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Arbutin in marjoram and oregano

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ABSTRACT

Arbutin is a hydroquinone derivative that has been found in species of several plant families. Within the genus *Origanum* the formation of arbutin is polymorphic, with arbutin present in considerable amounts (*O. dubium* 20.8 ± 15.3 mg/g; wild *O. majorana* 51.3 ± 15.4 mg/g, cultivated *O. majorana* 40.6 ± 11.2 mg/g), minor amounts (*O. microphyllum* 0.1 ± 0.1 mg/g, wild *O. onites* 0.3 ± 0.1 mg/g, cultivated *O. onites* 0.1 ± 0.1 mg/g, *O. saccatum* 0.1 ± 0.1 mg/g, *O. solymicum* 0.4 ± 1.0 mg/g) or completely absent (*O. husnucan-baseri*, *O. syriacum*, *O. vulgare*). Whereas the most important commercial oregano species (*O. onites* and *O. vulgare*) contain no or only minor amounts of arbutin, marjoram (*O. majorana*) has considerably high amounts. The high variability of arbutin in *O. majorana* would allow a selection into cultivars with high arbutin content and low arbutin varieties. In a segregating F_2-generation of a species crossing between *O. majorana* (high content of arbutin) and *O. vulgare* ssp. *vulgare* (free of arbutin), the presence of arbutin followed a Mendelian segregation of 3:1, indicating that only one gene is responsible for the polymorphism of arbutin in the genus *Origanum*. The absence of arbutin in *O. vulgare* ssp. *vulgare* or *O. syriacum* would even enable the breeding of marjoram with no arbutin at all.

1. Introduction

Arbutin (4-hydroxyphenyl-β-D-glucopyranoside) is a hydroquinone derivative that consists of a phenol molecule with a glucose moiety in the *para*-position. Arbutin has been found in species of several plant families, for example in the Rosaceae (*Pyrus communis* L. (Cui, Nakamura, Ma, Li, & Kayahara, 2005)), Lamiaceae (*Origanum majorana* L. (Assaf, Ali, & Makboul, 1987)), Myrothamnaceae (*Myrothamnus flabellifolia* Welw. (Suau, Cuevas, Valpuesta, & Reid, 1991)) and Ericaceae (e.g. *Vaccinium* spp. (Saario, Koivusalo, Laakso, & Autio, 2002; Wha, Sang, Soon, Kuk, & Won, 1999) or *Arctostaphylos uva-ursi* L. (Parejo, Viladomat, Bastida, & Codina, 2002)). Although in plants the content of arbutin can reach considerable amounts (up to 25% of the dry weight in leaves of *M. flabellifolia* (Bianchi et al., 1993; Suau et al., 1991) or up to 17% in the widely used *A. uva-ursi* (Hoppe, 1975; Hänsel, Keller, Rimpler, & Schneider, 1992; Parejo et al., 2002)) its physiological and ecological functions are still under discussion. As it is present in plant taxa capable of withstanding extreme low temperatures or extended drought, arbutin is thought to play an important role in resistance to such environmental stress (Hincha, Oliver, & Crowe, 1999; Oliver, Hincha, Tsvetkova, Vigh, & Crowe, 2001; Oliver et al., 2002). In *Pyrus* spp. hydroquinone formation from arbutin was found to be involved in fireblight resistance (Hildebrand, Powell, & Schroth, 1969; Smale & Keil, 1966).

In cosmetic preparations arbutin is widely used to lighten the skin (Lin, Chiang, Lin, & Wen, 2008; Parvez, Kang, Chung, & Bae, 2007). Arbutin is also well known for its diuretic and urinary anti-infective properties and the arbutin-rich leaves of *A. uva-ursi* (bearberry) are internally used for moderate inflammatory conditions of the urinary tract and bladder (Yarnell, 2002). In both cases the active principle is hydroquinone, a metabolite of arbutin. Arbutin, however, could also exhibit adverse effects, as its metabolites showed hepatotoxic, nephrotoxic, mutagenic and carcinogenic potentials in animal studies (Nowak, Shilkin, & Jeffrey, 1995; Peters, Jones, Monks, & Lau, 1997; Shibata et al., 1991). Furthermore, a hypothesis was published linking phenol and hydroquinone as causal factors for leukaemia (McDonald, Holland, Skibola, Duramad, & Smith, 2001). For this reason the therapeutic use of *A. uva-ursii* is always restricted to a short period.

