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Determination of hydroxycinnamic acids present in *Rhododendron* species

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ABSTRACT

Hydroxycinnamates including free hydroxycinnamic acids and their chlorogenic acid derivatives and glycosides have been profiled in leaf extracts of 98 *Rhododendron* species using LC-MS techniques. In total, 69 hydroxycinnamic acid derivatives were identified in the leaves of 98 *Rhododendron* species. Some derivatives serve as unique phytochemical marker for a single species, whereas other compounds are limited to certain subgenera. The distribution of compounds among six different subgenera of *Rhododendron* was studied using PCA and PLS-DA. This contribution presents data that provide unique metabolomic insight in the distribution of a class of secondary metabolites within a large selection of species from the botanically diverse plant genus *Rhododendron*.

1. Introduction

The classical discipline of chemotaxonomy is based on the assumption that classification of plants and animals can be based on similarities and differences in their biochemical composition. The discipline of chemotaxonomy has made significant advances in the field of protein composition showing that phylogenetically closely related organisms produce structurally related proteins.

In the field of secondary plant metabolites chemotaxonomy has been frequently used to support classification via the occurrence of compound classes unique for plant families such as glucosinolates in Brassiceae, quinolizidine alkaloids in Fabaceae, tropane alkaloids in Solanaceae, iridoids in Compositae or marker compounds for individual plant species such as morphine in *Papaver somniforum*, allicin in *Allium cepa* and many others (Wink, 2003; Hegnauer, 1986).

According to Erdtman (1955), 'a true chemotaxonomic classification of organisms will only become true when wide patterns of secondary metabolites are compared'. To the best of our knowledge such a pattern comparison has not been carried out yet and our data presented here form the first attempt of such a pattern classification using 98 closely related plant species. Additionally, modern LC-MS techniques allow for the first time the acquisition of comprehensive phytochemical data with acceptable experimental effort profiling in a single LC-MS experiment several hundred plant secondary metabolites creating the experimental basis for a pattern comparison. In this contribution we make a first attempt to create experimentally and interpret such a phytochemical pattern with the genus *Rhododendron* as a representative example as well focusing on a single but chemically diverse class of compounds, the hydroxycinnamates. Polyphenolic secondary metabolites have received mixed reviews with respect to their suitability in chemotaxonomic investigation. While Wink considered them, as of limited systematic value, we could show that sinapoyl quinic acids serve as useful marker compounds for *Coffea canephora* (Jaiswal and Kuhnert, 2010; Jaiswal et al., 2010).

Plants produce a diverse variety of polyphenolic secondary metabolites. Hydroxycinnamates are low molecular weight plant secondary metabolites that are believed to play a role in protecting

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plants from damaging UV irradiation, herbivores and pathogens (Jaiswal et al., 2011b). Some derivatives are reported to exert antimicrobial properties and in humans, anti-hypersensitive activity and preventive effects against cancer, type 2 diabetes and atherosclerosis have been reported (Jaiswal et al., 2011a,b, 2014a,b; Narváez-Cuenca et al., 2012). The compound class of hydroxycinnamates comprises of free hydroxycinnamic acids such as caffeic, ferulic and *p*-coumaric acids and their derivatives (Clifford, 2000). Among the derivatives, esters of hydroxyacids are commonly found in nature with esters of quinic acids also referred to as chlorogenic acids being the most common hydroxycinnamic derivatives found in plants. Additionally, esters of sugars and glycosides are frequently observed in plants and amides formed with amino acids or peptides encountered less frequently. The representative structures are shown in Fig. 1 (Jaiswal and Kuhnert, 2010; Jaiswal et al., 2011b).

