicrobial and antitumor action. The antimicrobial action was performed on 7 bacterial strains, reference strains and hospital strains, using aqueous extracts, hydroalcoholic extracts from the leaves and inflorescence and a concentrated aqueous solution of *Prunellae spica*. The results indicated that the antimicrobial action is influenced by the investigated plant product, the concentration of active ingredients and the type of solvent used. The determination of the antitumor action followed the potential of the concentrated aqueous solution of *Prunellae spica* on the tested cell line of breast adenocarcinoma (MDA-MB-231). The extract of the *Prunella vulgaris* L. species demonstrated a dose-dependent antitumor action.

In vivo studies on experimental models of ulcer and diabetes in rats were the consistent part of the pharmacological approach of biologically active compounds of *Prunella vulgaris* L. The antiulcer action was followed both preventively and curatively using two groups of experimental animals and different extract concentrations. The concentrated aqueous extract showed a therapeutic effect in phenylbutazone-induced ulcers, however, its preventive effect was modest. While curative treatment has been present, prolonged administration requires caution as it can lead to dysplasia. The determination of the antihyperglycemic action was performed using a concentrated extract of *Prunellae spica*. The study indicated that the preparation did not show antihyperglycemic action in animals, but, taking into account the antioxidant effect, it may be an adjunctive treatment in diabetes.

In conclusion, the doctoral research was completed with the following results that also represent the elements of originality of the thesis:

- contributions to the morphological and botanical knowledge of the plant in the Mureș County area
- the studies highlighted the antimicrobial and antitumor potential of the extracts, these studies were performed for the first time in Romania, being standardized regarding the content in active principles
- the research demonstrates that the extracts obtained from *Prunella vulgaris* L. have only modest effects in ulcer and experimental diabetes, the action being dose dependent.