Ultrasound-assisted extraction of cytotoxic alkaloids from *Caliphruria subedentata*: Comparative analysis of Bath and High-Intensity systems

Sergio Gonzalez-Lopez[1], Lina M. Trujillo Chacón[1], Edison Osorio[1*].

[1]Grupo de Investigación en Sustancias Bioactivas GISB, Facultad de Ciencias Farmacéuticas y Alimentarias, Universidad de Antioquia, Calle 70 No. 52-21, Medellín 0500100, Colombia.

*Corresponding author:
E-mail address: edison.osorio@udea.edu.co (E. Osorio).

Supplementary Material

Table S1. Coded input factors and levels defined for the experimental design with two factors and two levels.

Block	BUAE		HIUAE	
	Extraction time [a]	Solvent [b]	Extraction time [a]	Solvent [b]
1	-1	-1	-1	1
1	1	1	-1	-1
1	-1	1	1	1
1	1	-1	1	-1
2	1	1	-1	1
2	-1	1	1	-1
2	-1	-1	-1	-1
2	1	-1	1	1
3	1	-1	1	-1
3	-1	-1	1	1
3	1	1	-1	1
3	-1	1	-1	-1

[a] Min. [b] Methanol: Water (v/v)

Table S2. Factors and levels defined for the experimental design with two factors and two levels.

Factors	Level	BUAE[a]	HIUAE[b]
Extraction time (min)	1	10	7
	-1	60	13
Solvent [c]	1	1: 0	1: 0
	-1	1: 1	1: 1

[a] BUAE, Bath Ultrasound Assisted Extraction. [b] HIUAE, High Intensity Ultrasound Assisted Extraction. [c] Methanol: Water (v/v)

Table S3. Coded input factors and defined levels for HIUAE optimization.

	Block	Solvent*	Solid: Liquid
1	1	-1	0
2	1	0	1
3	1	1	0
4	1	-1	1
5	1	-1	-1
6	1	0	-1
7	1	1	1
8	1	0	0
9	1	0	0
10	1	1	-1
11	2	-1	0
12	2	0	1
13	2	1	0
14	2	-1	1
15	2	-1	-1
16	2	0	-1
17	2	1	1
18	2	0	0
19	2	0	0
20	2	1	-1
21	3	-1	0
22	3	0	1
23	3	1	0
24	3	-1	1
25	3	-1	-1
26	3	0	-1
27	3	1	1
28	3	0	0
29	3	0	0
30	3	1	-1

*Methanol: Water (v/v)

Table S4. Factors and levels defined for HIUAE optimization**.**

Level	Solid: Liquid	Solvent*
-1	1:10	7:3
0	1:20	8.5:1.5
1	1:30	1:0

*Methanol: Water (v/v)

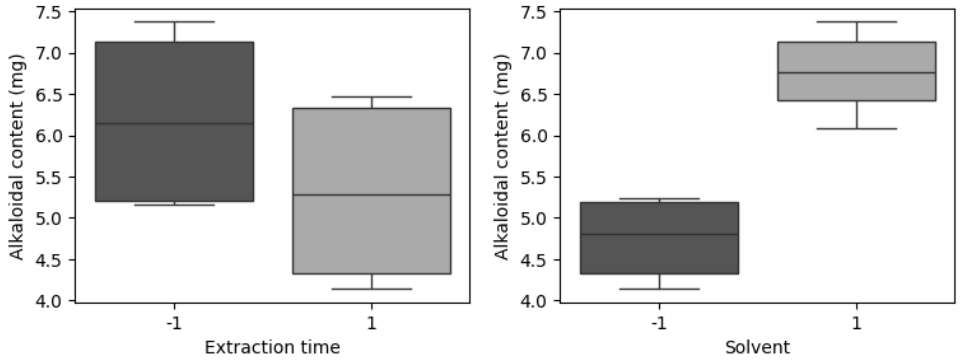

Figure S1. Effect of extraction time and solvent on alkaloidal extract yield

Table S5. Alkaloidal profile of *C. subedentata* obtained by Soxhlet, BUAE and HIUAE by GC-MS.

#	Compound	RT[a]	m/z	µg Gal/g AE[b]		
				Soxhlet	***BUAE***	***HIUAE***
			Galanthamine type			
1	Galanthamine	15.76	287(M^+, 81), 286(100), 216(36)	0.03	1.18	1.76
2	Narwedine	16.35	285 (M^+, 81), 284 (100), 199 (67)	nd	0.13	0.10
Haemantamine type						
3	8-*O*-Demethylmaritidine	16.86	273 (M^+, 100), 201 (95), 189 (62)	0.06	0.66	0.53
4	Haemantamine	17.81	301 (M^+, 13), 272 (100), 181 (30)	nd	0.32	0.68
Tazettine type						
5	6-*O*-Methylpretazettine	17.54	345 (M^+, 18), 261 (100), 239 (31)	nd	nd	0.05
6	Tazettine	17.90	331(M^+, 27), 298(21), 247(100)	0.02	0.14	0.89
Narciclasine type						
7	Trisphaeridine	15.97	223(M^+, 100) 222(38), 138(30)	0.03	0.40	0.52
Lycorine type						
8	Lycorine	18.68	287(M^+, 21), 227(64), 226(100)	0.03	0.13	0.54
9	Anhydrolycorine	16.97	251(M^+, 42) 250(100), 191(18)	0.02	0.04	0.84
10	11-12-dehydrolycorene	17.16	253(M^+, 51) 252(100), 224(12)	nd	0.61	0.80
11	Homolycorine type alkaloid	19.03	108 (M^+, 11), 250(1), 109(100).	0.02	0.69	1.94

[a] RT, Retention time (min). [b] Quantitative values obtained by response factor of the external Galanthamine standard (microgram of galanthamine per gram of alkaloid extract). nd, Not detected.