APPENDIX P I: TEST OF CYTOTOXICITY (KATEŘINA SKOPALOVÁ)

Test of cytotoxicity

Date: 22.9.2022

Perfom by: Kateřina Skopalová

Cell line	Mouse embryonic fibroblast cell line (ATCC CRL-1658 NIH/3T3, USA)
Medium	The ATCC–formulated Dulbecco's Modified Eagle's Medium (PAA Laboratories GmbH, Austria) containing 10% of calf serum (BioSera, France) and 1 % Penicillin/Streptomycin (GE Healthcare HyClone, United Kingdom), was used as the culture medium.
Cultivations condition	Cells were incubated at 37°C in 5% CO2 in humidified air.
Extract preparation	Extract should be prepared according to ISO standard 10993-12; 0.2g/1mL of media. However, the methodology had to be adjusted to a concentration of 0.066 g/1mL of media. The tested material was incubated in DMEM medium with CS for 24 hours at 37°C with stirring. The parent extracts (100 %) were then diluted in culture medium to obtain a series of dilutions with concentrations of 75, 50, 25, 10, and 1 %. All extracts were used up to 24 hours.
Determination of cell viability	Tetrazolium (MTT cell proliferation assay kit, Duchefa Biochemie,Netherlands) was used to determine cell viability. The absorbance was measured at 570 nm and the reference wavelength was adjusted on 690 nm. The results are presented as reduction of cell viability in percentage when compared to cell cultivated in medium without the extracts of tested materials.
Microscopic observation	Morphology of cells from the culture plates was observed using an inverted Olympus phase contrast microscope (IX 81).
Samples	TGO, BGO, SGO, HGO

Day -1	Preparation of extracts. Cells were seeded to pre incubate in the microtitration test plates dishes (TPP, Switzerland). Concentration $1x10^5$ cells per mL.
Day 0	Extract were diluted with medium to obtain following concentration: 100, 75, 50, 25, 10 and 1% of parent extract. All assays were performed in quadruplets. The medium were sucked up and replaced by individual extracts.
Day 1	The cells were observed by microscopy. MTT proliferation assay test were performed.

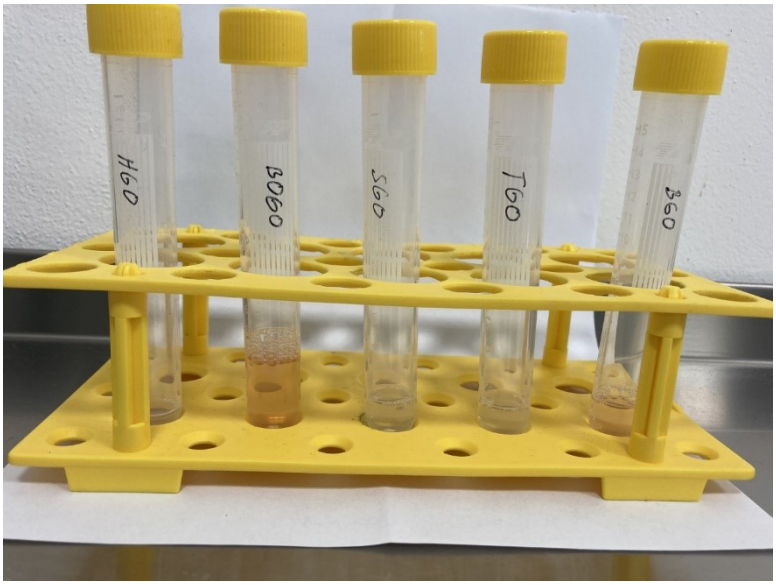

Figure 1: *Extracts of samples*

Results:

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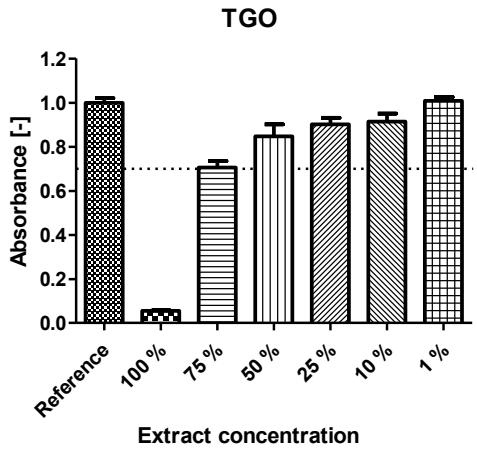