Rhododendron species are found all over the world (except some regions in America and Africa) from temperate cold climates to tropical regions. They are a diverse group of woody plants that belong to the genus *Ericaceae* and are well-known for their colorful flowers. Among the woody plants, *Rhododendron* is believed to be the most diverse genus with more than 1200 species and countless cultivars described. *Rhododendron* species containing low amount of grayanotoxins have been used in traditional medicine in some countries (Jaiswal et al., 2012). *Rhododendron* species have formed the basis for traditional medicines and have been used for many years for the treatment of inflammation, skin or gastrointestinal tract disorders (Kim et al., 2011; Khan et al., 2010). The crude extracts and isolated compounds of different *Rhododendron* species have been tested and proven to have antibacterial activity against different bacteria (Hakeem Said et al., 2017; Shrestha et al., 2017; Rezk et al., 2015). Through a correlation of phytochemical profiles with antimicrobial susceptibility, cytotoxicity and phylogenetic analyses, we identified potentially antimicrobial secondary metabolites compounds in various species (Hakeem Said et al., 2017; Grimbs et al., 2017). For this reason the genus *Rhododendron* forms a fascinating model system for studying the genetic and phytochemical diversity of plants and as well might serve as a source of promising medicinally relevant plant secondary metabolites.

2. Results and discussion

In this study, an extensive hydroxycinnamate profile was created for the leaves of 98 *Rhododendron* species from six different subgenera. The number of *Rhododendron* species analyzed is given in Table 1. The GenBank® number and herbarium number of the *Rhododendron* species are mentioned in supplementary

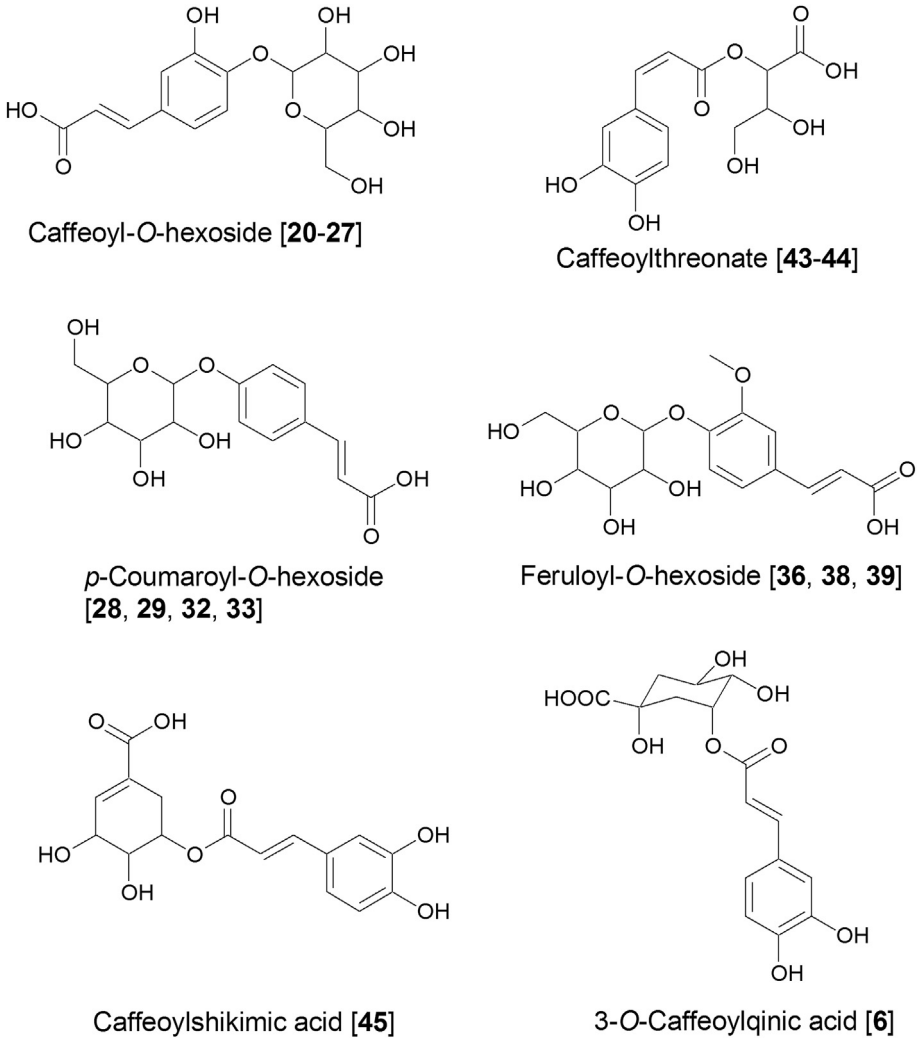

Fig. 1. Representative examples of hydroxycinnamic acid derivatives from *Rhododendron* species.