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Conference presentation

Biological activity and phytochemical analysis of homeopathic preparations of *Viscum album* L.

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Abstract

Several *in vitro* and *in vivo* studies investigated the biological properties of mistletoe preparations [1-4]. In Europe, where phytochemical constituents of aqueous *Viscum album* extracts were previously described, the most widespread and common form of mistletoe is the white-berried species (*Viscum album*) [5]. The aim of the present study was to analyze the biological activity and phytochemical features of different *Viscum album* ssp mother tinctures collected in 2 different harvesting periods.

Leaves, stems and berries of *Viscum album* samples were subjected to ethanolic extraction (45% v/v) following the homeopathic pharmacopeia [6,7]. The mother tinctures (MT) prepared were: *V. album* (from 3 host trees *Malus domestica*, *Quercus* sp, *Ulmus* sp), *V. album* ssp. *austriacum* (host tree *Pinus sylvestris*) and *V. album ssp. abietis* (host tree *Abias pectinata*). All samples were collected at Höfli farm (Switzerland) in July and August and subjected to chemical analysis by means of high-performance liquid chromatography-mass spectrometry (HPLC-MS/MS) and Thin-Layer Chromatography (TLC). Tumor (Yoshida and Molt4) and non-tumor cells (Ma104) were incubated with MT (1; 0.1; 0.01%) for 4, 24 and 48 hours at 37ºC. Proliferation was indirectly measured using WST-1 (tumor cells) and MTT (non-tumor cells). All cells (1.5 x 10^4/100 µL) were plated in triplicate in 96-well plates. The cell proliferation inhibition rate was calculated as percent inhibition relative to control cells treated with the MT solvent (45% ethanol v/v). Apoptosis/necrosis was measured using 2 x 10^5 cells incubated with Annexin V-FITC and 7-AAD at room temperature in the dark, and analyzed in flow cytometer [2]. Antimicrobial activity was assessed for *Candida albicans, Cryptococcus neoformans*, *Escherichia coli* and *Staphylococcus aureus* following the Clinical Laboratory Standards Institute manuals [8-9] and compared with 45% ethanol v/v (MT solvent).

The chromatographic assays of MT samples showed phenolic acids and lignans as main chemical classes identified. WST-1 and MTT experiments revealed different antitumor potential of MT. In general, Yoshida cells were more sensitive than Molt4. The maximal cytotoxic effect with 100% of mortality was attained by incubation of Yoshida and Molt-4 cells with 1% MT of *V. album ssp.*

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abietis (harvested in July) for 4 hours. Preliminary results with non-tumor cells indicate lower cytotoxicity compared to tumor cells. Annexin V/7ADD showed predominance of late apoptotic/necrotic events after 4 and 24 hours of incubation with all MT. The antimicrobial assay showed that MT of *Viscum album* ssp. austriacum (host tree *Pinus sylvestris*) exhibited minimum inhibitory concentration 7.25 mg/mL for the 2 tested bacterial strains.

The results of the present study suggest that *Viscum album* samples prepared according to the homeopathic pharmacopeia exhibit promising biological activities in a dose-dependent manner. The effects of potentiation will be assessed and compared to non-potentiated samples in future experiments. Therefore, the present study contributes with data on the main chemical components in homeopathic preparations of *Viscum album*, drawing attention to the relevance of extract solvent, seasonal aspects, host tree and harvested parts of plant harvested to the quality of thee mother tinctures

Keywords: Mistletoe, *Viscum album* L. cancer, homeopathy, phytochemistry; cytotoxicity

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