Polyphenols Communications 2006

August 22 - 25, 2006
Winnipeg, Manitoba, Canada

Editors
F. Daayf
A. El Hadrami
L. Adam
G. M. Ballance

P204. *RUMEX INDURATUS* LEAVES: PHENOLIC COMPOUNDS AND ANTIOXIDANT CAPACITY

Patrícia Valentão[a*], Vítor Ribeiro[a], Graciliana Lopes[a], Manuel Rodrigues[b], Angel Gil Izquierdo[c], Federico Ferreres[c], Rosa M. Seabra[a]

[a] REQUIMTE/ Serviço de Farmacognosia, Faculdade de Farmácia Universidade do Porto, R. Aníbal Cunha, 164, 4050-047 Porto, Portugal

[b] CIMO/ESAB, Quinta de Sta Apolónia, Apartado 1172, 5301-855 Bragança, Portugal

[c] Research Group on Quality, Safety and Bioactivity of Plant Foods, Dept. Food Science and Technology, CEBAS (CSIC), P.O. Box 164, 30100 Campus Univ. Espinardo (Murcia), Spain

* E-mail of the corresponding author: valentao@ff.up.pt

Abstract

The phenolic composition of *Rumex induratus* was determined by HPLC-DAD-MS/MS-ESI and HPLC/DAD. The results showed a profile composed by caffeoyl-hexoside, two *p*-coumaroyl-hexoside isomers, feruloyl-hexoside, sinapoyl-hexoside, 6-*C*-hexosyl-quercetin, 8-*C*-hexosyl-luteolin, 6-*C*-hexosyl-luteolin, 6-*C*-hexosyl-apigenin, 3-*O*-hexosyl-quercetin, 3-*O*-rutinosyl-quercetin, 7-*O*-hexosyl-diosmetin, 3-*O*-rutinosyl-isorhamnetin, 7-*O*-(acetyl)-pento-hexosyl-diosmetin, 6-*C*-hexosyl-genkwanin and four unidentified *O*-glycosyl-*C*-glycosylflavones. *R. induratus* was also investigated for its capacity to act as a scavenger of DPPH and superoxide radicals. Good antioxidative results were obtained against both radicals.

Introduction

The *Rumex* genus comprises several species, which leaves and roots have been used in traditional medicine as hepatoprotector, purgative, analgesic, and hypotensor. Due to their high oxalic acid content they have been implicated in oxalic intoxication, characterized by nauseas, vomits, colics and diarrhoea occurrence. *Rumex induratus*, usually called sorrel, is a spontaneous perennial herb, which grows in Northeast Portugal, where it is highly consumed. This species is very appreciated in salads and, to attenuate its acidity, is dressed with olive oil and sometimes mixed with boiled potatoes. Despite its high consumption, nothing has been reported about its nutritional value or biological potential. So, this work was developed in order to determine the phenolic compounds of *R. induratus* leaves and to evaluate its antioxidant activity.

Materials and Methods

Extraction. For the identification of the phenolic compounds in *R. induratus* leaves the lyophilised material (ca. 0.05 g) was thoroughly mixed with 1 ml methanol:water (1:1), ultra-sonicated, centrifuged and filtered. For the antioxidant capacity assays the extract was prepared by putting ca. 3.0 g of dried leaves in 500 ml of boiling water. The mixture was boiled for 30 min and then filtered over a Büchner funnel. The resulting aqueous extract was then lyophilised.

HPLC-DAD-MS/MS-ESI qualitative analysis of phenolics. Chromatographic separations were performed with a RP-18 LiChroCART. Elution was developed using acetic acid 1% (A) and methanol (B) as solvents, starting with 15% B and using a gradient to obtain 30% B at 20 min and 55% B at 40 min. The flow rate was 1 mL min^{-1}. The HPLC system was equipped with a diode array and a mass detector in series. Nitrogen was used as nebulizing gas at a pressure of 65 psi and the flow was adjusted to 11 L min^{-1}. The heated capillary and voltage were maintained at 350 °C and 4 kV, respectively. The full scan mass covered the range from *m/z* 90 up to *m/z* 2000. Collision-induced fragmentation experiments were performed in the ion trap using helium as collision gas, with voltage ramping cycles from 0.3 up to 2 V. MS data were acquired in the negative ionisation mode. MS^n data were achieved in the automatic mode on the more abundant fragment ion in MS^{n-1}.

HPLC-DAD quantitative analysis of phenolics. The extracts were analysed using an analytical HPLC unit, with a Spherisorb ODS2 column as reported before [1].

Antioxidant activity. DPPH [2] and superoxide radical [3] scavenging activities and the effect on xanthine oxidase activity [3] were performed according to described procedures.

