Electronic Supplementary Material (ESI) for New Journal of Chemistry.

Iron(III) Coordination Properties of Ladanein, a Flavone Lead with a Broad-Spectrum Antiviral Activity

X. Martin-Benlloch,[a] A. Novodomska,[a,b] D. Jacquemin,[c] E. Davioud-Charvet[a,*] and M. Elhabiri[a,*]

a. *UMR 7509 CNRS – Université de Strasbourg (ECPM), Laboratioey de Chimie Bioorgainque etMédicinale, 25 Rue Becquerel, 67087 Strasbourg, France. (ME) E-mail: elhabiri@unistra.fr; (EDC) E-mail: elisabeth.davioud@unistra.fr*

b. *Biochemistry Center (BZH), University of Heidelberg, Heidelberg, Germany*

c. *Ceisam Laboratory, UMR CNRS 6230. University of Nantes, 2, rue de la Houssinière, 44322 Nantes Cedex3, France*

Supporting Information

(pages S1-S12 including this one)

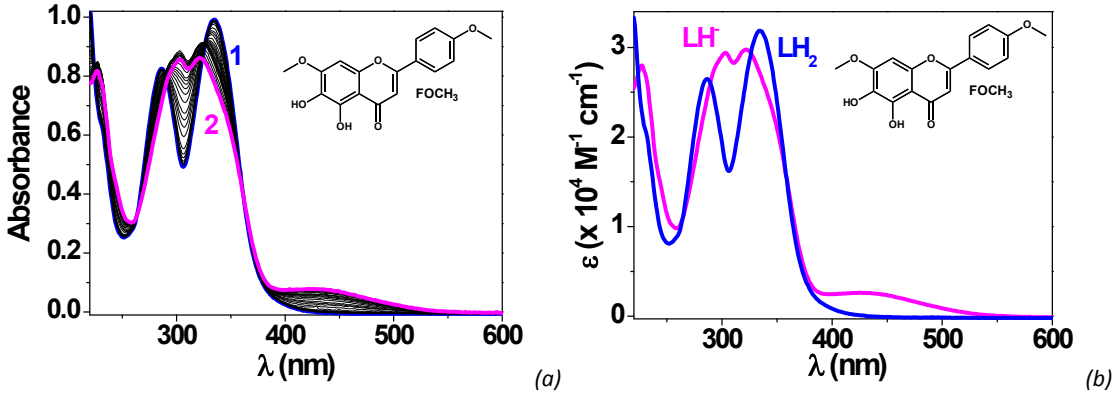

Figure S1. (a) Absorption spectrophotometric titration of ladanein (designated as **FOMe**) as a function of pH and (b) electronic absorption spectra of the protonated species of ladanein. Solvent: CH_3OH/H_2O (80/20 *w/w*); *I* = 0.1 M (NEt_4ClO_4); *T* = 25.0(2) °C; *l* = 1 cm; $[\mathbf{FOMe}]_{tot}$ = 3.12 x 10^{-5} M; (1) pH = 6.99; (2) pH = 12.15.

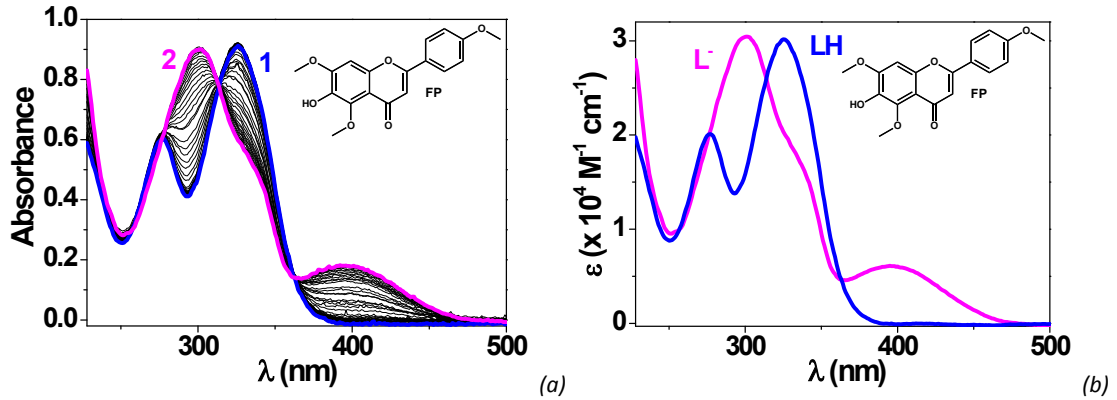

Figure S2. (a) Absorption spectrophotometric titration of the final precursor of ladanein (designated as **FP**) as a function of pH and (b) electronic absorption spectra of the protonated species of **FP**. Solvent: CH_3OH/H_2O (80/20 *w/w*); I = 0.1 M (NEt_4ClO_4); *T* = 25.0(2) °C; *l* = 1 cm; $[\mathbf{FP}]_{tot}$ = 3.05 x 10^{-5} M; (1) pH = 5.52; (2) pH = 11.85.

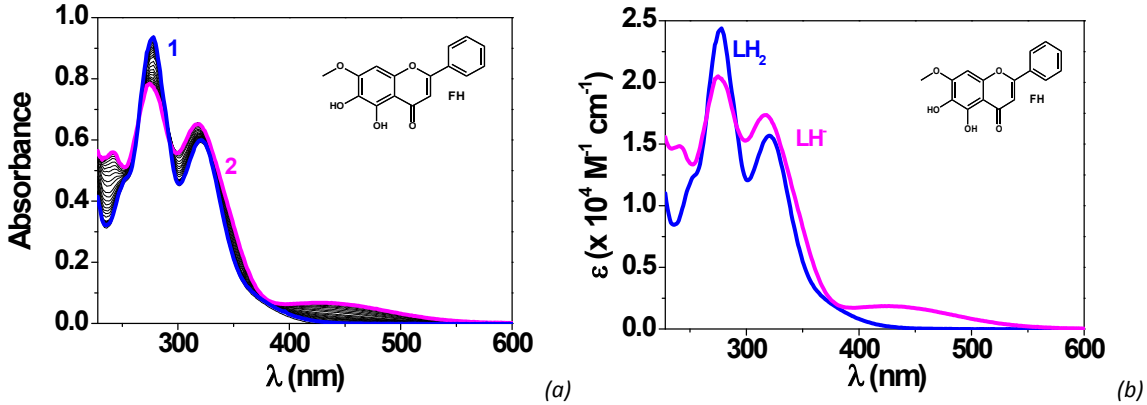

Figure S3. (a) Absorption spectrophotometric titration of negletein **FH** as a function of pH and (b) electronic absorption spectra of the protonated species of FH. Solvent: CH_3OH/H_2O (80/20 *w/w*); *I* = 0.1 M (NEt_4ClO_4); *T* = 25.0(2) °C; *l* = 1 cm; $[FP]_{tot}$ = 3.08 x 10^{-5} M; (1) pH = 5.13; (2) pH = 11.05.

