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MICROSCOPICAL TESTS FOR *MELILOTI HERBA*

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Keywords*:* light microscopy analysis; *Meliloti herba (flores)*; *Melilotus officinalis*; optical microscope; sweet yellow clover.

ABSTRACT

Yellow melilot, Melilotus officinalis, belongs to the Fabaceae family. It is abundantly found in Romania. The microscopic analysis of plant material from herbal tea was carried out in the current study. Microscopic evaluation based on the characteristic features of cells, calcium oxalate crystals, trichomes, fibres, vessels, etc. is useful in the initial identification of herbs, as well as for the detection of adulterants, followed by chemical qualitative and quantitative analysis of the raw plant material.

INTRODUCTION

Melilotus officinalis (sweet yellow clover, yellow melilot, or common melilot) is an herbaceous annual or biennial species in the Fabaceae family, about 50-100 cm tall at maturity, with alternate leaves composed of three leaflets and yellow flowers clustered in long racemes on branching stems, producing fruit in black pods containing just one seed. Plants have a characteristic sweet smell due to the presence of coumarin. In Romania it is widespread, it can be found in every county, from the plain up to 1200 (1400) m, on light soils, as ruderal or in hayfields. It is also cultivated as melliferous (honey production 130-300 kg/ha, Pârvu, 1991); as green manure; medicinal; or ornamental plant. It is used to extract coumarin for parfumery and in some cases to flavor tobacco leaves. Sweet yellow clover is a medicinal plant used in traditional medicine for its beneficial effects (for example anti-inflammatory effect, improvement of lymphatic circulation, reduction of high-protein edemas - EMA/HMPC/44165/2016). However, it contains coumarin in various amounts, which has hepatotoxic potential in man (Loprinzi et al. 1999) and carcinogenic effects in long-term studies in rats and mice. When the plant becomes moldy, coumarin converts to toxic dicoumarol, which is a powerful vitamin K antagonist and anticoagulant. According to Stegelmeier (2022) dicumarol concentrations of 20–30 mg/kg of hay ingested throughout several weeks are usually required to cause poisoning in cattle or other species of animals such as sheep, pigs, and horses. Clinical manifestation consist in bleeding abnormalities, as seen in anticoagulant rodenticide poisoning which have the same mode of action.

The current study presents a microscopic analysis of plant material from *Melilotus officinalis* herbal tea to record some of the parameters of taxonomic relevance using a simple method.

MATERIAL AND METHODS

The microscopic analysis was carried out at the Laboratory of Biology, Faculty of Biotechnologies of the University of Agronomic Sciences and Veterinary Medicine of Bucharest in July 2021. Surface preparations or samples of powdered plant material were obtained from *Melilotus officinalis* herbal tea (*Meliloti herba*) obtained commercially (Figure 1). To colour, toluidine blue was sometimes added. A Micros Austria optical microscope with ocular micrometer was used (calibration ratio was 1 µm for ob. 100×, 2.5 µm for ob. 40×, 10µm for ob. 10×). Microscopic images were photographed with a Sony Cyber-shot® digital camera (Carl Zeiss Vario-Tessar 5× zoom lens) and were later compared to descriptions found in the literature (Dordzhieva et al. 2019; Gîrd et al. 2009, Gîrd et al. 2010; Sheikh et al., 2016). For the pollen analysis the PalDat site was used (Halbritter et al. 2021).

RESULTS AND DISCUSSIONS

Microscopically several specific or common anatomical elements were identified in the powder, as follows: epidermal cells and anomocytic stomata (Figure 2); fibers with sheaths of calcium oxalate crystals (Figure 3); non-glandular trichomes with warty cuticle (Figure 4); xylem vessels with spiral thickening (Figure 5); xilem vessels pitted (Figure 6) and pollen grains - tricolporate, medium size, size of polar axis P = 34 µm, size of equatorial axis E = 24 µm; P/E = 1,41; prolate shape; isopolar, ornamentation not distinguishable (Figure 7).

