Phenolic profiles of *Viscum album* L. subspecies from different host trees

Polyphenolprofil in *Viscum album* L.-Subspezies verschiedener Wirtsbäume

*Tania Gärtner, Kerstin Link, Margit B. Müller, Florian C. Stintzing, Dietmar R. Kammerer*

**Summary**
Mistletoe samples collected from eight different host trees (*Abies, Crataegus, Malus, Pinus, Populus, Quercus, Salix, Tilia*) were characterized and assessed, whether a differentiation based on their profile and contents of phenolic compounds is possible. For this purpose, sample extracts were analyzed by TLC and HPLC-DAD-MS^n and also compared to the corresponding Iscucin® preparations. Certain phenolics were detected in all *Viscum* samples, whereas others were found to be specific to samples of the respective subspecies, i.e. plant material collected from either coniferous or deciduous trees. Quantitative differences of individual phenolic compounds were also observed, especially between samples of the aforementioned subspecies. These findings were not only true for *Viscum* extracts, but were mostly transferable to Iscucin® preparations. In summary, the determination of the phenolic profile of mistletoe extracts allows the delimitation of samples from either coniferous or deciduous trees, but fails to distinguish extracts from *Viscum* samples of different deciduous trees.

**Keywords:** Chemotaxonomy, host tree, HPLC, phenolic compounds, TLC.

**Zusammenfassung**

**Schlüsselwörter:** Chemotaxonomie, Wirtsbaum, HPLC, phenolische Verbindungen, DC.
Introduction

The availability of *Viscum album* L. (*Santalaceae*) preparations derived from different host trees markedly contributes to the broad applicability of mistletoe extracts, e.g., in complementary tumor therapy. *V. album* may be classified into three subspecies, which differ in their specificity for certain host trees, i.e., ssp. *abietis* (host tree *Abies*), ssp. *austriacum* (*Pinus*), and ssp. *album* (*Crataegus, Malus, Populus, Quercus, Salix, Tilia*) (Erhardt et al. 2008). Due to the hemiparasitic nature of *Viscum*, subspecies grown on different host trees are likely to differ in their profile of primary and secondary metabolites. This latter fact provides the opportunity to differentiate mistletoes and derived extracts obtained from different host trees based on specific analytical marker compounds.

In the past, mistletoe research has mainly been focused on predominant lectins and viscotoxins due to their cytotoxic effects on cancer cells, which are discussed to be crucial features in the context of complementary cancer therapy (Hunziker-Basler et al. 2007; Ramaekers et al. 2007). However, several further approaches have been published recently, aiming at the differentiation of host plant-specific preparations, such as the analysis of saccharides and sugar alcohols (Kohl 2012), nucleosides (Albrecht 2014), and minerals (Goedings 1995). Furthermore, phenolic compounds have been studied in this regard due to their plant-specific biosynthesis, which renders them particularly promising candidates for chemotaxonomic purposes, however with contradictory results in various *Viscum* subspecies (Becker, Exner 1980; Lorch 1993; Luczkiewicz et al. 2001; Zhao et al. 2011). Consequently, the present study aimed at a more detailed insight into the phenolic profile of mistletoe preparations derived from various host trees and to figure out analytical marker compounds allowing a clear-cut differentiation (Schlachtin 2013).

Furthermore, mistletoe plant material is commonly collected twice a year, in summer and in winter. Consequently, this study should also allow an evaluation, whether the harvest period affects the phenolic profiles and contents of the corresponding *Viscum* extracts.
Materials and Methods

Mistletoe samples were collected from eight different host trees (*Abies, Crataegus, Malus, Pinus, Populus, Quercus, Salix, Tilia*) both in summer and winter. Extracts were prepared from lyophilized and ground sample material. The corresponding extracts were analyzed by thin-layer chromatography (TLC) using silica gel plates and chlorofom/methanol/water (70:30:4, v/v/v) as mobile phase and acetic acid/chloro-sulfonic acid (2:1, v/v) for detection. Furthermore, samples were analyzed by HPLC-DAD-MS\textsuperscript{n}. Compound separation was achieved using an RP-C\textsubscript{18} Atlantis\textsuperscript{®} T3 stationary phase (Waters, Milford, USA) as well as 0.1 % formic acid in water (v/v) and acetonitrile as eluents. Detection was performed at 270, 300, 340 and 360 nm and spectra scanned in a wavelength range of 200–500 nm as well as by using a mass spectrometer (HCT Ultra ion trap mass spectrometer, Bruker Daltonik GmbH, Bremen, Germany). Individual compounds were characterized based on their chromatographic and mass spectrometric behavior. The phenolic profiles were compared to those of the corresponding Iscucin\textsuperscript{®} preparations obtained from these plant materials (strength H).