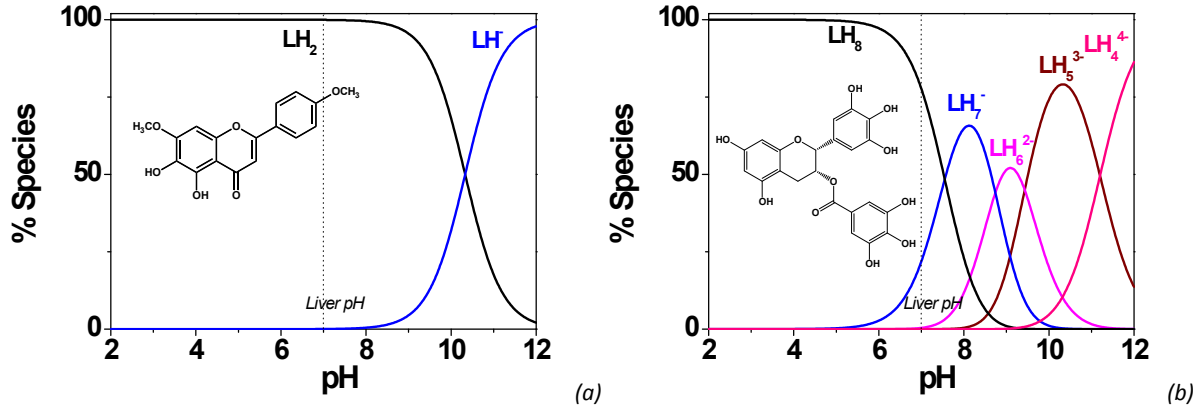

Figure S4. Distribution diagrams of the protonated species of *(a)* ladanein **FOMe** and *(b)* EGCG as a function of pH. [ligand] = 10^{-6} M (close to the anti-HCV EC_{50} values); *I* = 0.1 M; *T* = 25°C.

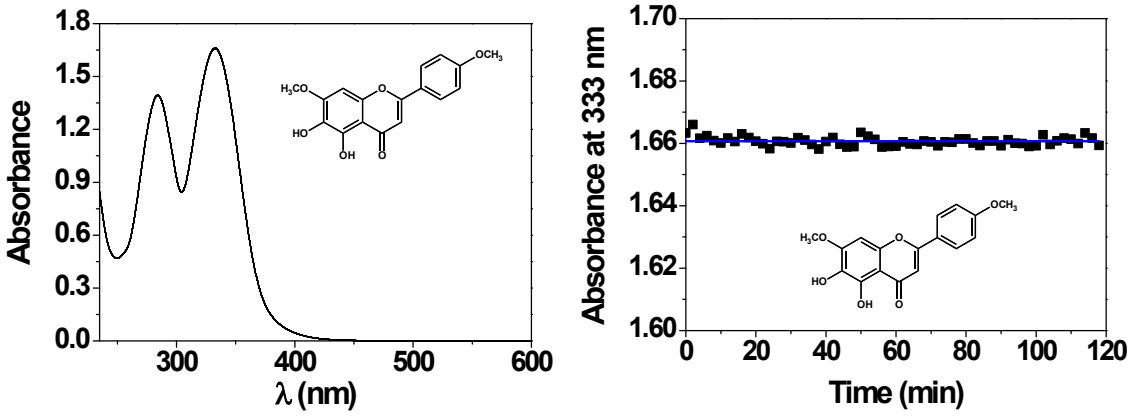

Figure S5. Absorption spectrophotometric spectra of solution of ladanein **FOMe** as a function of time (2 h). Solvent: CH_3OH/H_2O (80/20 *w/w*); *T* = 25.0(2) °C. $[\mathbf{FOMe}]_{tot}$ = 5.2 x 10^{-5} M; *l* = 1 cm.

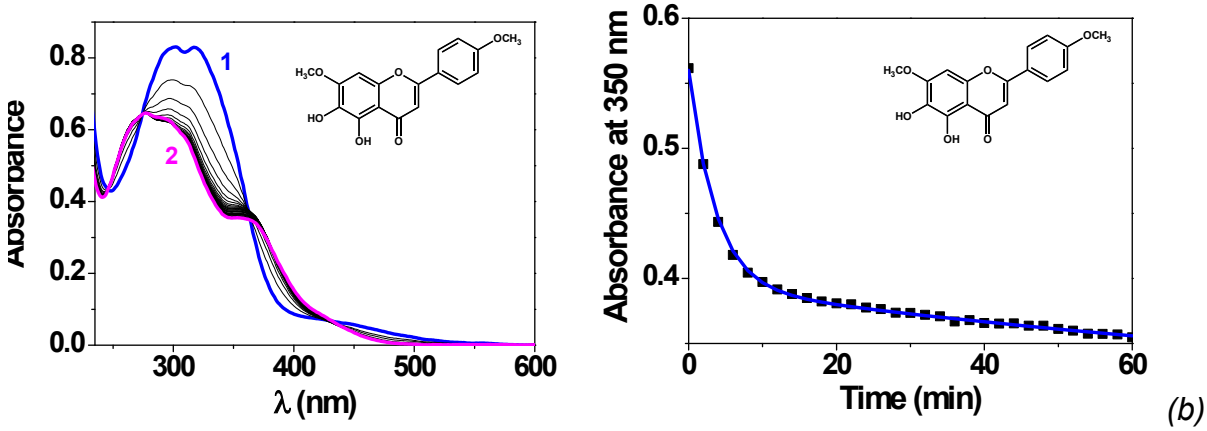

Figure S6. Absorption spectrophotometric spectra of solution of ladanein as a function of time (2 h) with the presence of base. Solvent: CH_3OH/H_2O (80/20 *w/w*); *I* = 0.1 M NEt_4ClO_4; *T* = 25.0(2) °C. (*a*) $[\mathbf{FOMe}]_{tot}$ = 4.83 x 10^{-5} M; $[NEt_4OH]_{tot}$ = 9.63 x 10^{-4} M; (*b*) $[\mathbf{FOMe}]_{tot}$ = 4.27 x 10^{-5} M; $[NEt_4OH]_{tot}$ = 8.75 x 10^{-3} M; *l* = 1 cm.

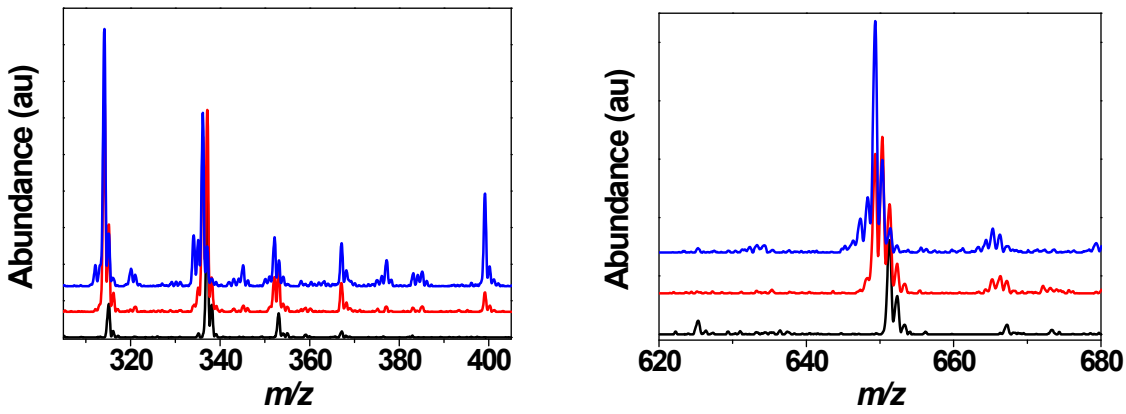

Figure S7. ESI-MS spectra of ladanein as a function of time (0 and 15 minutes) in the presence of base (NH_4OH). Solvent: CH_3OH/H_2O (80/20 *w/w*. (*a*) $[\mathbf{FOMe}]_{tot}$ = 5 x 10^{-5} M; NH_4OH (0.01 *v/v*). Fragmentor = 100 V.

