

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

CERASELA ELENA GÎRD*, LIGIA ELENA DUȚU, MARIA LIDIA POPESCU, MARIANA PAVEL, ALINA TITINA STERIE

University of Medicine and Pharmacy „Carol Davila“, Faculty of Pharmacy, Department of Pharmacognosy Phytochemistry Phytotherapy Traian Vuia no.6, Bucharest, Romania

**corresponding author: cerasela.gird@yahoo.com*

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 heat; pollen grains with smooth exine and 1- 2 germinal pores.

The raw material contains: flavones (0.180- 0.185 g%, expressed as rutin), polyphenolcarboxylic 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.

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 microscopic elementele anatomice caracteristice: fibre cu teci cristaligene, peri tectori verucoși, peri glandulari (cu picior scurt și glandă ovoidă), grăuncioare 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, sterolilor, carotenoidelor și a poliholozidelor mixte de tip mucilagii.

Comparativ cu materia primă vegetală s-a analizat și un extract fluid îmbogățit în derivați cumarinici.

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, trifoliolate 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 Arnov 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

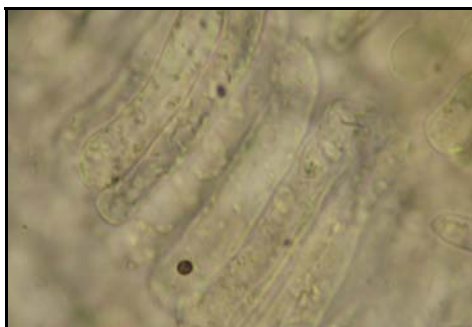
oxalate (fig. 2); chlorophyllian parenchyma associated with annularly thickened vessels (fig. 3); palisada (fig. 4, 5); covering trichomes with warty cuticle (fig. 6, 7, 8); leaf epidermis with stomata (fig. 9); glandular trichomes with a short stalk and an ovoid head (fig. 10); petal epidermis and pollen grains with smooth exine and 1 - 2 germinal pores (fig. 11, 12, 13); endothecium (fig. 14, 15).

