Anti-inflammatory potential and fast UHPLC-DAD-IT-TOF profiling of polyphenolic compounds extracted from green lettuce (Lactuca sativa L.; var. Maravilla de Verano)

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Fresh cut vegetables represent a widely consumed food worldwide. Among these, lettuce (Lactuca sativa L.) is one of the most popular and accessible on the market. The growing interest for this "healthy" food is related to the content of bioactive compounds, especially polyphenols, that show many beneficial effects. In this study, we report the anti-inflammatory and antioxidant potential of polyphenols extracted from green lettuce (var. Maravilla de Verano), in J774A.1 macrophages stimulated with Escherichia coli lipopolysaccharide (LPS) [1]. Lettuce extract significantly decreased reactive oxygen species and nitric oxide release and inducible nitric oxide synthase and cycloxygenase-2 expression. Moreover lettuce treatment also enhanced the cytoprotective heme-oxygenase-1 enzyme expression thus contributing to its beneficial effect during inflammation. Furthermore, a detailed quali/quantitative profiling of the polyphenolic content was carried out through a fast and accurate ultra high performance liquid chromatography-ion-trap-time of flight mass spectrometer (UHPLC-IT-TOF) platform [2]. In the extracts, hydroxycinnamic acid derivatives and flavon-3-ols were the most abundant compounds. The method showed fast separation (10 min), together with satisfactory retention time and peak area repeatability, with maximum RSD % values of 0.80 and 8.68, respectively, as well as good linearity ($R^2 \geq 0.999$) and mass accuracy (≤ 5ppm).

SAMPLE PREPARATION

The experiment was conducted in 2011 in a conventional farm (Gerstaberg) located in central Sweden. Green lettuce (Lactuca sativa L.), var. Maravilla de Verano was used. At harvest, five plant were randomly picked in the field, in order to minimize soil differences among the treatments and avoid the borderline effect. For each plant, outer leaves (second stage of leaves) were detached and slightly washed with distilled water to remove eventual residues. Five grams of fresh leaf taken from the central part were collected with a scalpel and placed in an extracting solution of 49 mL methanol + 1 mL HCl 37%. The solution was covered with parafilm to avoid evaporation, shaken at 100 rpm at 20°C in the dark for 45 min. The resulting extracts were filtered through 0.20 µm Minisart SFCA sterile filters and immediately stored at -20°C.

Peak	[M-H]⁻ observed	[M-H]⁻ calculated	Error (ppm)	MS² *m/z*	Quantity (µg g⁻¹)	RSD %	Compound
1	353.0883	353.0878	1.42	191 353	19.40	± 0.034	Chlorogenic acid
2	325.0566	325.0565	0.31	163 293	40.62	± 0.015	Feruloyl tartaric acid
3	367.1019	367.1035	-4.36	179 191	257.31	± 0.035	Feruloylquinic acid
4	473.0723	473.0725	-0.42	293 311	22.45	± 0.214	Chicoric acid
5	463.0874	463.0882	-1.73	301	97.87	± 0.072	Quercetin-3-O-glucoside
6	491.0826	491.0831	-1.02	271 301	46.79	± 0.101	Isorhamnetin-3-O-glucuronide
7	339.0720	339.0722	-0.59	161 219	33.53	± 0.069	Esculin
8	563.1027	563.1042	-2.66	463 531	-	-	unknown
9	477.1044	477.1038	1.26	287	-	-	unknown
10	475.0880	475.0882	-0.42	285 299	162.28	± 0.046	Kaempferide-3-O-glucuronide
11	529.1373	529.1352	-3.97	349 367	281.63	± 0.022	Caffeoylferuloylquinic acid
12	487.0869	487.0882	-2.67	293 325	37.68	± 0.114	Methylcaffeoylferuloyltartaric acid
13	301.0365	301.0354	-3.65	151 179	74.39	± 0.084	Quercetin
14	487.0881	487.0882	-0.21	293 325	151.11	± 0.117	Methylcaffeoylferuloyltartaric acid
15	501.1021	501.1038	-3.39	219 339	-	-	unknown
16	501.1017	501.1038	-4.19	219 339	-	-	unknown

Figure 1: UHPLC-PDA chromatogram of green lettuce polyphenolic extract; column: Kinetex C18, 150 x 4.6mm (L × I.D.), 2.6 µm, flow: 2.2 mL /min, Inj.vol 2 µL.