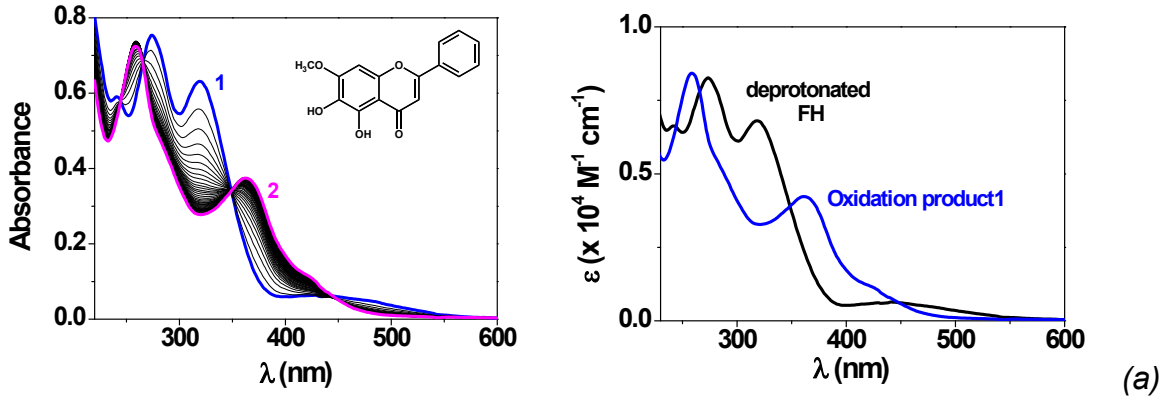

Figure S8. Variation of the absorption spectrophotometric spectra of solution of **FH** as a function of time (2 h) in the presence of base. Solvent: CH_3OH/H_2O (80/20 *w/w*); *I* = 0.1 M NEt_4ClO_4; *T* = 25.0(2) °C. (*a*) $[\mathbf{FH}]_{tot}$ = 4.83 x 10^{-5} M; $[NEt_4OH]_{tot}$ = 9.63 x 10^{-4} M; (*b*) $[\mathbf{FCF_3}]_{tot}$ = 4.27 x 10^{-5} M; $[NEt_4OH]_{tot}$ = 8.75 x 10^{-3} M; *l* = 1 cm.

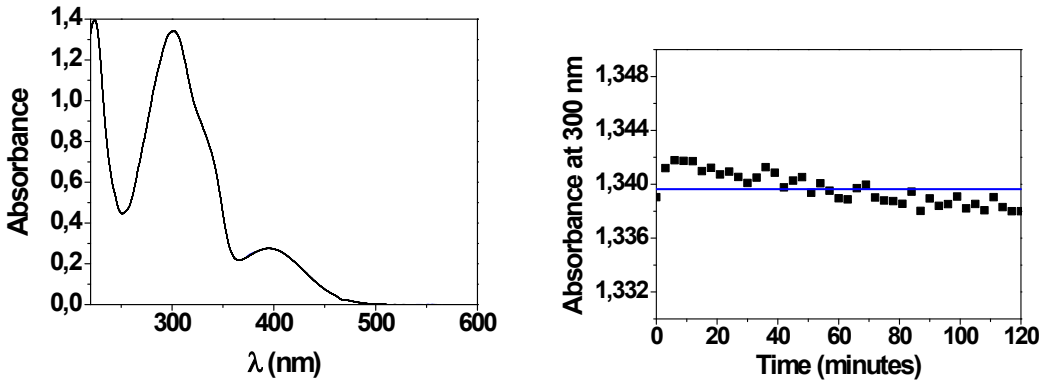

Figure S9. Absorption spectrophotometric spectra of solution of the final precursor **FP** as a function of time (2 h) with the presence of base. Solvent: CH_3OH/H_2O (80/20 *w/w*); *I* = 0.1 M NEt_4ClO_4; *T* = 25.0(2) °C. $[FP]_{tot}$ = 4.71 x 10^{-5} M; $[NEt_4OH]_{tot}$ = 9.42 x 10^{-4} M, *l* = 1 cm.

Table S1. ICP-MS data from the most active and the most inactive fractions along ladanein extraction.

Natural ladanein content	Natural ladanein-enriched fractions extracted from *M. peregrinum*				
	0%	0%	89%	83.1%	
Antiviral activity			+++	+++	
Metal content (mg/kg)	**LF-MPE1**	**S2F1**	**S3F3**	**S3F4**	
Mg	508	212	111	76	
Al	23	12	11	21	
Cr	<5	<5	<5	25	
Mn	<25	<25	<25	<25	
Fe	6	4	**34**	**158**	← **Fe-enriched active fraction**
Co	<5	<5	<5	<5	
Ni	<2	<2	7	15	
Cu	5	14	**49**	**24**	← **Cu-enriched active fraction**
Zn	42	305	55	87	
As	<5	<5	<5	<5	
Se	<5	<5	<5	<5	
Sr	<2	<2	<2	<2	
Zr	<25	<25	<25	<25	
Cd	<2	<2	<2	<2	
Sn	<2	<2	<2	<2	
Ba	2	<5	<5	<5	
Hg	<5	<5	693	<5	
Pb	<2	<2	10	18	
I	<60	<60	773	<60	

Abolishment of the antiviral activity of synthetic ladanein (BJ486K) after addition of an equimolar amount of Fe(III) chelator ferrioxamine B (DFO)

