Supporting Information

Deep Eutectic Solvents as an Eco-Efficient Alternative for the Sustainable Recovery of Multitarget Alkaloids from *Bocconia frutescens*

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TABLE S1. Statistical significance of the effect of extraction method and temperature on sanguinarine and chelerythrine recovery.

Extraction method	Sanguinarine 20 °C	Sanguinarine 40 °C	Chelerythrine 20 °C	Chelerythrine 40 °C
DES1	2.13 ± 0.04 ^{q}	5.16 ± 0.09 ^{p}	1.68 ± 0.03 ^{o}	3.80 ± 0.06 ^{mn}
DES2	6.02 ± 0.10 ^{op}	5.82 ± 0.09 ^{op}	3.21 ± 0.05 ^{n}	3.97 ± 0.06 ^{m}
DES3	7.18 ± 0.12 ^{n}	5.73 ± 0.09 ^{op}	6.45 ± 0.10 k	5.49 ± 0.09 ^{l}
DES4	12.89 ± 0.20 ^{k}	8.64 ± 0.14 ^{m}	10.28 ± 0.16 ^{i}	9.71 ± 0.15 ^{i}
DES5	6.27 ± 0.10 ^{o}	9.79 ± 0.16 ^{l}	5.39 ± 0.09 ^{l}	8.02 ± 0.13 ^{j}
DES6	17.00 ± 0.27 ^{j}	23.14 ± 0.37 ^{cd}	11.64 ± 0.19 ^{h}	13.68 ± 0.22 ^{ef}
DES7	22.88 ± 0.37 ^{d}	28.20 ± 0.45 ^{a}	14.49 ± 0.23 ^{cd}	16.65 ± 0.27 ^{a}
DES8	26.78 ± 0.43 ^{b}	26.90 ± 0.43 ^{b}	16.49 ± 0.26 ^{ab}	16.01 ± 0.26 ^{ab}
DES9	18.07 ± 0.29 ^{i}	23.91 ± 0.38 ^{c}	12.41 ± 0.21 ^{g}	14.83 ± 0.24 ^{c}
DES10	16.68 ± 0.27 ^{j}	20.73 ± 0.33 ^{fg}	12.07 ± 0.19 ^{gh}	14.20 ± 0.23 ^{cde}
DES11	19.73 ± 0.32 ^{h}	23.09 ± 0.37 ^{cd}	14.04 ± 0.23 ^{de}	15.95 ± 0.63 ^{b}
DES12	20.20 ± 0.32 ^{gh}	21.71 ± 0.35 ^{e}	13.55 ± 0.22 ^{ef}	13.30 ± 0.21 ^{f}
DES13	1.22 ± 0.02 ^{rs}	1.56 ± 0.02 ^{qr}	0.84 ± 0.01 ^{p}	1.55 ± 0.02 ^{o}
EtOH 96%	20.61 ± 0.33 ^{fg}	21.41 ± 0.34 ^{ef}	13.62 ± 0.22 ^{ef}	14.76 ± 0.24 ^{c}
Water	0.48 ± 0.01 ^{s}	0.54 ± 0.01 ^{s}	0.42 ± 0.01 ^{p}	0.42 ± 0.01 ^{p}

*Values are expressed as mean ± standard deviation (SD) of three replicate extractions (n = 3). Statistical analyses were performed separately for sanguinarine and chelerythrine using two-way ANOVA, with extraction method and temperature as fixed factors. A significant extraction method × temperature interaction was detected for both sanguinarine ($F(14,60) = 177.68$, $p < 0.0001$) and chelerythrine ($F(14,60) = 54.22$, $p < 0.0001$). Therefore, Tukey's HSD post hoc test was applied to

compare extraction method–temperature combinations within each alkaloid. Means followed by different letters are significantly different according to Tukey's HSD test ($p < 0.05$); means sharing at least one letter are not significantly different.

TABLE S2. Statistical significance of the effect of water addition on sanguinarine and chelerythrine recovery.

