

## SUPPLEMENTARY MATERIALS

---

# Enrichment in Antioxidant Flavonoids of Stamen Extracts from *Nymphaea lotus* L. using Ultrasonic-Assisted Extraction and Macroporous Resin Adsorption

Duangjai Tungmunnithum <sup>1,2,3,\*</sup>, Samantha Drouet <sup>2,3</sup>, Atul Kabra <sup>4</sup>, and Christophe Hano <sup>2,3,\*</sup>

<sup>1</sup> Department of Pharmaceutical Botany, Faculty of Pharmacy, Mahidol University, Bangkok 10400, Thailand; duangjai.tun@mahidol.ac.th (D.T.);

<sup>2</sup> Laboratoire de Biologie des Ligneux et des Grandes Cultures, INRA USC1328, University of Orleans, 45067 Orléans CEDEX 2, France; samantha.drouet@univ-orleans.fr (S.D.)

<sup>3</sup> Bioactifs et Cosmétiques, CNRS GDR 3711 Orleans, 45067 Orléans CEDEX 2, France

<sup>4</sup> School of Pharmacy, Raffles University, Neemrana 301705, Alwar, Rajasthan, India; atul.kbr@gmail.com

\* Correspondence: [duangjai.tun@mahidol.ac.th](mailto:duangjai.tun@mahidol.ac.th) (D.T.); [hano@univ-orleans.fr](mailto:hano@univ-orleans.fr) (C.H.); Tel.: +66-264-486-96 (D.T.); +33-237-309-753 (C.H.)

### CONTENT:

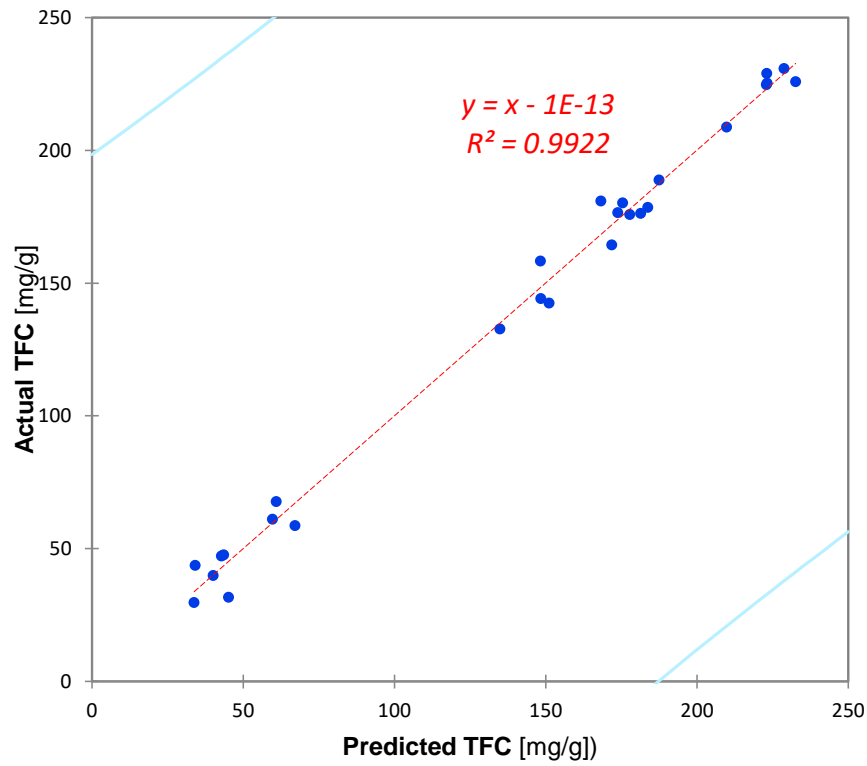
**Figure S1:** Biplot representation of the linear relation between predicted vs. measured TFC in the 27 *N. lotus* sample extracts – page 2.

**Table S1:** Conductivity and total reducing sugar contents in the different extracts from *N. lotus* stamen – page 3.

**Table S2:** Characteristic and tentative identification of flavonoids from *N. lotus* stamen extract – page 4.

**Table S3:** Relative quantification of the different flavonoid glucosides in the *N. lotus* stamen extracts – page 5.

**Figure S1:** Biplot representation of the linear relation between predicted vs. measured TFC in the 27 *N. lotus* sample extracts.



Light blue lines represented 95% confidence interval.

