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BOLETIN LATINOAMERICANO Y DEL CARIBE DE PLANTAS MEDICINALES Y AROMÁTICAS
19 (2): 161 - 166 (2020)
© / ISSN 0717 7917

Artículo Original | Original Article

Phenolic profile and antioxidant, acetylcholinesterase, and tyrosinase inhibitor activities of *Jurinea tzar-ferdinandii* Davidov, an endemic plant of the Balkans

[Perfil fenólico y actividad antioxidante, acetilcolinesterasa e inhibidora de tirosinasa de *Jurinea tzar-ferdinandii* Davidov, planta endémica de los Balcanes]

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Abstract: The methanol extract of the Balkan endemic species *Jurinea tzar-ferdinandii* Davidov demonstrated weak antioxidant activity against DPPH• and ABTS+• and low inhibitory potential against acetylcholinesterase (8.3% Inh.) and tyrosinase (IC_{50} = 208 ± 8 µg/mL) enzymes. Phytochemical investigation of the extract led to isolation and identification of apigenin, luteolin, apigenin-7-O-glucoside, apigenin-4'-O-glucoside, apigenin-7-O-gentiobioside, luteolin-4'-O-glucoside, rutin, narcissin, chlorogenic and 1,5-dicaffeoylquinic acid. With exception of apigenin and rutin, all isolated compounds are reported for the first time in the representatives of genus *Jurinea*. The distribution of flavonoids was discussed from chemotaxonomic point of view.

Keywords: *Jurinea tzar-ferdinandii*; Flavonoids; Antioxidant; Acetylcholinesterase; Tyrosinase.

Resumen: El extracto de metanol de la especie endémica de los Balcanes *Jurinea tzar-ferdinandii* Davidov demostró una actividad antioxidante débil contra DPPH• y ABTS+• y un bajo potencial inhibidor contra las enzimas acetilcolinesterasa (8.3% Inh.) tirosinasa (IC_{50} = 208 ± 8 µg/mL). La investigación fitoquímica del extracto condujo al aislamiento e identificación de apigenina, luteolina, apigenina-7-O-glucósido, apigenina-4'-O-glucósido, apigenina-7-O-gentiobiósido, luteolina-4'-O-glucósido, rutina, narcissin, clorogénico y ácido 1,5-dicafeoilquinico. Con excepción de la apigenina y la rutina, todos los compuestos aislados se informan por primera vez en el género *Jurinea*. La distribución de flavonoides se discute desde el punto de vista quimiotaxonómico.

Palabras clave: *Jurinea tzar-ferdinandii*; Flavonoides; Antioxidante; Acetilcolinesterasa; Tirosinasa

Recibido | Received: July 31, 2019
Aceptado | Accepted: October 20, 2019
Aceptado en versión corregida | Accepted in revised form: November 10, 2019
Publicado en línea | Published online: Marchr 30, 2020
Este artículo puede ser citado como / This article must be cited as: A Trendafilova, N Kutova, G Ozek, M Todorova, S Yur, T Ozek, D Dimitrov. 2020 Phenolic profile and antioxidant, acetylcholinesterase, and tyrosinase inhibitor activities of *Jurinea tzar-ferdinandii* Davidov, an endemic plant of the Balkans. **Bol Latinoam Caribe Plant Med Aromat** 19 (2): 161 – 166.

INTRODUCTION

Oxidative stress, defined as an imbalance between increased levels of free radicals and a low activity of antioxidant mechanisms, can induce damage to the cellular structure and potentially destroy tissues. Free radicals have become the culprit for influencing human health. Many studies have shown that oxidative stress plays a major role in ageing and various neurodegenerative disorders (Singh *et al*., 2019). Alzheimer's disease (AD) or dementia is characterized by progressive loss of cognitive and behavioural deterioration, which leads to the impairment of daily and routine activities. It is one of the most prevalent neurodegenerative disorders manifesting 45 million people worldwide. The inhibition of acetylcholinesterase (AChE) preserves the levels of acetylcholine and improves the cholinergic function, and therefore has become the standard approach in the symptomatic treatment of AD (Adewusi & Steenkamp, 2011). Parkinson's disease (PD) is the second most common neurodegenerative disorder after AD in elderly people. The role of tyrosinase in neuromelanin production and damage of the neurons related to Parkinson's disease has been extensively studied (Xu *et al*., 1997). Using natural plant products instead of synthetics has become an important issue in the world. The phenolic compounds and flavonoids take an important place among natural antioxidants and inhibitors of acetylcholine and tyrosinase enzymes (Şöhretoğlu *et al*., 2018; Szwajgier *et al*., 2018).

