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Original Article

# Total Phenolics, Flavonoids, Condensed Tannins Content of Eight *Centaurea* Species and Their Broad Inhibitory Activities against Cholinesterase, Tyrosinase, α-Amylase and α-Glucosidase

Gokhan ZENGIN[1]*, Marcello LOCATELLI[2,3]**, Simone CARRADORI[2], Andrei M. MOCAN[4], Abdurrahman AKTUMSEK[1]

[1]Selcuk University, Science Faculty, Department of Biology, Konya, Turkey; gokhanzengin@selcuk.edu.tr (*corresponding author); aktumsek@selcuk.edu.tr
[2]University "G. d'Annunzio" of Chieti-Pescara, Department of Pharmacy, 66100, Chieti, Italy; m.locatelli@unich.it (**co-corresponding author); simone.carradori@unich.it
[3]Interuniversity Consortium of Structural and Systems Biology, 00136, Rome, Italy; m.locatelli@unich.it
[4] "Iuliu Hatieganu" University of Medicine and Pharmacy, Department of Pharmaceutical Botany, 8 Victor Babes Street, Cluj-Napoca, Romania; mocan.andrei@umfcluj.ro

**Abstract**

Several bioactive compounds originate from natural sources and their uses are generally related with traditional or folk medicine. Synthetic drugs can have adverse side effects and, for this reason, the investigation of novel, safe, and natural-occurring products can account for the development of new drugs. The genus *Centaurea* L. is one of the most important genera of the Asteraceae family, containing more than 200 species in the Turkish flora, about 140 of which are endemic. The aim of the present work was to determine enzyme inhibitory potentials of two extracts (chloroform and ethyl acetate) from eight *Centaurea* species against cholinesterase, tyrosinase, amylase, and glucosidase. The total phenolics, flavonoids and condensed tannin contents were also reported for each extract. These components in the extracts varied according to species and extraction solvents as well as enzyme inhibitory effects. The highest level of phenolics was found to be in the chloroform extract of *C. pulchella* (119.23 mg GAEs/g extract). Generally, chloroform extracts exhibited stronger enzyme inhibitory effects as compared to ethyl acetate. Additionally, possible correlations with total phenolics, flavonoids, and condensed tannins content were also highlighted. This paper is the first report of the inhibitory capacities of the eight *Centaurea* species on the selected enzymes. The present results may be a valuable starting point in the development of new bioactive formulations.

***Keywords:*** natural products, bioactive compounds, enzyme inhibition, Turkey

## Introduction

Up to date, the search of new biologically active agents from natural sources represents a promising area of investigation. Therefore, a remarkable number of modern drugs (about 40%) derive from plants well known for their uses in traditional or folk medicine (De Monte *et al.*, 2015). This is rooted in the cumulative evidence that connects synthetic drugs with adverse effects such as carcinogenic, hepatotoxic and gastrointestinal disorders (Pan *et al.*, 2013). Thus, the investigation of novel, safely, and natural-derived products can contribute for the development of new drugs in pharmaceutical areas (Locatelli *et al.*, 2012a; Carradori *et al.*, 2014).

The inhibition of key enzymes is one of the most accepted practices for major health problems including Alzheimer's disease (AD) and Diabetes mellitus (DM). According to the actually used therapy, the "inhibition of key enzymes" approach is an effective way for the treatment and management of these pathologies. For example, cholinesterase inhibitors increase brain acetylcholine levels, thus improving cognitive functions in AD patients (Mukherjee *et al.*, 2007). Carbohydrate digestive enzymes (α-amylase and α-glucosidase) inhibitors are important for the management of blood glucose levels in DM patients (Kumar *et al.*, 2013). Therefore, several synthetic inhibitors are used clinically, but they show limited effectiveness and some side effects (Orhan *et al.*, 2004; Tundis *et al.*, 2012, Gidaro *et al.*, 2015). For these reasons, effort has been made in order to screen and develop new inhibitors from natural sources with high efficacy and reduced side effects.