Results and Discussion

TLC analyses revealed differences in the occurrence and intensities of individual bands, especially between mistletoe samples from coniferous and deciduous trees, respectively, with higher intensities and differences in the phenolic profile being detected in *Viscum* extracts derived from coniferous trees (Fig. 1). Based on these phenolic profiles, a differentiation among samples derived from different deciduous trees was impossible. Furthermore, significant differences between the phenolic profiles of plant materials collected in summer and winter could not be observed.
These findings were further verified by HPLC analyses. Whereas certain phenolics, such as syringin and syringenin-4’-O-apiosylglucoside, were detected in all Viscum samples, other compounds were specific to individual subspecies or host trees, e.g. 5,7-dimethoxy-4’-hydroxyflavanone for V. album ssp. austriacum (host tree Pinus, Tab. 1). Interestingly, a number of phenolic compounds, such as gallic, rosmarinic, and ellagic acids, as well as quercetin, isoquercetin, kaempferol, hyperoside and homoe-ridictyol, which have previously been reported in the literature for mistletoe, were not detected in the samples investigated in the present study. Furthermore, caffeic acid, which has been reported to occur in all three Viscum subspecies (Becker, Exner 1980), was only detected in V. album Salicis plant material collected in summer (Tab. 1).

Quantitative differences were also observed, with the former two hydroxycinnamic alcohols being detected in significantly higher amounts in V. album ssp. abietis (Abies) and V. album ssp. austriacum (Pinus) compared to V. album ssp. album (deciduous trees). As an example, the syringin contents ranged from 0.2 to 0.5 mg/g dried plant material in mistletoes from deciduous trees, whereas the amounts ranged from 1.2 to 2.5 mg/g in the aforementioned conifers. Furthermore, the syringenin-4’-O-apiosylglucoside contents of dried plant material collected in winter are exemplified in Figure 2.
**Tab. 1: Occurrence of phenolic compounds in *V. album* from eight different host trees**

<table>
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<tr>
<th></th>
<th>Abies</th>
<th>Crataegus</th>
<th>Malus</th>
<th>Pinus</th>
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<th>Quercus</th>
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<td>Gallic acid</td>
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<td>Protocatechuic acid</td>
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<td>Chlorogenic acid</td>
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<td>Isoquercetin^b</td>
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<td>Naringenin</td>
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<td>5,7-Dimethoxy-4′-hydroxyflavone</td>
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☐ Plant material collected in summer, ☐ Plant material collected in winter
^aQuercetin 3-O-rhamnoside, ^bQuercetin 3-O-glucoside, ^cQuercetin 3-O-galactoside
According to the studies by Lorch (1993), the phenolic contents of mistletoes from coniferous trees are about ten times higher compared to those from deciduous trees. Even though such high differences were not observed in the present study, the general trend of higher phenolic amounts in Viscum samples from coniferous trees could be corroborated. Deviations from the aforementioned study may be ascribed to differences in the extract preparation, but are more likely to depend on to the specific characteristics of the plant material, since phenolic contents are known to depend among others on the place and time of harvest, climatic and phytosanitary conditions.

The above findings were obtained for samples collected in summer and winter and were mostly transferable to Iscucin® preparations. Quantitative differences may be explained by selectivities and extraction efficiencies of the solvents applied for obtaining the plant extracts and corresponding Iscucin® preparations (Vicas et al. 2011). Findings are exemplified for the HPLC chromatograms of Viscum album Pini plant material collected in summer and winter as well as of the corresponding Iscucin® Pini, strength H (Fig. 3).
Fig 3: HPLC chromatograms (270 nm) of extracts from plant material of *V. album Pini* collected in summer (a), winter (b) and of the corresponding Isucin® preparation, strength H (c).
Conclusions

The determination of the phenolic profile in mistletoe extracts allows the discrimination of the aforementioned subspecies, but fails to differentiate extracts from *Viscum* samples from various deciduous trees. Consequently, a combination of various analytical parameters also considering non-phenolic compounds appears to be constructive for future routine control analyses aiming at a clearcut differentiation of *Viscum* preparations obtained from various host trees.

References


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