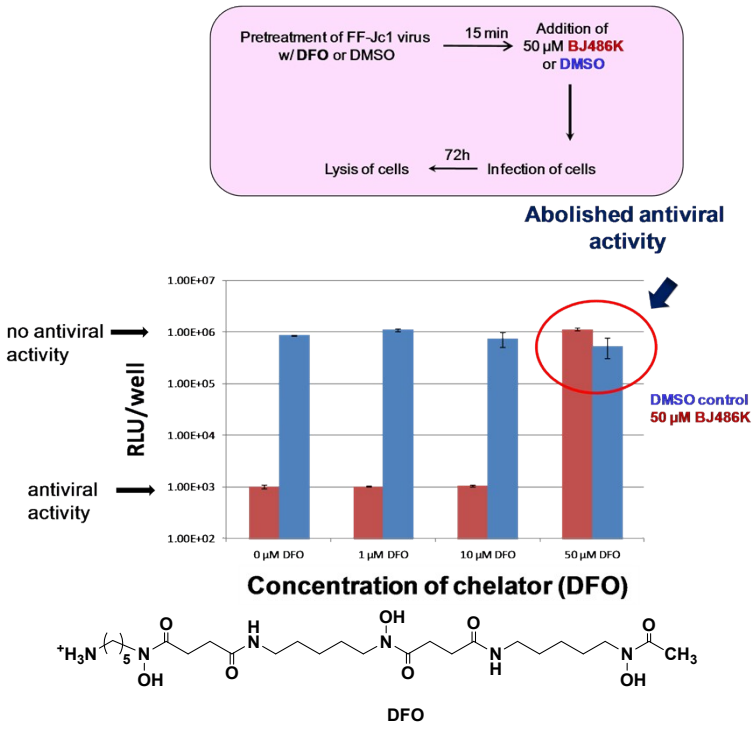

Figure S10. Influence of iron chelator DFO on antiviral activity of **BJ486K**.

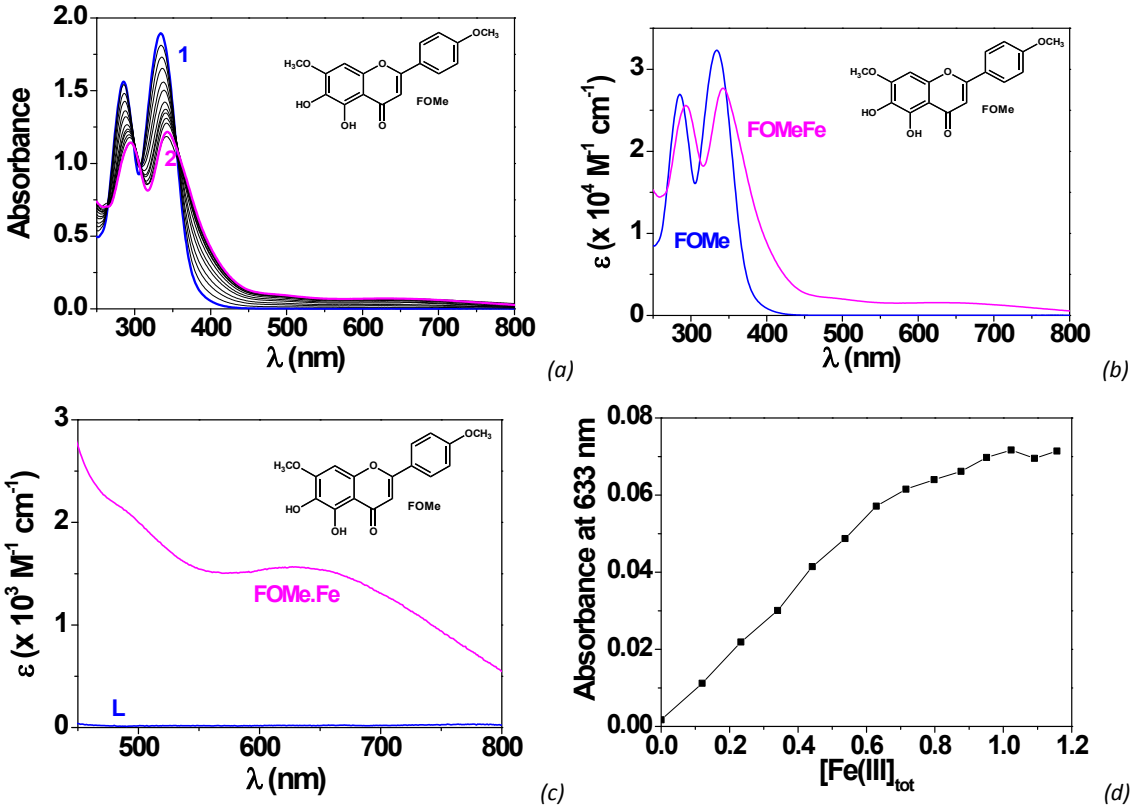

Figure S11. Absorption spectrophotometric titration of ladanein **FOMe** with Fe(III) at pH 2.0. (*a*) Absorption spectra versus $[Fe(III)]_{tot}$, (*b*) and (*c*) absorption electronic spectra of ladanein **FOMe** and its ferric complexes and (*d*) variation of the absorbance at 633 nm (LMCT) as a function of $[Fe(III)]_{tot}$. Solvent: CH_3OH/H_2O (80/20 by weight); *I* = 0.1 M (NEt_4ClO_4); *T* = 25.0(2) °C; pH = 2.0; *l* = 1 cm; (1) $[\mathbf{FOMe}]_{tot}$ = 5.92 × 10^{-5} M; (2) $[Fe(III)]_{tot}/[\mathbf{FOMe}]_{tot}$ = 1.60.

