Supplementary Material

Disposable Electrochemical Biosensor Based on Acetylcholinesterase for Inhibition Assays Using a Natural Substance and Plants Extracts

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S.1. Synthesis and Morphological Characterizations of AuNPs-GSH

The synthesis of AuNPs-GSH was performed according to the procedure developed by.[1] For this, 79.0 mg of $HAuCl_4.3H_2O$ and 30.8 mg of glutathione were used. Then they were mixed with 2.0 mL of concentrated acetic acid and 12.0 mL of methanol. In the next step, a $NaBH_4$ solution was prepared by diluting 120.0 mg of $NaBH_4$ in 6.0 mL of ultrapure water, which was added slowly to the previous solution under rapid stirring for 2 h.

The suspension was filtered with a 50 kDa molecular filtration membrane (previously washed with a 0.1 mol L^{-1} NaOH solution) and centrifuged at 3500 rpm. The AuNPs-GSH were washed four times with ultrapure water and resuspended in 15 mL of an aqueous solution of 20.0 mmol L^{-1} HEPES buffer (pH = 8.0). The AuNPs-GSH dispersion was then stored at 4 ºC, protected from light. After being synthesized, the AuNPs-GSH were morphologically characterized using TEM (Figure S1) and EDX analysis (Figure S2).

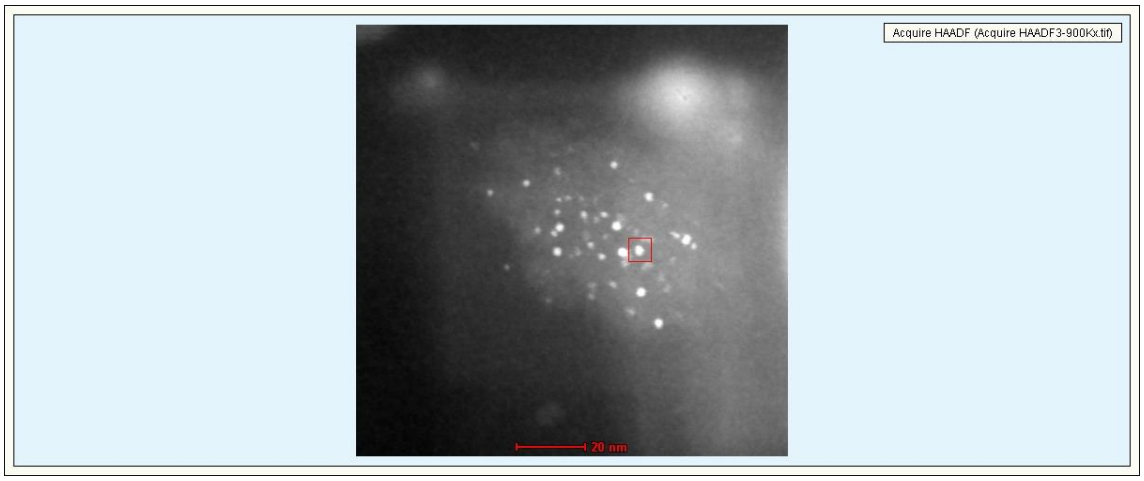

Figure S1. TEM images obtained for AuNPs.

