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Original article

Microscopical descriptions and chemical analysis by HPTLC of *Taraxacum officinale* in comparison to *Hypochaeris radicata*: a solution for mis-identification

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ABSTRACT

Taraxacum officinale F. H. Wigg, Asteraceae, is frequently misidentified or substituted with *Hypochaeris radicata* L., Asteraceae (false dandelion). To increase our knowledge of *T. officinale* and differentiate it from *H. radicata*, we investigated the two species using a combination of taxonomy, microscopy, and chromatographic studies via fingerprint profiles. Micromorphological characteristics were studied using scanning electron microscopy, while optic light microscopy was used for histochemical observations. Fingerprint profiles were constructed using HPTLC. *T. officinale* was found to have a morphologically distinct type of pluricellular trichomes that can be used to differentiate the two species, as these structures were not identified in *H. radicata* samples. Furthermore, two types of laticiferous vessels may also be distinctive characteristics of *T. officinale* at species level. In addition, the HPTLC data derived from methanolic extracts of *H. radicata* and *T. officinale* roots showed clearly different chemical profiles. Thus this study establishes the authenticity of *T. officinale*, and the observed parameters could help minimize drug substitutions in herbal medicines.

Introduction

The prevalent use and wide availability of herbal medicines has raised concerns about their quality, efficacy and safety. Correct species identification is paramount to quality assurance, as very few traditional herbs are cultivated and nearly all raw materials are obtained from natural stands of vegetation. In consequence, misidentifications or substitutions can easily occur. Dandelions, *Taraxaxum officinale* F. H. Wigg, Asteraceae, are widely spread throughout the world and traditionally used to stimulate dieresis, to increase bile flow and appetite, to treat dyspepsia, and to treat gastrointestinal ailments (Blumenthal et al., 1998; 2000; WHO, 2007; Yarnell and Abascal, 2009). However, *T. officinale* and the false dandelion, *Hypochaeris radicata* L., Asteraceae, are two closely related and morphologically similar species (Fig. 1) that are often misidentified or substituted with one another. The latter may cause pasture-associated stringhalt, an acquired equine disease characterized by peripheral neuropathy and hyperflexion of the pelvic limbs. The disease occurs mostly during periods of drought in horses grazing pastures that are heavily contaminated with

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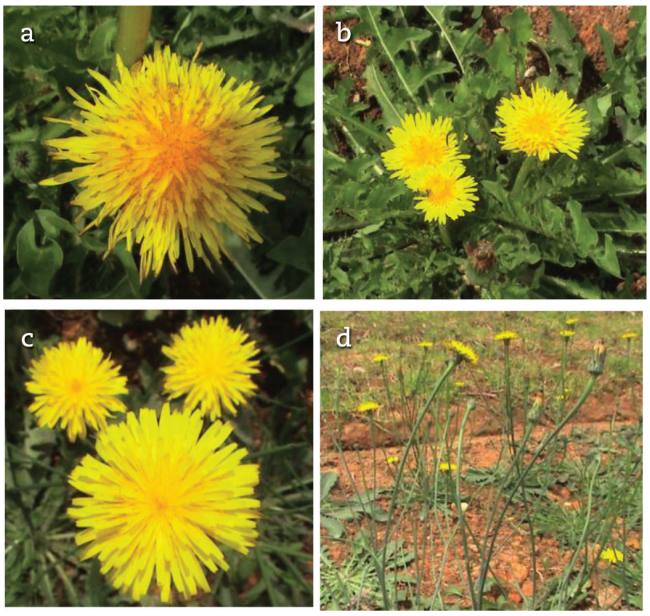


Figure 1 – *Taraxacum officinale*: open flower and a rosette with terminal inflorescence (a-b). *Hypochaeris radicata*: open flower and the long, branched inflorescences (c-d).

H. radicata (Huntington et al., 1989; Araújo et al., 2008; MacKay et al., 2013). In Eastern Antioquia (Colombia), misidentification of *T. officinale* and *H. radicata* based on traditional methods of authentication via morphology are common. Dandelion leaves and roots replacement with parts of the false dandelion occur approximately two-thirds of the time; in fact *H. radicata* is one of the most used plants in the region in comparison with *T. officinale*; however, its bioactive and toxic compounds are unknown. As a result, clinical application of *T. officinale* in herbal medicine is in fact combining the effects of the chemical composition of both species.

