

Supplementary Materials

Novel Butyrylcholinesterase Inhibitors Alkaloids from *Cannabis sativa* Roots: Bioguided Isolation and *In Silico* Study [†]

Javier E. Ortiz^{1,*}, Camila W. Adarvez-Feresin², Olimpia Llalla-Cordova^{1,3}, Diego Cristos⁴, Adriana Garro² and Gabriela E. Feresin^{1,3,*}

¹ Instituto de Biotecnología, Facultad de Ingeniería, Universidad Nacional de San Juan, Av. General San Martín 1109 (oeste), San Juan, CP 5400, Argentina, jortiz@unsj.edu.ar, gferesin@unsj.edu.ar, olimpiallcr@gmail.com

² IMIBIO-SL CONICET, Facultad de Química, Bioquímica y Farmacia, UNSL, Ejército de Los Andes 950, San Luis, Argentina, CP:5700. camilaadarvezferesin@gmail.com, adrianagarros@gmail.com

³ Consejo Nacional de Ciencia y Tecnología (CONICET CCT San Juan), Av. General San Martín 1109 (oeste), San Juan, CP 5400, Argentina, gferesin@unsj.edu.ar, olimpiallcr@gmail.com

⁴ Instituto Tecnología de Alimentos, Centro de Investigación de Agroindustria (CIA-INTA), Nicolas Repetto y de los Reseros s/n (1686), Hurlingham, Buenos Aires, Argentina, cristos.diego@inta.gob.ar

* Correspondence: J.E. Ortiz: jortiz@unsj.edu.ar and G.E. Feresin: gferesin@unsj.edu.ar

[†] In Memoriam: Dr. Francisco Cecati (1984–2025), esteemed researcher and professor at the Universidad Nacional de San Luis. His passionate dedication to the study of medicinal cannabis and his unwavering commitment to teaching will continue to inspire future generations of scholars and researchers.

