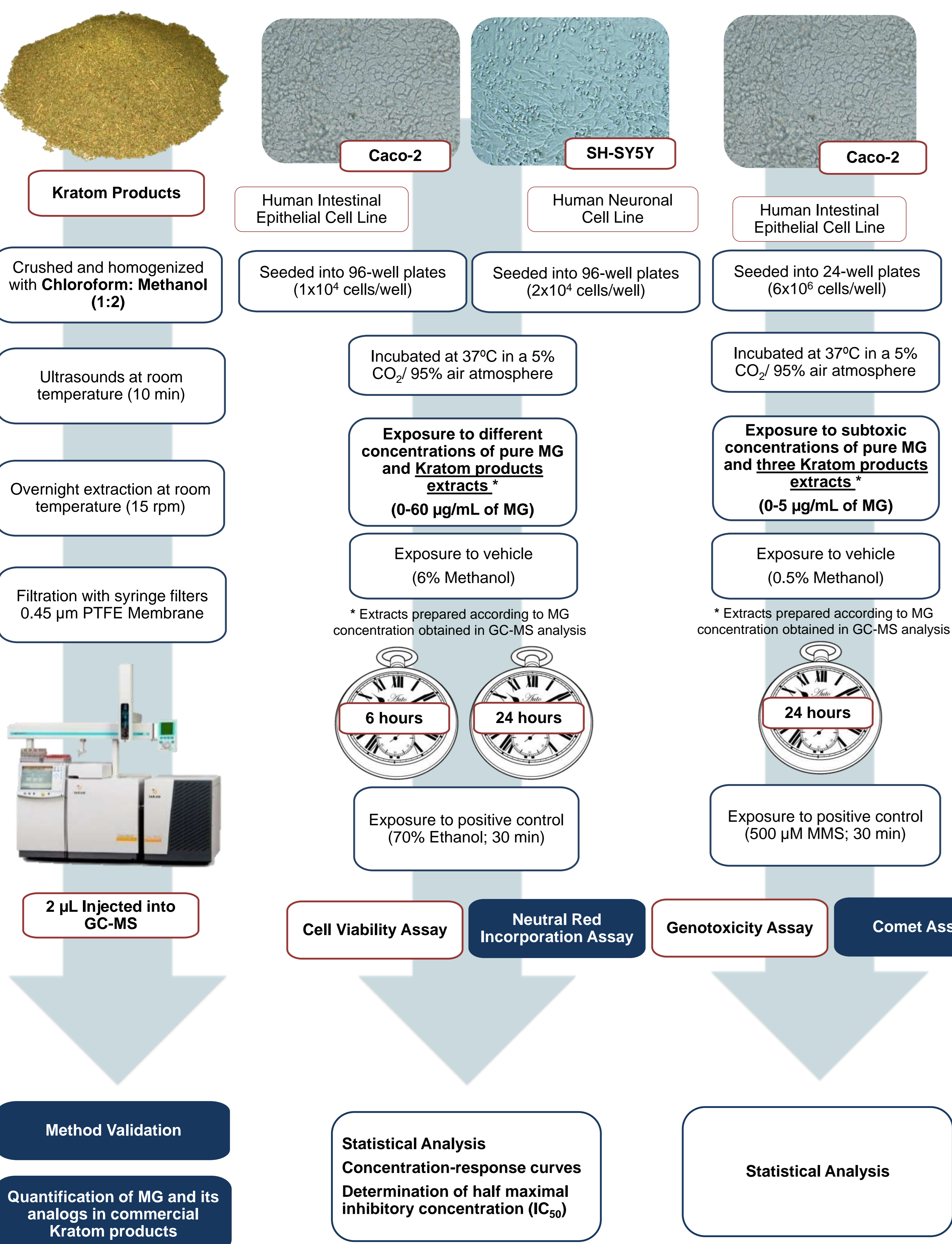


Introduction

The traditional market of recreational drugs has been suffering drastic changes in the last few years in consequence of the emergence of numerous new psychoactive substances (NPS), also entitled "legal highs". In recent years, one of the most popular legal high worldwide among young people, is the Kratom - a psychotropic product extracted from the *Mitragyna speciosa* plant. The increasing use of Kratom for recreational purposes and for self-treatment of withdrawal symptoms by opioid consumers has alerted risk assessment bodies due to absence of information its real composition and potential health risks. In the present study, we aimed to determine and compare the alkaloid content of 13 commercial Kratom products, purchased from different sources (smartshops and online), by Gas Chromatography – Mass Spectrometry (GC-MS) analysis. We also assessed the cytotoxicity induced by pure MG or Kratom extracts (2.5 - 60 µg/mL of MG) in *in vitro* models of human intestinal (Caco-2) and neuronal (SH-SY5Y) cells after 6 h and 24 h. Genotoxicity of pure MG and the most consumed and cytotoxic Kratom extracts was further evaluated in intestinal Caco-2 cells following 24 h of exposure to subtoxic concentrations (2.5 and 5 µg/mL of MG), using the comet assay.

