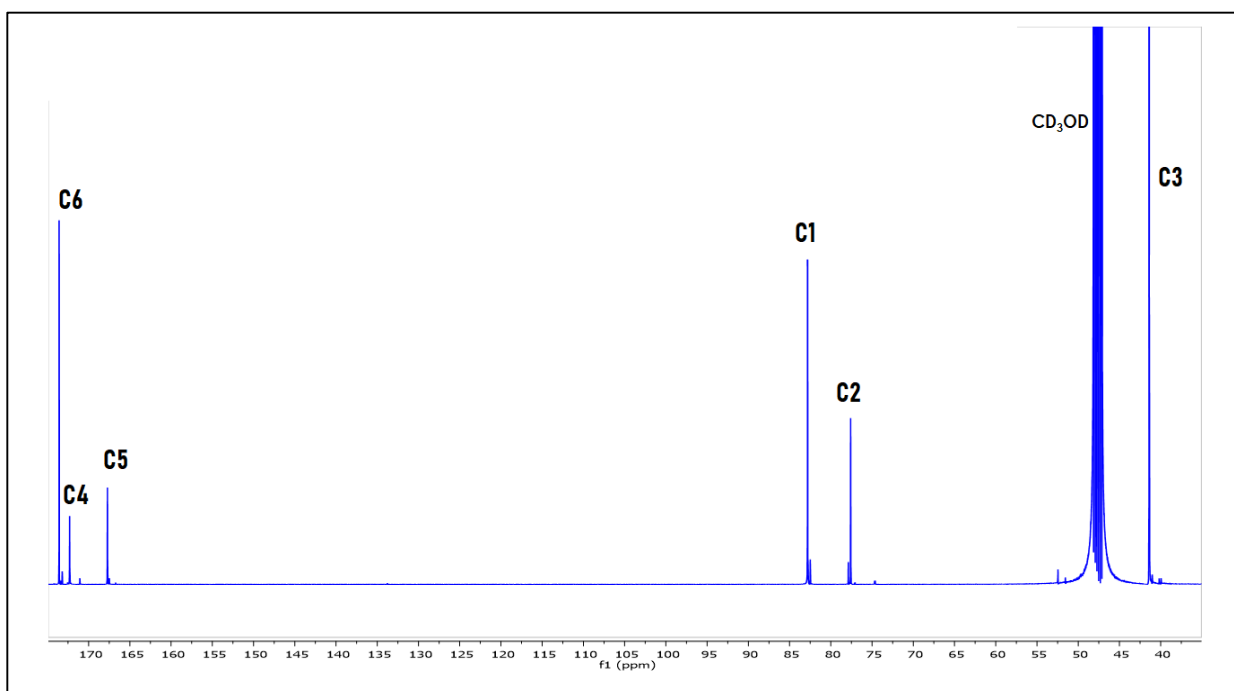
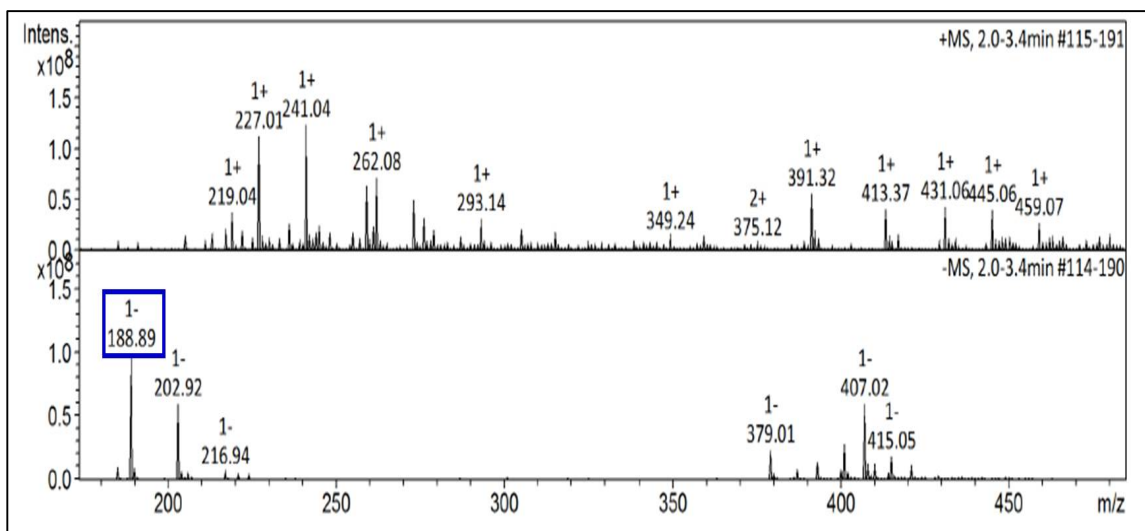