**Figure 2**

Fibres with crystals of calcium oxalate sheath

**Figure 3**

Chlorophyllian parenchyma and annularly thickened vessels

**Figure 4**

Palisada

**Figure 5**

Palisada

**Figure 6**

Epidermis, palisada and covering trichomes with warty cuticle

**Figure 7**

Covering trichome with warty cuticle



Figure 8
The base of covering trichome

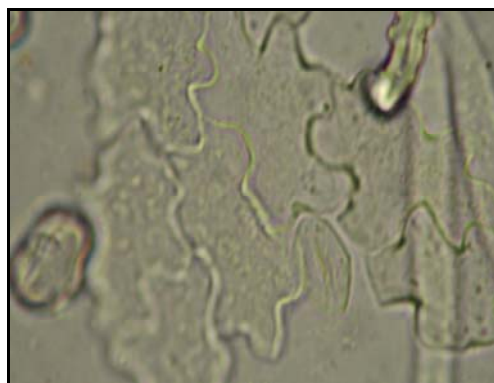


Figure 9
Epidermis with stomata



Figure 10
Glandular trichome



Figure 11
Petal epidermis and pollen grains

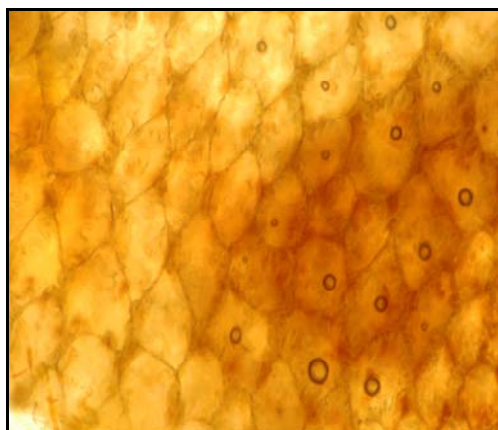


Figure 12
Petal epidermis

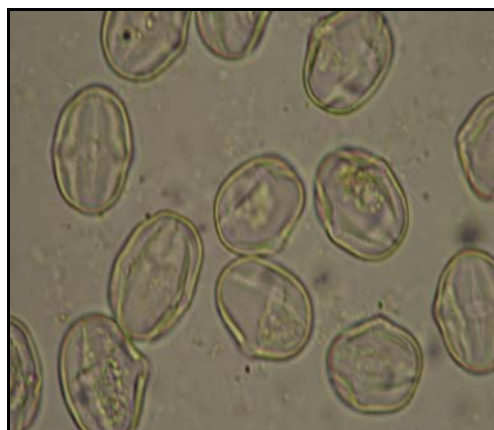


Figure 13
Pollen grains with smooth exine and 1 – 2
germinal pores

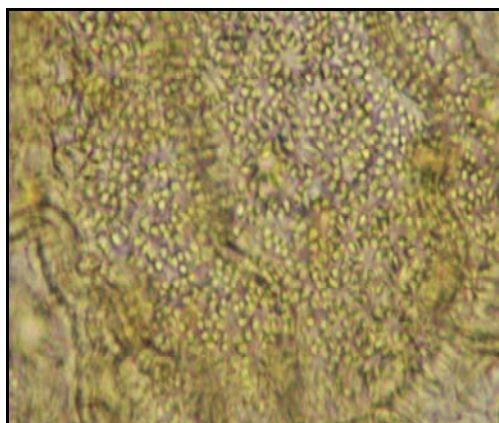


Figure 14
Endothecium

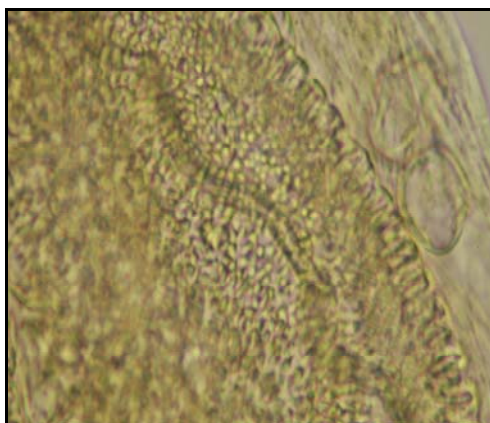


Figure 15
Endothecium

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.

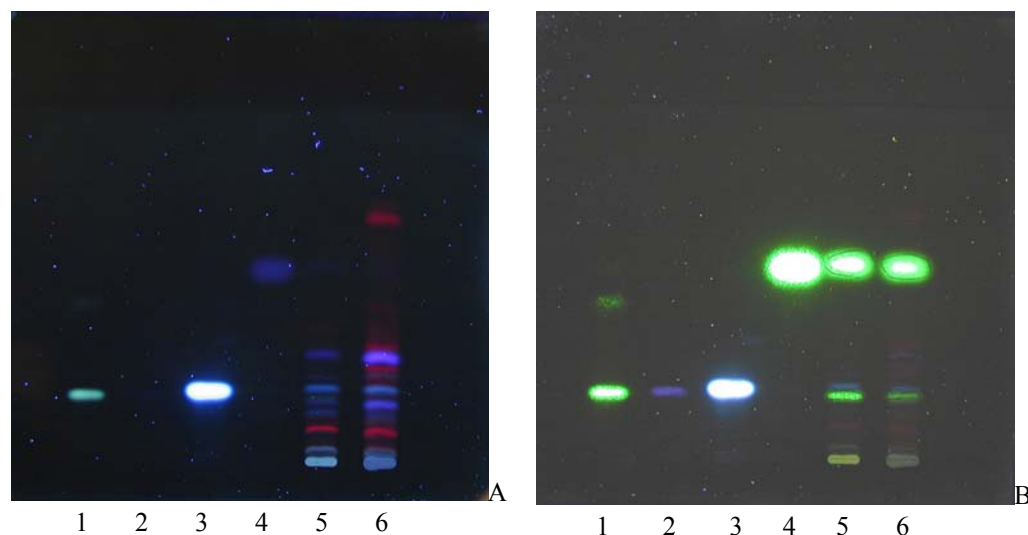
Table I
Quantitative analysis

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

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

The following compounds were identified by TLC technique, in both samples solutions (fig. 16):

- coumarin ($R_f = 0.54$, blue fluorescent area, which changed to yellow after spraying the plate with the solution of alcoholic potassium hydroxide);
- umbeliferone ($R_f = 0.19$; blue fluorescent zone before and after spraying the plate with the solution of alcoholic potassium hydroxide);
- *o*-coumaric acid ($R_f = 0.17$, blue fluorescent zone, which became yellow after spraying the plate with the alkaline solution).

**Figure 16**

TLC chromatograms (A - before spray; B - after spray; 1 - *o*-coumaric acid; 2 - *p*-coumaric acid; 3 - umbeliferone; 4 - coumarin; 5 - fluid extract; 6 - methanolic extractive solution of vegetal drug)

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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