The genus *Jurinea* Cass. (family Asteraceae, tribe *Cardueae*) comprising about 250 species is widespread in Southwest and Central Asia, the Mediterranean and the Balkan regions of Europe (Susanna *et al*., 2006). This genus is represented by 10 taxa in Bulgaria and five of them are local or Balkan endemics (Stoyanov *et al*., 1967; Assyov *et al*., 2006). The literature survey showed that only a few species of genus *Jurinea* have been investigated so far. *Jurinea* species are rich in sesquiterpene lactones and triterpenes (Singh *et al*., 2016; Trendafilova *et al*., 2018). Recently, a study of *J. dolomiaea* Boiss. roots demonstrated the presence of caffeic acid, apigenin, catechin and rutin (Shah *et al*., 2014). It is worth to mention that the number of articles reporting the isolation of individual phenolic components from *Jurinea* species is also limited. In fact, there are only two reports describing the isolation of arctigenin and arctiin from the fruits of *Jurinea mollis* Wettst. (Könye *et al*., 2016) and four flavones from the aerial parts of *J. tzar-ferdinandii* Davidov (Trendafilova *et al*., 2018). The total phenolic and flavonoid contents in *J. consanguinea* DC., *J. dolomianea* and *J. humilis* DC. extracts have been recently reported in relation to their antioxidant capacity, anticholinesterase and antibacetrial activities (Öztürk *et al*., 2011; Shah *et al*., 2014; Singh *et al*., 2015; Ayad *et al*., 2017).

Jurinea tzar-ferdinandii is a Balkan endemic plant with limited distribution in Bulgaria and Romania. Recently, we have started phytochemical investigation of this species and 26 compounds (triterpenoids, sesquiterpene lactones and nonpolar flavonoids) were isolated and identified in the chloroform extract (Trendafilova *et al*., 2018). In this study, the total chloroform extract and the sesquiterpene lactone onopordopicrin have shown good inhibitory activity against bacterial lipase from *Candida rugosa* and a lipase from porcine pancreas. These promising results prompted us to continue the investigation of this endemic plant. Here in, we report phenolic constituents, antioxidant and enzyme inhibitory activities of the methanol extract of *J. tzar-ferdinandii.*

MATERIALS AND METHODS

Plant material

Wild growing *Jurinea tzar-ferdinandii* Davidov was collected in full flowering stage in July 2016 from Chepan mountain in Bulgaria. A voucher specimen (SOM 1356) has been deposited in the Herbarium of the Institute of Biodiversity and Ecosystem Research, BAS.

Extraction and isolation

Dried and powdered aerial parts of *J. tzar-ferdinandii* (25 g) were successively extracted with chloroform and methanol at room temperature (2 times, for 12 hrs each). After filtration, the combined extracts were concentrated under vacuum to yield the chloroform (2.5 g) and methanol (1.7 g) extracts. The methanol extract was dissolved in CH_3OH and subjected to column chromatography (CC) on Sephadex LH 20 (CH_3OH) to give 8 fractions. Fractions F4-F8 enriched in phenolic compounds (TLC monitoring before and after spraying with NP/PEG reagent and UV visualization at 366 nm) were separated by PTLC and CC. CC (RP18, CH_3OH/H_2O, 1:1) of F4 (86 mg) afforded 12 subfractions. Preparative thin layer

chromatography (PTLC) (RP18, CH_3OH/H_2O, 1:1) of F4/6 gave 2.1 mg of rutin (**7**). PTLC ($CHCl_3/CH_3OH$, 3:1) of F4/8 yielded **3** (4.09 mg) and **5** (1.98 mg). PTLC ($CHCl_3/CH_3OH$, 3:1) of F4/8 gave **4** (4.45 mg). F4/11 (1.38 mg) contained one TLC spot, identified as narcissin (**8**). CC (RP18, CH_3OH/H_2O, 7:3, 1:1 and 2:3) of F6 (71 mg) gave 5 subfractions. PTLC ($CHCl_3/CH_3OH$, 5:1) of F6/3 gave **6** (5.98 mg). PTLC ($CHCl_3/CH_3OH$, 3:1) of F7 (6 mg) afforded **1** (1.2 mg) and **2** (1.0 mg). The presence of compounds **9** and **10** in F3 and F5 was confirmed by comparison with authentic standards.

Determination of total phenolic content (TPC)

Total phenolic content (TPC) was measured using Folin–Ciocalteu method with slight modifications (Miliauskas *et al*., 2004). Gallic acid was used as a standard compound and TPC was expressed as mg gallic acid equivalents (GAE) per 1 g of dry extract.

