

CONTRIBUTIONS TO THE PHARMACOGNOSTICAL AND PHYTOBIOLOGICAL STUDY ON *LEONURUS CARDIACA* L. (LAMIACEAE)

MARIA-LIDIA POPESCU*, MIHAELA DINU, OLIMPIA TOTH

University of Medicine and Pharmacy „Carol Davila“, Faculty of
Pharmacy, 6 Traian Vuia street, Bucharest, Romania

*corresponding author: lidiapopescu24@yahoo.com

Abstract

The paper presents our experimental results regarding a comparative pharmacognostical and phytobiological study of *Leonurus cardiaca* L., motherwort (Lamiaceae), the vegetal drugs *herba*-, *folium*-, *flos*-, and *caulis*-type. Microscopically we identified the following specific anatomical elements: unicellular trichomes, with warty cuticle and pluricellular trichomes, with smooth cuticle for *Leonuri folium*; unicellular trichomes, with thin and smooth cuticle for *Leonuri flos* respectively. The chemical analysis showed the presence of flavonoids ($2.04\text{--}2.21 \times 10^{-3}$ g% in herb, $5.82\text{--}6.74 \times 10^{-3}$ g% in leaves, $1.14\text{--}1.63 \times 10^{-3}$ g% in flowers, and $0.83\text{--}1.02 \times 10^{-3}$ g% in stems; expressed as rutin), polyphenolcarboxylic acids ($62.34\text{--}65.12 \times 10^{-3}$ g% in herb, $89.91\text{--}93.43 \times 10^{-3}$ g% in leaves, $36.47\text{--}40.18 \times 10^{-3}$ g% in flowers, and $12.41\text{--}15.64 \times 10^{-3}$ in stems; expressed as caffeic acid), iridoides, triterpenes, tannins, sterols, carotenoids, monosaccharides, and polysaccharides. *Triticum* bioassay revealed the *up and down* (two ways) effect of the tested solutions. The aqueous extracts 5.00-1.66% had concentration-dependent mitoinhibitory effect and the diluted solutions (0.33%) had a stimulating effect on radicular elongation of *Triticum vulgare*.

Rezumat

Lucrarea prezintă rezultatele experimentale privind studiul farmacognostic și fitobiologic comparativ al produselor vegetale de tip *herba*, *folium*, *flos* și *caulis*, obținute de la specia *Leonurus cardiaca* L., talpa găștii (Lamiaceae). Microscopic s-a constatat că elementele specifice pentru *Leonuri folium* sunt perii tectori unicelulari verucoși și pluricelulari, cu cuticula netedă, iar pentru *Leonuri flos* perii tectori unicelulari, cu cuticula netedă și subțire. Prin examenul chimic s-a stabilit prezența flavonozidelor ($2,04\text{--}2,21 \times 10^{-3}$ g% în herba, $5,82\text{--}6,74 \times 10^{-3}$ g% în frunze, $1,14\text{--}1,63 \times 10^{-3}$ g% în floare și $0,83\text{--}1,02 \times 10^{-3}$ g% în tulpini; exprimare în rutozidă), acizilor polifenolcarboxilici ($62,34\text{--}65,12 \times 10^{-3}$ g% în herba, $89,91\text{--}93,43 \times 10^{-3}$ g% în frunze, $36,47\text{--}40,18 \times 10^{-3}$ g% în floare și $12,41\text{--}15,64 \times 10^{-3}$ g% în tulpini; exprimare în acid cafeic), iridoidelor, triterpenelor, taninurilor, sterolilor, carotenoidelor, ozelor și poliholozidelor. Testul *Triticum* a relevat efectul bisens al soluțiilor testate. Extractele apoase de concentrație 5,00% - 1,66% au acțiune mitoinhibitoare (direct proporțională cu concentrația), iar extractele mai diluate, de concentrație 0,33% au efect de stimulare a alungirii radiculare.

Key words: *Leonurus cardiaca* L., polyphenols, mitoinhibitory activity

Introduction

Leonurus cardiaca L., motherwort (*Lamiaceae*), has a long history of medicinal use. The herbal product consists of aerial (above-ground) parts collected during the flowering season. Motherwort herb contains diterpene bitter principles (leocardin), labdane-type diterpenes (15-O-ethylleopersin C, 15-O-methylleopersin C, and 15-EPI-O-methylleopersin C), iridoide monoterpenes (ajugoside, ajugol, galiritoside, reptoside), flavonoids (rutin, quercitrin, isoquercitrin, hyperoside, genkwanin), betaine (stachydrine), caffeic acid derivatives, tannins, triterpenoids of ursane class (ursolic acid, ilelatifol D, corosolic acid and euscaphic acid), volatile oil [1, 2, 7, 9, 12].