(a)



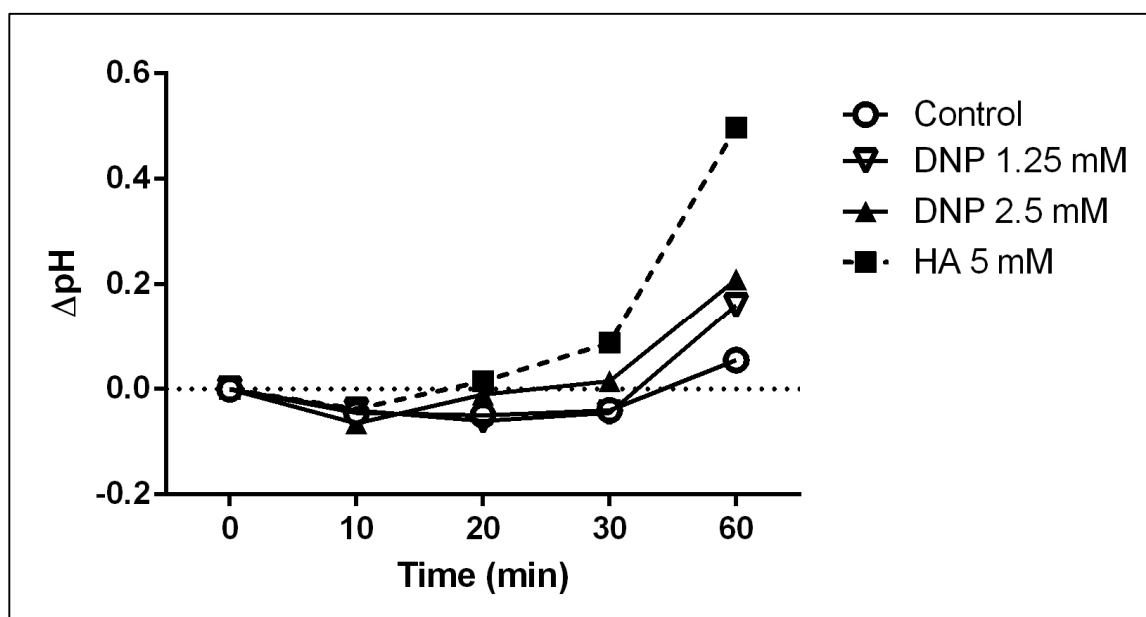
(b)



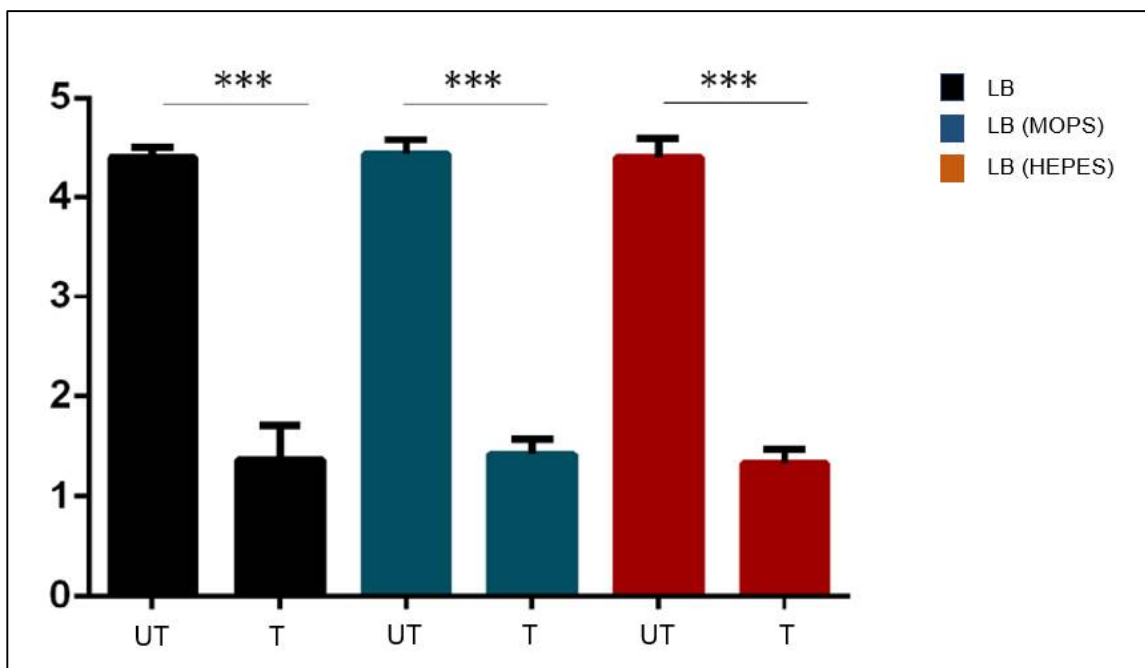
(c)

