



Original research article

DNA-based identification of *Peucedanum ostruthium* specimens and detection of common adulterants by high-resolution melting curve analysis

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ABSTRACT

Masterwort (*Peucedanum ostruthium*, syn. *Imperatoria ostruthium*, Apiaceae) is an old economic plant in Alpine countries cultivated as ornamental plant and used for spirits and in folk medicine. *P. ostruthium* is a species that has often been confused with related Apiaceae species or morphologically similar roots or tubers resulting in products of minor quality. Masterwort can be distinguished from other Apiaceae species by nrDNA (ITS1 and ITS2). The analysed chloroplast markers (trnK 5' intron, trnT-trnL, and psbA-trnH), however, showed no species-specific mutations. With the application of two primer pairs amplifying parts of ITS and developed for high-resolution melting curve analysis (HRM) the target species was distinguishable from the other *Peucedanum* and Apiaceae species of our reference set. A multiplex PCR/HRM was developed to detect adulterations with *Gentiana* spp., *Aconitum napellus* and *Veratrum album*.

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1. Introduction

Masterwort (*Peucedanum ostruthium* (L.) Koch, syn. *Imperatoria ostruthium* L., Apiaceae) has been used in Austrian folk medicine since ancient times against various human diseases like common cold, inflammatory diseases, stomach pain and ulcer, dorsalgia and for treatment of cattle diseases like claw disorders, gastrointestinal diseases or ulcerous wounds [1] and [2]. The rhizomes and leaves contain essential oil and coumarins and possess antiphlogistic and antipyretic properties [3]. Masterwort is also traded as ornamental plant, wild types and varieties with variegated leaves are available.

Peucedanum is often considered as a relatively large genus with more than 100 species distributed in Europe, Asia and Africa [4] and [5]. The Flora Europaea [6] lists 29 species and the flora of Austria, South Tyrol and Liechtenstein [7] lists 10 species. The genus is taxonomically complex and several phylogenetic studies of *Peucedanum* and related genera were published in the last decades. Analysis of nrDNA revealed that *Peucedanum* s. l. is polyphyletic and that the African and several Eurasian species would need to be

shifted to other genera [8] [9], and [10]. According to Spalik et al. [9] the segregation of *Peucedanum* s. l. into *Cervaria*, *Holandrea*, *Imperatoria*, *Oreoselinum*, *Peucedanum* s. str., *Pteroselinum*, *Thomasinia*, *Thysselinum* and *Xanthoselinum* results with the exception of *Cervaria* and *Holandrea* in a group of monophyletic genera and retaining such a delimitation of the genus would be advantageous from the point of view of nomenclatural stability. For this reason and according to The Plant List [11], several investigated species are treated in this paper as *Peucedanum* but common or suggested synonyms [9] and [11] are given in Table 1.

Besides the problems of taxonomy, the correct identification of plant material used in trade, food or medicine is of utmost importance. Nowadays existing methods for identification are increasingly complemented by molecular approaches. DNA based methods can get very helpful where morphological features are limited, like in roots, seeds or in cut or ground plant material, or where phytochemical analysis show only limited differentiation. *P. ostruthium* is a species that has often been confused in the past with similar looking roots or tubers that led to products of minor quality. According to Berger [12] and List and Hörhammer [13] confusions or admixtures of masterwort roots (*Radix Imperatoriae*) with *Peucedanum officinale* L., *Angelica archangelica* L., *Gentiana lutea* L., *Gentiana purpurea* L., *Gentiana punctata* L. or *Gentiana*

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Table 1

Peucedanum and out-group samples and their origin used for analysis.