Quality control is crucial to ensure the safety and correct handling of herbal medicines. The quality parameters for herbal drugs are usually specified and implemented in pharmacopoeias, including national pharmacopoeias and the European Pharmacopoeia. However, a monograph of *T. officinale* is not available for quality control purposes in pharmacopoeias, except for the WHO Monographs on Selected Medicinal Plants, which only describes the physicochemical parameters and recommends that chemical testing be established in accordance with national requirements (WHO, 2007). Other pharmacopoeias, such as the British Herbal Pharmacopoeia (1990) (Hoffmann, 2003) and the German Commission E (Blumenthal et al., 2000), only present pharmacological monographs of the dandelion without quality parameters. Given that *T. officinale* has received surprisingly little research attention (Yarnell and Abascal, 2009), a monograph of this particular herb that adheres to modern quality standards should be developed to facilitate and encourage safe use by practitioners for authorized products. Detailed exomorphology and micromorphological studies of *T. officinale* have been performed (Popescu et al., 2010; Sudo et al., 2012). However, modern methods described in pharmacognostical reports for determining microscopical standards and for the identification and quantification of active constituents in plant material may be useful for the standardization of *T. officinale* identification and may solve problems related to misidentification or substitution. This study provides criteria for the correct identification of *T. officinale* and *H. radicata* species for fresh, dry or powdered samples. Because of the importance of quality control, careful attention was given to identity establishing criteria using a combination of taxonomy, microscopy and chromatographic studies via HPTLC fingerprint profiles.

Material and methods

Plant material

Taraxaxum officinale F. H. Wigg, Asteraceae, and *Hypochaeris radicata* L., Asteraceae, were botanically identified, and voucher specimens were deposited at the Herbarium of Universidad de Antioquia HUA (*T. officinale*: Alzate 3444, *H. radicata*: Alzate 3443). Two *T. officinale* specimens, D4 and D5, were harvested from two agroecological locations in Antioquia Department (Colombia), Rio Negro (RN) and Guarne (G), respectively, and evaluated. Two different origins of *H. radicata* were also evaluated: D2 and D3 were cultivated in the same locations as *T. officinale*. Leaf and root samples were dried at 45% in an air-forced dryer (Dies, Medellín, Colombia) and grounded using an excelsior mill (IKA A11 Basic, IKA Works, Inc. United States). The powder was stored in sealed vessels for further use.

Chemicals

Chloral hydrate and other chemicals used for microscopic studies were analytical grade. Analytical grade ethyl acetate, formic acid and glacial acetic acid were purchased from Merck. HPLC grade methanol was purchased from Merck (Darmstadt, Germany). Water was purified using a Milli-Q system. Dandelion (*T. officinale*) root E standard was purchased from ChromaDex, Inc. (United States).

Microscopic observation

For the histochemical observations, powdered leaves and roots of *T. officinale* and *H. radicata* were clarified with a chloral hydrate solution (800 g/l) and observed with a Nikon E200 microscope (ob. 10× and 40×) coupled to a Nikon DS-Fi1 digital camera. The images were analyzed using the NIS-Elements software (Nikon). The micromorphology of samples was analyzed using a scanning electron microscope (SEM) model JEOL JSM 6490 LV. The SEM analyses were performed under vacuum conditions using backscatter electrons with a distance of 10 mm and an accelerating voltage of 20 kV. The samples were coated with Au over 60 s, using the Denton Vacuum Desk IV equipment as in typical SEM imaging processes.