The genus *Centaurea* L. is one of the most important genera of the Asteraceae family, and is represented by approx. 500 species (Garci-Jacas *et al.*, 2000). Turkey flora gathers more than 200 species of which about 140 are considered endemic (Davis, 1988). Members of *Centaurea* have been used in Anatolian traditional medicine for the treatment of various ailments such as diarrhea and wound healing (Karamenderes *et al.*, 2006; Shoeb *et al.*, 2007). The uses of these species in traditional medicine is also highlighted by the high number of studies concerning members of this genus (Zengin *et al.*, 2010; Jemia *et al.*, 2012; Aktumsek *et al.*, 2013; Ertas *et al.*, 2014; Maggio *et al.*, 2014). Many bioactive constituents were identified in these plants such as terpenes, phenolics, coumarins and lignans. However, literature is scarce about their enzyme inhibitory effects (only anticholinesterase) (Aktumsek *et al.*, 2013; Ertas *et al.*, 2014).

Continuing our studies (Genovese *et al.*, 2010; Locatelli *et al.*, 2011; Locatelli *et al.*, 2012b; De Monte *et al.*, 2014; Zengin *et al.*, 2015), and the partial information available in literature

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regarding these species, the aim of this study was to investigate, for first time, the potential correlation between total bioactive components and biological activities on key enzymes involved in AD, DM (type 2), and skin disorders of eight *Centaurea* species from Turkish spontaneous flora.

## Materials and Methods

*Plant material*

Eight *Centaurea* species (1 - *C. depressa*; 2 - *C. drabifolia* subsp. *detonsa*; 3 - *C. kotschyi* var. *persica*; 4 - *C. patula*; 5 - *C. pulchella*; 6 - *C. tchihatcheffi*; 7 - *C. triumfettii*; 8 *C. urvillei* subsp. *hayekiana*) were collected from Konya and Ankara (Golbası), in Turkey. The senior taxonomists Dr. Murad Aydın Sanda and Dr. Tuna Uysal from the Department of Biology (Selcuk University), confirmed the taxonomic identification of the raw plant material, and voucher specimens were deposited in the Herbarium of the Department of Biology (Selcuk University, Konya-Turkey).

*Preparation of the extracts*

Raw collected materials were air-dried at 45 °C (±1 °C) for 48 hours in the dark. In this way, as also reported in literature (Anwar *et al.*, 2013; Khoddami *et al.*, 2013) it is possible to obtain the higher phenolics preservation before solvent extraction.

To produce solvent extracts, air-dried samples (10 g) from the aerial part of *Centaurea* species were macerated with 200 mL of ethyl acetate or chloroform at room temperature (25 °C ± 1 °C) for 24 h in the dark, then the solvents were removed to dryness using a rotary evaporator. Extracts obtained using organic solvents were redissolved in methanol and then filtered. All extracts were stored at -20 °C until analyses.

*Determination of total bioactive components*

*Total phenolics content*

The total phenolics content was determined by employing the methods described in the literature (Slinkard and Singleton, 1977) with slight modifications. Sample solutions (0.25 mL) were mixed with diluted Folin-Ciocâlteu reagent (1 mL, 1:9, *v:v*) and shaken vigorously. After 3 minutes, $Na_2CO_3$ solution (0.75 mL, 1%) was added, and the sample absorbances were read at 760 nm after 2 hours of incubation, at room temperature (RT, 25 °C ± 1 °C). The total phenolics content was expressed as equivalents of gallic acid (GAEs) according to the equation obtained from the standard gallic acid graph.

*Total flavonoids content*

The total flavonoids content was determined using the Dowd method as adapted by Berk *et al.* (2011). Briefly, the sample solutions (1 mL) were mixed with the same volume of aluminum trichloride (2%) in methanol. Similarly, blank samples were prepared by adding sample solutions (1 mL) to methanol (1 mL) without $AlCl_3$. The absorbances of samples and blanks were read at 415 nm after 10 minutes of incubation at RT (25 °C ± 1 °C). The blank sample absorbances were subtracted from the sample, and the total flavonoids content was expressed as equivalents of rutin (REs) according to the equation obtained from the standard rutin graph.