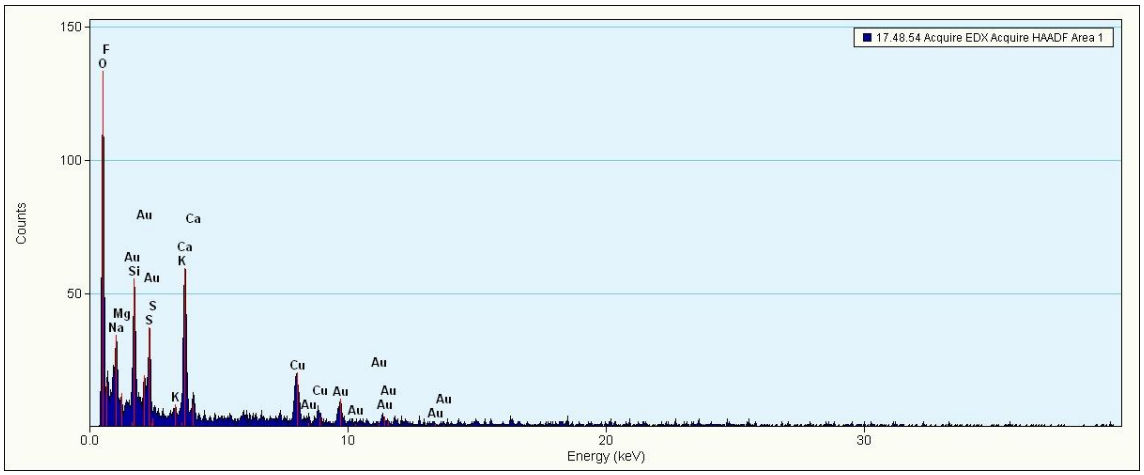

Figure S2. EDX analysis for the AuNPs-GSH.

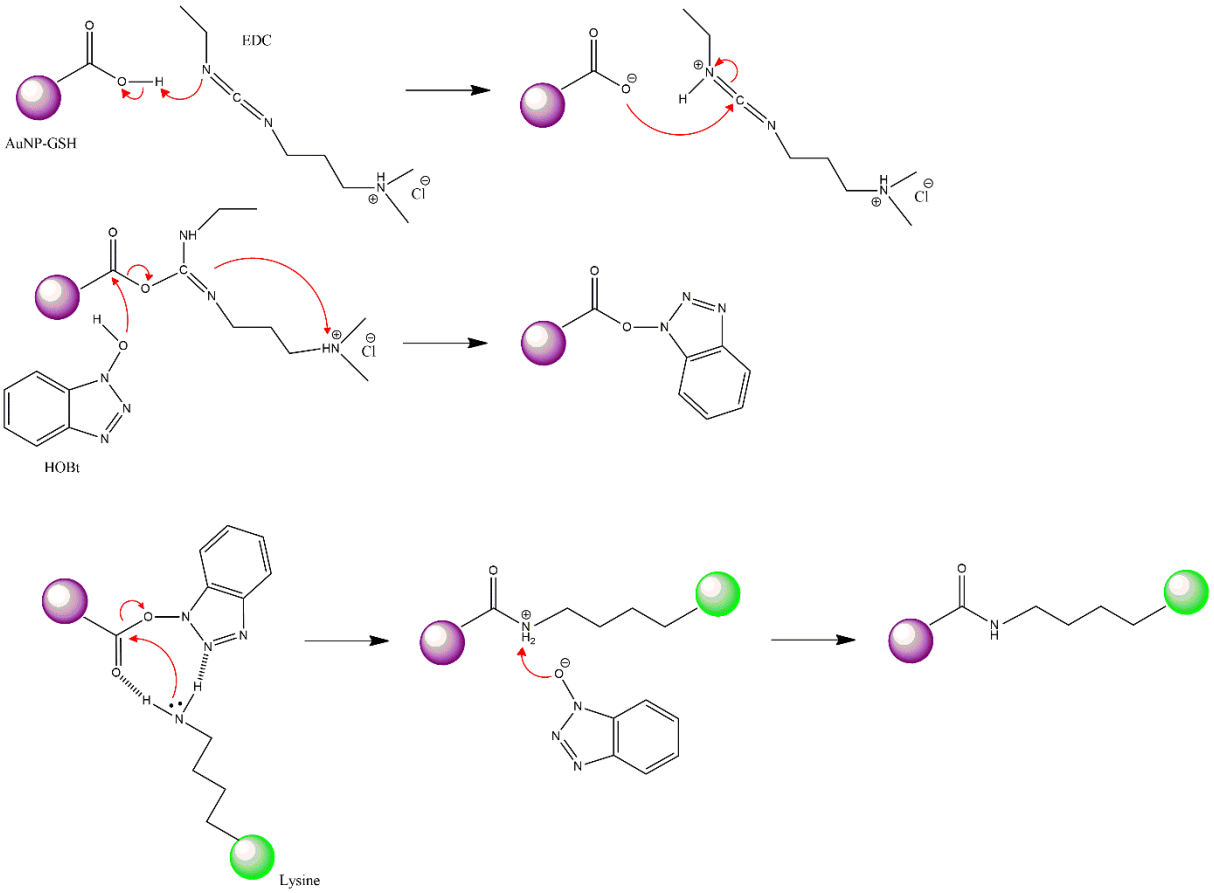

Figure S3. EDC-HOBt reaction for activation of the carboxylic group of AuNPs-GSH and reaction with the amino group of an amino acid present in the enzyme. Adapted to Fiaromonte *et al.*[2]

