

CONTRIBUTION TO THE AUTHENTICATION OF *ARNICA*
MONTANA FLOWER-BASED HERBAL DRUGS USING
HIGH-PERFORMANCE THIN-LAYER
CHROMATOGRAPHY (HPTLC) ANALYSIS

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Abstract

Arnica flower (*Arnica montana* L.) is a valuable ingredient in many herbal and pharmaceutical products with anti-inflammatory properties. However, the pharmacopoeial herbal substance is often replaced by flower heads from other species, most often *Heterotheca inuloides* Cass. According to the European Pharmacopoeia one of the ways to distinguish drugs containing these two species is by TLC analysis of the rutin content in an aqueous ethanol extract. The present work proposes a method of distinguishing the two species based on exudate flavonoid profile, detected by HPTLC. Exudate flavonoid profiles of both species are distinguishable visually under UV light based on the different colouration of the main components. Methylated derivatives mainly of flavones were identified as the main component in the exudate of *A. montana*, whereas methylated flavonol derivatives were detected in the exudate of *H. inuloides*. The main components in *H. inuloides* are flavonoid aglycones coloured yellow (quercetin-3',3,7-trimethyl ether, quercetin-3,3',4',7-tetramethyl ether) or orange (quercetin, quercetin-3,7-dimethyl ether) under UV light. On the other hand, the main components found in *A. montana* (scutellarein-6-methyl ether, scutellarein-6,4'-dimethyl ether) are predominantly brown coloured. The

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proposed method is suitable for rapid identification of the *Arnica* flower containing drugs based on the profile of exudate flavonoid aglycones by the HPTLC technique.

Key words: *Arnica montana*, *Heterotheca inuloides*, exudate flavonoids, quercetin derivatives, scutellarein derivatives

Introduction. *Arnica montana* flower heads are the main ingredient of numerous drug preparations, having anti-inflammatory, antiseptic, antifungal, antimicrobial, antibiotic and immunomodulatory activities [1]. Different products of *Arnica* are applied externally to treat rheumatic illnesses, sprains, contusions, hematomas, and superficial skin inflammation [2]. Unfortunately, for many years the pharmacopoeial raw material of *A. montana* has been a subject of adulteration most often by *Heterotheca inuloides* Cass. and *Calendula officinalis* L. [3]. Adulteration of *Arnica* has been reported with species from the genera *Inula* (*Inula britannica* L., *I. helenioides* DC, *I. helvetica* Weber, *I. montana* L., *I. salicina* L.), *Doronicum* (*D. carpatanum* Boiss. & Reut. Ex Willk. & Lange, *D. grandiflorum* Lam. and *D. pardalianches* L.), *Senecio* (*Senecio doronicum* (L.) L., *S. jacobaea*), *Crepis* (*C. paludosa* (L.) Moench, *C. vesicaria* L. subsp. *taraxacifolia* (Thuill.) Thell. and many more [1, 4–6].

Thin layer chromatography (TLC) has proved to be an excellent technique for solving important scientific and practical issues [7,8]. The authentication of a great variety of drugs is made using TLC technique as it has been established as a gold standard by many pharmacopoeias. High-performance thin-layer chromatography (HPTLC) is an analytical technique based on TLC but with better resolution [8].

Exudate flavonoids and their distribution among the plant kingdom have been comprehensively studied by WOLLENWEBER [9]. Representatives of the Asteraceae are rich in exudate flavonoids and they are one of the most studied species for external flavonoid aglycones [10,11]. Exudate profiles of many species are used in chemotaxonomic studies, indicating that they are an important marker related to species identity [12, 13]. The largest advantage when dealing with exudate flavonoids is that they isolate very quickly and easily and this is very useful in case a rapid assessment of the quality of plant material is needed.

We found that herbal supplements in the form of raw herbal drugs of *Heterotheca inuloides* instead of *Arnica montana* are sold massively in drug stores and pharmacies in Bulgaria and other European countries. In some cases, the trade name “Arnica” is accompanied by the correct Latin name of the source species *Heterotheca inuloides*. However, in most cases, this clarification is not provided, or it is even miswritten as *Arnica montana*.

The present work aims to propose a method for distinguishing *Arnica montana* and *Heterotheca inuloides* raw materials based on their exudate flavonoid profiles by HPTLC.