HPTLC analysis

Extracts were prepared using 200 mg of powdered material and extracted with 3 ml of methanol for 5 min in a hot water bath at 60°C. One milliliter of extract was recovered for analysis. Pre-coated HPTLC silica gel 60 F-254 aluminum plates (20 × 10 cm;

250 µm thicknesses; Merck, Germany) were used. Samples were applied with a 100 µl sample microsyringe (Hamilton - Bonaduz Schweiz, Camag, Switzerland) using a Linomat 5 system (Camag, Switzerland). Five microliters of the samples were applied as 5 mm bands in tracks 1-10 in the following sequence on all plates: *T. officinale* standard extract (1-2), *H. radicata* D2 extract (3-4), *H. radicata* D3 extract (5-6), *T. officinale* D4 extract (7-8) and *T. officinale* D5 extract (9-10). Plates were developed in a vertical glass chamber (20 x 10 cm; Camag, Muttenz, Switzerland) using ethyl acetate:formic acid:glacial acetic acid:water (100:11:11:26 v/v/v/v) as the mobile phase. The optimized chamber saturation time for the mobile phase was 10 min at room temperature (25° ± 2°C). The solvent front moved 7 cm over approximately 20 min. The plates were dried after development, and the components were visualized by UV irradiation at 254 nm. All measurements were performed using winCATS version 1.4.4.6337 software (Camag, Muttenz, Switzerland). The plates were derivatized with either of the following spray reagents for visualization: (i) anisaldehyde-sulfuric acid or (ii) vanillin-sulfuric acid. Each analysis was carried out in duplicate.

Results and discussion

Macroscopic characteristics

The World Health Organization (WHO, 1998) recommends that medicinal plant materials be categorized according to both macroscopic and microscopic characteristics. Therefore, macroscopic and microscopic identification are the first steps in quality control assessment. *T. officinale*, an herbaceous plant, has deeply serrated large leaves that are either light or dark green and are clustered in a rosette at the base of the plant. The flowering stalks are long and upstanding and carry a solitary terminal inflorescence (Fig. 1 a-b). The inflorescence ranges from 7 to 15 mm in diameter and is composed of 140-400 yellow ligulate florets. The fruits are conical brown achenes that are crowned by a white, hairy pappus that allows the seeds to be distributed by wind (Schütz et al., 2006). In contrast, the invasive *H. radicata*, classified as a noxious weed, is a short-lived perennial with plumed seeds that also allows long-distance wind dispersal (Soons et al., 2004). It has a rosette of hirsute basal leaves, a flowering stalk that can reach 45 cm in height, and well-dispersed plumed achenes (Schoenfelder et al., 2010). Therefore, *H. radicata* is easily distinguishable from *T. officinale* by its long, branched inflorescences (Fig. 1 c-d).

Microscopic evaluation of T. officinale *and* H. radicata

Microscopy is one of the easiest and cost-effective methods for correct identification (Kumar et al., 2012). Because of the lack of previous microscopy studies to differentiate *T. officinale* and *H. radicata*, the present study aimed to establish standards that could be used to identify differences between the two species. Analysis of the raw plant material by optical microscopy found that both the epidermal tissue and the morphology of the stomata in the leaves of both species are very similar. These similarities can be observed in Fig. 2. The stomatal apparatus consists of two guard cells bounding a lenticular pore, the orientation of which is largely parallel to the guard cells. The stomata of both *T. officinale* and *H. radicata* are distributed on both the adaxial and abaxial leaf surfaces; however, they occur more frequently on the lower surface. The stomata are mainly anomocytic and similar to the stomatal apparatus of other Asteraceae species (Adedeji and Jewoola, 2008).