*Total condensed tannins content*

The total condensed tannins content was determined by the vanillin method (Bekir *et al.*, 2013) with slight modifications. Sample solutions (0.5 mL) were mixed with vanillin reagent (1.5 mL, 1% in 7M $H_2SO_4$) in an ice bath and then vigorously shaken. Similarly, blank samples were prepared by adding sample solution (0.5 mL) to 7M $H_2SO_4$ (1.5 mL). The sample and blank absorbances were read at 500 nm after 15 minutes of incubation at RT (25 °C ± 1 °C). The blank sample absorbances were subtracted from the sample, and the total condensed tannins content was expressed as equivalents of (+)-catechin (CEs) according to the equation obtained from the standard (+)-catechin graph.

*Enzyme inhibitory activity*

*Cholinesterase inhibition*

Cholinesterase (ChE) inhibitory activity was measured using Ellman's method, as previously reported (Aktumsek *et al.*, 2013) with slight modifications. Sample solution (2 mg/mL, 50 µL) was mixed with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB, 125 µL) and AChE (or BChE) solution (0.26 u/mL, 25 µL) in Tris-HCl buffer (pH 8.0) in a 96-well microplate and incubated for 15 minutes at 25 °C (± 1 °C). The reaction was started with the addition of acetylthiocholine iodide (ATCI) or butyrylthiocholine chloride (BTCl) (25 µL). Similarly, blank sample was prepared by adding sample solution to all reaction reagents without enzyme (AChE or BChE). The samples and blank absorbances were read at 405 nm after 10 minutes of incubation at 25 °C (± 1 °C). The blank sample absorbance was subtracted from the sample, and the cholinesterase inhibitory activity was calculated according to the following equation:

$$I(\%) = \frac{(A_0 - A_1)}{A_0} \cdot 100 \qquad (1)$$

where $A_0$ is the absorbance of the control, and $A_1$ is the absorbance of the extract/standard (galanthamine).

*Tyrosinase inhibition*

Tyrosinase inhibitory activity was measured using the modified dopachrome method with L-3,4-dihydroxyphenylalanine (L-DOPA) as substrate (Zengin *et al.*, 2014) with slight modifications. Sample solution (2 mg/mL, 25 µL) was mixed with tyrosinase solution (200 u/mL, 40 µL) and phosphate buffer (100 µL, pH 6.8) in a 96-well microplate, and then incubated for 15 minutes at 25 °C (± 1 °C). The reaction was started with the addition of L-DOPA (40 µL). Similarly, a blank sample was prepared by adding sample solution to all reaction reagents without enzyme (tyrosinase). The samples and blank absorbances were read at 492 nm after 10 minutes of incubation at 25 °C (± 1 °C). Kojic acid (0.1 mg/mL) was used as a reference standard. The blank sample absorbance was subtracted from the sample, and the results were reported as percentage inhibition and calculated according to (1) (Table 2).

*α-Amylase inhibition*

The α-amylase inhibitory activity was performed using Caraway-Somogyi iodine/potassium iodide (IKI) method (Zengin *et al.*, 2014) with slight modifications. Sample solutions (2 mg/mL, 25 µL) were mixed with α-amylase solution (10 u/mL, 50 µL) in phosphate buffer (pH 6.9, 6 mM sodium chloride) in a 96-well microplate and incubated for 10 minutes

Table 1. Total bioactive components of the different solvent extracts of *Centaurea* species