**Table S1:** Conductivity and total reducing sugar contents in the different extracts from *N. lotus* stamen.

<b>Extract</b>	<b>Conductivity</b> ( $\mu\text{S}/\text{cm}$ )	<b>Reducing sugar content</b> ( $\text{AU}/\text{mL}^1$ )
HRE	$0.039 \pm 0.005$	$0.056 \pm 0.011$
USAE	$0.122 \pm 0.010$	$0.065 \pm 0.010$
MPR	$0.044 \pm 0.012$	$0.007 \pm 0.006$

<sup>1</sup> AU/mL: expressed in absorbance unit per mL of extract; HRE: *N. lotus* extract obtained by HRE; USAE: *N. lotus* extract obtained by USAE; MPR: *N. lotus* extract obtained by USAE followed by DAX-8 MPR purification step.

**Table S2:** Characteristic and tentative identification of flavonoids from *N. lotus* stamen extract

Peak number	Retention time (min)	$\lambda_{max}$ (nm)	[M-H] <sup>-</sup>	Tentative identification	Commercial standard	Reference
1	29.11	263,349	479	Myr 3-O-Gal	+ (ES)	Zhu et al., 2012
2	31.67	254,305,366	449	Myr 3'-O-Xyl	+ (ES)	Zhu et al., 2012
3	32.26	257,348	447	Que-3-O-Rha	+ (SA)	Zhu et al., 2012; Yin et al., 2015
4	32.87	250,366	433	CNar-2''-O-Gal	-	Zhu et al., 2012; Yin et al., 2015
5	33.52	265,343	447	Kae-3-O-Gal	+ (SA)	Zhu et al., 2012
6	33.91	254,366	433	Que-3'-O-Xyl	+ (SA)	Zhu et al., 2012
7	34.71	268,350	477	Iso-7-O-Gal	-	Zhu et al., 2012; Yin et al., 2015
8	35.51	252,268,352	447	Iso-7-O-Xyl	-	Zhu et al., 2012; Yin et al., 2015
9	36.57	265, 343	447	Iso-3-O-Xyl	-	Yin et al., 2015

Myr: myricetin; Que: quercetin; CNar: chalcononaringenin; Kae: kaempferol; Iso: isorhamnetin; Gal: galactoside; Xyl: xyloside; Rha: rhamnoside. Standards: + = available ; - = not available ; ES = commercial standard from Extrasynthese (Genay, France) ; SA = commercial standard from Sigma-Aldrich (Saint-Quentin Fallavier, France) ; References: Zhu et al., 2012 (reference 14, main manuscript) : Zhu, M., Zheng, X., Shu, Q., Li, H., Zhong, P., Zhang, H., ... & Wang, L. (2012). Relationship between the composition of flavonoids and flower colors variation in tropical water lily (*Nymphaea*) cultivars. *PLoS one*, 7(4); Yin et al., 2015 (reference 4, main manuscript): Yin, D. D., Yuan, R. Y., Wu, Q., Li, S. S., Shao, S., Xu, Y. J., ... & Wang, L. S. (2015). Assessment of flavonoids and volatile compounds in tea infusions of water lily flowers and their antioxidant activities. *Food chemistry*, 187, 20-28.

**Table S3:** Relative quantification of the different flavonoid glucosides in the *N. lotus* stamen extracts;

Peak number	Tentative identification	HRE	USAE	MPR
1	Myr 3-O-Gal	21.1 ± 1.3	28.3 ± 1.1	76.9 ± 3.8
2	Myr 3'-O-Xyl	34.4 ± 1.7	45.7 ± 2.1	129.3 ± 6.7
3	Que-3-O-Rha	62.1 ± 3.1	86.3 ± 1.4	244.2 ± 5.1
4	CNar-2''-O-Gal	16.4 ± 1.3	22.9 ± 1.3	64.7 ± 3.6
5	Kae-3-O-Gal	54.7 ± 2.3	73.4 ± 3.2	200.2 ± 5.4
6	Que-3'-O-Xyl	29.1 ± 1.5	41.2 ± 2.1	117.2 ± 2.4
7	Iso-7-O-Gal	24.8 ± 1.6	33.6 ± 1.7	90.4 ± 3.3
8	Iso-7-O-Xyl	41.4 ± 2.4	55.4 ± 2.3	144.2 ± 5.7
9	Iso-3-O-Xyl	13.2 ± 1.4	19.7 ± 1.1	56.6 ± 1.7

Expressed in absorbance unit per g DW; HRE: *N. lotus* extract obtained by HRE; USAE: *N. lotus* extract obtained by USAE; MPR: *N. lotus* extract obtained by USAE followed by DAX-8 MPR purification step.