In folk medicine motherwort is used for heart complaints, bronchial asthma, climacteric symptoms, and amenorrhea. Indications approved by European Commission are nervous heart complaints and thyroid dysfunction. Homeopathic treatments include its use for cardiac complaints, flatulence, and hyperthyroidism [12].

The objectives of this study were to establish the microscopic characteristics, the main active principles and to test the activity on plant cell division of the vegetal drugs *herba*-, *folium*-, *flos*-, and *caulis*-type from indigenous *Leonurus cardiaca* L.

Material and methods

The raw material consists of *Leonuri herba*, *Leonuri folium*, *Leonuri flos* and *Leonuri caulis*, harvested at their full maturity in 2006, from Hofigal S.A. The herbal products were naturally dried in the shadow and stored in controlled laboratory conditions.

For the identity checking and quality determination the pharmacognostic analysis was applied. The identity was assessed using a macroscopic examination (the morphological and organoleptic characters), a microscopic examination (using powder clarified with 800 g/L chloral hydrate solution and a Labophot 2-Nikon microscope with digital photo camera) and a qualitative chemical analysis. For the qualitative analysis the raw material was successively extracted with different solvents (ethyl ether, methanol, water). Half of the above alcoholic and aqueous solutions were hydrolyzed. Specific reactions were carried out in initial and hydrolyzed solutions, in order to identify the main classes of natural compounds [5, 6, 8].

In order to evaluate the product quality it was performed an assay for flavonoids (using a spectrophotometric method based on the chelating reaction with aluminium chloride, according to the Romanian Pharmacopoeia 10 th edition – *Cynarae folium* monograph) and for polyphenolcarboxylic acids (using a spectrophotometric method based on

the formation of oxymes in the presence of the sodium nitrite and sodium molybdate). The results of the quantitative chemical research were calculated on a dried substance [5, 10, 11].

For the phytobiological research (Constantinescu method, *Triticum* bioassay) we used as a biological reagent embryonic roots of wheat (*Triticum vulgare* Mill.). The wheat caryopses were provided from the Institute for Agricultural Research - Fundulea, germinated and treated in laboratory conditions. For testing, 5.00% (expressed as plant product/mL) infusions were prepared from the aerial part (H), leaves (Fz), flowers (Fl), stems (T) respectively. From the primary 5.00% extract (samples H1, Fz1, Fl1 and T1) three serial dilutions were made, resulting solutions of 2.50% (samples H2, Fz2, Fl2 and T2), 1.66% (samples H3, Fz3, Fl3 and T3) and 0.33% (samples H4, Fz4, Fl4 and T4) [3, 4].

Results and discussion

The macroscopic examination established the morphologic characteristics and confirmed the identity of the raw material (based on the correspondence of the raw material morphological characters with these described by scientific literature) (Fig. 1).



Figure 1. *Leonuri herba*

The microscopic examination showed the following diagnostic characters: conical, uniseriate, unicellular trichomes covered with warty cuticle (Fig. 2) or composed of 2 to 4 cells with smooth cuticle (Fig. 3) for leaves; unicellular trichomes covered with a thin cuticle (Fig. 4) for flowers; lignified fibres for stem. Some other anatomical elements common for the flowering aerial parts of *Lamiaceae* species were identified: spirally and annularly thickened vessels (Fig. 5), glandular trichomes with a short unicellular stalk and a globular head composed of 8 cells (Fig. 6), endothecium (Fig. 7), stomata of the diacytic type, and papillae with striated cuticle, from the corolla (Fig. 8) or

papillae with smooth cuticle from the stigma (Fig. 9), spherical pollen grains, with three germination pores and a smooth exine. These characters are mentioned for *Leonuri herba*, according to scientific sources [9, 10].

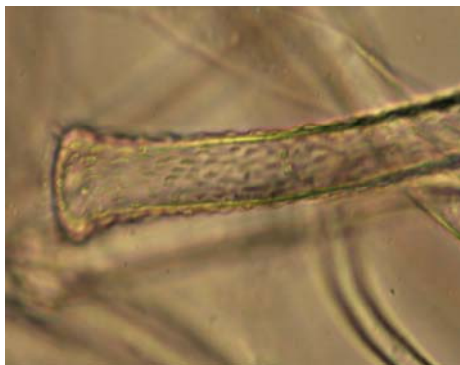


Figure 2

Leaf trichomes with warty cuticle (ob.40x)

