Cytisus multiflorus: source of antioxidant polyphenols

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ABSTRACT

The present study investigates the phenolic composition and the antioxidant capacity of an ethanolic extract from flowers of *C. multiflorus*. As determined by HPLC-DAD, ESI-MS^n and NMR combined analysis, this extract was mainly composed of chrysin-7-*O*-β-D-glycopyranoside, and also contained considerable amounts of rutin, a dihydroxyflavone isomer of chrysin and glycosidic derivatives of luteolin and apigenin. The *C. multiflorus* ethanolic extract exhibited high DPPH scavenging activity and reducing power with EC_{50} of 13.4 ± 0.6 and 95.7 ± 4.6 µg/mL, respectively. The exposure of human hepatoblastoma HepG2 cells to the non-toxic concentrations of the extract (50 or 200 µg/mL) resulted in a decreased rate of ROS production in a concentration dependent manner. Moreover, the mixture of standards that simulated the phenolic composition of the plant afforded a protection of about 50% in intracellular ROS production. Hence, the gathered results suggest that *C. multiflorus* polyphenols are closely associated to its antioxidant properties.

1. INTRODUCTION

Cytisus multiflorus is an endemic shrub of the western Iberian Peninsula characterized by white flowers that are consumed as tea infusions due to its beneficial effects [1]. The therapeutic properties of this species, enclosing diuretic, anti-inflammatory, anti-hypertensor and antidiabetic effects, have been associated to the antioxidant properties of its polyphenols [2]. However, the phenolic characterization of this plant species is poorly studied and there are few scientific works supporting its ethnopharmacological uses. In this sense, this study focus on the phenolic composition of *C. multiflorus*, as well as on the evaluation of the antioxidant ability of the polyphenols present in this plant.

2. MATERIALS AND METHODS

2.1 Preparation of plant extracts and phenolic composition determination

The ethanolic extract from flowers of *C. multiflorus* was prepared by extraction with an 80% ethanolic solution (*v/v*), as previously described [3]. The phenolic composition of the extract was separated by means of HPLC-DAD, which was performed on a RP-C18 column 250 mm× 4 mm id, 5 µm bead diameter (temperature of 30ºC, flow rate of 1 mL/min) and a mobile phase comprising (A) 0.1% formic acid in water and (B) acetonitrile. The phenolic compounds were identified by ESI-MS and MS^n analyses in the negative ion mode, through direct injection of the collected HPLC fractions into the ESI source at a flow rate of 8 µL min^{-1}. Typical ESI conditions were similar to those previously described [3]. The quantification of individual polyphenols was performed at 280 nm by HPLC-DAD using the external standard method.

2.2 Antioxidant activity

The antioxidant properties of the *C. multiflorus* ethanolic extract was evaluated through the chemical models of 2,2-diphenyl-1-picrylhydrazyl radical ($DPPH^{\bullet}$) scavenging potential [4] and reducing power [5], as well as on human hepatoblastoma HepG2 cell cultures. The protective effects against the generation of reactive oxygen species (ROS) induced by potassium dichromate (5 and 25 µM) in human hepatoblastoma HepG2 cells was measured by flow cytometry using dichlorofluorescein diacetate, after treatment with the extract for 48 h [6]. ROS measurements were also performed with a mixture of the main polyphenols (apigenin, chrysin, luteolin, quercitin) that resemble their levels in the extract.

3. RESULTS AND DISCUSSION

3.1 Polyphenols of the *C. multiflorus* extract

The total quantified polyphenols in the ethanolic extracts of *C. multiflorus* accounted for 130.0 ± 7.2 mg/g extract. Chrysin-7-*O*-β- D-glycopyranoside was the major compound and it represented approximately 38% of these phenolics (49.4 ± 7.3 mg/g extract). The extract also contained considerable amounts of a dihydroxyflavone isomer of chrysin (21.8 ± 3.8 mg/g extract), rutin (14.1 ± 1.7 mg/g extract), 2″-*O*-pentosyl-8-*C*-hexosyl-luteolin (10.7 ± 0.8 mg/g extract), 2″-*O*-pentosyl-6-*C*-hexosyl-luteolin (10.2 ± 1.2 mg/g extract) and 6″-*O*-(3-hydroxy-3-methylglutaroyl)-2″-*O*-pentosyl-*C*-hexosyl-apigenin (11.2 ± 2.1 mg/g extract) [2].