**Supplementary Figure S1. NMR and mass spectrum data for purified hibiscus acid.** (a) <sup>1</sup>H NMR spectrum (600 MHz, CD<sub>3</sub>OD) of a sample of hibiscus acid. (b) <sup>13</sup>C NMR (150 MHz, CD<sub>3</sub>OD) spectrum of hibiscus acid. (c) Positive (up) and negative (down) mass spectrum (ESI) of hibiscus acid.

Supplementary Figure 2



**Supplementary Figure S2. Effect of HA and DNP on extracellular pH in *Salmonella* cultures.** Represented is the difference in pH ( $\Delta$ pH) between the starting point when the compounds were added (0 min) and the indicated time point for each case. Cultures were incubated at 25 °C in agitation (120 rpm) and pH was determined every 10 min as described in the Methods section. Results are the average of two independent experiments made in duplicates. 4% DMSO was used as a control.



**Supplementary Figure S3. *Salmonella* motility in buffered media.** Bars represent bacterial mobility in semisolid LB media (black bars) or with MOPS (green bars) and HEPES (red bars) pH buffers. Motility of *Salmonella* decreases in the media treated (T) with 0.5 mg/mL of *Hibiscus sabdariffa* L. extract compared with untreated media (UT). The trial was conducted in triplicate and the bar represents the standard deviation.  $P > 0.05$  was considered as a significant difference.

**Supplementary Table S1.** Composition of the mobile phase used for the fractionation of the total extract of *H. sabdariffa* L. by column chromatography and biological activity.

Mobile phase	Vol. (mL)	Fractions	Inhibition of <i>Salmonella</i> motility
Hexane	200	1-4	Negative
Hexane-ethyl acetate 9:1	100		
Hexane-ethyl acetate 8:2	100		
Hexane-ethyl acetate 7:3	100	5-6	Negative
Hexane-ethyl acetate 6:4	100		
Hexane-ethyl acetate 5:5	100		
Hexane-ethyl acetate 3:7	100	7-10 ("Fraction A")	Positive
Hexane-ethyl acetate 1:9	100		
Ethyl acetate-methanol 9:1	100		
Ethyl acetate-methanol 8:2	100		
Ethyl acetate-methanol 7:3	100	11-14	Negative
Ethyl acetate-methanol 6:4	100		
Ethyl acetate-methanol 5:5	100		
Ethyl acetate-methanol 3:7	100		
Methanol	500		ND*

\*. Not determined

**Supplementary Table S2.** Composition of the mobile phase used for the fractionation of the "Fraction A" by column chromatography and biological activity.

Mobile phase	Vol. (mL)	Fractions	Inhibition of <i>Salmonella</i> motility
Hexane	100	1-8	Negative
Hexane-ethyl acetate 9:1	100		
Hexane-ethyl acetate 8:2	200		
Hexane-ethyl acetate 7:3	100	9-13	Negative
Hexane-ethyl acetate 6:4	100		
Hexane-ethyl acetate 5:5	100		
Hexane-ethyl acetate 3:7	100	14-17 ("Fraction B")	Positive
Hexane-ethyl acetate 1:9	100		
Ethyl acetate-methanol 9:1	200	18-46	Negative
Ethyl acetate-methanol 8:2	200		
Ethyl acetate-methanol 7:3	100	47-54	Negative
Ethyl acetate-methanol 6:4	100		
Ethyl acetate-methanol 5:5	100	55-69	Negative
Ethyl acetate-methanol 3:7	100		
Methanol	500		ND*

\*. Not determined

**Supplementary Table S3.** Composition of the mobile phase used for the fractionation of "Fraction B" by column chromatography and biological activity.

Mobile phase	Vol. (mL)	Fractions	Inhibition of <i>Salmonella</i> motility
Hexane	100	1-2	Negative
Hexane-ethyl acetate 5:5	100		
Hexane-ethyl acetate 1:9	100	3-5 ("Fraction C")	Positive
Ethyl acetate-methanol 8:2	100		
Ethyl acetate-methanol 6:4	100	6-9	Negative
Ethyl acetate-methanol 3:7	100		
Ethyl acetate-methanol 2:8	100		
Methanol	300	10-15	Negative