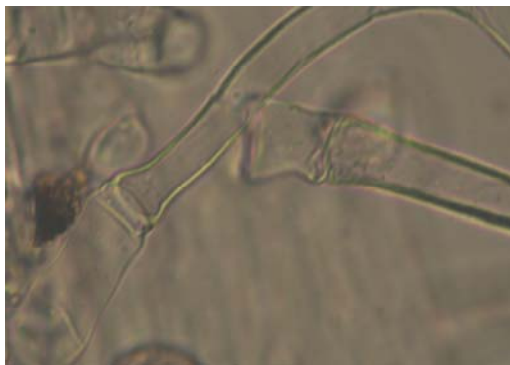


Figure 3

Leaf trichomes with smooth cuticle (ob.40x)

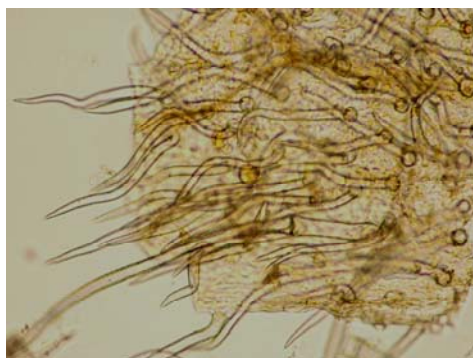


Figure 4

Flower trichomes (ob.10x)



Figure 5

Annularly thickened vessels (ob.10x)

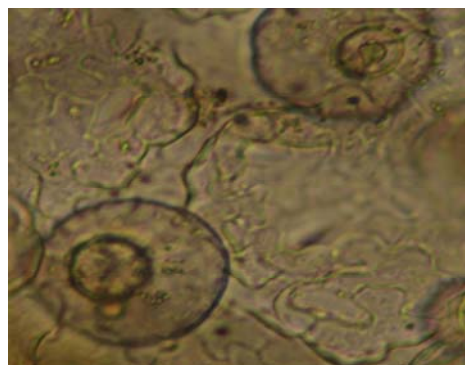


Figure 6

Glandular trichomes (ob. 40x)

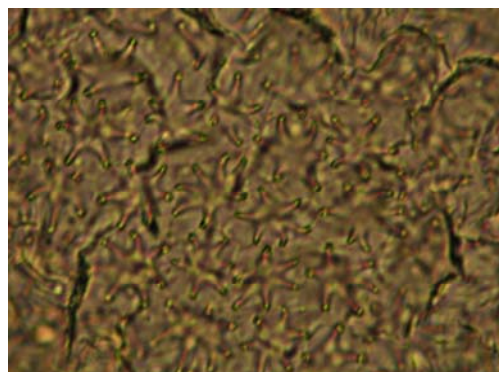


Figure 7

Endothecium (ob. 40x)

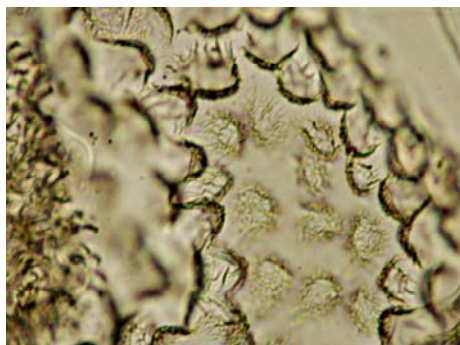


Figure 8
Corolla papillae (ob. 10x)

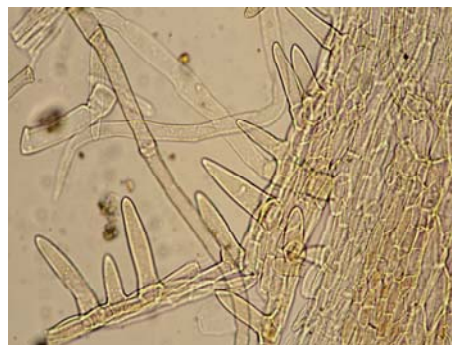


Figure 9
Stigma papillae (ob. 10x)

The following compounds were identified in the extractive solutions from herb, leaves, flowers, and stalks of *Leonurus cardiaca* using specific chemical reactions: flavonoids (aglycones and glycosides), polyphenolcarboxylic acids, iridoides, triterpenes (aglycones and glycosides), tannins, sterols, carotenoids, monosaccharides, polysaccharides (mucilages) and non-alkaloid nitrogen containing compounds. These compounds are mentioned as being present in *Leonuri herba*, according to scientific sources which we have consulted [2, 7, 9, 12].

The content of flavonoids and polyphenolcarboxylic acids are presented in the table I.

