ORIGINAL ARTICLE

EVALUATION OF POLYPHENOLIC PROFILE AND ANTIOXIDANT ACTIVITY FOR *CYTISUS NIGRICANS* AND *CYTISUS ALBUS*

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Abstract

The aim of this study was to assess the polyphenolic composition and antioxidant capacity of *Cytisus nigricans* L. (lack broom) and *Cytisus albus* Link (white broom) (*Fabaceae*). A qualitative and quantitative characterization of the main phenolic compounds from ethanolic extracts of aerial parts was carried out by a HPLC/UV/MS method. The total polyphenolic, flavonoids and caffeic acid derivatives content were measured by spectrophotometric methods. The *in vitro* antioxidant activity of these extracts was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent antioxidant capacity (TEAC) and an electron paramagnetic resonance (EPR) based methods. The polyphenolic profile of these species revealed the common components (gentisic acid, isoquercitrin, rutin, quercitrin, luteolin, apigenin) and specific other compounds (*p*-coumaric acid, ferulic acid, kaempferol) for *C. nigricans.* The highest antioxidant capacity and total polyphenolic content were revealed by *C. nigricans.* Both species contain significant amounts of polyphenolic compounds and they may be considered an important source of natural antioxidants for pharmaceutical uses.

Rezumat

Acest studiu a evaluat compuşii polifenolici din speciile de *Cytisus nigricans* L. (lemnul bobului) şi *Cytisus albus* Link, drob alb (*Fabaceae*) precum şi capacitatea lor antioxidantă. Caracterizarea calitativă şi cantitativă a principalilor compuşi fenolici din extractele etanolice obţinute din părţile aeriene a fost realizată printr-o metodă HPLC/UV/MS. Concentraţiile de polifenoli totali, flavonoide şi derivaţi de acid cafeic au fost determinate prin metode spectrofotometrice. Activitatea antioxidantă *in vitro* a acestor extracte a fost evaluată prin testele 2,2-difenil-1-picrilhidrazil (DPPH), TEAC (*trolox equivalent antioxidant capacity*), precum şi printr-o metodă bazată pe rezonanţă electronică pramagnetică (EPR). Profilul polifenolic al acestor specii evidenţiază componente comune (acid gentisic, izoquercitrina, rutozida, quercetina, luteolina, apigenina), precum şi compuşi specifici pentru *C. nigricans* (acid *p*-cumaric, acid ferulic, kempferol). Extractul de *C. nigricans* prezintă capacitate antioxidantă şi conţinut polifenolic superioare speciei *C. albus*. Cele două specii de *Cytisus*, prin conţinutul ridicat de compuşi polifenolici, pot fi considerate surse importante de antioxidanţi naturali, pentru domeniul farmaceutic.

Keywords: *Cytisus nigricans*, *Cytisus albus*, polyphenols, antioxidant

Introduction

In recent decades, many herbs and natural compounds have increasingly been receiving public interest as complementary and alternative medicines. The polyphenolic compounds represent one of the largest, most widespread and functionally important groups of secondary plant metabolites. In recent years, these compounds have attracted great interest due to their antioxidative capacity, which confers them a valuable therapeutic potential in treating a large array of free-radical mediated diseases [2]. *Cytisus* Desf. (*Fabaceae*) is a large and diversified genus including approximately 60 species, which are particularly abundant around the Mediterranean Sea, although they are found in distinct geographic regions such as the north and south of Africa, the western and central Europe, the Black Sea and Turkey to the East [1, 3, 10]. Plants of this genus exhibit bioactive properties, including antioxidant, diuretic, hypnotic, anxiolytic, antiparasitic and anti-diabetic activities [4, 9]. The therapeutic properties and, in particular, the antioxidant activity of *Cytisus* is related to their high concentration of phenolic compounds, including flavonoids. *Cytisus scoparius* Link contain 6'-O-acetyl-scoparin, kaempferol, rutin,

quercetin, quercitrin and isorhamnetin, genistein and sarothamnoside, while the species *Cytisus nigricans* L. and *Cytisus albus* Link were reported to contain the isoflavones ononin and genistin [7, 9]. As previous studies mentioned, *C. albus* and *C. nigricans* can be considered among other *Fabaceae* species natural sources of isoflavones. Ononin was the only isoflavone reported for *C. nigricans* while daidzin, genistin, ononin and genistein were identified in *C. albus*. Even though *C. albus* presents a richer isoflavone spectrum, a larger quantity of ononin was reported for *C. nigricans* [7].