DPPH radical cation scavenging activity (DPPH)

Free DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity of the extract was performed in 96-well plate according procedure proposed by Lee *et al*. (1998). The absorbance was read at 517 nm using a spectrophotometer. Data were expressed as the mean ± standard deviation (± SD).

Trolox Equivalent Antioxidant Capacity (TEAC)

ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) free radical cation scavenging activity of the sample was tested according to the procedure described by Re *et al*. (1999) and expressed as Trolox equivalent antioxidant capacity (TEAC). The absorbance was read at 734 nm using a spectrophotometer. Data were expressed as the mean ± standard deviation (± SD).

Microtiter assay for determination of acetylcholinesterase inhibition (AChE)

AChE inhibition of the sample was evaluated using Ellman's method (Ellman *et al*., 1961). Galanthamine hydrobromide from *Lycoris* sp. (0.1 mg/mL) was used as positive control.

Microtiter assay for determination of tyrosinase inhibition (TYR)

TYR inhibiton was assessed using the 96-well microplate method reported by Masuda (Masuda *et al*., 2005). Kojic acid (0.01-0.1 mg/mL in buffer) was used as a positive control. Data obtained from *in vitro* enzyme inhibition assays were expressed as the mean ± standard error (±SEM).

Table No. 1
Total phenolic content (TPC), DPPH scavenging activity (DPPH), Trolox equivalent antioxidant capacity (TEAC), acetylcholinesterase (AChE) and tyrosinase (TYR) enzymes inhibition of *J. tzar-ferdinandii* methanol extract

Sample	TPC [mgGAE/gE]	DPPH [%]	TEAC [µg/mL]	AChE [Inh %]	TYR IC_{50} [µg/mL]
MeOH extract	**54.7±0.4**	**35.7±0.4**	**259±8**	**8.3**	**208±8**
Gallic acid (5 µg/mL)*		**54.4±0.5**			
Kojic acid**					**16±2**
Galanthamine***				**98.6**	

***Positive control for DPPH; **Standard inhibitor of tyrosinase; ***Standard inhibitor of acetylcholinesterase**

RESULTS AND DISCUSSION

Initially, methanol extract obtained from the aerial parts of *J. tzar-ferdinandii* was analyzed for its total phenolic content and free radical scavenging properties (Table No. 1). It was found that the extract contained 54.7 ± 0.4 mg GAE/g extract. The inhibition of DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals (%) at a concentration of 200 µg/mL of the extract was only 35.7 ± 0.4 %. TEAC of the sample (1 mg/mL) towards to $ABTS^{\bullet+}$ was 259 ± 8 µg/mL extract. It is difficult to compare the obtained results with those published for other *Jurinea* species, because of different methods of testing and presentation of the results. However, the amount of

total phenolics found in *J. tzar-ferdinandii* is quite low and similar to that in the methanol extract obtained from *J. consanguinea* aerial parts (47.95 µg pyrocatechol equivalents/mg extract) (Öztürk *et al*., 2011). In contrast, *J. dolomiaea* and *J. humulis* aerial parts were rich in polyphenolic compounds and their TPC was 193.3 and 124.4 mg GAE/g, respectively (Singh *et al*., 2015; Ayad *et al*., 2017). The relatively low content of phenolic compounds reflected in the antioxidant capacity of the studied extract. Comparison with the results for other *Jurinea* species showed that the methanol extract from *J. consanguinea* used in the same concentration of 200 µg/mL inhibited 80% of DPPH radicals (Öztürk *et al*., 2011). Better DPPH radical scavenging activity was observed for *J. humulis* (IC_{50} = 0.36 mg/mL) (Ayad *et al*., 2017) and *J. dolomiaea* aerial parts (IC_{50} = 102.2 µg/mL) (Singh *et al*., 2015) and roots (IC_{50} = 194.7 ± 1.3 µg/mL) (Shah *et al*., 2014). Further, *J. dolomiaea* root extract also demonstrated high TEAC in ABTS assay (IC_{50} = 93.3 ± 2.2 µg/mL) (Shah *et al*., 2014), which was higher than that found for *J. tzar-ferdinandii*.