Table I
The results of quantitative chemical analysis

No.	Sample	Flavonoids g% (expressed as rutin)	Polyphenolcarboxylic acids g% (expressed as caffeic acid)
1.	<i>Leonuri herba</i>	$2.04-2.21 \times 10^{-3}$	$62.34-65.12 \times 10^{-3}$
2.	<i>Leonuri folium</i>	$5.82-6.74 \times 10^{-3}$	$89.91-93.43 \times 10^{-3}$
3.	<i>Leonuri flos</i>	$1.14-1.63 \times 10^{-3}$	$36.47-40.18 \times 10^{-3}$
4.	<i>Leonuri caulis</i>	$0.83-1.02 \times 10^{-3}$	$12.41-15.64 \times 10^{-3}$

This study showed that leaves of motherwort have a higher content of active principles than herb and flowers. The lower content of active principles is in stems. These results suggest that only the leaves should be harvested and used in therapy.

The results of the phytobiological study (Constantinescu method, *Triticum* bioassay) are presented in fig. 11 and fig. 12.

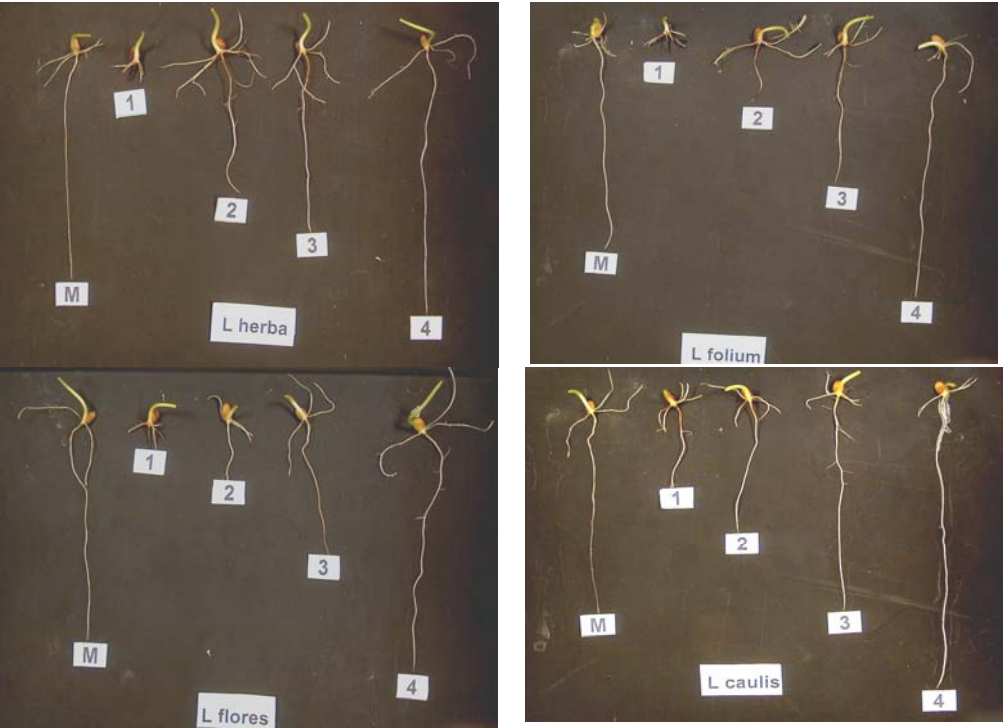


Figure 11
Influence of *Leonurus* extracts on embryonic *Triticum* root elongation in the 5th day

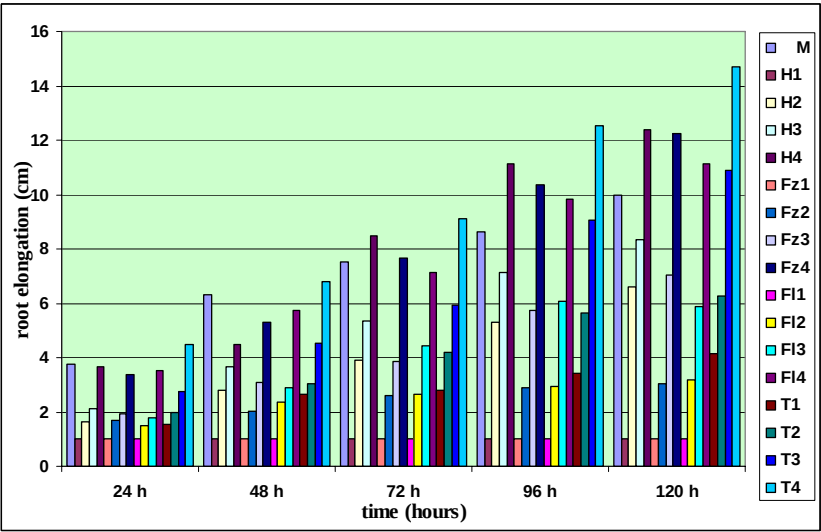


Figure 12
The root elongation values for each sample tested, in each of the five days [aerial part (H), leaves (Fz), flowers (FI), stems (T)]