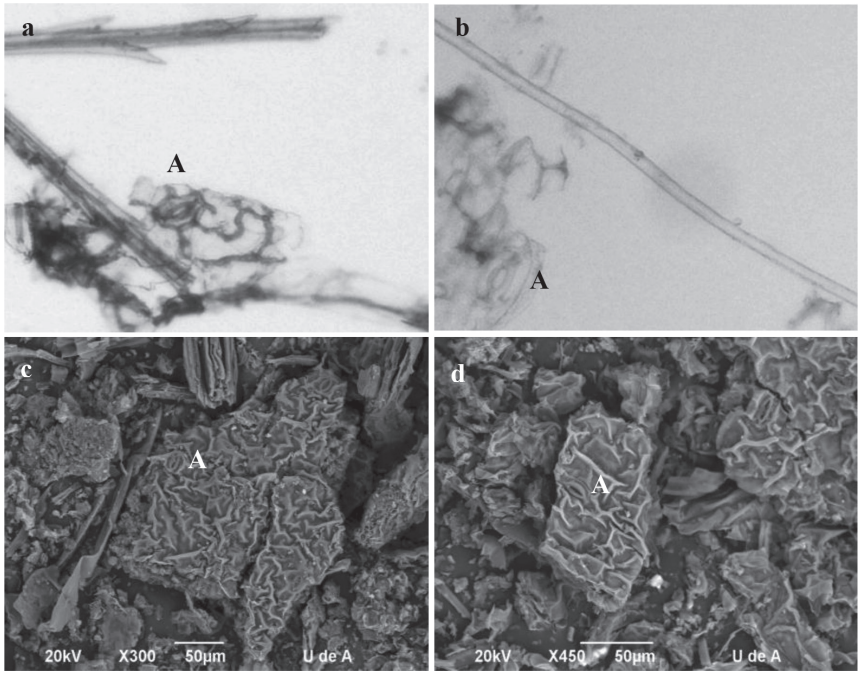


Figure 2 – Microphotographs showing the anatomical elements of *T. officinale* and *H. radicata*. Light micrographs of *T. officinale* (a) and *H. radicata* (b) with stomata (A) in D4 and D2 leaves samples, respectively (10× magnification). Scanning electron microscopy (SEM) micrographs showing A on D4 (c) and D2 (b) leaves. (—) Scale bar in (b) 50 µm.

The indumentums of *H. radicata*, however, consists of multicellular epidermal hairs (Fig. 3), a feature only found in this species. Trichomes can also be important for discrimination among taxa and play a key role in plant taxonomy (Giuliani et al., 2008). Thus, the trichome morphology on *T. officinale* leaf surfaces was studied using optical microscopy. One morphologically distinct type of pluricellular trichomes, characterized by sharp and irregular segments, was observed (Fig. 3c), indicating that trichome morphology could be used to differentiate between the two species as these structures were not identified in *H. radicata* samples.

Using optical microscopy, continuous xylem was clearly evident in root samples as ladder-like xylem vessels in *T. officinale*, (Fig. 4a) (Popescu et al., 2010). Scanning electron micrographs were also used to compare the structural

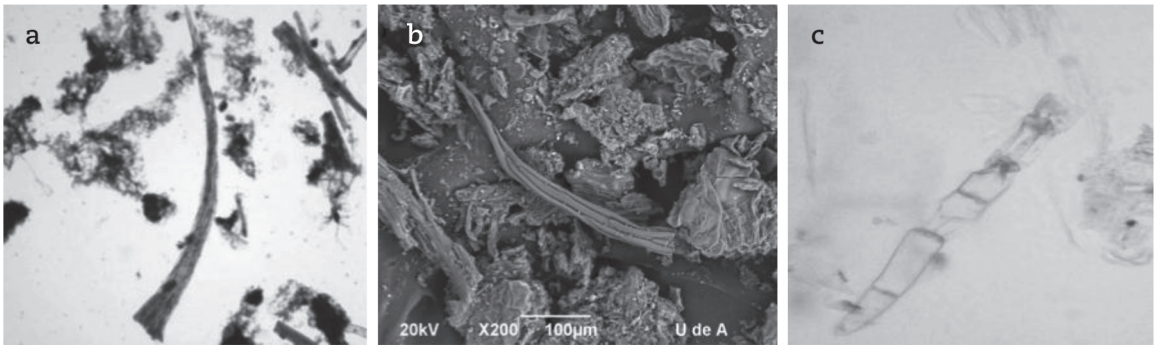


Figure 3 – Epidermal hair observed in *H. radicata*. Photo obtained via optical microscope of one multicellular epidermal hairs (10 × magnification) (a). Scanning electron microscopy (SEM) image showing detail of epidermal hair (b). (—) Scale bar in 100 µm. Micrographs of the leaf trichomes of *T. officinale* (10 × magnification) (c).