Figure 1. Herbal tea used in the present study (*Meliloti herba*)

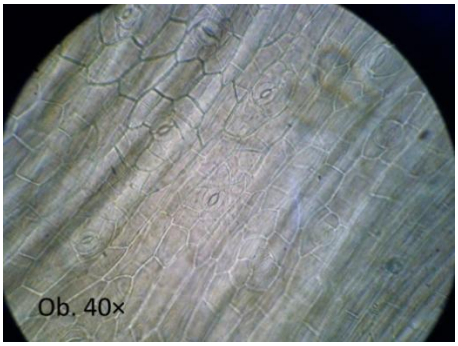


Figure 2. Epidermal cells and anomocytic stomata (*Meliloti herba*)

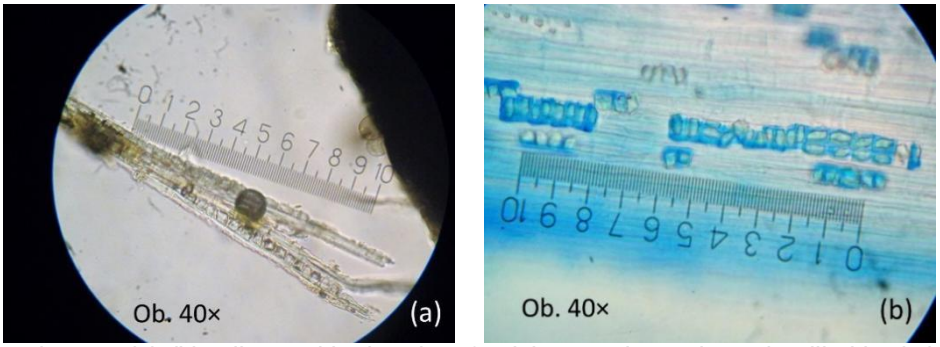
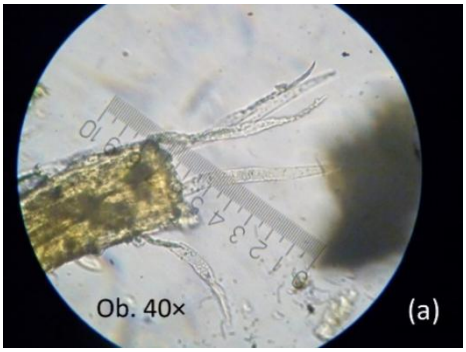
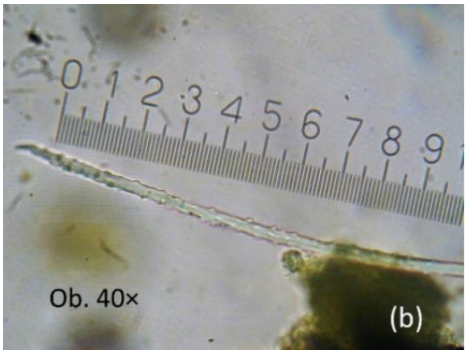


Figure 3 (a)-(b). Fibers with sheaths of calcium oxalate prisms (*Meliloti herba*)





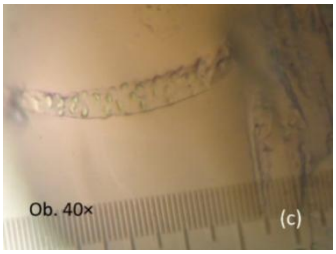


Figure 4 (a)-(c). Non-glandular trichomes with warty cuticle (*Meliloti herba*)

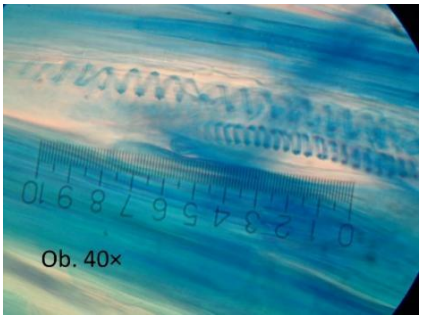


Figure 5. Xylem vessels with spiral thickening (*Meliloti herba*)