Material and methods. Plant material. Herbal substances of *H. inuloides* incorrectly labelled *A. montana* were obtained from herbal pharmacies in Bulgaria and Spain. The reference herbal substance of *A. montana* originated from the experimental plots of the Department of Industrial and Medicinal Plants of the University of Life Sciences in Lublin (Poland).

Dried flowering heads of *A. montana* and *H. inuloides* were analyzed macroscopically and compared to reference sources [14–17].

Extraction. Air-dried plant material of studied samples (not powdered, 1 g) was briefly (2–3 min) rinsed with acetone (20 mL) at room temperature to dissolve the flavonoid aglycones accumulated on the plant surface – in thin epicuticular layer and glandular hairs. After filtration and evaporation of the acetone, the residue was re-dissolved in 250 μ L methanol and then subjected on HPTLC.

Chromatographic equipment and conditions. Exudates (5 μ L) were applied to 10 $\times$ 20 cm aluminium-backed HPTLC plates coated with 0.2 mm layers of silica gel 60 F₂₅₄ 5548 (Merck). Plant samples were spotted in triplicate. Toluene-dioxan-acetic acid (95:25:4, v/v/v) was applied for the development of the flavonoid mixture. The migration distance was 90 mm. Compounds were visualized by spraying with ‘Naturstoffreagenz A’ reagent. The fluorescence emission of flavonoid aglycones was recorded under UV radiation at 336 nm through a digital camera.

In addition to the thin-layer chromatography conditions cited above, TLC analysis was performed on DC-Alufolien Polyamid 11 F₂₅₄ plates as stationary phase and toluene-methylethylketone-methanol (60:25:15, v/v/v) as mobile phase to identify the compounds.

Results and discussion. Pharmacognostic analysis. Prior to HPTLC analysis of *A. montana* and *H. inuloides* exudates a detailed macroscopic examination of both raw materials was performed to confirm their botanical authenticity (Fig. 1, 2). The flower head characteristics of *A. montana* differed significantly from those of *H. inuloides*. The capitulae measured approximately 15 mm in *A. montana* and 20 mm in *H. inuloides*. The involucre of *A. montana* was composed of lanceolate bracts with acute apices, arranged in two rows whereas those of *H. inuloides* consisted of narrow elongated bracts in several rows. The abaxial surface of the bracts of *A. montana* was green with reddish colouring and abundant bright hairs in the central longitudinal section whereas that of *H. inuloides* was beige, hairy. The receptacle of *A. montana* was convex, alveolate and covered with hairs whereas that of *H. inuloides* was flat, without any hairs. The ray florets of *A. montana* were about 20 whereas those of *H. inuloides* were about 40. The tubular corollas ended with five reflexed lobes of *A. montana* whereas those of *H. inuloides* with straight lobes. The tubes of both ray and tubular florets of *A. montana* were densely covered with trichomes from the middle down whereas those of *H. inuloides* were with fewer and shorter hairs. The pappus of *A. montana* consisted of whitish bristles whereas that of *H. inuloides* was rosy brown in



Fig. 1. Macromorphological characteristics of the herbal substance Arnicae flos: A – general view of the raw material; B – cypsel with pappus and tubular floret; C – single tubular floret; D – single ray floret; E – convexed and hairy receptacle; F – involucre; G – fragmented flowering head; H – abaxial view of involucre bracts

colour, varying from lighter to more intense shade. Cypselas of *A. montana* were narrow, light to dark brown, with continuously hairy indumentum whereas those of *H. inuloides* varied in length and thickness of the indumentum. In *H. inuloides* the cypselas of the ray florets were shorter and with scarce or absent trichomes compared to those of the tubular florets.

High-performance thin layer chromatography analysis. Exudate flavonoid profiles of the studied samples analyzed by HPTLC are presented in Fig. 3. The differences in colouration of the individual compounds in the profiles obtained for the two species are clearly visible. TLC analysis of different sorbents silica gel and polyamide allowed the identification of the individual compounds. Methylated derivatives mainly of flavones were identified as the main component in the exudate of *A. montana*, whereas methylated flavonol derivatives were detected in the exudate of *H. inuloides*. The main components in *H. inuloides* are flavonoid aglycones coloured yellow (quercetin-3',3,7-trimethyl ether, quercetin-3,3',4',7-tetramethyl ether) or orange (quercetin, quercetin-3,7-dimethyl ether) under UV light. On the other hand, the main components found