In the *Triticum* bioassay a total inhibitory effect on the radicular elongation was observed only for the 5.00% aqueous extracts of *Leonuri herba*, *Leonuri folium*, and *Leonuri flos*. The 2.50-1.66% aqueous extracts of these products and 5.00-1.66% aqueous extracts of *Leonuri caulis* have a moderate mitoinhibitory effect compared with the control sample. At 0.33% concentration a stimulation effect on the radicular elongation produced by all types of *Leonurus cardiaca* herbal extract tested was observed.

The microscopic examination of the embryonic wheat roots treated confirmed the total mitoinhibitory effect (chromatoclastic action) of the higher concentration (5.00%) aqueous extracts of *herba*, *folium*, and *flos*, (nuclei with 1-2 hypertrophied nucleoli, troubled metaphases). For the 5.00 - 1.66% aqueous extracts from *caulis* and for the lower concentrations (2.50 - 1.66%) from *herba*, *folium*, and *flos* a moderate mitodepressive effect could be observed (numerous interphases and prophases; frequent metaphases). This behavior could be explained by the chemical constituents of the plant (iridoides, triterpenes, tannins, quite well-known for their mitoinhibitory effect). At the 0.33% concentration a stimulation effect on cell division was observed.

Conclusions

Leonuri folium has a higher content of active principles than *Leonuri herba* and *Leonuri flos*. The phytobiological assay revealed for *herba*, leaves, flowers and stems aqueous extracts obtained from *Leonurus cardiaca* an *up and down* effect (mitoinhibitory effect of cell division for the 5.00 - 1.66% concentrations and a stimulation one for 0.33%).

References

1. Agnihotri V.K., Elsohly H.N., Smillie T.J. et al. - New labdane diterpenes from *Leonurus cardiaca*, *Planta Med.*, 2008, 74(10), 1288-90
2. Ali M.S., Ibrahim S.A., Jalil S., Choudhary M.I. - Ursolic acid: a potent inhibitor of superoxides produced in the cellular system, *Phytother Res.*, 2007, 21(6), 558-61
3. Ancuceanu R.V., Istudor V., Dinu M., Codreanu M.V. - Contributions to the study of some *Cuscuta* species. Note 1. Study of the correlations between the metabolisms of *Cuscuta campestris* Yunck. and its hosts, by the Constantinescu bioassay (*Triticum* test), *Farmacia*, 2005, 53 (4), 28-38
4. Constantinescu D.Gr., Retezeanu M., OȚleanu R., Constantinescu M., Die Phytobiologische Prüfung der cytostatischen Wirkung in der Chemotherapie des Krebses angewendeten Äthylenimino- benzochinonen- (1,4), *Die Pharmazie*, 1963, 18, 699
5. Gîrd C.E., Duțu L.E., Popescu M.L., Pavel M., Iordache A.T., Tudor I. - Bazele teoretice și practice ale analizei farmacognostice, vol. I, Ed. Curtea Veche, București, 2008, 33, 104-105, 114-115, 134-137, 228-230

6. Gîrd C.E., Duțu L.E., Popescu M.L., Pavel M., Iordache A. – Researches about obtaining of active extracts polyphenolic-standardized, Note I. *Hyperici herba* – pharmacognostic analyse of raw material, *Farmacia*, 2009, 57(1), 35-42
7. Matkowski A., Piotrowska M. - Antioxidant and free radical scavenging activities of some medicinal plants from the *Lamiaceae*, *Fitoterapia*, 2006, 77 (5), 346-53
8. Popescu M.L., Dinu M. - Contributions to the pharmacognostical and phytobiological study on *Forsythia viridissima* L. (*Oleaceae*), *Farmacia*, 2008, 56(3), 267 – 274
9. Wichtl M., Anton R. - Plantes therapeutiques, TEC & DOC, Paris, 2003, 333-334
10. xxx - European Pharmacopoeia, 5 th edition, Council of Europe, Strassbourg, 2006, 2063
11. xxx - Farmacopeea Română ediția a X-a. Ed. Medicală, București, 1993, 335, 1016
12. xxx - PDR for herbal medicines, Ed. Thomson, Montvale, 2004, 573-4

Manuscript received: 12.12.2008