The aim of this paper was to determine the polyphenolic content, and to evaluate the antioxidant activity of two Romanian *Fabaceae* species, *C. albus* Link and *C. nigricans*. L. To our concern, none of these plants have been subjects to previous detailed studies that reveal their polyphenolic content or antioxidant activity.

Materials and Methods

Chemicals and Apparatus

Chlorogenic acid, *p*-coumaric acid, caffeic acid, rutin, apigenin, quercetin, isoquercitrin, quercitrin, hyperoside, kaempferol, myricetol, fisetin were purchased from Sigma (St. Louis, MO, USA), ferulic acid, sinapic acid, gentisic acid, gallic acid, patuletin, luteolin from Roth (Karlsruhe, Germany), and cichoric acid, caftaric acid from Dalton (Toronto, ON, Canada). Sodium molybdate dihydrate, sodium nitrite, sodium hydroxide, sodium carbonate were purchased from Sigma-Aldrich (Steinheim, Germany). HPLC grade methanol, analytical grade orthophosphoric acid, hydrochloric acid, aluminium chloride, sodium acetate, ethanol and Folin-Ciocalteu reagent were purchased from Merck (Darmstadt, Germany). DPPH·, $ABTS^{+}$ was obtained from Alfa-Aesar (Karlsruhe, Germany). All spectrophotometric data were acquired using a Jasco V-530 UV-vis spectrophotometer (Jasco International Co., Ltd., Tokyo, Japan).

Plant material and extraction procedure. The aerial parts of these two species were collected in 2015, during the blooming period (June-July) from the North - West of Romania. Voucher specimens (No. 959, 960) were deposited in the Herbarium of the Department of Pharmacognosy of the Faculty of Pharmacy, Cluj-Napoca, Romania. The vegetal material was air dried at room temperature in shade, separated and grinded to fine powder (300 µm). The material was extracted with 70% ethanol for 30 min on a water bath, at 60°C. The samples were then cooled down and centrifuged at 4500 rpm for 15 min, and the supernatant was recovered [12, 13].

HPLC Analysis. The identification and quantification of polyphenols was achieved using an Agilent Technologies 1100 HPLC Series system coupled with an Agilent 1100 mass spectrometer (LC/MSD Ion Trap VL) equipped with degasser, binary gradient pump, column thermostat, autosampler and detector. For the separation, a reverse-phase analytical column was employed. The detection of the compounds was performed on both UV and MS mode. There were used as reference standards the following phenolic compounds: chlorogenic, *p*-coumaric, caffeic, cichoric, caftaric, ferulic, sinapic, gentisic gallic acids, quercetin, isoquercitrin, quercitrin, hyperoside, rutin, kaempferol, myricetol, fisetin, patuletin, apigenin, luteolin. Calibration curves in the 0.5 - 50 µg/mL range with good linearity ($R^2 > 0.999$) for a five point plot were used. The chromatographic data were processed using ChemStation and DataAnalysis software from Agilent [5].

The total phenolic content (TPC) of the extracts was determined using the Folin-Ciocalteu method, with some modifications. The absorbance was measured at 725 nm and the results were expressed in gallic acid equivalents on dry material plant (GAE; mg/g sample) [8, 14].

The quantitative determination of flavonoids was performed using the spectrophotometric aluminium chloride method and the results was expressed in rutin equivalents (RE; mg/g sample) [8, 14].

The caffeic acid derivatives content was determined using the Arnow's spectrophotometric method. The percentage of phenolic acids was expressed as caffeic acid (CAE; mg/g sample) [8, 14].

Antioxidant Activity Test

DPPH free radical method is an antioxidant assay based on electron-transfer. The decrease in the absorbance was measured at 517 nm. The anti-radical activity was expressed as IC_{50} (µg/mL), the concentration of vegetal material required to cause a 50% DPPH inhibition [14].