| Extracts | Species | Total phenolics (mg GAEs/g extract)[1] | Total flavonoids (mg REs/g extract)[2] | Total condensed tannins (mg CEs/g extract)[3] |
|---|---|---|---|---|
| Ethyl acetate | *C. depressa* | 60.66 ± 8.07$^{c}$ | nd | 7.95 ± 2.48$^{c}$ |
| | *C. drabifolia* subsp. *detonsa* | 67.60 ± 1.48$^{c}$ | 16.61 ± 0.13$^{b}$ | 15.64 ± 0.65$^{b,c}$ |
| | *C. kotschyi* var. *persica* | 98.74 ± 0.31$^{a}$ | 5.76 ± 1.65$^{c}$ | 7.88 ± 0.86$^{c}$ |
| | *C. patula* | 81.00 ± 0.17$^{b}$ | nd | 41.15 ± 1.18$^{a}$ |
| | *C. pulchella* | 48.07 ± 2.31$^{d}$ | nd | 18.46 ± 2.91$^{b}$ |
| | *C. tchihacheffii* | 83.65 ± 0.01$^{b}$ | 3.06 ± 0.55$^{d}$ | 22.57 ± 2.69$^{b}$ |
| | *C. triumfettii* | 90.29 ± 0.65$^{a,b}$ | nd | 22.95 ± 1.51$^{b}$ |
| | *C. urvillei* subsp. *hayekiana* | 100.22 ± 1.44$^{a}$ | 25.57 ± 0.51$^{a}$ | 20.90 ± 3.34$^{b}$ |
| Chloroform | *C. depressa* | 88.77 ± 0.70$^{c}$ | nd | 24.63 ± 1.08$^{c}$ |
| | *C. drabifolia* subsp. *detonsa* | 58.40 ± 0.52$^{e}$ | 1.48 ± 1.02$^{c}$ | 49.22 ± 2.91$^{a}$ |
| | *C. kotschyi* var. *persica* | 104.64 ± 0.70$^{b}$ | nd | 26.08 ± 0.11$^{c}$ |
| | *C. patula* | 91.46 ± 0.92$^{c}$ | 1.87 ± 0.47$^{c}$ | 15.80 ± 2.58$^{d}$ |
| | *C. pulchella* | 119.23 ± 1.44$^{a}$ | nd | 34.76 ± 0.75$^{b}$ |
| | *C. tchihacheffii* | 80.47 ± 1.18$^{d}$ | nd | 17.17 ± 0.43$^{d}$ |
| | *C. triumfettii* | 43.19 ± 1.79$^{f}$ | 6.33 ± 1.78$^{b}$ | 15.26 ± 2.69$^{d}$ |
| | *C. urvillei* subsp. *hayekiana* | 27.36 ± 0.09$^{g}$ | 18.92 ± 0.68$^{a}$ | 5.36 ± 0.97$^{e}$ |

Data represent mean values ± standard deviation (n = 3). In the same extract, data marked with different letters indicate significant difference ($p < 0.05$). [1]GAEs, gallic acid equivalents; [2]REs, rutin equivalents; [3]CEs, catechin equivalents; nd = not detected.

Table 2. Enzyme inhibitory activities of the different solvent extracts of *Centaurea* species (%)