Solvent system	Temperature (°C)	Sanguinarine (mean ± SD)	Chelerythrine (mean ± SD)
DES7	20	22.87 ± 2.37 ^{cd}	14.50 ± 1.49 ^{abcd}
	40	28.20 ± 2.38 ^{ab}	16.66 ± 1.35 ^{ab}
DES7:water 75:25	20	24.73 ± 0.42 ^{bc}	13.92 ± 0.93 ^{bcd}
	40	30.03 ± 0.83 ^{a}	17.10 ± 0.80 ^{a}
DES7:water 50:50	20	12.90 ± 1.54 ^{g}	10.28 ± 0.97 ^{e}
	40	17.00 ± 0.75 ^{efg}	11.64 ± 0.48 ^{de}
EtOH 96%	20	20.60 ± 1.14 ^{cde}	10.28 ± 0.97 ^{e}
	40	21.40 ± 2.43 ^{cde}	14.76 ± 1.65 ^{abc}
EtOH 75%	20	18.77 ± 1.98 ^{def}	13.97 ± 1.43 ^{bcd}
	40	19.23 ± 1.76 ^{def}	14.34 ± 1.13 ^{abcd}
EtOH 50%	20	14.93 ± 1.36 ^{fg}	12.60 ± 0.95 ^{cde}
	40	15.37 ± 0.67 ^{fg}	13.49 ± 0.70 ^{cd}
water	20	0.50 ± 0.10 ^{h}	0.42 ± 0.09 ^{f}
	40	0.53 ± 0.42 ^{h}	0.41 ± 0.30 ^{f}

*Values are expressed as mean ± standard deviation (SD) of three replicate extractions (n = 3). Statistical analyses were performed separately for sanguinarine and chelerythrine using two-way ANOVA, considering solvent system and temperature as fixed factors. Because significant solvent × temperature interactions were observed, Tukey's HSD post hoc test was used to compare individual solvent–temperature combinations. Within each alkaloid column, means sharing at least one letter are not significantly different, whereas means with no letters in common are significantly different according to Tukey's HSD test ($p < 0.05$). Letters should be interpreted independently for sanguinarine and chelerythrine.

TABLE S3. Statistical significance of the effect of time extraction on sanguinarine and chelerythrine recovery at 40 ^{0}C

Extraction time (min)	Sanguinarine (mean ± SD)	Chelerythrine (mean ± SD)
30	26.75 ± 0.90[a]	16.51 ± 0.90[a]
60	25.34 ± 0.90[a]	15.34 ± 0.90[a]
90	25.05 ± 0.90[a]	15.22 ± 0.90[a]
120	24.76 ± 0.91[a]	14.58 ± 0.91[a]
150	24.76 ± 0.91[a]	14.15 ± 0.92[a]

*Values are expressed as mean ± standard deviation (SD) of three replicate extractions (n = 3). Statistical analyses were performed separately for sanguinarine and chelerythrine using one-way ANOVA, with extraction time as the independent factor. No significant effect of extraction time was observed for either sanguinarine or chelerythrine. Tukey's HSD post hoc test was used for multiple comparisons among extraction times. Means sharing the same letter are not significantly different ($p > 0.05$). Statistical comparisons were performed independently for each alkaloid.

TABLE S4. Statistical Significance of the Effect of solid: DES7-water 75:25 ratio

SOLID:DES7-water 75:25 RATIO	Chelerytrine mg/g dry weigh
(1:10)	5.543 ± 0.042[a]
(1:20)	3.147 ± 0.006[b]
(1:30)	2.103 ± 0.006[c]
(1:50)	1.360 ± 0.105[d]
(1:100)	1.207 ± 0.025[e]
(1:200)	0.773 ± 0.006[f]
(1:250)	0.730 ± 0.020[f]

*Values are expressed as mean ± standard deviation (SD) of three replicate extractions (n = 3). Different superscript letters indicate statistically significant differences among solid-to-solvent ratios according to Tukey's HSD post hoc test ($p < 0.05$) following one-way ANOVA. Means sharing the same letter are not significantly different. The solid-to-solvent ratio had a significant effect on chelerythrine recovery ($F(6,14) = 4493.41$, $p < 0.0001$).