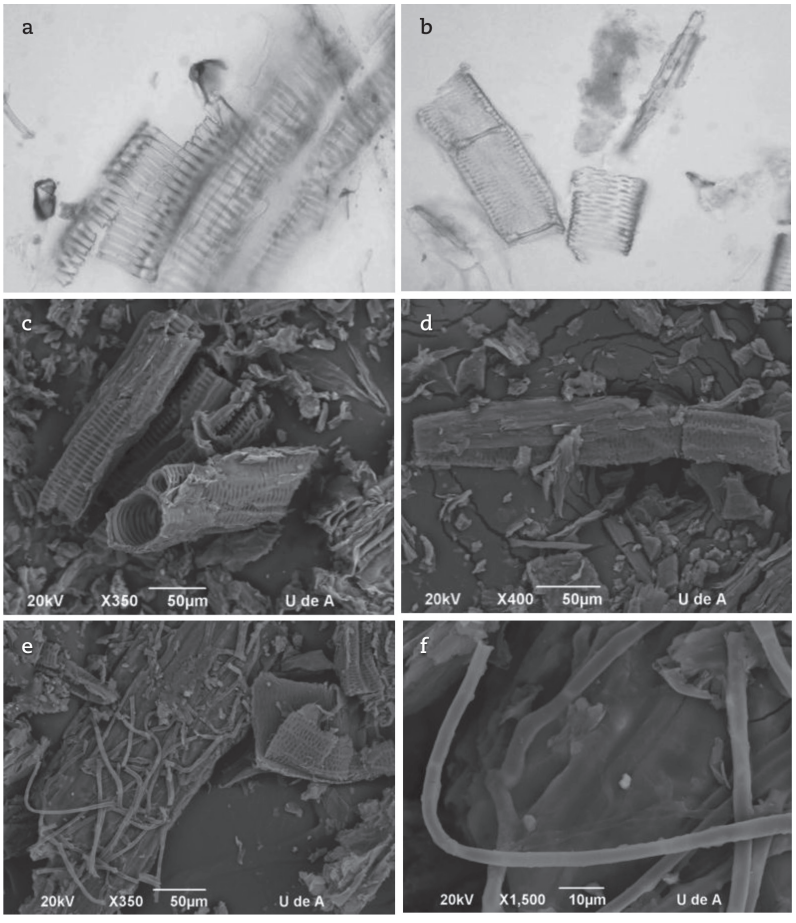


Figure 4 – Conducting system in *T. officinale* and *H. radicata* roots. Optical microscope images of xylem vessels in *T. officinale* and *H. radicata* (a and b, respectively) (10× magnification). Scanning electron microscopy (SEM) images showing the details of groups of laticiferous tubes arranged in several interrupted rings (c). (—) Scale bar in 50 µm. SEM image of xylem vassels in *H. radicata* (d). (—) Scale bar in 50 µm. SEM images of flagellar long trichomes in *H. radicata* roots (e-f). (—) Scale bar in (e) 50 µm, (—) Scale bar in (f) 10 µm.

morphology characteristics. In D4 and D5 samples, we observed driving hoops formed from groups of laticiferous tubes arranged in several interrupted rings (Fig. 4c) (WHO, 2007), while we observed a characteristic conduction system of substances within root vessels in *H. radicata* using both types of microscopy (Fig. 4b and 4d). In the samples of *H. radicata* roots it was possible to identify flagellar structures as long trichomes, which were considered an important differential characteristic since this element was not identified in *T. officinale* and could be key in the microscopical analysis of *H. radicata* (Fig. 4e and 4f).

HPTLC analysis

HPTLC method optimization

The WHO has emphasized the need to ensure the quality of medicinal plants using modern controlled techniques (Sharma et al., 2010). Therefore, HPTLC is a valuable tool for reliable identification, as it can provide chromatographic fingerprints that can be visualized and stored as electronic images (Johnson et al., 2011; Sampathkumar and Ramakrishnan, 2011). Several runs for HPTLC analysis were performed using mobile phases containing solvents of varying polarity and concentrations to obtain high resolution and reproducible peaks. A 100:11:11:26 v/v/v/v mixture of ethyl acetate:formic acid:glacial acetic acid:water yielded good resolution, well-defined zones throughout and clear definition of the profiles of *H. radicata* and *T. officinale* (Fig. 5). The two species were run in parallel (tracks 3-6 and 7-10) on all plates and displayed distinct chemical profiles. Derivatization with vanillin-sulfuric acid solution followed by visualization under white light was best suited for band detection. For this study, the chromatograpic plates were scanned at 254 nm before spraying, and the HPTLC profile of a methanolic root extract from *H. radicata* revealed eight spots with R_f values of 0.18 and 0.97 (Table 1). The methanolic extract of *T. officinale* roots showed five spots with R_f values in the range of 0.18 to 0.93 (Table 1, Fig. 5). In general more degree of chemical diversity has been observed in *H. radicata* parts when compared with *T. officinale* parts. Furthermore, comparison of the two origins of *T. officinale* revealed they were qualitatively similar in general chemical compositions as illustrated by the HPTLC chromatograms. This could mean that environmental changes in the crop do not affect greatly their chemical profiles, at least in the studied environments. On the other hand, the dandelion standard (tracks 1-2) showed some variations in the zone intensities.