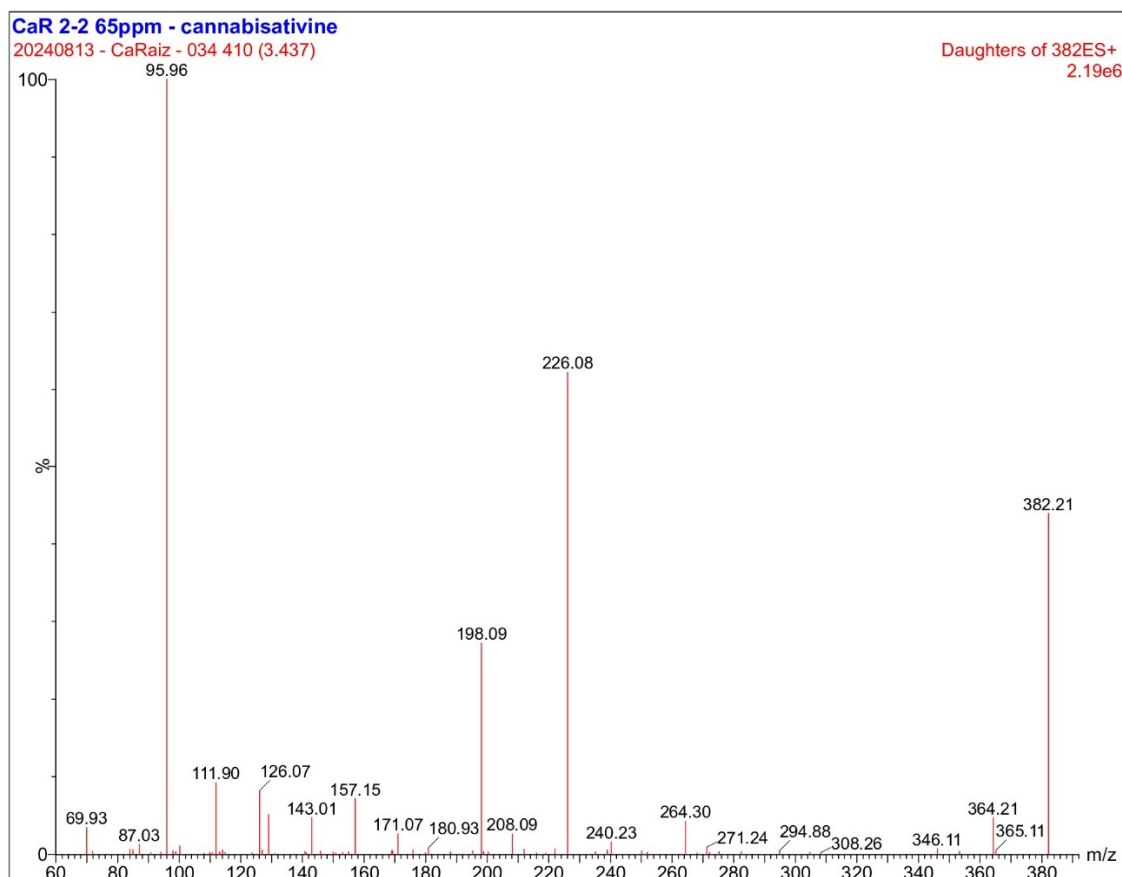


Figure S1. Cannabisativine (CS) MS/MS spectrum (daughters of 382).

