1. **Viscum album L*.* ethanolic extracts from different host trees show distinct metabolome and antitumor activity through glycolytic pathway**

**Pharmacology of Traditional Medicine**

**Background**:Antitumoral efficacy of *Viscum album* L is attributed to the aqueous extracts and presence of lectins and viscotoxins. However, previous studies conducted by our group showed antitumoral activity of European *V. album* ethanolic extracts (VAE).

**Aims**: Evaluate the metabolic profile of fifty VAE harvested in summer and winter time and its antitumoral activity through 2D and 3D models.

**Methodology**: The following VAE were prepared by homeopathic extraction method, using maceration with ethanol: *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 (LC-HRMS). Mass spectra were processed using the MSDial software 4.0 and the resulting data table was submitted to multivariate statistical analysis using Partial Least Squares Discriminant Analysis (PLS-DA) in the Metaboanalyst 4.0 platform. The antitumor potential of selected VAE was evaluated in 2D and 3D models (MDA-MB-231 cancer cells) by MTT, crystal violet and glycolytic pathway analysis.

**Results**: The first 3 principal components in PLS-DA analyses explained about 60% and 40% of data variation in positive and negative electrospray ion modes, respectively. VAE samples were clustered into three groups and showed chemical similarity among *V. album* subspecies. Compounds responsible for group separation were tentatively identified as: pinobankasin or naringenin hexoside; isorhamnetin-3-hexoside, meglutol and different amino acids. Summer VAE at 0.5% v/v induced higher cytotoxic damage than winter preparations, and *Abies alba* and *Quercus* sp*.* VAE, promoted 49% and 42% tumor viability reduction in 3D model (72h incubation), respectively. MDA-MB-231 glycolytic pathway in 2D model shows a decrease in the glucose consumption and extracellular lactate production. Also, PFK (6-phosphofructo-1-kinase) and PK (Pyruvate kinase) activities were inhibited by *Abies alba* and *Quercus* sp. VAE at 48h of incubation.

Conclusions: Chemical analyses were important to distinguish the ethanolic extracts from *V. album* subspecies. The glycolytic pathway (PFK and PK enzymes) should be an important target involved with the growth tumoral inhibition induced by VAE.

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