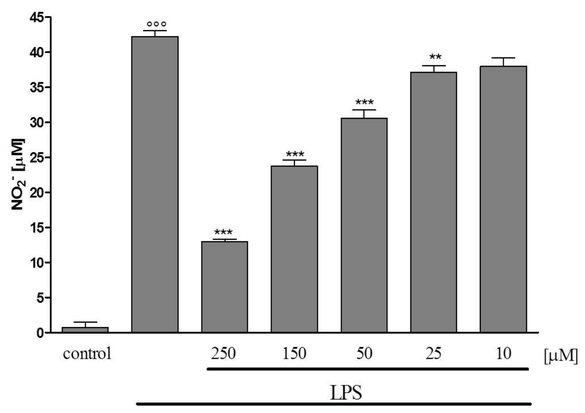

Figure 2: *Effect of green lettuce extracts (250–10 µg/mL) on NO release, evaluated as NO_2^- (µM), by macrophages J774A.1 stimulated with LPS.*

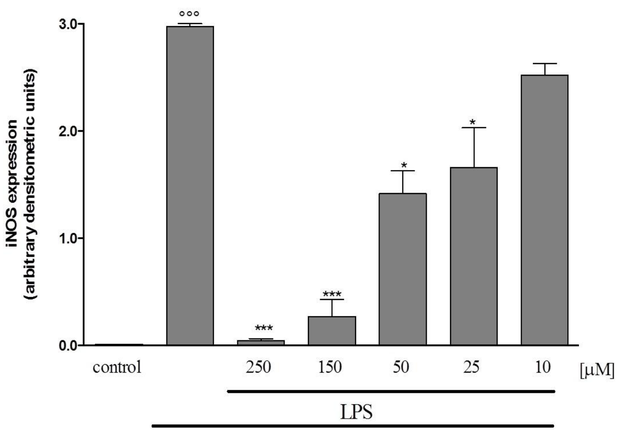

Figure 3: *Densitometric analysis of the concentration dependent effect of green lettuce extracts (250-10 µg/mL) on LPS-induced iNOS expression in J774A.1 macrophages.*

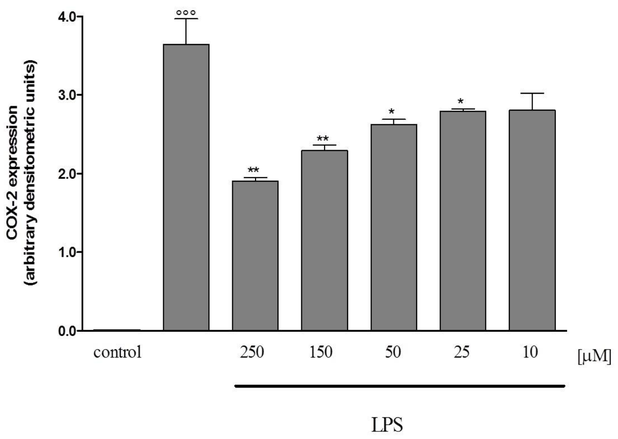

Figure 4: *Densitometric analysis of the concentration dependent effect of green lettuce extracts (250-10 µg/mL) on LPS-induced COX-2 expression in J774A.1 macrophages.*

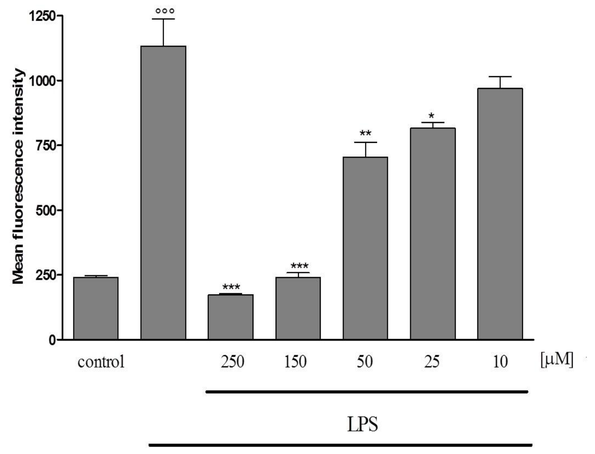

Figure 5: *Effect of green lettuce extracts (250-10 µg/mL) on ROS formation, evaluated by means of the probe 2',7' dichlorofluorescein-diacetate (H2DCF-DA), in LPS-stimulated J774A.1 macrophages.*

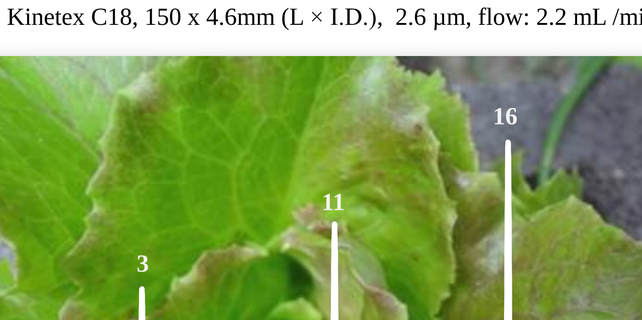

Figure 6: *Densitometric analysis of the concentration dependent effect of green lettuce extracts (250-10 µg/mL) on LPS-induced OH-1 expression in J774A.1 macrophages*

CONCLUSION

Green lettuce is an important dietary leafy vegetable and our results have shown that it exterts strong antioxidant and anti-inflammatory activities. The analytical method, based on a fast UHPLC-DAD-IT-TOF platform, was able to characterize the polyphenolic compounds in a very short time, with respect to other methods reported. The analytical analysis let us to hypothesize that biological activity of green lettuce could be addressed to the presence of high amounts of hydroxycinnamic acids derivatives, flavonols and coumarins, suggesting that its nutraceutical potential could provide important health-promoting benefits.

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