Table S1. Relative Standard Deviation (RSD) for intra-day and inter-day Bio−AChE under conditions optimized for the bioassays

Parameter	Bio−AChE	RSD / %
Repeatability	Intra-day	1.46
	Inter-day	0.90

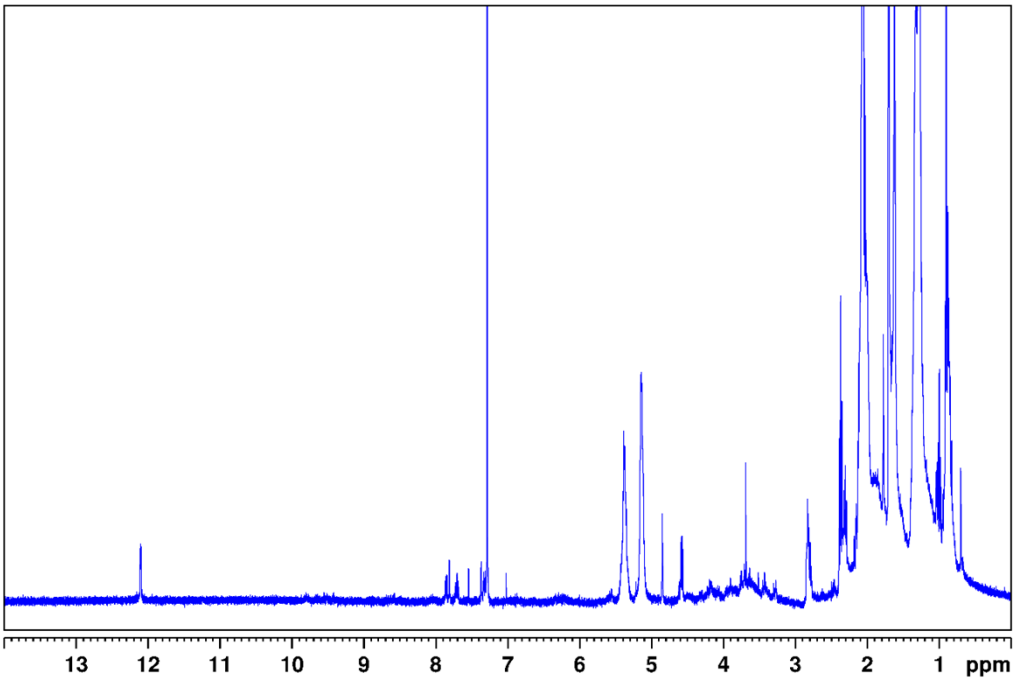

Figure S4. ^{1}H NMR spectrum of *Picramnia riedelli* extract in $CDCl_3$ (400 MHz).

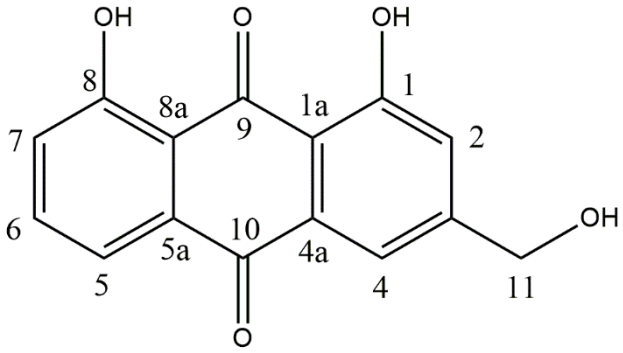

Figure S5. Anthraquinone aloe-emodin isolated from *Picramnia riedelli* and *Cape aloe* with numbered carbons.[3,4]

Table S2. ^{1}H NMR data (400 MHz, $CDCl_3$) of the structure of Aloe-emodin (Figure S5 (1) in and of the literature referring to anthraquinone (2) [3].