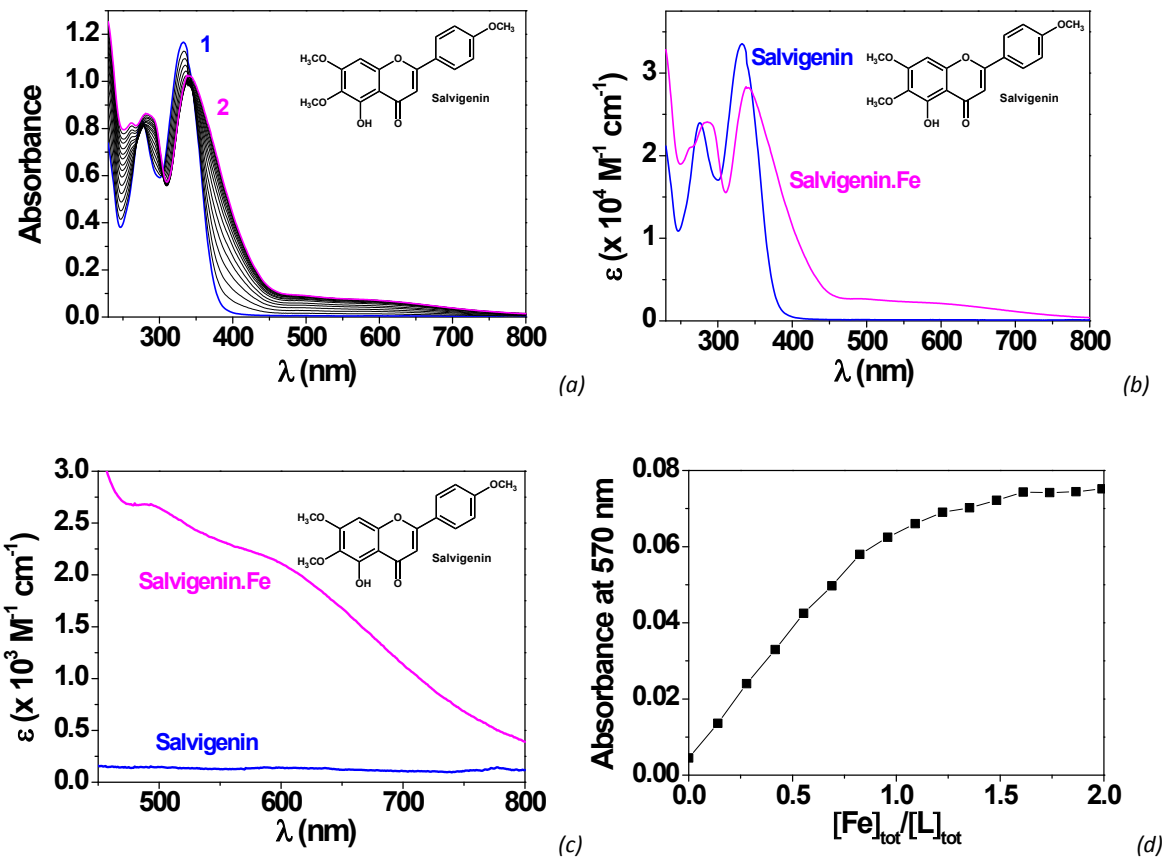

Figure S12. Absorption spectrophotometric titration of salvigenin with Fe(III) at pH 2.0. (*a*) Absorption spectra *versus* $[Fe(III)]_{tot}$, (*b*) and (*c*) absorption electronic spectra of salvigenin and its ferric complexes and (d) variation of the absorbance at 570 nm (LMCT) as a function of $[Fe(III)]_{tot}$. Solvent: CH_3OH/H_2O (80/20 by weight); I = 0.1 M (NEt_4ClO_4); T = 25.0(2) °C; pH = 2.0; l = 1 cm; (1) $[salvigenin]_{tot} = 3.48 \times 10^{-5}$ M; (2) $[Fe(III)]_{tot}/[salvigenin]_{tot}$ = 2.0.

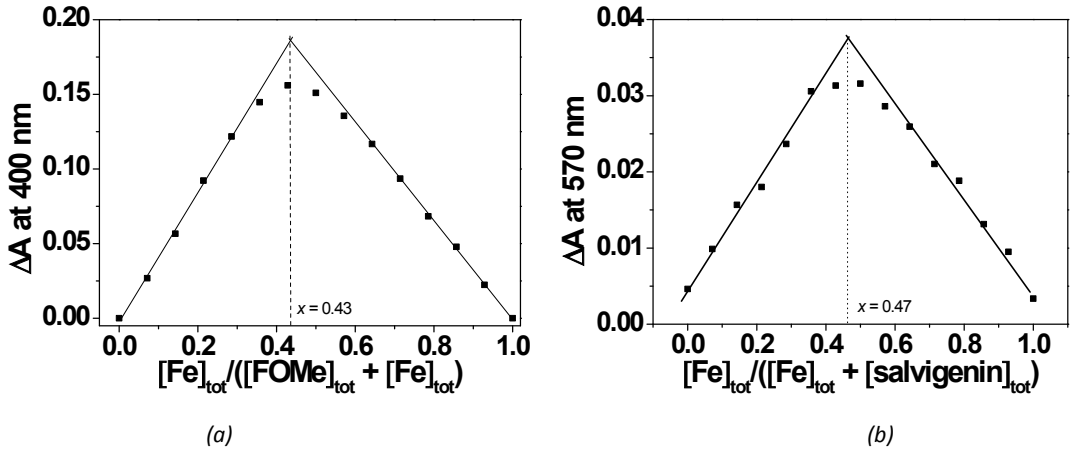

Figure S13. Job plots obtained upon mixing (a) ladanein and Fe^{3+} ($\Delta A/\Delta A_{max}$ at 400 nm) and (a) salvigenin and Fe^{3+} ($\Delta A/\Delta A_{max}$ at 570 nm). Solvent: CH_3OH/H_2O (80/20 by weight); I = 0.1 M (NEt_4ClO_4); T = 25.0(2) °C; pH = 2.0; $([\mathbf{FOMe}]_{tot} + [Fe(III)]_{tot}) = 4.94 \times 10^{-5}$ M; $([salvigenin]_{tot} + [Fe(III)]_{tot}) = 3.48 \times 10^{-5}$ M; l = 1 cm.

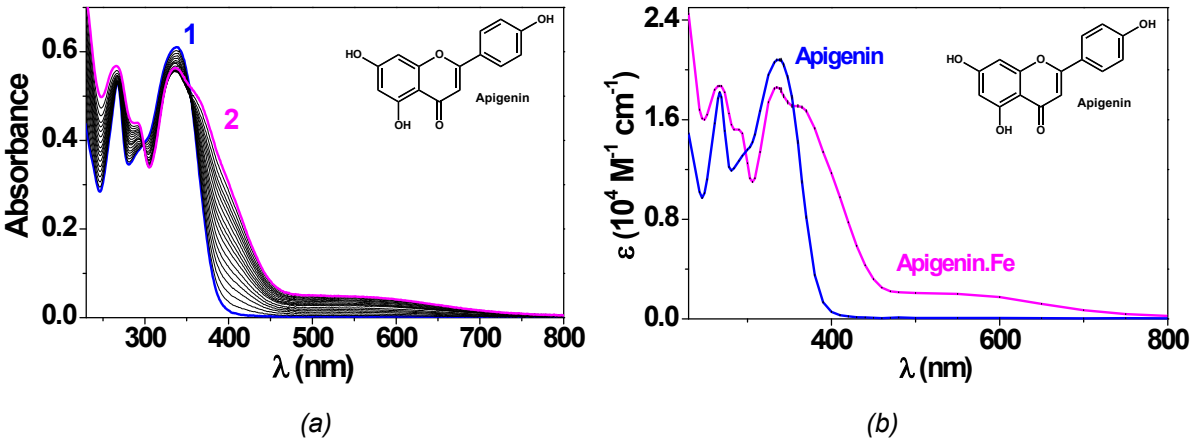

Figure S14. Absorption spectrophotometric titration of apigenin with Fe(III) at pH 2.0. (*a*) Absorption spectra *versus* $[Fe(III)]_{tot}$ and (*b*) absorption electronic spectra of apigenin and its ferric complexes. Solvent: CH_3OH/H_2O (80/20 by weight); *I* = 0.1 M ($NaClO_4$); *T* = 25.0(2) °C; pH = 2.0; *l* = 1 cm; (1) $[apigenin]_{tot}$ = 2.91 × 10^{-5} M; (2) $[Fe(III)]_{tot}/[apigenin]_{tot}$ = 1.6. (from Carrër, C. "Chélation de métaux de transition par des polyphénols du régime alimentaire", PhD Thesis from University Louis Pasteur of Strasbourg, January 28th, 2005)