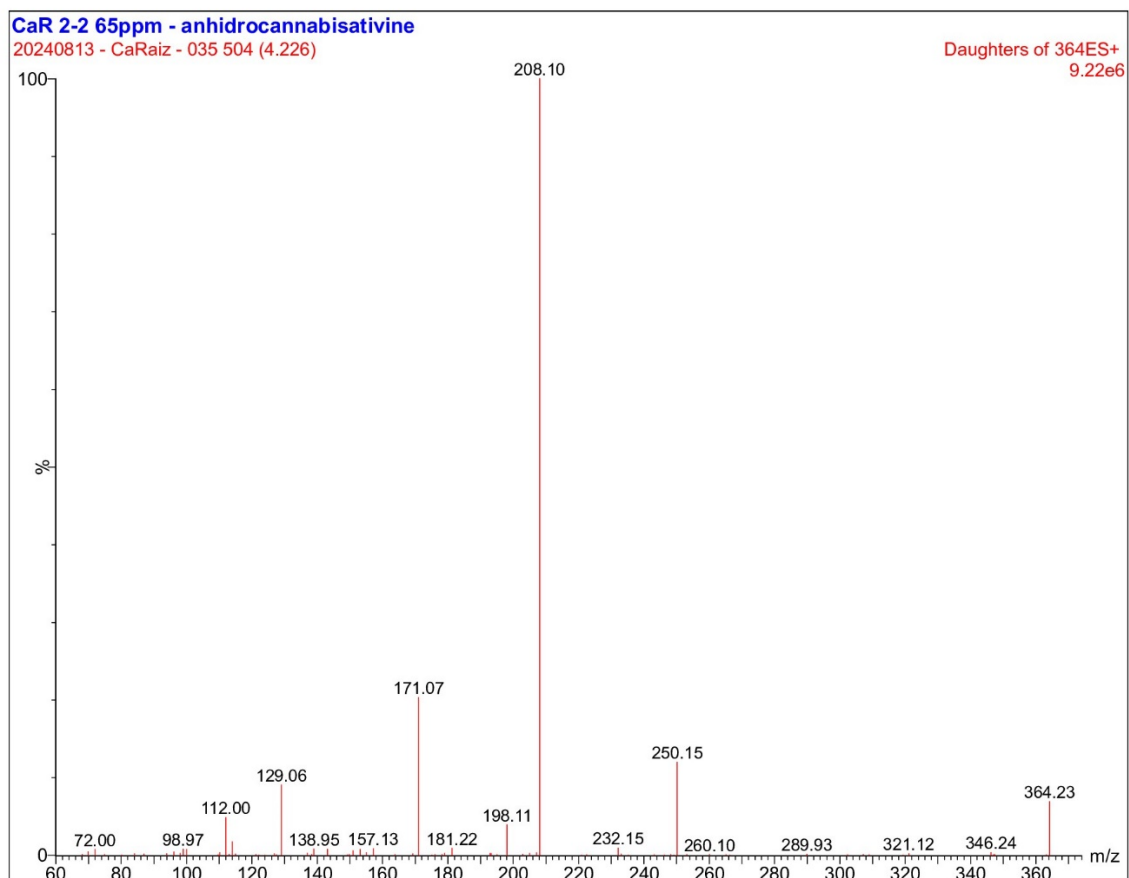


Figure S2. Anhydrocannabisativine (ACS) MS/MS spectrum (daughters of 364).

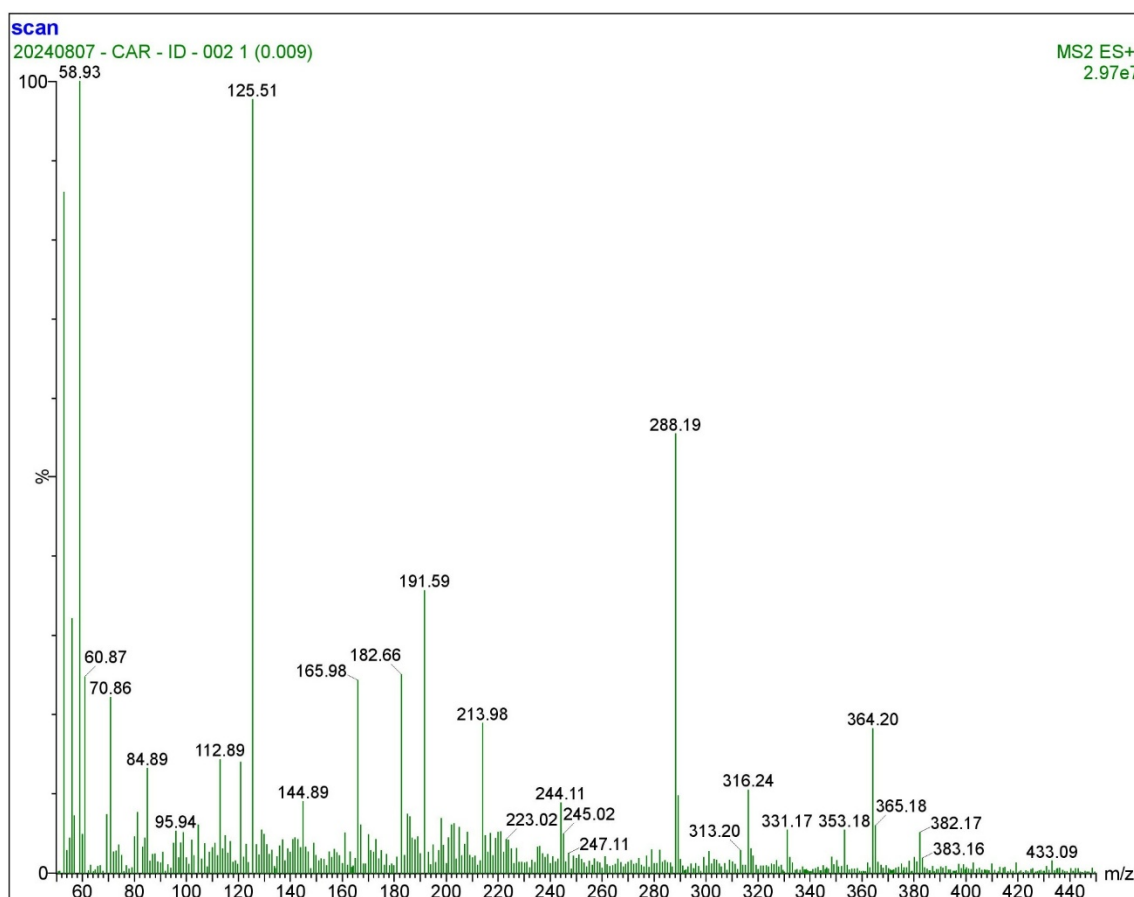


Figure S3. MS spectrum of the alkaloid-enriched extract from *C. sativa* roots (DI-ESI-MS). Red arrows indicate $[M+H]^+$ species of CS and ACS, while blue arrows their $[M+2H]^{2+}$ species.