Results and Discussion

Fifteen phenolic compounds were partially identified and quantified by HPLC-DAD-MS/MS-ESI and HPLC-DAD, respectively (Fig. 1): caffeoyl-hexoside, two *p*-coumaroyl-hexoside isomers, feruloyl-hexoside, sinapoyl-hexoside, 6-*C*-hexosyl-quercetin, 8-*C*-hexosyl-luteolin, 6-*C*-hexosyl-luteolin, 6-*C*-hexosyl-apigenin, 3-*O*-hexosyl-quercetin, 3-*O*-rutinosyl-quercetin, 7-*O*-hexosyl-diosmetin, 3-*O*-rutinosyl-isorhamnetin, 7-*O*-(acetyl)-pento-hexosyl-diosmetin and 6-*C*-hexosyl-genkwanin. Four unidentified *O*-glycosyl-*C*-glycosylflavones were also detected. 6-*C*-Hexosyl-luteolin was the main compound.

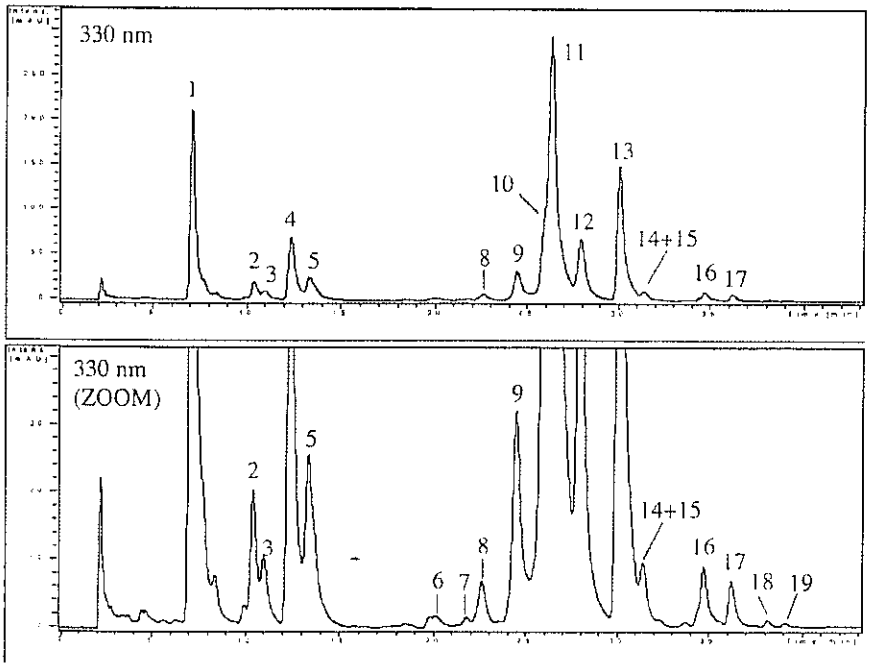

Fig. 1. HPLC phenolic profile of *Rumex induratus* leaves. Peaks: (1) Caffeoyl-hexoside; (2) *p*-Coumaroyl-hexoside isomer; (3) *p*-Coumaroyl-hexoside isomer; (4) Feruloyl-hexoside; (5) Sinapoyl-hexoside; (6) Unidentified *O*-glycosyl-*C*-glycosylflavone; (7) 6-*C*-Hexosyl-quercetin; (8) Unidentified *O*-glycosyl-*C*-glycosylflavone; (9) 8-*C*-Hexosyl-luteolin; (10) Unidentified *O*-glycosyl-*C*-glycosylflavone; (11) 6-*C*-Hexosyl-luteolin; (12) Unidentified *O*-glycosyl-*C*-glycosylflavone; (13) 6-*C*-Hexosyl-apigenin; (14) 3-*O*-Hexosyl-quercetin; (15) 3-*O*-Rutinosyl-quercetin; (16) 7-*O*-Hexosyl-diosmetin; (17) 3-*O*-Rutinosyl-isorhamnetin; (18) 7-*O*-(Acetyl)-pento-hexosyl-diosmetin; (19) 6-*C*-Hexosyl-genkwanin.

The lyophilised aqueous extract of *R. induratus* leaves displayed an effective antioxidant capacity in the DPPH assay, in a concentration-dependent manner, with an IC_{50} at 150 μg mL^{-1}.

R. induratus leaves extract revealed to be a potent scavenger of superoxide radical generated in the enzymatic system, and the effect was concentration-dependent (IC_{50} at 67 μg mL^{-1}). In addition, it exerted some inhibitory effect on xanthine oxidase, which was also concentration dependent (IC_{25} at 709 μg mL^{-1}). Therefore, it was not possible to show a clear-cut scavenging effect on superoxide radical. The capacity of the lyophilised extract to strongly scavenge superoxide radicals in a concentration-dependent way was confirmed when this radical was generated by a chemical system, and an IC_{50} at 337 μg mL^{-1} was found. So, *R. induratus* leaves lyophilised extract exhibits antioxidant activity, achieved by its capacity to act as both superoxide radical scavenger and as xanthine oxidase inhibitor.

Acknowledgements
Patrícia Valentão is indebted to Fundação Calouste Gulbenkian for the grant.

References

[1] Valentão *et al.* (2005) *J. Agric. Food Chem.* 53: 4925-4931.

[2] Silva *et al.* (2004) *J. Agric. Food Chem.* 52: 4705-4712.

[3] Valentão *et al.* (2001) *J. Agric. Food Chem.* 49: 3476-3479.