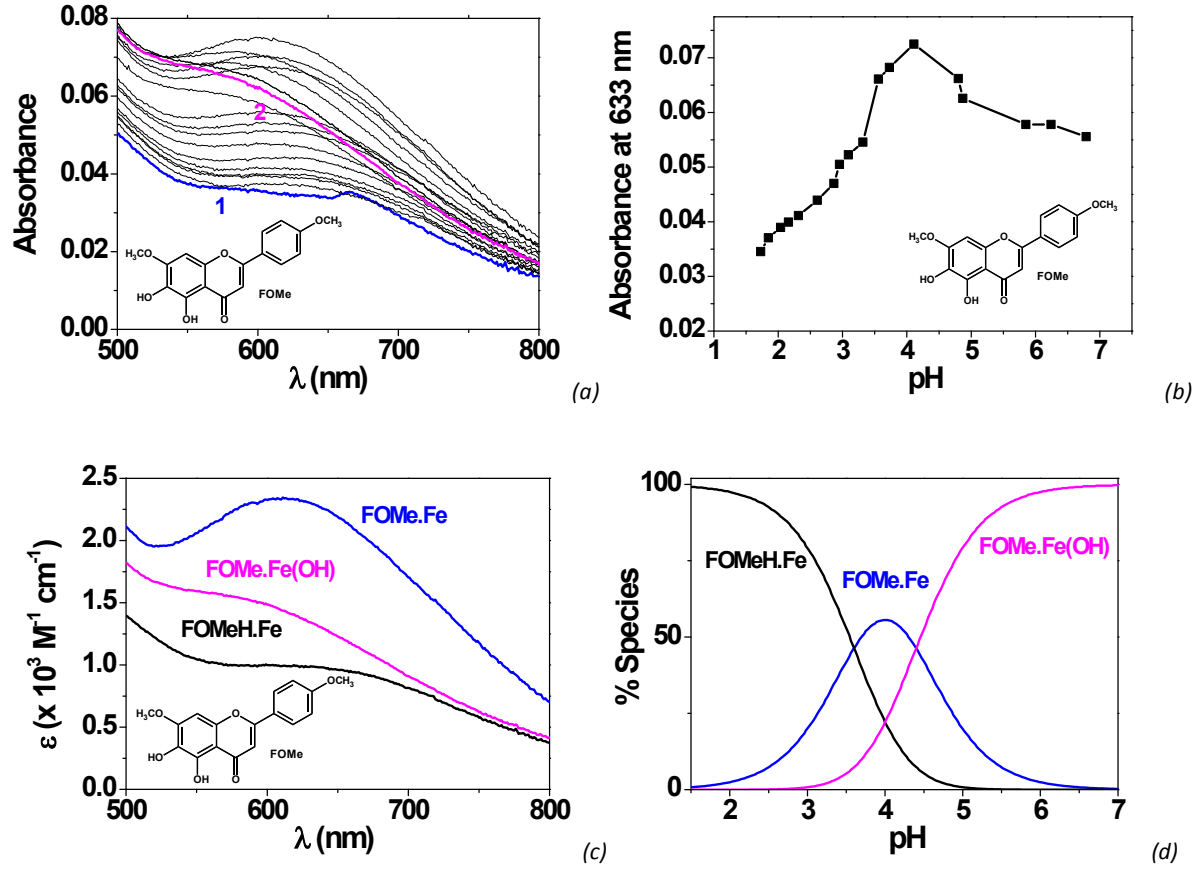

Figure S15. Absorption spectrophotometric titration of the ferric complexes of ladanein as a function of pH. (a) spectral variation of the LMCT recorded, (b) variation of the absorbance at 633 nm as a function of pH, (c) electronic spectra of the ferric complexes and (d) distribution diagrams of the ferric complexes of ladanein. Solvent: CH_3OH/H_2O (80/20 by weight); *I* = 0.1 M (NEt_4ClO_4); *T* = 25.0(2) °C; *l* = 1 cm; $[\mathbf{FOMe}]_{tot}$ = 4.55 x 10^{-5} M; $[Fe(III)]_{tot}$ = 4.14 × 10^{-5} M;.(1) pH = 1.72; (2) pH = 6.79.

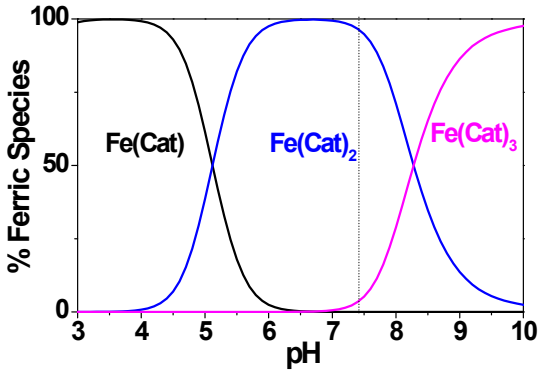

Figure S16. Distribution diagrams of the ferric complexes with catechin (noted Cat). Solvent: H_2O; *I* = 0.1 M ($NaClO_4$); *T* = 25.0(2) °C; $[Cat]_{tot}$ = 1.5 x 10^{-4} M; $[Fe(III)]_{tot}$ = 5 × 10^{-5} M. (from Elhabiri, M.; Carrër, C.; Marmolle, F. and Traboulsi, H. *Inorg. Chim. Acta* **2007**, *360*, 353.)

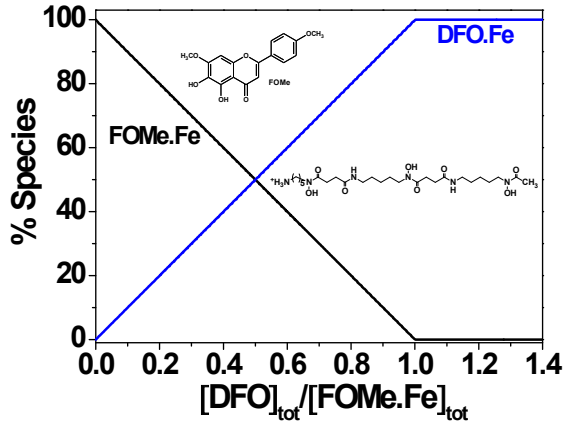

Figure S17. Distribution diagrams of the ferric complexes with ladanein **FOMe** and DFO showing the competition between these two chelators for Fe(III). Solvent: H_2O; *I* = 0.1 M; *T* = 25.0(2) °C; $[\mathbf{FOMe}]_{tot}$ = 5 x 10^{-5} M; $[Fe(III)]_{tot}$ = 5 × 10^{-5} M.