TEAC assay is based on the scavenging ability of antioxidants to the radical anion $ABTS^{\bullet+}$. The results was converted in Trolox equivalents by using of a calibration curve ($R^2 = 0.998$) made with 2 - 10 mg/L Trolox standards [11].

EPR measurements were performed on a Bruker Elexsys E500 spectrometer. A solution of DPPH was added in liquid samples of antioxidant extracts and quickly mixed with 10 µL of extract and transferred in EPR quartz capillary to record the EPR spectra. The rate of reaction between anti-oxidant samples and DPPH radical was expressed by integral intensity (I) [6].

Results and Discussion

The HPLC profile of the polyphenolic compounds (Table I, Figure 1) revealed the presence of 11 phenolic compounds for *C. nigricans* and only 7 compounds for *C. albus.* In *C. nigricans* and *C. albus* extracts we have identified five phenolic acids

(gentisic, caffeic, chlorogenic, ferulic, *p*-coumaric acids), three flavonoid glycosides (isoquercitrin, rutin, quercitrin) and four flavonoidic aglycones (quercetin, luteolin, kaempferol and apigenin). Rutin has been detected in highest amounts both in *C. nigricans* and *C. albus* (213.60 ± 0.78 µg/g and 221.02 ± 0.56 µg/g, respectively). Quercetin has been detected only in *C. albus* (112.91 ± 0.03 µg/g). In both extracts there were found free flavonoidic aglycones in small amounts, i.e. quercetin, luteolin, and apigenin and kaempferol only in *C. nigricans*. Gentisic, caffeic and chlorogenic acids have been also identified in too low concentrations. Ferulic and *p*-coumaric acids have been identified only in *C. nigricans* (14.84 ± 0.12 µg/g and 12.39 ± 0.18 µg/g, respectively). So, the *C. nigricans* and *C. albus* species are the high amount of quercetin derivatives (isoquercitrin, rutin, quercitrin), while other related studied species e.g., *C. multiflorus* proved to be mostly rich in chrysin derivatives, in particular the flavone chrysin-7-O-ß-D–gluco-pyranoside [10].

Table I

Polyphenolic compounds HPLC analysed

Polyphenolic compounds	*m/z* value	t_R ± SD (min)	*C. nigricans* µg/g plant material	*C. albus* µg/g plant material
Gentisic acid	179	3.52 ± 0.04	< 0.2	< 0.2
Caffeic acid	179	5.60 ± 0.04	< 0.2	NF
Chlorogenic acid	353	5.62 ± 0.05	< 0.2	NF
p-coumaric acid	163	9.48 ± 0.08	14.84 ± 0.12	NF
Ferulic acid	193	12.8 ± 0.10	12.39 ± 0.18	NF
Isoquercitrin	463	19.60 ± 0.10	84.20 ± 0.45	78.04 ± 0.25
Rutin	609	20.20 ± 0.15	213.60 ± 0.78	221.02 ± 0.56
Quercitrin	447	23.64 ± 0.13	24.26 ± 0.55	19.59 ± 0.56
Quercetin	301	26.80 ± 0.15	NF	112.91 ± 0.03
Luteolin	285	29.10 ± 0.19	3.72 ± 0.45	12.71± 0.41
Kaempferol	285	32.48 ± 0.17	1.75 ± 0.05	NF
Apigenin	279	33.10 ± 0.15	2.55 ± 0.65	2.16 ± 0.05

Note: NF - not found below limit of detection. Values are the mean ± SD (n = 3)

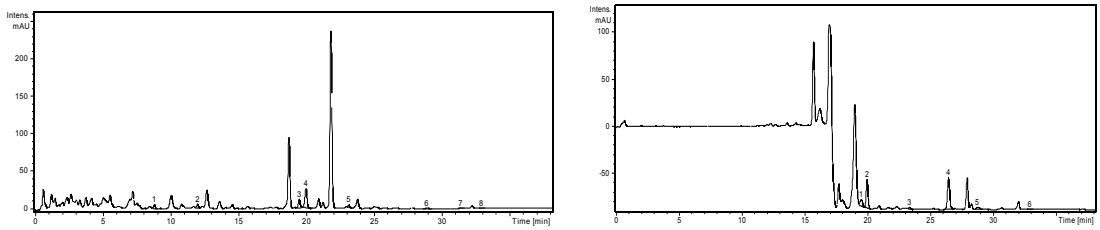

Figure 1.

