EXPERIMENTAL RESEARCH REGARDING THE ACTIVE EXTRACTS, POLYPHENOLS-STANDARDIZED. NOTE II. MELILOTI HERBA – PHARMACOGNOSTIC ANALYSIS OF THE RAW MATERIAL

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Abstract
Flowering tops of melilot (Melilotus officinalis (L.) Pallas) were studied, in order to obtain a selective extract, enriched in coumarins. Macroscopic exam, microscopic exam and a chemical exam (qualitative and quantitative) were performed for this vegetal raw material. The following anatomic characteristics were identified: fibres with thick walls and surrounded by a sheath containing prism crystals of calcium oxalate; covering trichomes with warty cuticle; glandular trichomes with a short stalk and an ovoid head; pollen grains with smooth exine and 1-2 germinal pores. The raw material contains: flavones (0.180-0.185 g%, expressed as rutin), polyphenolcarboxilic acids (0.956-1.066 g%, expressed as caffeic acid), coumarines, triterpenic saponins, tannins, sterols, carotenoids, polysaccharides (mucilages). Also an enriched coumarine extract was analysed in comparison with the raw material.

Keywords: Meliloti herba; pharmacognostic study

Rezumat
S-au luat în studiu somitățile florale de sulfină, în vederea obținerii unui extract îmbogățit în cumarine. Materia primă vegetală a fost caracterizată macroscopic, microscopic, chimic calitativ și cantitativ. Au fost decelate microscopice elementele anatomiche caracteristice: fibre cu teici cristaligene, peri tectori verucoși, peri glandulari (cu picior scurt și glandă ovoidă), grăuncoare de polen cu exina netedă și 1-2 pori germinativi. Prin examenul chimic s-a stabilit prezența flavonozidelor (0.180-0.185 g%, exprimate în rutozidă), a polifenolilor de tip acid cafeic (0.956-1.066 g%, exprimate în acid cafeic), a cumarinelor, saponozidelor triterpenice, taninurilor, sterilor, carotenoidelor și a poliholozidelor mixte de tip mucilagii. Comparativ cu materia primă vegetală s-a analizat și un extract fluid îmbogățit în derivați cumariniici.

Keywords: Meliloti herba; pharmacognostic study
Introduction

*Meliloti herba* is defined as the dried aerial parts (especially – flowering tops) of *Melilotus officinalis* (L.) Pallas, (*Fabaceae*), generally named melilot [1, 3, 5, 7].

*Melilotus officinalis* is an herbaceous plant, with a branched stem, trifoliate leaves with lanceolate stipules, and raceme inflorescences containing yellow small flowers, with papilionate corolla. It is known to contain coumarinic-derivatives (melilotoside, melilotin, melilotic acid, melilotin-coumaric acid). The odour of the dried herb (aromatic, pleasant, sweet) is due to these compounds; melilotoside yields coumarine upon enzymatic hydrolysis and lactonization [5, 7, 10].

In phytotherapy, *Meliloti herba* is a well-known vegetal drug for its therapeutic effects: anti-oedematous, increasing the venous and lymphatic flow rate, astringent, anti-inflammatory, capillary-protective, antispasmodic, and anticoagulant (it is used for treating the symptoms of capillary fragility, varix, and minor circulatory disorders). In folk medicine it is used for treating jaundice [5, 7, 10].

The aim of the present study is a pharmacobotanic, pharmacognostic and phytochemical examination of the aerial parts of melilot, which will be the raw material used in order to obtain (in future) a lot of selective extracts enriched in coumarins.

Materials and methods

The raw material is the flowering tops of *Melilotus officinalis* (L.) Pallas, harvested in 2006, from Hofigal S.A. culture, and fluid extract manufactured by the University of Bucharest Romania, Faculty of Biology.

The pharmacognostic analysis was performed in order to establish the identity and the quality of the vegetal drug, and also the chromatographic analysis, in order to identify the coumarine derivatives [2, 3, 6].

The identity of the vegetal drug was established using:

- a macroscopic exam (to verify the morphologic characteristics);
- a microscopic exam (using clarified surface - preparations and powder - preparations, Carl Zeiss microscope, Imager.D1);
- qualitative chemical exam (to identify the main active principles in etheric, alcoholic and aqueous extractive solutions) [2, 3].