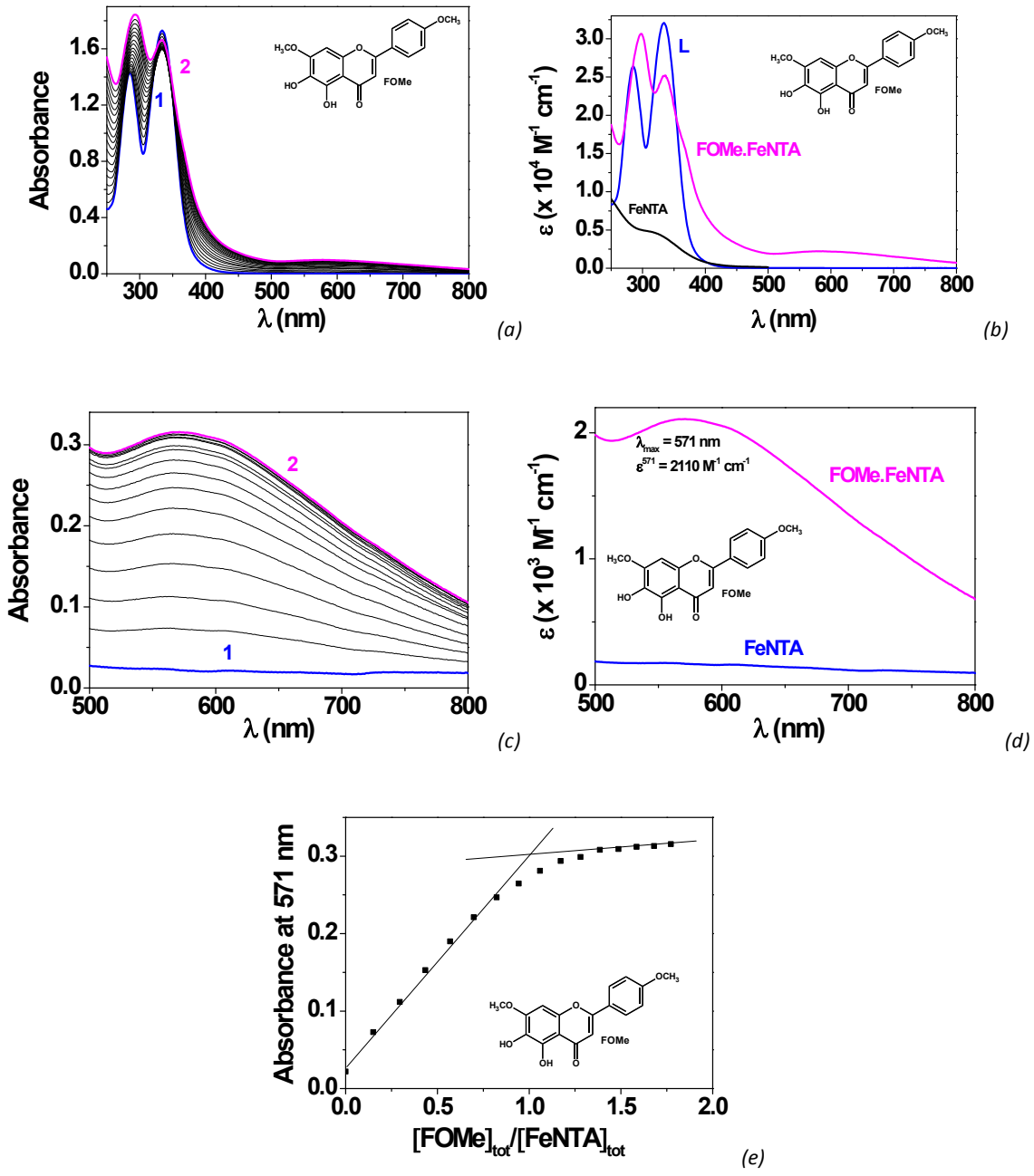

Figure S18. Absorption spectrophotometric titration of ladanein **FOMe** with Fe**NTA** at pH 7.4. (*a* and *c*) Absorption spectra *versus* [Fe**NTA**]$_{tot}$, (*b* and *d*) absorption electronic spectra of ladanein and its ferric ternary complexes with **NTA** and (*e*) variation of the absorbance at 571 nm (LMCT) as a function of the [**FOMe**]$_{tot}$/[Fe**NTA**]$_{tot}$ ratio. Solvent: CH_3OH/H_2O (80/20 by weight); pH = 7.4 (Hepes buffer); *I* = 0.1 M (Hepes); *T* = 25.0(2) °C; *l* = 1 cm; (a) (1) [**FOMe**]$_{tot}$ = 5.41 × 10^{-5} M; (2) [Fe**NTA**]$_{tot}$/[**FOMe**]$_{tot}$ = 2.0; (b) (1) [Fe**NTA**]$_{tot}$ = 2.30 × 10^{-4} M; (2) [**FOMe**]$_{tot}$/[Fe**NTA**]$_{tot}$ = 1.51.

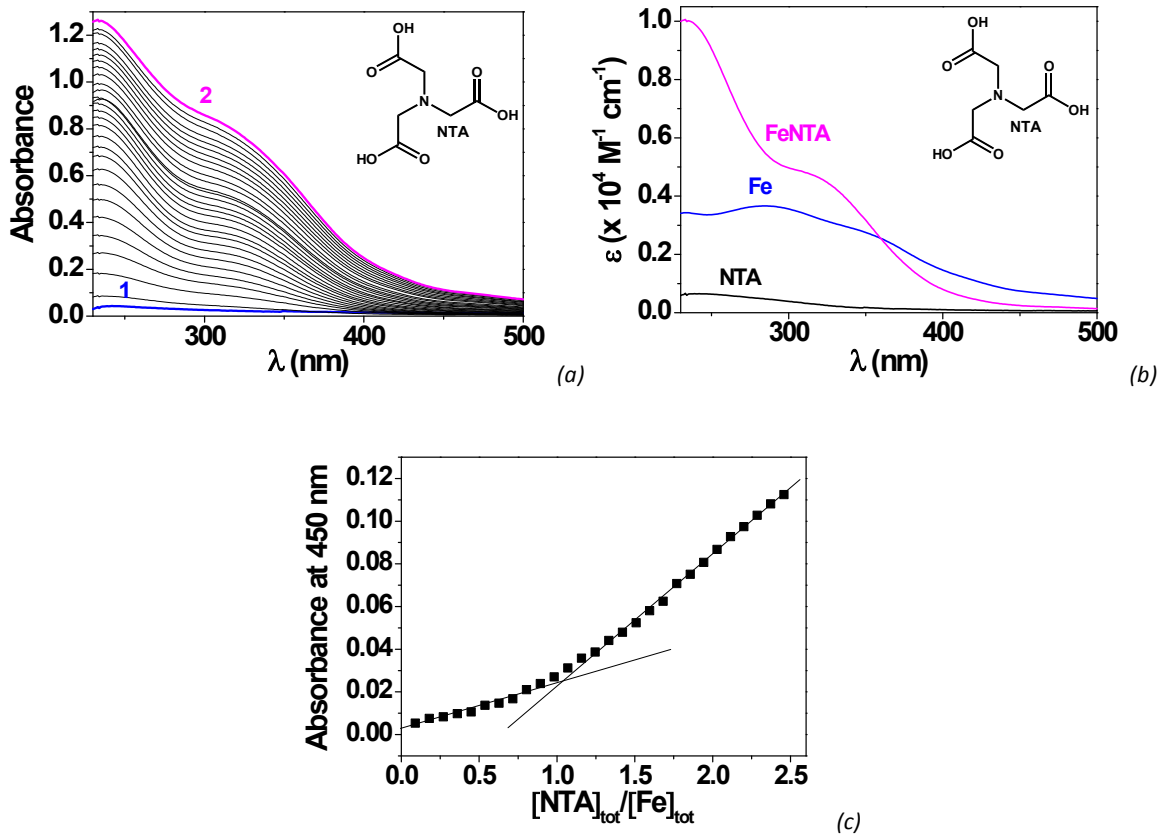

Figure S19. (*a*) Absorption spectrophotometric titration of **NTA** with Fe(III) at pH 7.4, (*c*) electronic spectra of the ferric **NTA** complexes and (c) variation of the absorbance at 450 nm suggesting the formation of the monoferric monochelate Fe**NTA** complex.. Solvent: CH_3OH/H_2O (80/20 by weight); *I* = 0.1 M (Hepes); pH 7.4; *T* = 25.0(2) °C; *l* = 1 cm; (1) $[\mathbf{NTA}]_{tot}$ = 8.37 x 10^{-5} M; (2) $[Fe(III)]_{tot}/[\mathbf{NTA}]_{tot}$ = 2.52.

