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. *abieters* (host tree *Abies 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 37°C. Proliferation was indirectly measured using WST-1 (tumor cells) and MTT (non-tumor cells). All cells (1.5 x 10⁴/100 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 10⁵ 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

**References**


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