Methods

GC-MS Analysis → Cytotoxicity → Genotoxicity



Results

GC-MS Analysis of MG

Table 1: Concentration of MG in the Kratom commercial products (mg/g sample)

Product Type	Product Name	Sample	MG
Shredded Leaves	Kratom Bali Shredded	Kr01BL	2.57 ± 0.12
	Kratom Thai Shredded	Kr03TL	3.04 ± 0.26
	Kratom Herbal	Kr05TL	2.64 ± 0.38
	Kratom Leaves	Kr08L	0.640 ± 0.015 ^b
Powdered Leaves	Kratom Herbal	Kr06TP	2.53 ± 0.04
	Kratom Malaysia Power	Kr07MP	2.58 ± 0.15
	Kratom Balinese	Kr09BP	2.07 ± 0.18 ^c
Resin	Kratom Bali Resin	Kr10BR	0.768 ± 0.042 ^d
	Kratom Bali Extract 15x	Kr02BE15x	4.30 ± 0.22 ^a
	Kratom Thai Extract 15x	Kr04TE15x	2.82 ± 0.10
	Kratom Bali Extract 15x	Kr11BE15x	2.66 ± 0.09
	Kratom Gold Extract	Kr12BEg	187 ± 17 ^a
Powdered Extracts	Kratom Red Extract 25x	Kr13BEr25x	2.70 ± 0.20

Genotoxicity – DNA damage parameters

Table 2: Comet assay analysis of DNA damage in Caco-2 cells exposed for 24h to the pure MG and Kratom products extracts (Kr10BR, Kr11BE15x and Kr12BEg)

	Tail Intensity (%)	Olive Tail Moment
Control	6.24 ± 2.20	1.05 ± 0.17
Vehicle (0.5% MeOH)	6.16 ± 0.55	0.92 ± 0.11
Positive Control (MMS 500 µM)	67.00 ± 4.89 ****	21.20 ± 5.27 ****
MG (µg/mL)		
2.5	6.68 ± 0.72	1.00 ± 0.16
5	5.64 ± 0.37	0.83 ± 0.07
Kr10BR (µg/mL)		
2.5	24.52 ± 15.09	4.28 ± 1.26
5	48.21 ± 9.34 ***	12.37 ± 1.45 ***
Kr11BE15x (µg/mL)		
2.5	47.90 ± 2.26 **	10.83 ± 1.03 **
5	47.69 ± 3.68 **	10.74 ± 1.35 **
Kr12BEg (µg/mL)		
2.5	13.21 ± 3.64	2.05 ± 0.63
5	38.10 ± 4.20 ***	7.92 ± 1.61 ***

Data are expressed as mean ± standard deviation (SD), n=3. Data was analyzed using the one-way ANOVA analysis followed by Tukey's multiple comparison test. ^a p < 0,0001 vs. All; ^b p < 0,0001 vs. All, except Kr10BR; ^c p < 0,0001 vs. Kr03TL; ^d p < 0,01 vs. Kr04TE15x; ^e p < 0,05 vs. Kr05TL, Kr11BE15x, Kr13BEr25x; ^f p < 0,0001 vs. All, except Kr08L.

Data represents the mean ± standard deviation (SD) of three independent experiments (n=2 per group). The analysis was performed using the Kruskal-Wallis test followed by Dunn's test. ** p < 0,01, *** p < 0,001, **** p < 0,0001 vs. control.