| Extracts | Species | Acetylcholinesterase | Butyrylcholinesterase | Tyrosinase | α-Amylase | α-Glucosidase |
|---|---|---|---|---|---|---|
| | | (2 mg/mL concentration level) | | | | |
| Ethyl acetate | *C. depressa* | 67.79 ± 2.39$^{b,c}$ | 49.15 ± 0.42$^{b,c}$ | 9.45 ± 1.28$^{c}$ | 36.93 ± 0.97$^{b}$ | 46.11 ± 0.97$^{c}$ |
| | *C. drabifolia* subsp. *detonsa* | 69.14 ± 0.09$^{a,b,c}$ | 82.23 ± 0.48$^{a}$ | 15.53 ± 0.23$^{a}$ | 25.58 ± 0.38$^{d}$ | 43.10 ± 2.41$^{c}$ |
| | *C. kotschyi* var. *persica* | 72.18 ± 2.61$^{a,b,c}$ | 59.31 ± 5.74$^{b}$ | 11.53 ± 0.45$^{a,b,c}$ | 36.16 ± 0.13$^{b}$ | 42.35 ± 2.22$^{c}$ |
| | *C. patula* | 68.61 ± 0.09$^{b,c}$ | 51.48 ± 0.80$^{b,c}$ | 15.21 ± 2.04$^{a,b}$ | 31.70 ± 0.04$^{c}$ | 54.88 ± 1.11$^{b}$ |
| | *C. pulchella* | 73.76 ± 1.87$^{a,b}$ | 56.36 ± 5.92$^{b,c}$ | 9.39 ± 0.01$^{c}$ | 21.54 ± 0.04$^{e}$ | 35.59 ± 0.58$^{d}$ |
| | *C. tchihacheffii* | 66.53 ± 1.14$^{c}$ | 46.70 ± 0.16$^{c}$ | 11.26 ± 1.43$^{b,c}$ | 29.89 ± 1.01$^{c}$ | 58.23 ± 0.53$^{b}$ |
| | *C. triumfettii* | 70.15 ± 0.57$^{a,b,c}$ | 50.11 ± 0.05$^{b,c}$ | 11.15 ± 0.23$^{b,c}$ | 42.84 ± 0.34$^{a}$ | 69.88 ± 1.16$^{a}$ |
| | *C. urvillei* subsp. *hayekiana* | 76.08 ± 2.91$^{a}$ | 85.23 ± 0.72$^{a}$ | 10.73 ± 0.98$^{c}$ | 43.20 ± 0.59$^{a}$ | 67.66 ± 0.05$^{a}$ |
| Chloroform | *C. depressa* | 67.27 ± 1.79$^{d}$ | 70.75 ± 2.60$^{b}$ | 12.54 ± 0.08$^{a}$ | 43.97 ± 0.92$^{b}$ | 53.45 ± 1.98$^{b,c}$ |
| | *C. drabifolia* subsp. *detonsa* | 68.88 ± 0.09$^{d}$ | 58.14 ± 0.80$^{d}$ | 10.09 ± 1.43$^{a}$ | 25.28 ± 0.38$^{e}$ | 36.03 ± 0.24$^{e}$ |
| | *C. kotschyi* var. *persica* | 76.98 ± 0.01$^{b}$ | 69.90 ± 1.99$^{b}$ | 10.67 ± 0.15$^{a}$ | 42.72 ± 0.17$^{b}$ | 49.42 ± 0.92$^{c}$ |
| | *C. patula* | 71.95 ± 0.09$^{c}$ | 66.89 ± 0.64$^{b,c}$ | 1.28 ± 1.06$^{c}$ | 33.30 ± 0.04$^{d}$ | 56.11 ± 0.24$^{a,b}$ |
| | *C. pulchella* | 95.93 ± 0.07$^{a}$ | 95.69 ± 0.06$^{a}$ | 4.54 ± 0.68$^{b}$ | 59.54 ± 0.59$^{a}$ | 60.31 ± 2.13$^{a}$ |
| | *C. tchihacheffii* | 72.29 ± 0.38$^{c}$ | 49.58 ± 0.16$^{e}$ | 0.91 ± 0.08$^{c}$ | 40.26 ± 0.29$^{c}$ | 53.45 ± 1.40$^{b,c}$ |
| | *C. triumfettii* | 60.04 ± 0.09$^{e}$ | 63.37 ± 0.37$^{c,d}$ | 4.32 ± 0.38$^{b}$ | 22.40 ± 0.17$^{f}$ | 41.12 ± 0.77$^{d}$ |
| | *C. urvillei* subsp. *hayekiana* | 72.65 ± 0.30$^{c}$ | 43.94 ± 1.51$^{g}$ | na | 17.53 ± 0.08$^{g}$ | 43.65 ± 0.39$^{d}$ |
| Reference Standards | Galanthamine (5 μg/mL) | 78.56 ± 1.05 | 40.87 ± 1.55 | - | - | - |
| | Kojic acid (0.1 mg/mL) | - | - | 59.34 ± 0.60 | - | - |
| | Acarbose (1 mg/mL) | - | - | - | 50.51 ± 0.25 | 44.16 ± 0.34 |

Data represent mean values ± standard deviation (n = 3). In the same extract, data marked with different letters indicate significant difference ($p < 0.05$); na = not active.

at 37 °C (± 1 °C). After pre-incubation, the reaction was started by the addition of starch solution (50 μL, 0.05%). Similarly, blank samples were prepared by adding sample solution to all reaction reagents without the enzyme. The reaction mixture were incubated 10 minutes at 37 °C (± 1 °C). The reaction was then stopped with the addition of HCl (25 μL, 1M) followed by iodine-potassium iodide solution addition (100 μL). The samples and blank absorbances were read at 630 nm. Acarbose (1 mg/mL) was used as a reference standard. The blank sample absorbance was subtracted from the sample, and the results were reported as percentage inhibition and calculated according to (1).