Next, the inhibitory effect of the extract on acetylcholinesterase (AChE) and tyrosinase enzymes was also evaluated (Table No. 1). It was found that the extract at a concentration of 3 mg/mL inhibited only 8.3% of AChE. Comparing with galanthamine (0.1 mg/mL) used as positive control (98.6% inhibition) as well as with that published for different *J. consanguinea* extracts (24-28% at concentration of 200 µg/mL) (Öztürk *et al*., 2011) the studied sample demonstrated a weak anti-AChE activity. The methanol extract demonstrated low inhibitory potential against tyrosinase enzyme (IC_{50} = 208 ± 8 µg/mL) in comparison with kojic acid used as a positive control (IC_{50} = 16 ± 2 µg/mL).

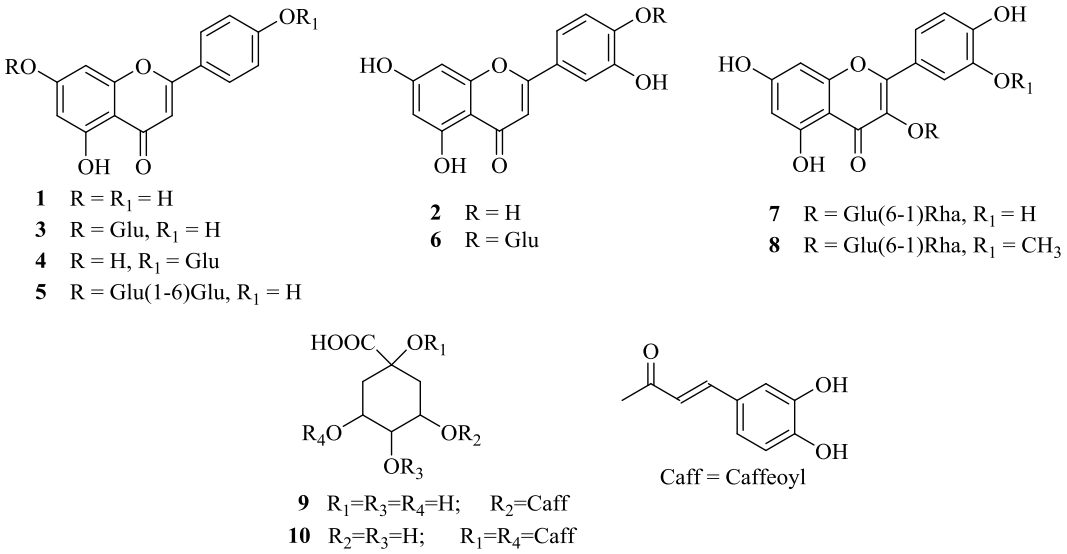

Figure No. 1
Structures of the isolated compounds

Apigenin (**1**), luteolin (**2**), apigenin-7-O-glucoside (**3**), apigenin-4'-O-glucoside (**4**), apigenin-7-O-gentiobioside (**5**), luteolin-4'-O-glucoside (**6**), rutin (**7**), narcissin (**8**), chlorogenic acid (**9**) and 1,5-dicaffeoylquinic acid (**10**) (Figure No. 1) were further isolated from the methanol extract. The identification of the compounds was achieved by comparison of their spectral data (NMR, MS and UV) with those published in the literature and/or by comparison with authentic standards (Buckingham *et al*., 2015). As can be seen, *J. tzar-ferdinandii* methanol extract contained predominately C-4'- and C-3-glycosyl substituted flavones and flavonols. The lack of 3',4'-dihydroxy groups in the B ring in most compounds and/or 3-OH group, which are important for the effective radical scavenging activity could explain the moderate activity of the extract against DPPH and ABTS radicals. Probably, chlorogenic and 1,5-

dicaffeoylquinic acids play significant role for the biological activity.

CONCLUSION

The present work is the first report on phenolic constituents and biological activity of the *J. tzar-ferdinandii* methanol extract. It is worth to mention that all isolated compounds, with exception of apigenin and rutin (Shah *et al*., 2014) are described for the first time in the species of genus *Jurinea*. Apigenin, luteolin, their glycosides and rutin are common components in the representatives of the tribe *Cardueae* such as *Onopordon* and *Centaurea* genera (Bruno *et al*., 2011; Formisano *et al*., 2012). 1,5-Dicaffeoylquinic acid is a characteristic compound for the species of genus *Cynara*, which also belongs to this tribe (Pandino *et al*., 2009). The flavonoids described above support the hypothesis for a biogenetic relationship of genus *Jurinea* with *Onopordon* and *Centaurea* genera, which has been recently proposed on the base of the structural type of the isolated sesquiterpene lactones from *J. tzar-ferdinandii* (Trendafilova *et al*., 2018).

ACKNOWLEDGEMENTS

Authors are thankful to Bulgarian Academy of Sciences and TUBITAK (Project No 116S021) for the partial financial support.

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