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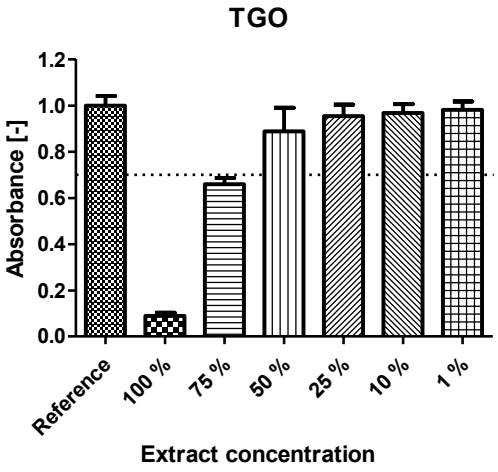

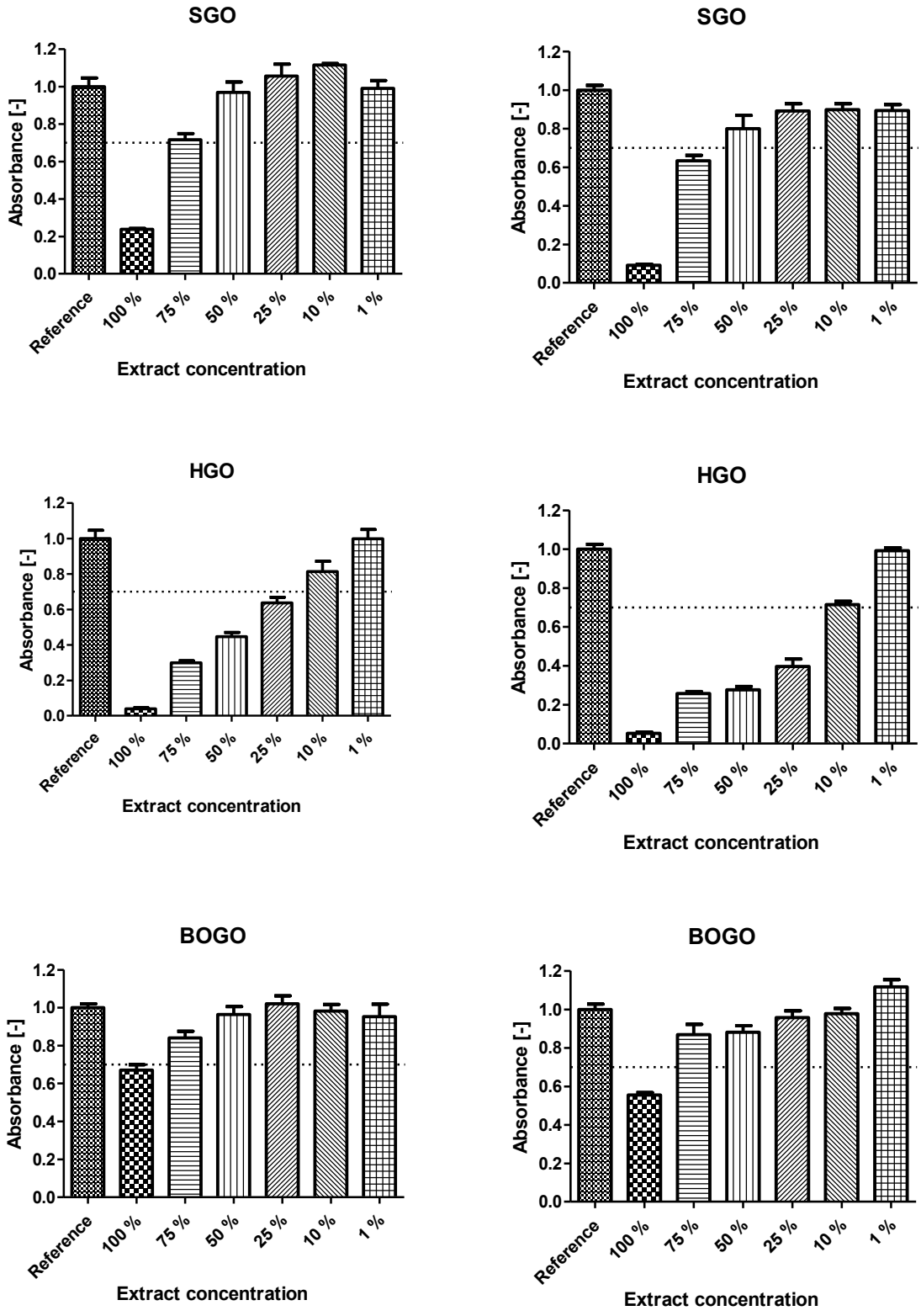

Figure 2: *Cell viability of individual samples extracts in various concentrations.*