In order to evaluate the quality, the contents of active principles (flavones and polyphenolcarboxylic acids) were investigated. Preliminary, the parameter „loss on drying” was established. Spectrometric methods were used to assay the flavones (the chelating reaction with aluminium chloride solution) and polyphenolcarboxylic acids (the forming of oxime
derivatives with Arnow reagent) [2, 4, 9]. Standard rutin - and caffeic acid - curves, and a UV-VIS Spectrophotometer Jasco V-530 were used.

**Chromatography for coumarines** was also performed. The experimental conditions were:

- mobile phase: toluene / ether (1 / 1, v/v), saturated with 100g/L acetic acid solution;
- stationary phase - silica gel G F254;
- test solutions - 5% extractive solution (from vegetal drug) in methanol, and fluid extract;
- reference solutions: umbeliferone, coumarin, p-coumaric acid, o-coumaric acid solutions in methanol;
- detection reagent: 100 g/L alcoholic potassium hydroxide solution;
- UV examination of the plate (365 nm), before and after spraying the plate with detection reagent [8].

**Results and discussion**

The following characteristics were observed: cylindrical, glabrous and finely branched stems; long - petiolate, trifoliate, alternate leaves, with elongate to ovate leaflets, and lanceolate stipules; the edge is dentate, and the veins are penate; racemic inflorescences containing yellow and small flowers, with papilionate corolla, axillary arranged (fig. 1); aromatic, pleasant and sweet odour. These characteristics, observed on our raw material, are the same with the ones that are mentioned in the literature for Meliloti herba [2, 7].

Figure 1

Meliloti herba

The following microscopic diagnostic characters were identified: fibres with thick walls and surrounded by a sheath containing prism crystals of calcium
oxalate (fig. 2); chlorophyll parenchyma associated with annularly thickened vessels (fig. 3); palisada (fig. 4, 5); covering trichomes with warty cuticle (fig. 6, 7, 8); leaf epidermis with stomata (fig. 9); glandular trichomes with a short stalk and an ovoid heat (fig. 10); petal epidermis and pollen grains with smooth exine and 1 - 2 germinal pores (fig. 11, 12, 13); endothecium (fig. 14, 15).
Figure 8
The base of covering trichome

Figure 9
Epidermis with stomata

Figure 10
Glandular trichome

Figure 11
Petal epidermis and pollen grains

Figure 12
Petal epidermis

Figure 13
Pollen grains with smooth exine and 1 – 2 germinal pores
The following active principles were identified: flavone aglycones, coumarinic aglycones, carotenoids and sterols (in etheric extractive solution), flavones, saponins, tannins, polyphenolcarboxylic acids (caffeic acid-type), reducing-compounds (in alcoholic solution) and mucilages (in aqueous extractive solution).

The results of the quantitative analysis are summarized in table I.

### Table I
Quantitative analysis

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sample</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Meliloti herba - herbal drug</td>
<td>Meliloti herba - fluid extract</td>
</tr>
<tr>
<td>Flavones</td>
<td>0.180 – 0.185 g% (w/w, expressed as rutin) *</td>
<td>0.010 – 0.013 g% (w/v, expressed as rutin)</td>
</tr>
<tr>
<td>Polyphenolcarboxylic acids</td>
<td>0.956 – 1.066 g% (w/w, expressed as caffeic acid) *</td>
<td>0.043 – 0.048 g% (w/v, expressed as caffeic acid)</td>
</tr>
</tbody>
</table>

*Note:* * - the result is reported to the dried drug

The following compounds were identified by TLC technique, in both samples solutions (fig. 16):
- coumarin (Rf = 0.54, blue fluorescent area, which changed to yellow after spraying the plate with the solution of alcoholic potassium hydroxide);
- umbeliferone (Rf = 0.19; blue fluorescent zone before and after spraying the plate with the solution of alcoholic potassium hydroxide);
- o-coumaric acid (Rf = 0.17, blue fluorescent zone, which became yellow after spraying the plate with the alkaline solution).
Conclusions

The identity and the quality of the raw material were established using a macroscopic, microscopic and a chemical exam (qualitative and quantitative). Coumarine derivatives were identified using TLC method.

We conclude that the analysed raw material can be used in future in order to obtain selective pharmacologic active extracts.

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