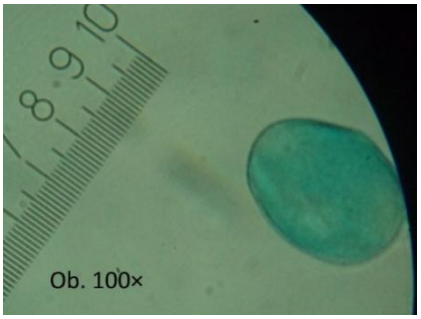
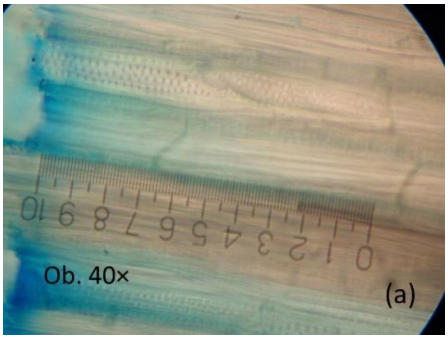


Figure 7. Pollen grain of *Melilotus officinalis*



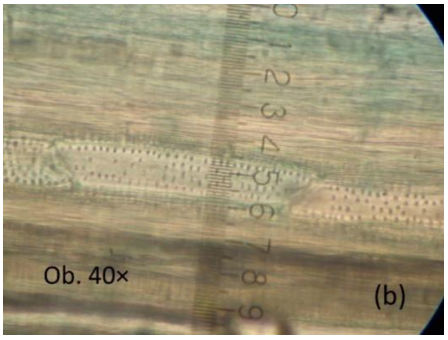


Figure 6 (a)-(b). Xilem vessels pitted (*Meliloti herba*)

Microscopic images of *Melilotus officinalis* leaf showed: upper epidermis with anomocytic stomata (Figure 8); lower epidermis with anomocytic stomata (Figure 9); lignified spiral xylem vessels (Figure 10); lignified pitted xylem vessels (Figure 11); fibers with crystalline sheaths (Figure 12); pluricellular globular gland (Figure 13).