The genus *Origanum* comprises 43 species (Ietswaart, 1980; Skoula & Harborne, 2002), mainly distributed in the Eastern Mediterranean region. Due to their high content of essential oil, species of the genus have traditionally been collected for centuries, for the flavouring of traditional dishes as well as for several purposes in traditional medicine. Today two aromatic qualities, marjoram and oregano, are commercially traded and widely used all over the world as popular herbs. The four closely-related species of section Majorana (*O. onites* L., *O. syriacum* L., *O. majorana* L. and *O. dubium* Boiss.), are amongst the most important species of the

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genus. Carvacrol-rich *O. onites* (Turkish oregano) possesses the typical oregano flavour and is, beside *O. vulgare* L., one of the most traded and consumed *Origanum* species. In 1999, Turkey exported more than 7500 tons of oregano, more than 80% of the exported plant material derived from *O. onites* (Baser, 2002). *O. syriacum* (Israeli oregano) is not extensively traded on the world market but is a crop of local importance as it is a basic ingredient of za'atar which has traditionally been used throughout the Arab world for the seasoning of meats and vegetables (Fleisher & Fleisher, 1988). *O. majorana* is cultivated for the production of marjoram in several European and African countries (Sarlis, 1994). Carvacrol-rich *O. dubium* (White oregano) is, due to its high essential oil content, the preferred plant material for the industrial production of essential oregano oil in Turkey (export volume > 30 tons per year) (Baser, 2002).

Within the genus *Origanum* the formation of arbutin seems to be polymorphic, with arbutin present in considerable amounts (*O. majorana*) or completely absent (*O. vulgare*) (Assaf et al., 1987; Kraus, Koch, & Hoffstetter-Kuhn, 1996). So far there is no information available whether the occurrence of arbutin is a special characteristic of *O. majorana* or whether other *Origanum* species contain this compound. The aim of this study was to give a first outlook about the intra-generic and intra-specific variability of arbutin in the genus *Origanum*. Due to their commercial importance and the affiliation of a species rich in arbutin a special focus was given to section Majorana (*O. onites*, *O. syriacum*, *O. majorana* and *O. dubium*). Additionally, one population of *O. microphyllum* (Bentham) Vogel and single plant individuals of *O. husnucan-baseri* Duman, Ayataç et Duran, *O. saccatum* Davis and *O. solymicum* Davis were analysed. Moreover, samples of commercial marjoram and oregano were included in the analysis. To study the segregation pattern and to discuss possible approaches for the breeding of arbutin-low or even arbutin-free marjoram, an inter-specific crossing between arbutin-rich marjoram (*O. majorana*) and arbutin-free oregano (*O. vulgare* ssp. *vulgare*) was analysed.

2. Material and methods

2.1. Plant material

For the investigation of intra-generic and intra-specific variability four populations of *O. majorana* L. (maj1 = seeds from a natural population on Cyprus; cultivars 'Erfo', 'G' and 'Miraz' = seeds from a commercial source), one population of *O. microphyllum* (Bentham) Vogel (seeds collected from a natural population in Crete) and one population of *O. onites* L. (seeds from a commercial source) were grown in the greenhouse of the Institute for Applied Botany, University of Veterinary Medicine, Vienna. Additional plants of *O. syriacum* L., *O. onites* L., *O. majorana* L., *O. dubium* Boiss., *O. saccatum* Davis, *O. solymicum* Davis and *O. husnucan-baseri* Duman, Ayataç et Duran were collected in Syria (May 2006, beginning to full bloom), Turkey (June 2006, beginning to full bloom), Cyprus (July 2006, full bloom) and Greece (October 2007, seed ripening), from their natural habitats. Details about their geographic origin, the identification number of the sampled populations and the number of individuals investigated are provided in Table 1. The species were identified by following the identification key in the latest taxonomic revision of the genus *Origanum* (Ietswaart, 1980). For the delimitation of *O. dubium* and *O. majorana* the Flora of Cyprus (Ietswaart, 1985) was used as a second reference. Voucher specimens representing wild and greenhouse populations were deposited in the Herbarium of the Institute for Applied Botany and Pharmacognosy, University of Veterinary Medicine, Vienna. The plants grown in the greenhouse were harvested in full bloom and dried at 35 °C in a drying chamber (Memmert, Schwabach, Germany). The plant material collected from the natural populations was dried at room temperature.