Because herbal medicines can be quite specific and complex, chemical markers can help ensure and demonstrate the quality of these products. The selection of chemical markers is crucial for quality control and authentication of genuine species (Li et al., 2008). Markers of chemically defined constituents or groups of constituents in a herbal medicinal product are of interest for quality control purposes regardless of whether the products possess any therapeutic activity (EMA, 2008). Ideally, chemical

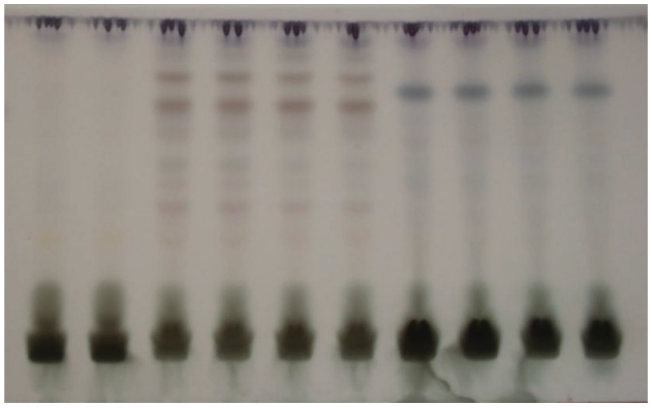


Figure 5 – HPTLC comparisons of *H. radicata* and *T. officinale* extracts from roots collected from two geographic locations in the Antioquia Department (Colombia): Rio Negro (RN) and Guarne (G). Tracks 1 and 2 = Dandelion standard; 3 and 4 = *H. radicata*, RN; 5 and 6 = *H. radicata*, G; 7 and 8 = *T. officinale*, RN; 9 and 10 = *T. officinale*, G. Derivatisation with anisaldehyde–sulfuric acid solution and visualization under white light.

Table 1
HPTLC profiles of *H. radicata* and *T. officinale* root extracts.

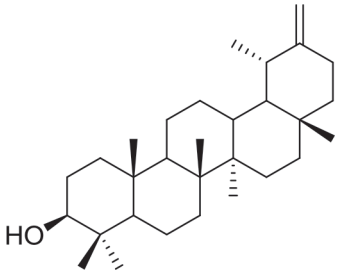
H. radicata			*T. officinale*		
Peak	Rf	UV spectra (λ nm)	Peak	Rf	UV spectra (λ nm)
1	0.18	259	1	0.18	248
2	0.36	284	2	0.65	248, 330, 426
3	0.44	273	3	0.74	247
4	0.55	278	4	0.9	249, 332, 433
5	0.69	265	5	0.93	279, 329, 414
6	0.77	282	6	0.97	Solvent front
7	0.86	283, 330, 429	-	-	-
8	0.90	245,330, 433	-	-	-
9	0.97	Solvent front	-	-	-