Sample (synonyms)	Reference no	Origin
<i>Peucedanum ostruthium</i> (L.) W.D.J.Koch (<i>Imperatoria ostruthium</i> L.)	Peu17 Peu49 Peu54, 130–4 Peu58–63, 148–52 Peu66–7, 69 Peu73–7 Peu78–81 Peu84–6, 116– 20 Peu88–9 Peu92–5 Peu99–101 Peu102–5 Peu107–9 Peu112–5 Peu146 Peu147 Peu155–60 Peu177 Peu164–5 Peu46 Peu01–3	WU: Austria, C, Hafnergruppe Austria, LA, Wechsel trade samples (seeds) trade samples (nursery/roots and rhizomes) Austria, St, Rosenkogel Austria, N-T, Kühtai Austria, N-T, Fotscher Bach Austria, Va, Zug im Lechtal Austria, C, Kleiner Pal Austria, C, Turracher Höhe Austria, C, Nockberge Austria, St, Gaberl Austria, N-T, Fotscher Bach Austria, Va, Lech am Arlberg Austria, S, Katschberg Austria, S, Weißpriachtal Italy, Friuli, Laghi di Fusine RBGE (E00043120): Andorra, El Serrat RBGE (E00253236, E00253239): Serbia, Uzice Austria, V, Stammersdorf WU: Austria, LA, Wildendürnbach/Baden/ Gutenstein
<i>Peucedanum aegopodioides</i> (Boiss.) Vandas (<i>Aegopodium involucreatum</i> Orph. Ex Boiss.)	Peu164–5	RBGE (E00253236, E00253239): Serbia, Uzice
<i>Peucedanum alsaticum</i> L. (<i>Xanthoselinum alsaticum</i> (L.) Schur)	Peu46	Austria, V, Stammersdorf
<i>Peucedanum austriacum</i> (Jacq.) W. D. J. Koch (<i>Pteroselinum austriacum</i> (Jacq.) Rchb.)	Peu01–3	WU: Austria, LA, Wildendürnbach/Baden/ Gutenstein
<i>Peucedanum carvifolia</i> (<i>Holandra carvifolia</i> (Vill.) Reduron, Charpin et Pimenov, <i>Dichoropetalum carvifolia</i> (Vill.) Pimenov & Kljuykov)	Peu04 Peu167	WU: Bulgaria RBGE (E00265976): Serbia
<i>Peucedanum</i> cf. <i>carvifolia</i>	Peu166	RBGE (E00043050): France, Haut-Rhin
<i>Peucedanum cervaria</i> (L.) Lapeyr. (<i>Cervaria rivini</i> Gaertn.)	Peu05–7 Peu08 Peu18, 47 Peu71–2 Peu168 Peu169 Peu170 Peu171 Peu09, 172	WU: cultivated/Austria, LA, Großes Erlaufthal/ Eichkogel WU: Italy, Toscana, Ortaglia WU: Austria, LA, Pottenstein/Bisamberg Austria, LA, Gainfarn RBGE (E00328523): Taiwan, Hsiulin Hsiang RBGE (E00043049): Portugal, Minho RBGE (E00134254): Spain, Granada RBGE (E00019242): Taiwan, Chimeiyu WU: cultivated/RBGE (E00043048): Yugoslavia, Crna Gora RBGE (E00043100): Bulgaria, Burgas
<i>Peucedanum obtusifolium</i> Sm. (<i>Ferula obtusifolia</i> (Sm.) Spreng.)	Peu173	RBGE (E00043100): Bulgaria, Burgas
<i>Peucedanum officinale</i> L.	Peu10 Peu11–3 Peu178 Peu174	WU: Austria, B, Götschlacke WU: France, Villeneuve/Bulgaria, Sophia/ cultivated Kosovo, Prizren, Mirusha RBGE (E00043066): Macedonia, Bitola
<i>Peucedanum oligophyllum</i> (Griseb.) Vandas (<i>Dichoropetalum oligophyllum</i> (Griseb.) Pimenov & Kljuykov, <i>Seseli oligophyllum</i> Griseb.)	Peu14 Peu15 Peu175–6	WU: Austria, B, Hirmer Wald WU: Austria, LA, Pfaffenberg RBGE (E00043140): Italy, Bergamo/ (E00249801): Spain, Cataluña
<i>Peucedanum oreoselinum</i> (L.) Moench (<i>Oreoselinum nigrum</i> Delarbre)	Peu16 Peu162–3 Peu110 Peu19	WU: Austria, LA, Göpfritz a.d. Wild Kew Gardens (TCMK 546, 589): China Austria, T, Nassereith WU: Korea