*α-Glucosidase inhibition*

The α-glucosidase inhibitory activity was performed accordingly to a previous published method (Zengin et al., 2014) with slight modification. Sample solution (2 mg/mL, 50 μL) were mixed with glutathione (50 μL), α-glucosidase solution (0.4 u/mL, 50 μL) in phosphate buffer (pH 6.8) and *p*-nitrophenyl-β-D-glucopyranoside (PNPG, 10 mM, 50 μL) in a 96-well microplate and incubated for 15 minutes at 37 °C (± 1 °C). Similarly, blank samples were prepared by adding sample solutions to all reaction reagents without enzyme. The reaction was stopped with the addition of sodium carbonate (50 μL, 0.2 M). The sample and blank absorbances were read at 400 nm. Acarbose was used as a reference standard (1 mg/mL). The blank sample absorbance was subtracted from the sample and the results were reported as percentage inhibition and calculated according to (1) (Table 2).

## Results and Discussion

*Total phenolics, flavonoids, and condensed tannins content*

Phenolic compounds are important plants secondary metabolites and have remarkable biological activities such as anti-oxidant, anti-carcinogenic and anti-inflammatory (Tsao, 2010). Hence, it is important to quantify phenolics content and to assess their contribution to enzyme inhibitory activity. Table 1 shows total phenolics, flavonoids and condensed tannins content of *Centaurea* extracts. The chloroform extract of *C. pulchella* had the highest total phenolics content (119.23 mg GAEs/g extract), followed by the ethyl acetate extracts of *C. urvillei* subsp. *hayekiana,* and *C. kotschyi* var. *persica*. These values are in accordance with those already reported in literature for other *Centaurea* species such as *C. pulchella* (55.00 mg GAEs/g extract by Zengin *et al.* (2010) and, *C. antalyense* (227.12 mg GAEs/g extract by Aktumsek *et al.* (2013)).

The total flavonoids content was measured using the aluminium chloride colorimetric assay. Among *Centaurea* samples, both ethyl acetate and chloroform extracts of *C. urvillei* subsp. *hayekiana* contained the highest concentration of flavonoids with 25.57 and 18.92 mg REs/g extracts, respectively. However, the presence of flavonoids was not detectable in all *Centaurea* extracts (Table 1).

Regarding total condensed tannins content, the chloroform extract of *C. drabifolia* subsp. *detonsa* had the highest total condensed tannins content with a value of 49.22 mg CEs/g extract, followed by the ethyl acetate extract of *C. patula* and the chloroform extract of *C. pulchella* (Table 1).

Ethyl acetate (polarity 6) and chloroform (polarity 4.8) were used in this work as extraction solvents in order to characterize the phenolics extraction yields in less polar solvents, as observed by Anwar *et al.* (2013). These authors reported that extraction yields tend to increase from water (polarity 80) to ethanol (polarity 24), while acetone (polarity 21) and methanol (polarity 33) present similar extraction yields.

*Enzyme inhibitory activities*

AD is a form of dementia, gradually and irreversibly evolving in a loss of memory and other mental abilities (Scarpini *et al.*, 2003). Low levels of acetylcholine in synaptic cleft characterize AD, and in this context, the cholinergic hypothesis is the most accepted strategy for the treatment of AD. Accordingly, the inhibition of cholinesterase, which catalyzes acetylcholine hydrolysis increase the level of acetylcholine in the brain (Murray *et al.*, 2013).

The evaluation of cholinesterase inhibitors is one of the most important topics in pharmaceutical areas and, particularly, in medicinal chemistry. Most drugs commonly used for AD treatment are cholinesterase inhibitors, namely tacrine, galantamine and donezepil. However, they present several side effects such as gastrointestinal disorders (Chopra *et al.*, 2011). In this context, the discovery of new, safe, effective, and naturally occurring cholinesterase inhibitors from plants might be an important strategy for the management of symptoms associated with AD.

In this work, the anticholinesterase activities of *Centaurea* extracts were investigated using Ellman's spectrophotometric method and the results are depicted in Table 2. The chloroform extract of *C. pulchella* exhibited the highest inhibitory activity against both AChE and BChE. This extract had excellent inhibition value on AChE (95.93%) and BChE (95.69%) at the concentration of 2 mg/mL, and had the highest level of phenolics amongst the studied samples. Therefore, phenolic components present in the extract might be responsible for the observed anticholinesterase activity. Several authors reported previously a strong relationship between phenolic content and anticholinesterase activity (Orhan *et al.*, 2012; Hlila *et al.*, 2015). Moreover, anticholinesterase activities of some *Centaurea* species such as *C. antalyense*, *C. balsamita, C. iberica* were also reported recently in literature (Aktumsek *et al.*, 2013; Ertas *et al.*, 2014).