markers should be unique components that contribute to the therapeutic effects of an herbal medicine. However, it is very difficult to identify the correct marker compounds for all traditional herbal medicines, as some have unknown active constituents or multiple active constituents. The active ingredients in *T. officinale* are found in both the roots and leaves. The leaves contain bitter sesquiterpene lactones, such as taraxinic acid, and triterpenoids, such as cycloartenol, taraxasterol (**1**) and Ψ–taraxasterol (**2**). The roots, in addition to these compounds, contain phenolic acids, inulin and others sesquiterpenes, including the eudesmanolides, tetrahydroridentin B (**3**) and taraxacolide-*O*-β-glucopyranoside (**4**); the guaianolides 11β,13-dihydrolactucin and ixerin D; three germacranolide esters, taraxinic acid β-glucopyranoside (**5**), its 11,13-dihydro-derivative (**6**) and ainslioside (**7**); and various triterpenes, their acetates and 16-hydroxy derivatives (Kisiel and Barszcz, 2000; WHO, 2007; González-Castejón et al., 2012). However, characteristic markers for *T. officinale* are not commercially available. Therefore, the compounds with the R_f values 0.69 and 0.77, which were unique to the root of *H. radicata*, and a characteristic peak with an R_f value of 0.74 in *T. officinale* (Fig. 6) might be suitable as marker compounds for the quality control of the two herbs. These compounds could be subjected to a process of isolation and characterization based on HPTLC screening and spectroscopic methods, for the research of new marker compounds, at least to solve this particular problem. Other compounds may also be subject to this analysis; in particular, the compounds responsible for the biological activity of *T. officinale*.

In conclusion, this study has revealed that the methanolic extracts of *T. officinale* and *H. radicata* have clearly distinct chemical profiles according to HPTLC analysis. The congruency of the chemical profiles of the two origins of *T. officinale* might be useful in distinguishing the presence of chemical components from adulterant sources of *H. radicata*. This study shows that HPTLC fingerprinting is a precise and accurate method for *T. officinale* identification and can be used for the authentication of this medicinally important plant. While further work is needed to characterize active chemical constituents and perform quantitative estimation of marker compounds, our data can be used, along with microscopy identification, to determine standards for this plant. In this regard, the morphological analysis of inner structures in the samples of roots and leaves from *T. officinale* and *H. radicata* provides also a strong evidence to identify both species.

Authors' contributions

NC (MSc student) contributed in sample preparation, running the laboratory work, analysis of the data and draft of the paper. CM contributed to performance the laboratory work, analysis of the data and draft of the paper. KM contributed to chromatographic analysis. JD, RS and DG contributed to plant collection and critical reading of the manuscript, and EO designed the study, lead and advised the laboratory work and performed the analysis of the data and drafted the manuscript. All the authors have read the final manuscript and approved the submission.



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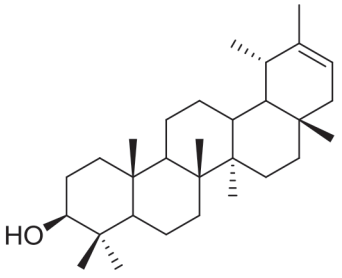

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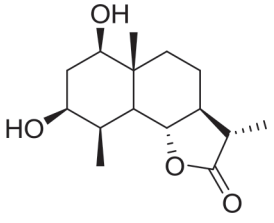

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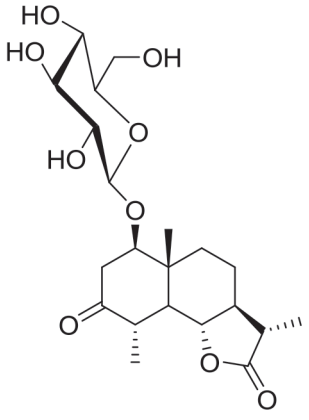

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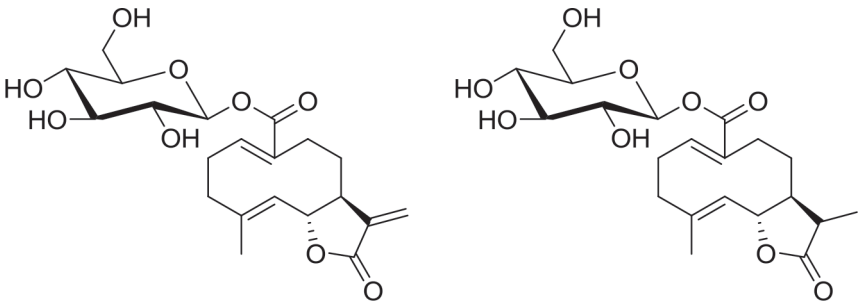