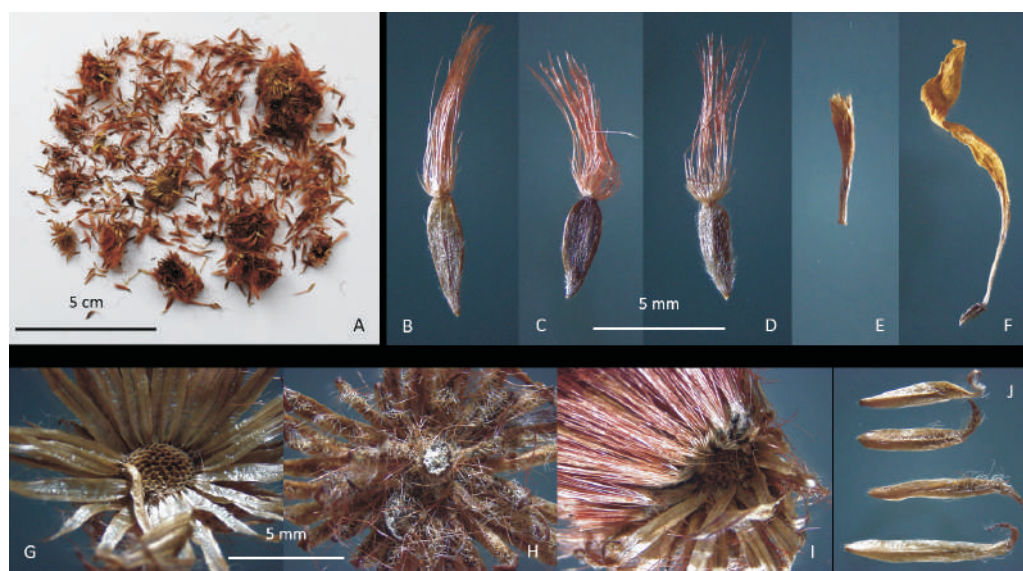


Fig. 2. Macromorphological characteristics of dried flowering heads of *Heterotheca inuloides* Cass.: A – general view of the raw material; B–D – cypsellas with pappus; E – single tubular floret; F – single ray floret; G – flat and bare receptacle; H – involucre; I – fragmented flowering head; J – abaxial view of involucre bracts

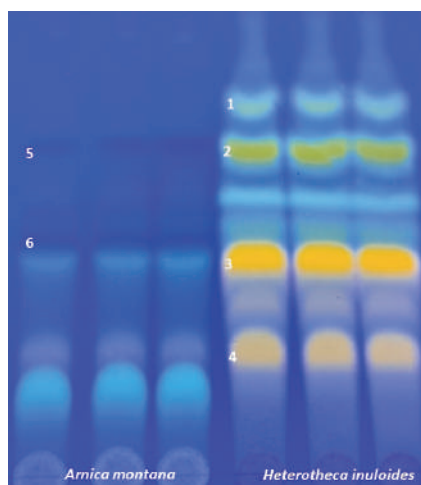


Fig. 3. HPTLC chromatogram of flavonoid aglycones profiles of *Arnica montana* and *Heterotheca inuloides*: 1. quercetin-3,3',4',7-tetramethyl ether; 2. quercetin-3',3,7-trimethyl ether; 3. quercetin-3,7-dimethyl ether; 4. quercetin; 5. scutellarein-6,4'-dimethyl ether; 6. scutellarein-6-methyl ether

in *A. montana* (scutellarein-6-methyl ether, scutellarein-6,4'-dimethyl ether) are predominantly brown coloured.

The European Pharmacopoeia differentiates *A. montana* from *H. inuloides* through TLC analysis of rutin in ethanol extracts [3]. The approach presented in this work complements the reported differences with data relating to flavonoid aglycones. Exudate flavonoids are extracted quickly, which makes the method faster and the differentiation is based on the flavonoid aglycone profile. The

exudate flavonoid profile of *A. montana* remains consistent across different geographic origins [18,19]. Moreover, the exudate flavonoid profiles of different plant species have been used as chemotaxonomic markers that show their importance as a characteristic feature of the species [12,13,20].

Conclusion. The current work presents a rapid HPTLC method for distinguishing *Arnica montana* from *Heterotheca inuloides* based on their exudate flavonoid profiles. Given the frequent mislabelling of Arnica-based products, this technique has significant potential for enhancing regulatory quality control. Standardizing HPTLC-based authentication methods will be a crucial step toward improving herbal drug quality worldwide. This study highlights inadequate regulation of herbal drugs in commercial markets.

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