Compound name: Caffeine
 Correlation coefficient: $r = 0.994352$, $r^2 = 0.988735$
 Calibration curve: $199931 * x + -62742.5$
 Response type: External Std, Area
 Curve type: Linear, Origin: Include, Weighting: $1/x$, Axis trans: None

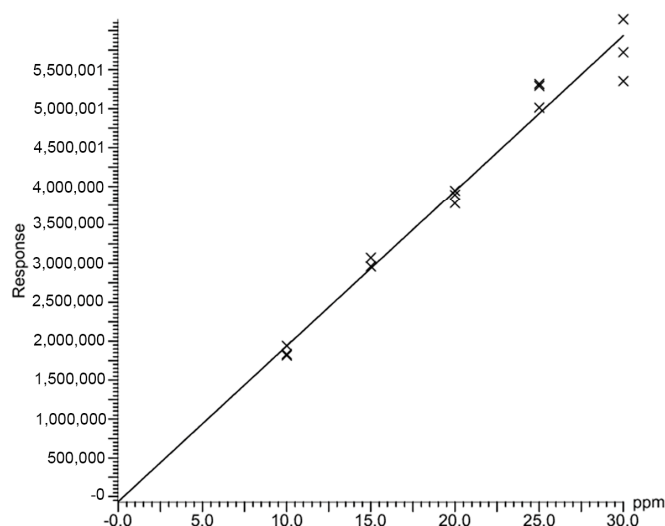


Figure S4. Caffeine calibration curve for CS and ACS quantification.

Table S1. Proposed ESI(+)-MS/MS main fragments structures for cannabissativine.

Structure	Fragment Mass	Ion	Chemical Formula
	382	$[M+H]^+$	$C_{21}H_{40}N_3O_3^+$
	364	$[M+H-18]^+$	$C_{21}H_{38}N_3O_2^+$
	226	$[M+H-156]^+$	$C_{13}H_{28}N_3^+$
	157	$[M+H-225]^+$	$C_8H_{17}N_2O^+$

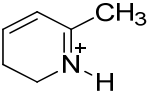
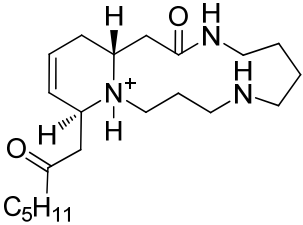
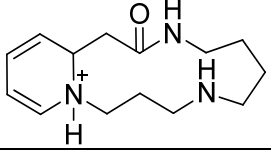
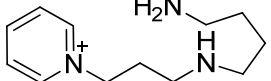
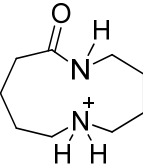
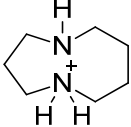
	96	[M+H-286] ⁺	C ₆ H ₁₀ N ⁺
---	----	------------------------	---

Table S2. Proposed ESI(+)-MS/MS main fragments structures for anhydrocannabisativine.

Structure	Fragment Mass	Ion	Chemical Formula
	364	[M+H] ⁺	C ₂₁ H ₃₈ N ₃ O ₂ ⁺
	250	[M+H-114] ⁺	C ₁₄ H ₂₄ N ₃ O ⁺
	208	[M+H-156] ⁺	C ₁₂ H ₂₂ N ₃ ⁺
	171	[M+H-193] ⁺	C ₉ H ₁₉ N ₂ O ⁺
	129	[M+H-235] ⁺	C ₇ H ₁₇ N ₂ ⁺