The synthesis and spread use of melanin play a main role in skin color and pigmentation. Tyrosinase, a copper-containing mono-oxygenase, is a key enzyme in melanin biosynthesis (Kim and Uyama, 2005). Skin disorders such as scarce, melasma (facial pigmentation), and freckles are related to excessive melanin biosynthesis. Thus, tyrosinase inhibitors are used to control or treat pigmentation disorders, and are widely used in the cosmetic industry. In fact, some tyrosinase inhibitors such as kojic acid and hydroquinones are nowadays commercially produced, but they can present severe side effects for example skin inflammation (Loizzo *et al.*, 2012). Hence, in recent years, more attention has been paid to the use of natural plant extracts as a safe and alternative source of tyrosinase inhibitors for cosmetic purposes.

*Centaurea* extracts had low tyrosinase inhibitory activities (0.91-15.53%, at the concentration of 2 mg/mL), as reported in Table 2. The ethyl acetate extract of *C. drabifolia* subsp. *detonsa* presented the highest tyrosinase inhibition, with a value of 15.53%. Chloroform extract of *C. urvillei* subsp. *hayekiana* showed no inhibitory effect against tyrosinase. Additionally, chloroform extract of *C. pulchella* had the highest concentration of phenolics, but exhibited lower inhibitory effect on tyrosinase (4.54%). This demonstrates that the phenolic compounds from the tested *Centaurea* samples have none or low inhibitory effects.

DM is one of the most common metabolic diseases and is characterized by insulin secretion problems and defects of carbohydrate, lipid, and protein metabolism. Thus, type 2 DM is generally associated with chronic hyperglycemia. For this reason, the decrease of high blood glucose level is an important factor in development of DM. Two enzymes, namely α-amylase and α-glucosidase, are involved in the hydrolysis of starch and oligosaccharides increasing considerably blood glucose levels. In this sense, amylase and/or glucosidase inhibitors are valuable agents in the treatment and management of hyperglycemia (Exteberria *et al.*, 2012). Synthetic inhibitors, such as acarbose, are widely used as oral anti-diabetic drugs. Nevertheless, they also cause side effects including diarrhea and flatulence (Dong *et al.*, 2012; Saha and Verma, 2012).

α-amylase and α-glucosidase inhibitory effects of *Centaurea* extracts are shown in Table 2.

All *Centaurea* extracts were able to inhibit the activity of both enzymes at the concentration of 2 mg/mL (Table 2). Regarding α-amylase inhibitory effects, the chloroform extract of *C. pulchella* was the most active (59.54%) followed by the chloroform extract of *C. depressa* (43.97%) and the ethyl acetate extract of *C. urvillei* subsp. *hayekiana* (43.20%). As observed for the anti-glucosidase effect, the ranked order of inhibition was: ethyl acetate extract of *C. triumfetti* (69.88%) > ethyl acetate extract of *C. urvillei* subsp. *hayekiana* (67.66%) > chloroform extract of *C. pulchella* (60.31%). However, acarbose (50.51% for α-amylase and 44.16% for α-glucosidase) showed the highest

inhibitory effect already at 1 mg/mL in comparison to the other herein studied *Centaurea* extracts.

## Conclusions

Based on the reported inhibition activities and biologically active compounds (phenolics, flavonoids and condensed tannins), *Centaurea* species can be considered as a valuable starting point in the development of new bioactive formulations or dietary supplements that can support the biological effects of a "classical" therapy for AD or DM disease without the adverse effects generally observed for synthetic drugs. Moreover, evidence that anticholinesterase, α-amylase and α-glucosidase inhibitory activities might be related with the total phenolics content in the studied extracts studied was shown. To the best of our knowledge, this is the first report of the *in vitro* anticholinesterase, tyrosinase, α-amylase and α-glucosidase inhibitory effects of the studied *Centaurea* species. However, further investigations are necessary in order to elucidate the mechanisms of the *in vivo* pharmacological activities, bioavailability and involved metabolic pathways.

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