	1	2
Position	δH (J in Hz)	δH (*J* in Hz)
2	7,36 *sl*	7, 35 *sl*
4	7,81 *sl*	7,79 *sl*
5	7,85 *d* (7,3)	7,81 *d* (8,8)
6	7,70 *dd* (7,3-8,4)	7,70 *t* (8,8)
7	7,32 *d* (8,4)	7,30 *d* (7,4)
11	4,74 *d*	4,74 *d* (8,0)
OH	12,10 *s*	12,11 *s*
-	12,09 *s*	12,06 *s*

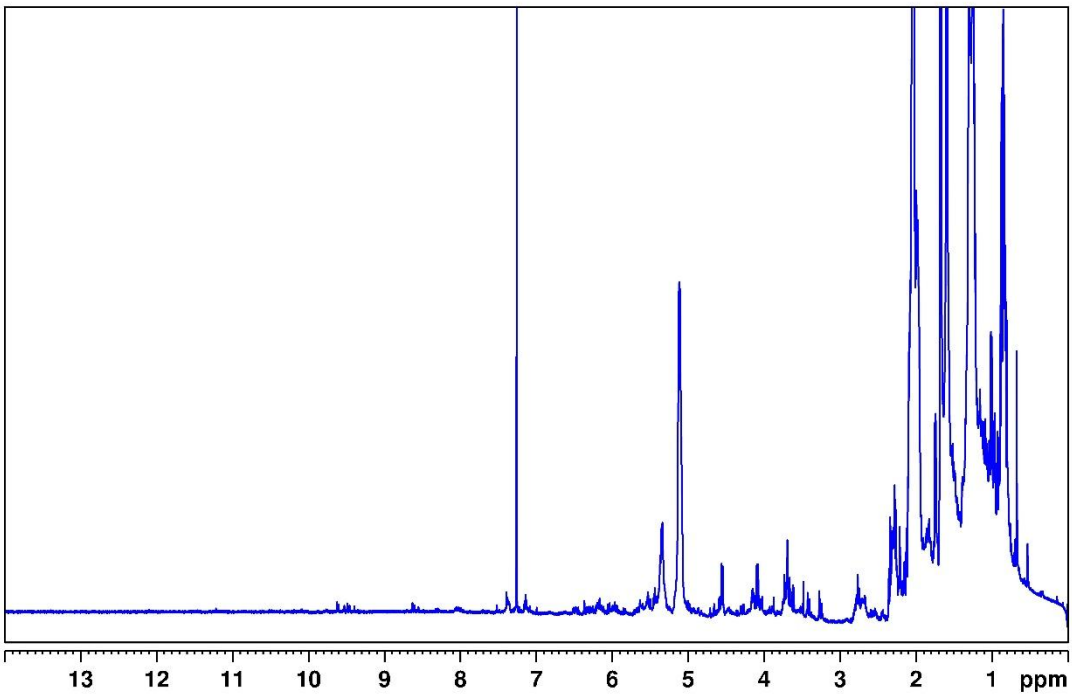

Figure S6. 1H NMR spectrum of *Toona ciliata* extract in CDCl3 (400 MHz).

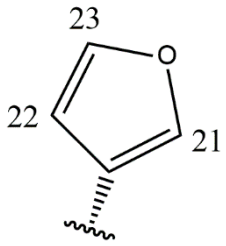

Figure S7. Furan ring structure with the usual numbers for the carbons present in it.

The signs at 7.39, 7.14, and 6.36 ppm belong to the hydrogens in the furan ring, at positions 21, 23, and 22 (usual numbering for the carbons in this ring) respectively (Figure S7). Similar displacements for these hydrogens have already been observed in the literature.[5-7] Signals between 1.21 and 0.85 ppm, in comparison with the literature, refer to the methyl hydrogens found in the structure of limonoids.[7,8]

References

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