The samples of commercial marjoram and oregano (usually made up of plant material cut into small pieces) were purchased from local markets or supermarkets in several European countries. The geographical origin of the commercial plant material (as indicated on the packaging) is provided in Table 2. An assignment of the plant material was performed on the basis of intact calyces (according to Ietswaart, 1980) that were isolated from the samples. The commercial oreganos from Italy, Peru, Slovenia, Tunisia and those two of unknown origin were identified as *O. vulgare*. In Turkish oregano calyces with a shape typical for *O. onites* were present. According to calyx shape, sensorial quality and with the knowledge about the geographical origin, the oregano sample from Cyprus was identified as *O. dubium*.

The three bearberry samples were obtained from Viennese pharmacies.

For the inheritance study a segregating F_2-generation was established by the selfing of a naturally occurring hybrid between the species *Origanum majorana* L. and *Origanum vulgare* L. ssp. *vulgare* detected in a population survey of oregano (Marn, Novak, & Franz, 1999; Novak, Gimplinger, & Franz, 2002). The hybrid nature was unambiguously identified by the shape of the calyx (Novak et al., 2002). The plants were grown in the greenhouse and 106 individuals were harvested at the beginning of bloom and dried at 35 °C in a drying chamber.

2.2. Extraction

Leaves and flowers of each plant were separated from the stem and ground to a fine powder in a micro hammer mill (Culatti, Zürich, Switzerland). For the extraction of the traded samples of marjoram and oregano a representative quantity of each sample was ground.

For TLC analyses 0.5 g of the powder were extracted with 5 ml methanol:water (50:50) under reflux for 15 min. The extracts were filtered immediately through a 'fast' folded filter (Whatman Ltd., Springfield Mill, England) and 0.2 ml of a 9.5% lead acetate solution were added to the filtrate. After a second filtration the extracts were used for TLC (Kraus et al., 1996).

For HPLC analyses 0.05 g of powdered plant material were extracted with 25 ml methanol:water (50:50) for 30 min at 25 °C in an ultrasonic water bath (Parejo, Viladomat, Bastida, & Codina, 2001; the methanol:water ratio was modified). The extracts were filtered through a microfilter with 2 μm pore size (Minisart RC 25, Sartorius, Göttingen, Germany).

2.3. TLC

The reference substance was 10 mg of arbutin (Roth, Karlsruhe, Germany) dissolved in 10 ml methanol. A stationary phase of HPTLC, silica gel 60 F254 nm (Merck, Darmstadt, Germany) with a mobile phase of acetic acid ethyl ester/methanol/water (77:13:10) was used. Detection was with dibromchinochlorimide with inspection in visible light; R_f-value of arbutin: 0.47 (Kraus et al., 1996). Absence or presence of arbutin was scored as 0 or 1, respectively.

2.4. HPLC

HPLC analysis was carried out on a Waters modular system (626 pump, in-line degasser AF, photodiode array detector PDA996; Waters, Milford, MA) using a Symmetry C18 column (5.0 μm, 4.6 × 150 mm). The mobile phase was water:methanol (95:5) at a flow rate of 2 ml/min. The injection volume was 10 μl and UV detection was at 220 nm. The concentration of arbutin was deter-