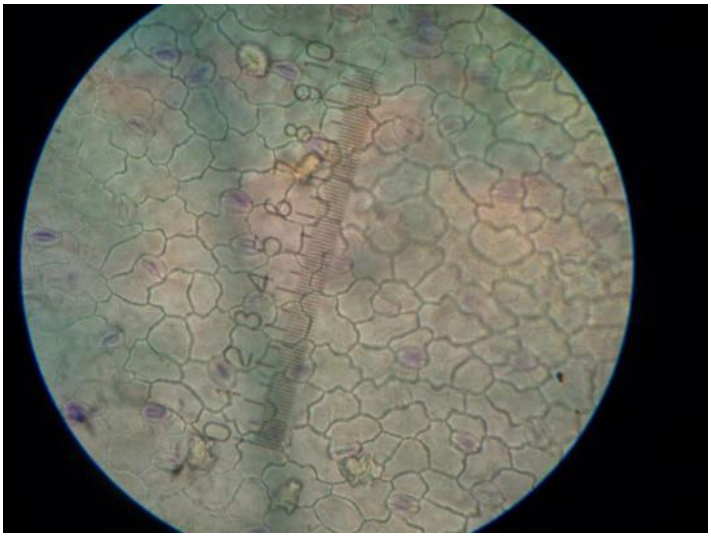


Figure 8. Upper epidermis showing anomocytic stomata in sweet yellow clover leaf

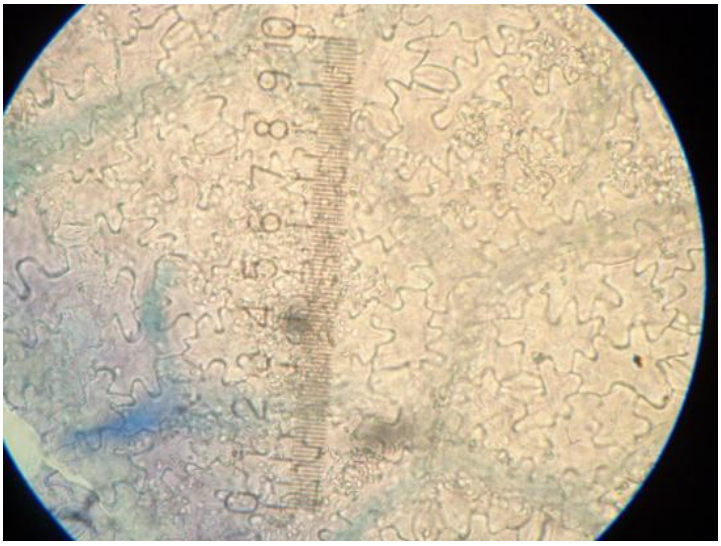


Figure 9. Inferior epidermis with anomocytic stomata in sweet yellow clover leaf

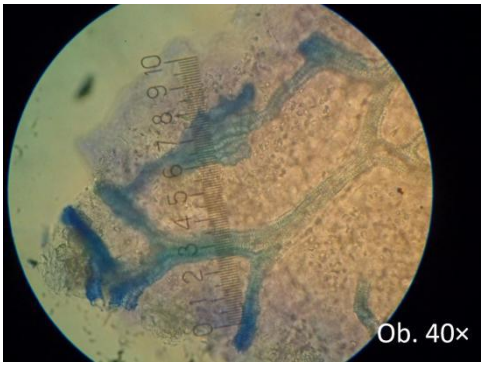


Figure 10. Lignified spiral wooden vessels in sweet yellow clover leaf, ortocromatic coloring with toluidine blue

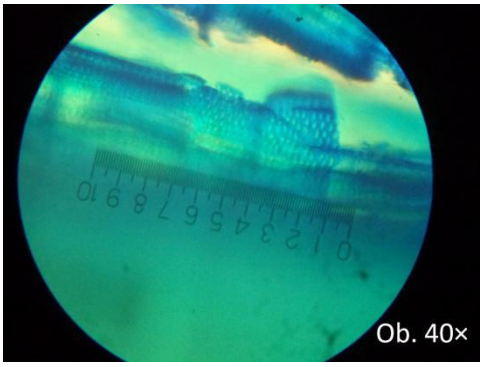


Figure 11. Lignified pitted wooden vessels in sweet yellow clover leaf

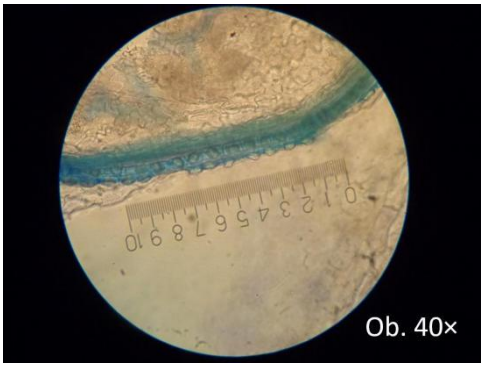


Figure 12. Fibers with crystalline sheaths in sweet yellow clover leaf

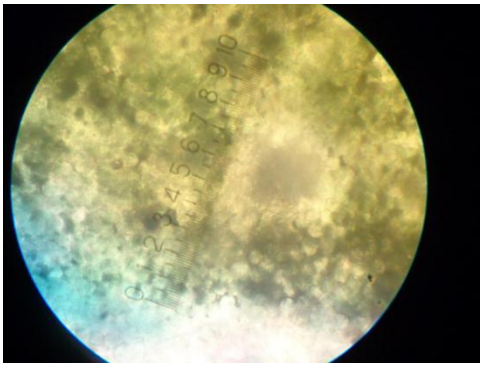


Figure 13. Pluricellular globular gland in sweet yellow clover leaf

CONCLUSIONS

Powder microscopy showed epidermal cells and anomocytic stomata; fibers with crystalline sheaths; non-glandular trichomes with warty cuticle; spiral xylem vessels; xilem vessels pitted and tricolporate pollen grains. The leaf also showed epidermis with anomocytic stomata; lignified spiral xylem vessels; lignified pitted xylem vessels; fibers with crystalline sheaths; and also pluricellular globular gland. Pollen grains were also seen, but no other flower parts. The preliminary work presented here included some of the characteristics described in the literature for the dried aerial parts containing flowering tops of *Melilotus officinalis*.

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