Abstract

Metabolomic Analysis of *Viscum album* L Homeopathic Tinctures and Antitumor Studies in 3D Spheroid Models

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Background The antitumoral efficacy of aqueous *Viscum album* extracts is attributed to the presence of lectins and viscotoxins. However, previous studies demonstrated an antitumoral activity of European *V. album* ethanolic homeopathic tinctures (VAHT) prepared according to homeopathic methodology. Aims To investigate the seasonal influences (summer and winter) in the metabolomic profile of *V. album* ssp. homeopathic mother tinctures (VAHT) and to evaluate the antitumoral activity of some VAHT in 3D tumor spheroid models. Methodology The following VAHT were prepared by ethanolic maceration: *V. album* ssp. *album* growing on *Malus domestica*, *Quercus* sp. and *Ulmus* sp.; *V. album* ssp. *austriacum* from *Pinus sylvestris*, *V. album* ssp. *abietis* from *Abies alba*. Chemical analyses were performed using liquid chromatography coupled to high-resolution mass spectrometry. Data was submitted to multivariate statistical analysis using principal component analysis (PCA) and Partial Least Squares Discriminant Analysis (PLSDA) in Metaboanalyst platform. The antitumor potential of VAHT (0.5% v/v) was conducted in 3D tumor spheroid models (MDA-MB-231 cell line) by MTT for 72 hours. Results and discussion The PCA analysis explained 40% of data variation and clustered VAHT samples into 3 groups, emphasizing the chemical similarity between the botanical subspecies of *V. album*. Some key compounds were mainly responsible for this separation: pinobankasin hexose-pentose (*V. album* ssp. *abietis*); citric acid (*V. album* ssp. *austriacum*); malic acid (*V. album* ssp. *album*). The chemical differences among summer and winter samples, detected by PLSDA, were related to the *Viscum album* host trees. A significant reduction of 50% and 41% (p<0.05) was detected in tumor cell viability when VAHT from *Abies alba* and *Quercus* sp. were compared to ethanol control, respectively. Conclusion The present study shows, for the first time, an overview of metabolomic profile from different homeopathic mother tinctures of European *Viscum album* and their promising antitumor activity in 3D spheroid model.

Keywords: *Viscum album*, homeopathic tinctures, antitumoral, metabolomic, spheroid

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Cite as: Int J High Dilution Res. 2019; 18(2): 18-18
https://doi.org/10.51910/ijhdr.v18i02.996

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