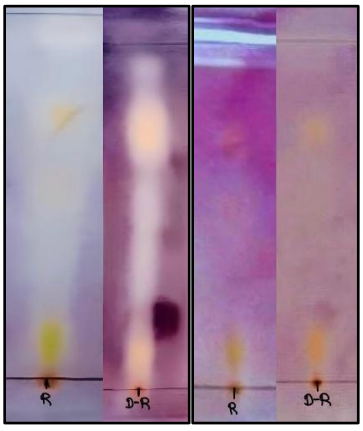
TABLE S5. Exact mass data and the principal MS/MS fragment ions of sanguinarine and chelerythrine

Peak tr (min)	$[M]^+$ / $[M+H]^+$	MS/MS	Compound [Ref]
4.9	332.056 / 333.069	*m/z* 317, 304, 274, 246, 218	Sanguinarine [20-22]
5.2	348.096/ 349.100	m/z 333, 304, 290, 274	Chelerythrine [20-22]

TABLE S6. Analytical Method Validation Parameters for Chelerythrine Quantification

Analytical parameter	Result
Calibration levels	8
Calibration range	0.2–1000 ppm
Independent injections per level	3
Regression equation	$y = 0.174245x + 0.283888$
R^2	0.999967
Instrumental precision (RSD)	1.20–1.94%
Estimated instrumental LOD	0.156 ppm
Estimated instrumental LOQ	0.472 ppm
Recovery	Not determined

FIGURE S1. Direct bioautography TLC plates of *Bocconia frutescens* root extracts evaluated for cholinesterase inhibitory activity.

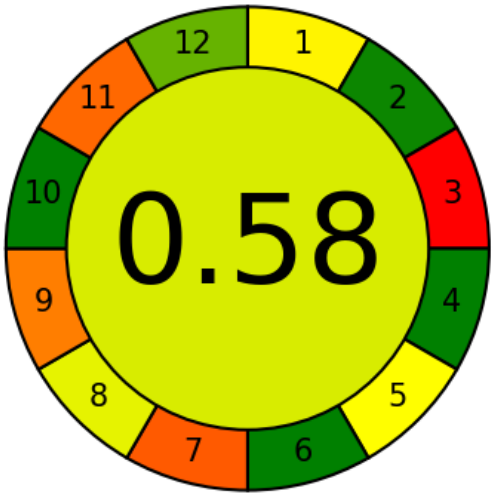


A **B**

R: ethanolic root extract; D-R: root extract obtained using the citric acid–glucose (1:1) deep eutectic solvent (DES). **(A)** Acetylcholinesterase (AChE) inhibition assay; **(B)** Butyrylcholinesterase (BuChE) inhibition assay.

Figure S2
Analytical Greenness report sheet

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Criteria	Score	Weight
1. Direct analytical techniques should be applied to avoid sample treatment.	0.48	2
2. Minimal sample size and minimal number of samples are goals.	0.98	2
3. If possible, measurements should be performed in situ.	0.0	2
4. Integration of analytical processes and operations saves energy and reduces the use of reagents.	1.0	2
5. Automated and miniaturized methods should be selected.	0.5	2
6. Derivatization should be avoided.	1.0	2
7. Generation of a large volume of analytical waste should be avoided, and proper management of analytical waste should be provided.	0.18	2
8. Multi-analyte or multi-parameter methods are preferred versus methods using one analyte at a time.	0.55	2
9. The use of energy should be minimized.	0.25	2
10. Reagents obtained from renewable sources should be preferred.	1.0	2
11. Toxic reagents should be eliminated or replaced.	0.21	2
12. Operator's safety should be increased.	0.8	2