<i>Peucedanum palustre</i> (L.) Moench (<i>Thysselinum palustre</i> (L.) Hoffm.)	Peu20–1	WU: Austria, LA, Pernitz/Haselrast
<i>Peucedanum praeruptorum</i> Dunn	Peu51 Peu33 Peu22–3, 136, 153–4 Peu34 Peu161 Peu35 Peu37, 53 Peu36 Peu140 Peu38 Peu24, 142 Peu39 Peu40 Peu143 Peu41 Peu26 Peu141, 145	Austria, LA, Ybbsitz Austria, LA, Heiligenkreuz trade samples Austria, V, Anton-Schmid-Promenade Kew Gardens (TCMK 483): China Germany, Baden-Württemberg, Memmingen Austria, V, Sulzwiese/LA, Semmering Austria, V, Süttelstraße trade sample cultivated cultivated Austria, LA, Bisamberg Austria, V, Prater cultivated Austria, V, Reichsbrücke cultivated trade samples
<i>Peucedanum</i> sp.	Peu110	Austria, T, Nassereith
<i>Peucedanum terebinthaceum</i> (Fisch. ex Trevir.) Ledeb. (<i>Kitagawia terebinthacea</i> (Fisch. ex Trevir.) Pimenov, <i>Selinum terebinthaceum</i> Fisch. ex Trevir.)	Peu19	WU: Korea
<i>Peucedanum verticillare</i> (L.) W. D. J. Koch ex DC.) (<i>Tommasinia verticillaris</i> (L.) Bertol.)	Peu20–1	WU: Austria, LA, Pernitz/Haselrast
<i>Aegopodium podagraria</i> L.	Peu51	Austria, LA, Ybbsitz
<i>Aethusa cynapium</i> L.	Peu33	Austria, LA, Heiligenkreuz
<i>Angelica archangelica</i> L.	Peu22–3, 136, 153–4 Peu34 Peu161 Peu35 Peu37, 53 Peu36 Peu140 Peu38 Peu24, 142 Peu39 Peu40 Peu143 Peu41 Peu26 Peu141, 145	trade samples Austria, V, Anton-Schmid-Promenade Kew Gardens (TCMK 483): China Germany, Baden-Württemberg, Memmingen Austria, V, Sulzwiese/LA, Semmering Austria, V, Süttelstraße trade sample cultivated cultivated Austria, LA, Bisamberg Austria, V, Prater cultivated Austria, V, Reichsbrücke cultivated trade samples
<i>Angelica decursiva</i> (Miq.) Franch. et Sav. (<i>Peucedanum decursivum</i> (Miq.) Maxim.)	Peu161	Kew Gardens (TCMK 483): China
<i>Angelica sylvestris</i> L. (<i>Peucedanum angelicifolium</i> Turcz.)	Peu35 Peu37, 53 Peu36 Peu140 Peu38 Peu24, 142 Peu39 Peu40 Peu143 Peu41 Peu26 Peu141, 145	Germany, Baden-Württemberg, Memmingen Austria, V, Sulzwiese/LA, Semmering Austria, V, Süttelstraße trade sample cultivated cultivated Austria, LA, Bisamberg Austria, V, Prater cultivated Austria, V, Reichsbrücke cultivated trade samples
<i>Anthriscus cerefolium</i> (L.) Hoffm.	Peu36	Austria, V, Süttelstraße
<i>Apium graveolens</i> L.	Peu140	trade sample
<i>Berula erecta</i> (Huds.) Coville	Peu38	cultivated
<i>Carum carvi</i> L.	Peu24, 142	cultivated
<i>Chaerophyllum bulbosum</i> L.	Peu39	Austria, LA, Bisamberg
<i>Chaerophyllum tremulum</i> L.	Peu40	Austria, V, Prater
<i>Conium maculatum</i> L.	Peu143	cultivated
<i>Daucus carota</i> L.	Peu41	Austria, V, Reichsbrücke
<i>Eryngium campestre</i> L.	Peu26	cultivated
<i>Foeniculum vulgare</i> Mill.	Peu141, 145	trade samples

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