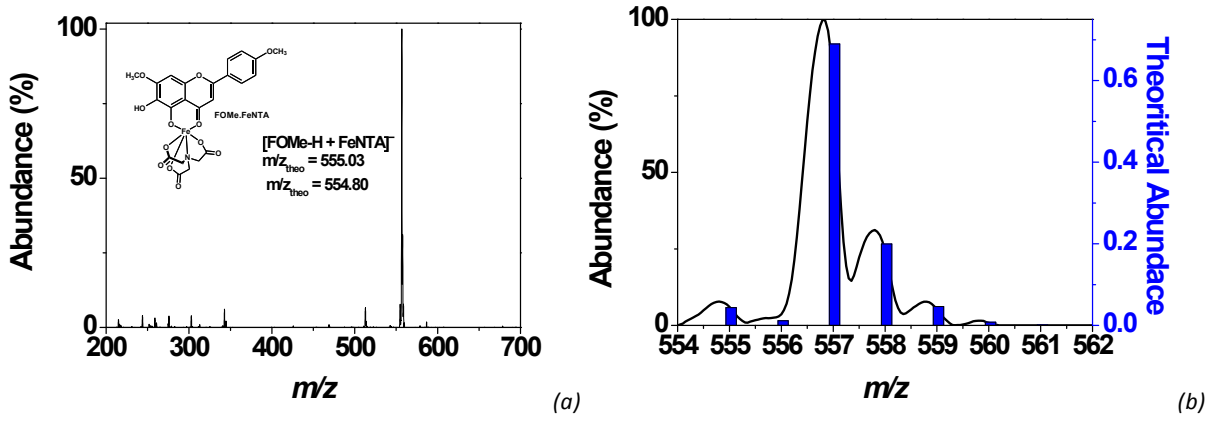

Figure S20. Electrospray mass spectra of ladanein ferric complex in the presence of **NTA**. Solvent: CH_3OH/H_2O, capillary voltage = 4000 V. $[\mathbf{FOMe}.Fe\mathbf{NTA}]_{tot}$ = 10^{-4} M; negative mode; Fragmentor = 150 V.

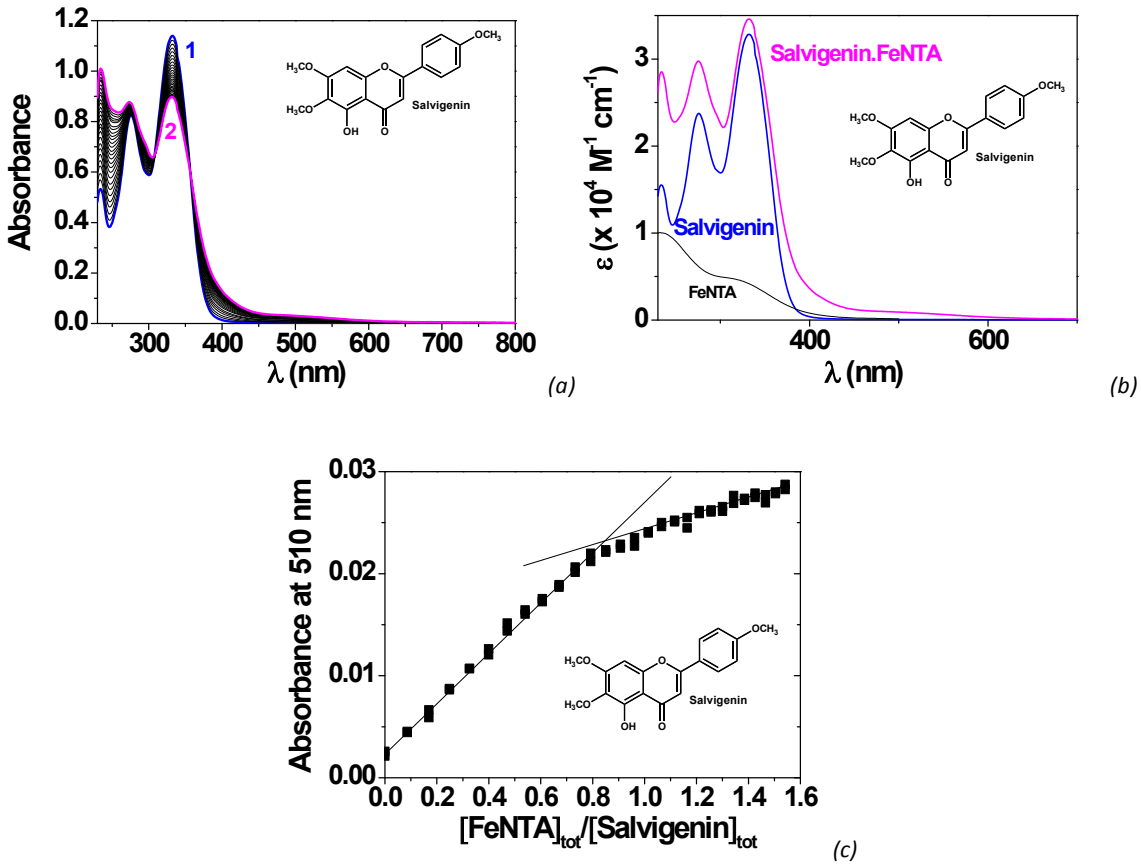

Figure S21. Absorption spectrophotometric titration of salvigenin with FeNTA at pH 7.4. (*a*) Absorption spectra *versus* $[FeNTA]_{tot}$, (*b*) absorption electronic spectra of salvigenin and its ferric ternary complexes with NTA and (*c*) variation of the absorbance at 510 nm (LMCT) as a function of the $[Fe\mathbf{NTA}]_{tot}/[salvigenin]_{tot}$ ratio. Solvent: CH_3OH/H_2O (80/20 by weight); pH = 7.4 (Hepes buffer); *I* = 0.1 M (Hepes); *T* = 25.0(2) °C; *l* = 1 cm; (a) (1) $[\mathbf{FOMe}]_{tot} = 3.48 \times 10^{-5}$ M; (2) $[Fe\mathbf{NTA}]_{tot}/[salvigenin]_{tot} = 1.54$.