

## SUPPLEMENTARY MATERIAL

### **A new ultra-high pressure liquid chromatography method for the determination of antioxidant flavonol aglycones in six *Lysimachia* species**

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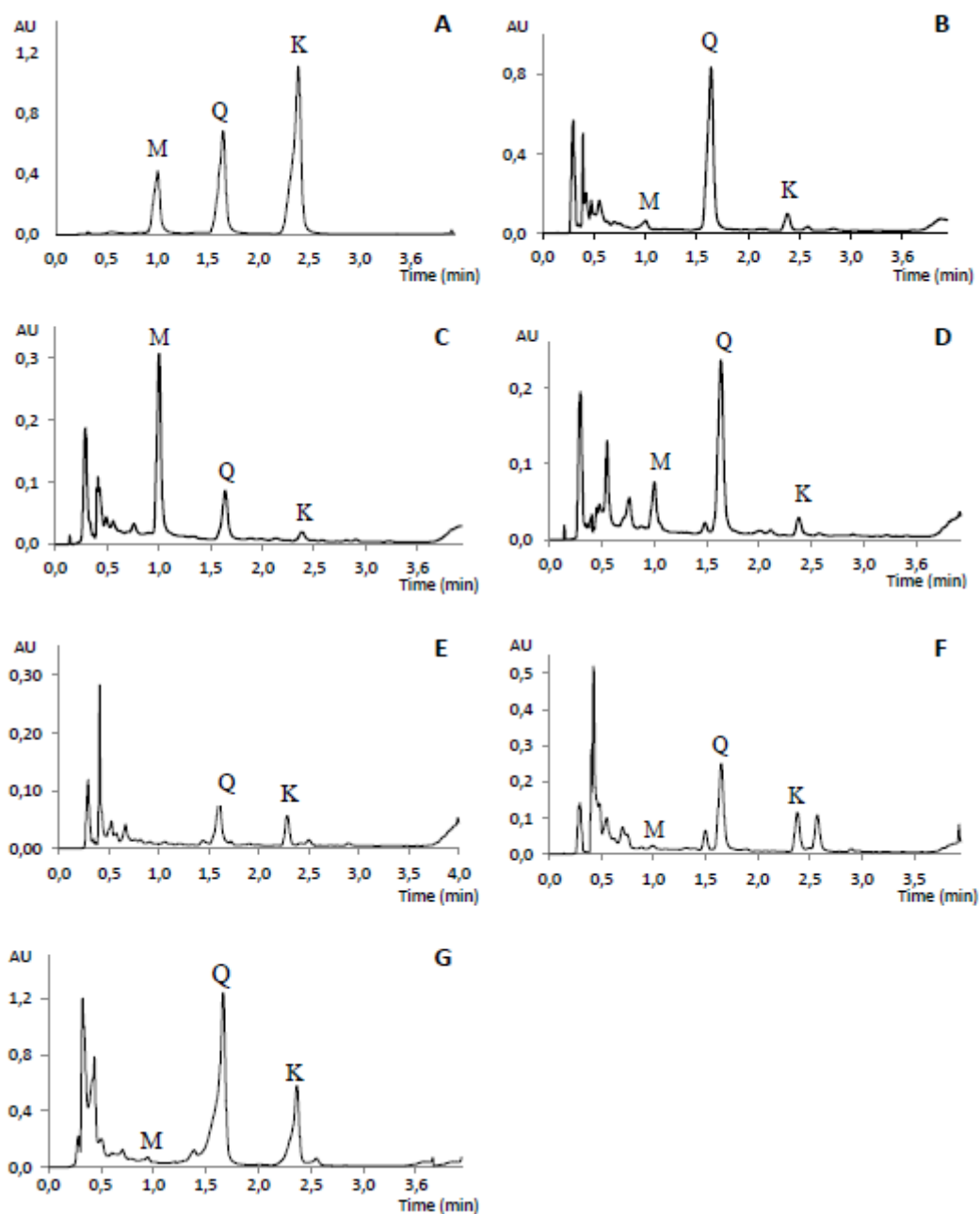
#### **Abstract**

UPLC-DAD method was developed and validated for the quantitative determination of free flavonol aglycones (kaempferol, quercetin and myricetin) after acidic hydrolysis in six *Lysimachia* species. Quantitative analyses showed that the amounts of various flavonol aglycones were significantly different in *L. vulgaris*, *L. nummularia*, *L. punctata*, *L. christinae*, *L. ciliata* and *L. clethroides*. The *L. clethroides* sample was found to be the richest in kaempferol ( $25.77 \pm 1.29$   $\mu\text{g}/\text{mg}$  extract) and quercetin ( $97.67 \pm 4.61$   $\mu\text{g}/\text{mg}$  extract), while the *L. nummularia* sample contained the highest amount of myricetin ( $20.79 \pm 1.00$   $\mu\text{g}/\text{mg}$  extract). The antioxidant capacity of hydrolyzed extracts was evaluated using *in vitro* DPPH<sup>•</sup> (2,2-diphenyl-1-picrylhydrazyl) and ABTS<sup>•+</sup> [2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid)] decolourization tests. The observed radical scavenging capacities of the extracts showed a relationship with the measured flavonol aglycone content and composition.

The acidic treatment resulted in an increased free radical scavenging activity compared to the untreated methanol extract.

**Keywords:** UPLC, *Lysimachia*, flavonoid, antioxidant, loosestrife

**Figure S1.** UPLC-DAD chromatograms of flavonoid standard mixture: myricetin, quercetin, kaempferol (A) and hydrolyzed flavonoid extracts of six *Lysimachia* species: *Lysimachia vulgaris* (B), *L. nummularia* (C), *L. punctata* (D), *L. christinae* (E), *L. ciliate* (F), *L. clethroides* (G). Abbreviations: K: kaempferol, M: myricetin, Q: quercetin



**Table S1. Regression, LOQ and LOD of the quantitative methods**

Standard	Regression equation	r <sup>2</sup>	regression range (µg/ml)	LOD (µg/ml)	LOQ (µg/ml)
kaempferol	y=14403x-4380.2	0.9999	1-500	0.10	0.31
quercetin	y=13710x-2568	1.0000	1-500	0.13	0.42
myricetin	y=9994.4x-9143	0.9999	1-500	0.15	0.50

**Table S2. Method validation: Precision and accuracy of the quantitative methods**

Nominal concentration (µg/ml)	Precision (RSD%)		Accuracy (%)	
	Intra-day	Inter-day	Intra-day	Inter-day
Kaempferol				
1	2.60	1.88	93.7	93.1
50	1.31	3.69	99.6	95.7
500	1.24	3.28	100.2	96.7
Quercetin				
1	3.44	2.86	107.6	107.8
50	3.58	2.29	101.7	100.9
500	1.19	1.37	100.7	99.6
Myricetin				
1	0.71	0.67	102.2	102.1
50	1.34	3.75	105.3	104.2
100	0.27	1.00	100.0	100.1