Cytotoxicity – IC₅₀ Values and Concentration-response curves

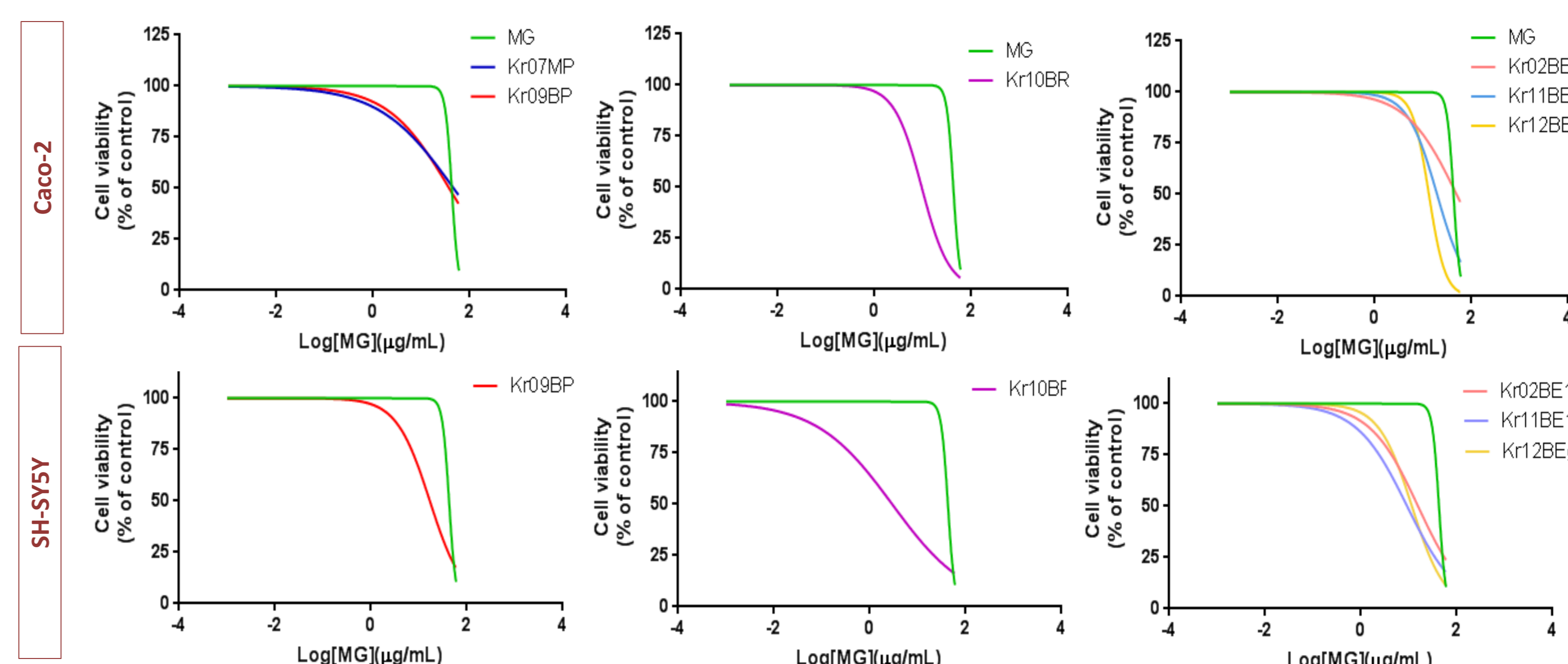


Figure 1: Cell viability concentration-response curves of Caco-2 and SH-SY5Y cells incubated with the pure MG, Kratom products extracts of powdered leaves (Kr07MP, Kr09BP), resin (Kr10BR) and "powered extracts" (Kr02BE15x, Kr11BE15x, Kr12BEg) for 24h, as assessed by the NR assay. Concentration-response curves were fitted using the least squares as the fitting method.

Table 3: Comparison between IC₅₀ values of the pure MG and Kratom products extracts concentration-response curves in Caco-2 and SH-SY5Y cells after 24h of exposure

Sample	MG	Kr02BE15x	Kr07MP	Kr09BP	Kr10BR	Kr11BE15x	Kr12BEg
IC₅₀ Caco-2 (µg/mL)	42,51	49,03	46,34	37,55	9,43	19,41	13,17
95% CI	41,04 – 44,04	40,60 – 59,20	35,60 – 60,36	28,78 – 48,99	8,50 – 10,47	18,02 – 20,91	12,13 – 14,29
IC₅₀ SH-SY5Y (µg/mL)	42,64	15,57	> 60	16,95	2,93	9,22	11,23
95% CI	41,21 – 44,13	12,22 – 19,84		13,69 – 20,99	1,80 – 4,78	7,28 – 11,68	9,30 – 13,57
Curve P value (comparison between the fitted curves)	-	< 0,0001	-	< 0,0001	< 0,0001	< 0,0001	< 0,0001
LogIC₅₀ P value (comparison between LogIC ₅₀ values)	-	< 0,0001	-	< 0,0001	< 0,0001	< 0,0001	< 0,0001

95% CI: 95% Confidence Interval. Concentration-response curves were fitted using least squares as the fitting method and the comparisons between curves and LogIC₅₀ were made using extra sum-of-squares F test. In all cases, p values < 0.05 were considered statistically significant.

Conclusions

- The results obtained by GC-MS revealed inconsistencies between the package label information of commercial Kratom products about the "power" and their real MG composition.
- A concentration-dependent decrease of cell viability was observed in both cell lines, SH-SY5Y cells being more sensitive.
- The resin and the "powered extracts" were the most cytotoxic, with IC₅₀ values significantly lower than the leaf extracts and the pure MG.
- Significant DNA damage was observed in Caco-2 cells exposed to all Kratom products extracts tested but not to the pure MG.

Cytotoxic and genotoxic effects observed appear not to be caused not only by MG but also by other substances present in the extracts or by interactions among the Kratom components.

Bibliography

Oliveira, Ana Sofia, et al. "Chemical characterization and *in vitro* cyto- and genotoxicity of 'legal high' products containing Kratom (*Mitragyna speciosa*)." *Forensic Toxicology* (2016):1-14. doi:10.1007/s11419-015-0305-6