C. nigricans - HPLC chromatogram (left); *C. albus* - HPLC chromatogram (right)

The highest amount of polyphenols and caffeic acid derivatives has been determined for *C. nigricans* (42.63 ± 0.31 mg/g, 24.58 ± 0.23 mg/g) followed by *C. albus* (27.33 ± 0.26 mg/g, 10.14 ± 0.29 mg/g), while the content of flavonoids has been richer in *C. albus* (24.92 ± 0.24 mg/g) than *C. nigricans* (7.6 ± 0.12 mg/g). Regarding the content of polyphenols in related species of genus, on can observed that the polyphenolic content of *C. nigricans* is comparable with *C. multiflorus* species (44.7 mg/g) [10].

Table II

The content of polyphenols in *C. nigricans* and *C. albus*

Samples	TPC (mg GAE/g plant material)	Flavonoids (mg RE/g plant material)	Caffeic acid derivatives (mg CAE/g plant material)
C. nigricans	42.63 ± 0.32	7.6 ± 0.16	24.58 ± 0.23
C. albus	27.33 ± 0.24	16.92 ± 0.34	10.14 ± 0.29

According to all the antioxidant used methods, *C. nigricans* presented higher antioxidant capacity than *C. albus* that is in agreement with TPC and caffeic acid derivatives values (Table II and III, Figure 2). Regarding the EPR method, the values of the integral intensity of these samples were compared with the DPPH radical standard. The rate of reaction between antioxidant compounds of the extracts and DPPH radical, were monitored using normalized double integrated residual EPR signal, which is correlated with the number of paramagnetic species (Figure 3). One can observe that the extract of *C. nigricans* had a higher antioxidant capacity than *C. albus* extract (Table III). The EPR results are in good concordance with the DPPH radical scavenging and TEAC assays results.

Table III

Antioxidant activity results obtained

Samples	DPPH IC_{50} (µg/mL)	TEAC (mmol Trolox/mg plant material)	EPR Integral intensity I_{DPPH} = 797.12
C. nigricans	88.44 ± 0.72	165.85 ± 5.01	52.16 ± 1.1
C. albus	131.13 ± 0.6	100.71 ± 4.81	292.26 ±2.1
Quercetin	5.60 ± 0.32	-	
BHT	16.1 ± 0.44	-	

Note: All assays were perform in triplicate

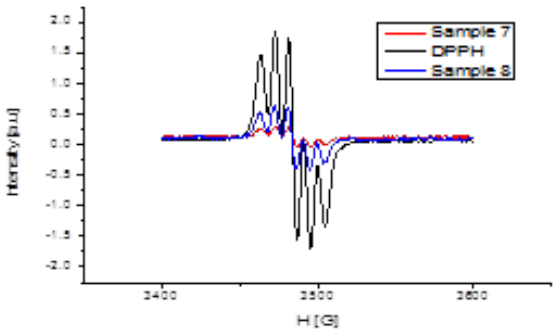

Figure 2.

EPR- the rate of interaction between the extracts and DPPH radical

Note: Sample 7 - *C. nigricans*, Sample 8 - *C. albus*

It can be noticed that the *C. nigricans* and *C. albus* species are the important sources of quercetin derivatives in particular rutin. The quercetin is a well-known natural antioxidant (Table III, DPPH IC_{50} = 5.60 ± 0.32), therefore the high level of antioxidant potential could be explained by its presence among other polyphenols in these tested extracts.

Conclusions

The polyphenolic profile and the antioxidant activity for two indigenous *C. nigricans* and *C. albus* species were analysed and the lack of literature data was completed. There are significant differences, both qualitative and especially quantitative, between these studied taxa. The systematically comparative study emphasized that these species represent a potent natural source of polyphenolic antioxidants.

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