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Quality control of commercial product Flos Arnicae by HPTLC analysis of surface flavonoid aglycones

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ABSTRACT

A convenient, rapid and accurate chromatographic method was developed for authentication and quality control of commercial product Flos Arnicae. Chromatography (HPTLC or TLC) was performed on silica gel 60 F_{254} (Merck) plates with toluene–dioxane –acetic acid (95:25:4) as mobile phase. Compounds were visualized by spraying with 'Naturstoffreagenz A' reagent. Detection was performed at λ=366 nm. Eight flavonoid aglycones were detected of the exudates of *Arnica montana* and *Arnica chamissonis* by co-chromatography with authentic markers. Differences in the fluorescence emission were observed in flavonoid profiles between *A. montana* and *A. chamissonis* under mentioned above conditions of analysis. Flavonoid profile of *Arnica montana* is presented by flavonoids with brown fluorescence while the flavonoid profile of *Arnica chamissonis* is composed of flavonoids with yellow-green fluorescence. These visually noted differences of the flavonoid profiles of the both species allow they to be quickly distinguished. The proposed method could be used for quality control of identity and purity of commercial product Flos Arnicae.

Introduction

Arnica montana L. (Asteraceae) is a rare medicinal plant under strict protection in several European countries (Lange, 1998). The alcohol extract of flowers traditionally used for treatment of skin wounds, bruises and contusions (Wijnsma, 1995). *Arnica montana* is very similar to *Arnica chamissonis* Less that is found in the United States and surrounding regions. *A. chamissonis* is considered an invasive species in Austria, Denmark, Finland, Norway and Sweden (CABI, 2013). Both species differ in morphological markers in fresh condition but when the plant material is in the dried form the differentiation is difficult. Although in a few countries such as Germany and Netherlands a mixture of inflorescences of *A. montana* and *A. chamissonis* is available on market as Arnica Flos (Evstatieva, 2009) in some cases, a more accurate assessment of the quality of drug by determining of individual species is needed. Moreover recent survey shows that the amount and range of sesquiterpene lactones - substances that determine the pharmacological action - are different in both species (Barron, 2010)

Thin layer chromatography (TLC) is widely used for the monitory the identity and purity of drug (Wagner and Bladt S 1996; Mohammad et al., 2010). TLC has the advantages of low cost, ease of maintenance, and good selectivity of detection. In addition, TLC can analyze several parallel samples in a single run (Braz, 2012).

The selection of chemical markers is crucial for the quality control of plant drugs (Ahmad 2010; Braz, 2012). Plant species shows a characteristic flavonoid profile as indicated by many chemotaxonomic studies (Emerencianoa et al., 2001; Güzel et al., 2011). The chemotaxonomic markers are used as indicators of botanical identity of plant material. Externally accumulated flavonoid aglycones can be valuable chemotaxonomic markers too (Wollenweber and Schneider, 2000; Valant-Vetschera et al., 2003). The largest advantage when dealing with surface flavonoids are that they isolate very quick and easy and this is very useful in case you need to make a rapid assessment of the quality of plant material.

The present work proposes convenient, rapid and accurate method for quality control of the commercial product Flos Arnicae on the base of externally accumulated flavonoid aglycones.

Material and Methods

Plant material

Plant material of *Arnica montana* and *A. chamissonis* was collected from the experimental fields of Institute of Biodiversity and Ecosystem Research, Bulgaria - Vitosha Mt., Zlatni mostove 1400 asl.,

Preparation of exudates

Air dried plant material (flowers) but not powdered (1 g) was briefly (2-3 min) rinsed with acetone (20 mL) at room temperature to dissolve the lipophilic components accumulated on the surface. After evaporation of the acetone the residues were redissolved in 250 µL methanol and then subjected on HPTLC.

Chromatographic equipment and conditions

Arnica montana and *A. chamissonis* exudates (5 µL) were applied to 10 cm × 20 cm aluminium-backed HPTLC plates coated with 0.2 mm layers of silica gel 60 F_{254} 5548 (Merck). Plant samples were spotted in triplicate. The mobile phase was toluene–dioxane –acetic acid, 95:25:4. 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, by means of a digital camera. The identification of the compounds was achieved by co-chromatography with authentic markers obtained from Prof. Eckhard Wollenweber.