3.2 Antioxidant capacity

The extract exhibited high DPPH scavenging activity and reducing power with EC_{50} of 13.4 ± 0.6 and 95.7 ± 4.6 µg/mL, respectively.

As determined by the MTT assay, the *C. multiflorus* ethanolic extract was not toxic for HepG2 cells up to concentrations of 200 µg/mL (results not shown). Hence, two distinct non-toxic concentrations (50 and 200 µg/mL) were chosen for further testing the protective

potential of *C. multiflorus* ethanolic extract against the generation of reactive oxygen species (ROS), induced by potassium dichromate.

The exposure of HepG2 cells to the *C. multiflorus* ethanolic extract at 50 and 200 µg/mL decreased the rate of ROS production, both under the basal condition (absence of potassium dichromate) or on the oxidative stress models (potassium dichromate at a concentration of 5 and 25 µM), in a concentration dependent manner (Table 1A). Additionally, the treatment of the cells with a mixture of standards which simulated the phenolic composition of this plant caused a decrease on the intracellular ROS formation by approximately 50% (Table 1B). These *in vitro* experiments support the importance of polyphenol content in the antioxidant activity of this plant. Since antioxidant activity is crucial in many health disorders, these results might support some of the traditional uses of *C. multiflorus*.

Table 1. Effect in ROS production induced by potassium dichromate at 5 and 25 µM of in human hepatoblastoma HepG2 cells treated during 48 h with (A) *C. multiflorus* ethanolic extract or (B) the mixture of phenolic standards.

(A) Extract (µg/mL)	DK0	DK5	DK25
0	100.0 ± 2.2	187.5 ± 10.0$^{###}$	289.6 ± 12.9$^{###}$
50	52.6 ± 9.9**	151.0 ± 17.9	187.1 ± 8.5***
200	46.8 ± 9.7***	102.9 ± 8.3***	150.1 ± 6.2***
(B) Standards (µg /mL)			
0	100.0 ± 4.7	338.2 ± 41.7$^{###}$	-
Mix	40.9 ± 3.1***	178.4 ± 19.2***	-

Cells were incubated in the absence (0) or presence of potassium dichromate (5 or 25 µM), co-incubated with of *C. multiflorus* ethanolic extract at 50 or 200 µg/mL (A) or the mixture that simulates the composition of the extract (B), for 48 h.

Values are represented as means ± S.E.M. of percentage of ROS production respect to control, from three different cultures carried out in triplicate per data point. Statistical analysis was performed by one-way ANOVA, followed by Dunnett's post test. DK0, without potassium dichromate; DK5, potassium dichromate at 5 µM; DK25, potassium dichromate at 25 µM; $^{###}P < 0.001$ (compared with the control); *** $P < 0.001$ (compared with cells without extract, in presence of DK0, DK5 or DK25).

4. CONCLUSIONS

In summary, these results demonstrate the high antioxidant activity of the enriched phenolic extract of *C. multiflorus*, suggesting the importance of polyphenols in this capacity.

More studies are now being undertaken in order to analyze the individual contribution of polyphenols in the *C. multiflorus* antioxidant ability.

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References

[1] T Rodriguez-Riano, FJ Valtuena, A Ortega-Olivencia, Ann Bot, 2006, 98,777-791

[2] A Luis, F Domingues, C Gil, AP Duarte J Med Plants Res, 2009, 3, 886-893

[3] OR Pereira, AMS Silva, MRM Domingues, SM Cardoso, Food Chem, 2012, 131, 652-659

[4] A Ferreira, C Proenca, MLM Serralheiro, MEM Araujo, Journal of Ethnopharmacol, 2006,108, 31-37

[5] M Oyaizu M, Jpn J Nutr, 1986, 44, 307-15

[6] MJ Perez, B Castaño, S Jimenez, MA Serrano, JM Gonzalez-Buitrago, JJG Marín, Toxicol Appl Pharm, 2008, 232, 327-336