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Optimization of extraction protocol for maximum recovery of triol ginsenoside from cell suspensions of *Panax sikkimensis* and *P. quinquefolium* as function of culture age

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In the present study, efforts were undertaken to optimize a chemical extraction protocol for *Ginseng* bioactives: triol ginsenosides, from suspension cultures of two *Panax* spp., and their quantification. Methanol (100%, 70% and 30%), water (hot and cold), water saturated butanol and butanol saturated water were the chosen solvents for ultrasonication - assisted extraction from callus. Alternately, cells were refluxed with 100% methanol in a soxhlet arrangement for extraction. Butanolic extracts after downstream processing, were vacuum evaporated and redissolved in methanol. HPTLC quantification was using TLC silica gel 60 F_{254} plates with chloroform:methanol:water; 13:7:2 and was scanned in a Camag TLC Scanner at 575 nm wavelength. It was observed that ginsenoside recovery was dependent on the choice of solvent rather than the method of extraction. Sonication – assisted extraction with 100% methanol was found to be the best method for maximum recovery of the triol ginsenoside. This protocol was utilized to determine ginsenoside production kinetics in the two cell lines over a period of 50 days at 7-10 day time intervals. Both the lines were found to be dominantly producing triol ginsenosides: Re and Rg1 with a total recovery of 0.57% DW in *P. quinquefolium* and 0.732% DW in *P. sikkimensis* cultures. Rg2 was found to be produced in *P. sikkimensis* cultures in an age dependent manner irrespective of biomass changes. Detailed data will be highlighted during the presentation.