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Results and discussion

The flowers of *A. montana* and *A. chamissonis* are difficult to distinguish in the dry state but often it is necessary in practice. The extracts of *A. montana* and *A. chamissonic* have been shown a similar TLC pattern of flavonoid glycosides so they can not be distinguished by analysis of these components (Bohm, 2001). The research of external flavonoid profiles of both species showed clear differences between them. The present study provides convenient and accurate HPTLC (or TLC) method for distinguishing the two species in commercial product Flos Arnicae

In the acetone exudate of *A. montana* were detected by co-chromatography with authentic markers four surface flavonoid aglycones - scutellarein 6-methyl ether (hispidulin) (**1**), scutellarein 6,4'-dimethyl ether (pectolinarigenin) (**2**), 6-OH luteolin 6-methyl ether (**3**) and kempferol-6-methyl ether (**4**). Thin layer chromatographic data – R_F (rate of flow) and color of detected flavonoid aglycones are presented at Table 1.

Table 1. Thin layer chromatographic data on detected flavonoid aglycones in the exudates of *Arnica montana* and *Arnica chamissonis*

Flavonoid aglycones	Thin layer chromatographic data – R_F (rate of flow) and color*
(**1**) scutellarein 6,4'-dimethyl ether (pectolinarigenin)	0.54 (brown/ brown)
(**2**) scutellarein 6-methyl ether (hispidulin)	0.33 (brown/ brown)
(**3**) kempferol-6-methyl ether	0.39 (brown / brownish-yellow)
(**4**) 6-OH luteolin 6-methyl ether	0.16 (brown / orange)
(**5**) apigenin-4'-methyl ether	0.50 (brown/ yellow)
(**6**) kempferol	0.31 (brownyellow / yellow green)
(**7**) luteolin	0.13 (brown/ yellow)
(**8**) unidentified compounds	0.30 (brown/ yellow)

* For chromatographic protocol see Chromatographic equipment and conditions

In the acetone exudate of *Arnica chamissonis* except scutellarein 6,4'-dimethyl ether (pectolinarigenin) (**2**) were detected apigenin-4'-methyl ether (**5**), kempferol (**6**), luteolin (**7**) and another unidentified compounds (**8**) with yellow green fluorescence.

Differences in flavonoid components of the both species correspond to differences in the fluorescence emission of the flavonoids in their profiles. The flavonoid aglycones of exudate of *A. montana* show brown fluorescence emission under the conditions of analysis while those of exudate of A. *chamissonis* were with yellow green color.

As shown in the photos presented in Figure 1, the both flavonoid profiles visually very clearly distinguished.

Legend: A - *Arnica montana* exudates; B - *Arnica chamissonis* exudates; scutellarein 6,4'-dimethyl ether (**1**); scutellarein 6-methyl ether (**2**) ; kempferol-6-methyl ether (**3**); 6-OH luteolin 6-methyl ether (**4**); apigenin-4'-methyl ether (**5**); kempferol (**6**); luteolin (**7**); unidentified compounds (**8**);

For chromatographic protocol see Chromatographic equipment and conditions

In conclusion the proposed method allows fast and easy to distinguish flowers of *A. montana* from *A. chamissonis*. It is especially useful when the flowers of the both species are in dry form and it is necessary to take into account the ratio of the two species in the commercial product.

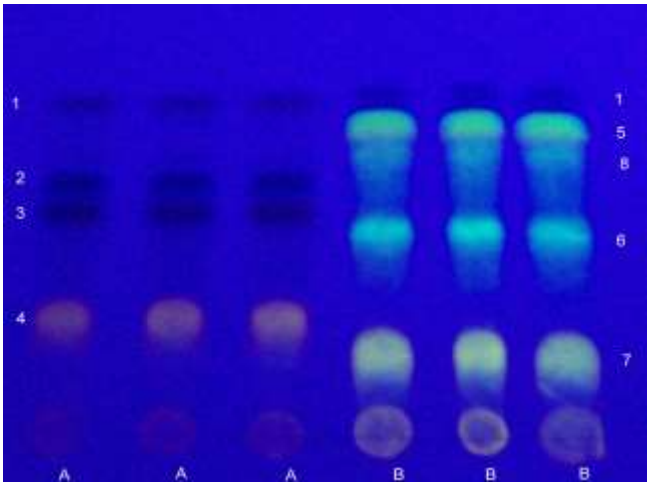

Figure 1. Chromatogram (HPTLC) of exudates of *A. montana* and *A. chamissonis*

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