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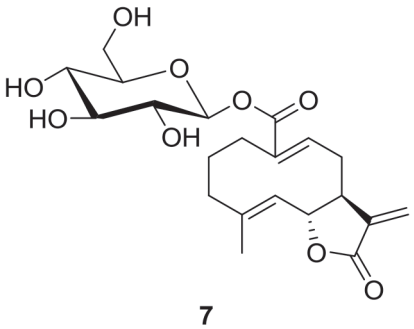

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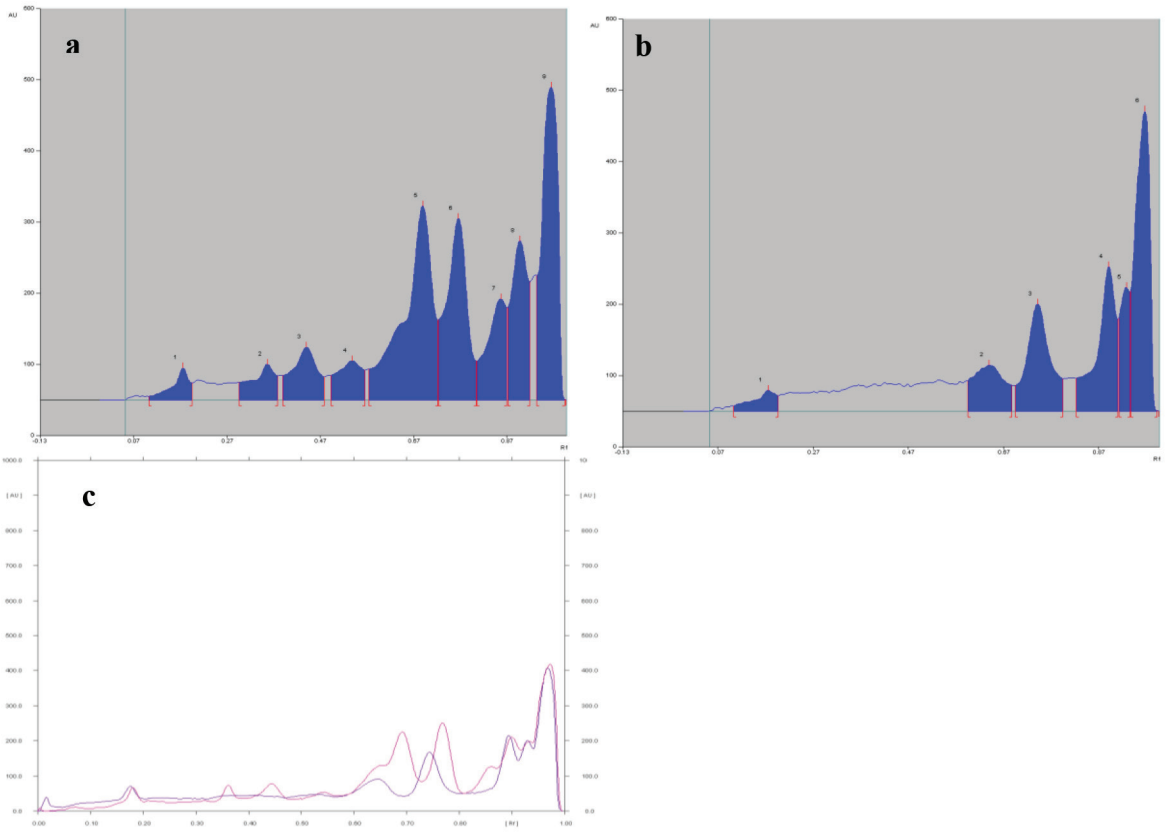

Figure 6 – HPTLC chromatogram of (a) *H. radicata* and (b) *T. officinale* root stem extract showing different peaks of phytoconstituents (scanned at 254 nm). (c) 2D display of a HPTLC chromatogram of *H. radicata* (pink) and *T. officinale* (blue) root stem extract. Mobile phase: ethyl acetate: formic acid: glacial acetic acid: water (100:11:11:26, v/v/v/v).

Conflicts of interest

The authors declare no conflicts of interest.

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