Journal of Pharmaceutical and Biomedical Analysis 149 (2018) 436–441

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Identification of phenolic components *via* LC–MS analysis and biological activities of two *Centaurea* species: *C. drabifolia* subsp. *drabifolia* and *C. lycopifolia*

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ARTICLE INFO

Article history:
Received 6 October 2017
Received in revised form
15 November 2017
Accepted 16 November 2017
Available online 20 November 2017

Keywords:
Centaurea
Phenolics
Biological effects
Natural bioactive agents
Functional products

ABSTRACT

The *Centaurea* genus has great potential in traditional systems and has attracted much interest in the design of novel drug formulations. The present study was focused on the chemical fingerprints and biological properties of *Centaurea drabifolia* subsp. *drabifolia* and *Centaurea lycopifolia* extracts. Spectrophotometric and LC–MS techniques were used to establish the chemical profiles of the studied extracts. Enzyme inhibitory potential was assessed against key enzymes linked to global health problems, namely neurodegenerative diseases (acetylcholinesterase), pigmentation (tyrosinase), and diabetes (α-amylase and α-glucosidase). The antimicrobial propensities of the extract were evaluated against 16 bacterial and fungal strains using the microdilution method. The antioxidant abilities were assessed using DPPH and ABTS radical scavenging, ferric, and cupric reducing powers, phosphomolybdenum, and ferrous metal chelation. The total phenolic compounds varied from 18.33 to 32.84 mgGAE/g extract. Total flavonoid content of the extracts were in the range of 2.88–22.39 mgRE/g extract. Methanol and water extracts showed stronger antioxidant abilities compared to the ethyl acetate extracts. However, the latter extracts were most efficient towards the target enzymes (except for tyrosinase). The water extracts also exerted considerable antimicrobial effects. Findings from the present work tend to support the idea that *C. drabifolia* subsp. *drabifolia* and *C. lycopifolia* may be utilized as effective bio-resources for designing novel health-promoting products or ingredients. It is anticipated that results amassed from this still will open new avenues for research and contribute towards establishing primary data on these species for designing novel phytopharmaceuticals.

1. Introduction

Since time immemorial, plants or plant products have been widely used by humans for several purposes including foods, drugs, or shelter. This fact has been growing day by day and the last century has witnessed a growing number of herbal consumers and could be considered as "herbal products era" against synthetics. From this point, global awareness on natural products have resulted in a plethora of investigations focused towards the discovery of novel and effective compounds from plants [1,2]. One famous example is the discovery of the antimalarial drugs, Artemisinin isolated from the traditionally used medicinal plant, *Artemisia annua*, which was awarded the 2015 Nobel Prize in Physiology or Medicine [3]. Moreover, several plant species have been suggested as reservoir of potential sources for designing health-promoting food products as witnessed by the growing number of publications in this area [4]. Within the framework of these information, new studies on plants, especially wild food plants are becoming one of the most attractive topics in the scientific area of natural products.

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https://doi.org/10.1016/j.jpba.2017.11.045
0731-7085/

Turkey is famous for its floral diversity, which contains about 12000 plant species. It has been suggested that this biodiversity offers an excellent repertoire of potential new bioactive candidates that remains largely unexplored [5]. The *Centaurea* genus is one of the largest genera in Turkish flora and it is represented by more than 200 species, with an endemism ratio >60%. The members of this genus are known as "Timur dikeni", "peygamber çiceği" or "pıtrak" and they are traditionally used for different purposes, including food, against hemorrhoids, cold, wound healing or gastrointestinal disturbances [6,7]. In this context, there has been an increasing amount of literature concerning chemical characterization and biological effects of different plants from *Centaurea* genus [8]. However, there is still a dearth of knowledge and a pressing need for comprehensive studies focused towards the chemical characterization and biological properties of *C. drabifolia* subp. *drabifolia* and *C. lycopifolia*. Hence, the main objective of this study is to establish the chemical profiles and biological activities of *C. drabifolia* subp. *drabifolia* and *C. lycopifolia* extracts. It is anticipated that results amassed from this study will open new avenues for research and contribute towards establishing primary data on these species for designing novel phytopharmaceuticals.

2. Materials and methods

2.1. Plant materials and extraction procedure

Aerial parts of two *Centaurea* species were collected in June 2015 and the locations are given below. Identification plants were performed by botanist Dr. Murad Aydin Sanda. Voucher specimens of the studied *Centaurea* species were stored at Department of Biology of Selcuk University, Turkey.

Centaurea drabifolia Sm. subsp.drabifolia Sm.

Between Avanos and Kayseri, 38°42′37"N, 34°53′52"E, 992 m.

Centaurea lycopifolia Boiss. et Kotschy

Kahramanmaraş, between Andırın and Kahramanmaraş, 37°33′59"N, 36°33′59"E, 1273 m.

The aerial parts of the selected species were analyzed. First, they were dried in the dark at room temperature. They were then ground to a fine powder using a laboratory mill. Samples (10 g) were stirred with ethyl acetate or methanol at room temperature for 24 h. Then, the extracts were concentrated to dryness using a rotary evaporator. As for the water extracts, 5 g of plant material was boiled in 100 mL water for 30 min. Then, the extracts were lyophilized. The extracts were then stored in dark glass at 4 °C until further analyses.

2.2. Quantification of total phenolics and flavonoids

The total bioactive compounds (phenolics and flavonoids content) of the studied *Centaurea* extracts were obtained using Folin-Ciocalteu and $AlCl_3$ methods, respectively [9]. The contents were expressed as gallic (mg GAEs/g extract) acid and rutin equivalents (mg REs/g extract), respectively.

2.3. Screening for biological activities

Antioxidant potentials, enzyme inhibitory effects, and antimicrobial properties were evaluated for the biological activities of the studied *Centaurea* species. Antioxidant methods used were: free radical scavenging (ABTS and DPPH), ferric (FRAP), and cupric reducing power (CUPRAC); phosphomolybdenum, and metal chelating assays. The antioxidative potentials were expressed as trolox equivalents (EDTA was only used for metal chelating assays). Anti-cholinesterase, anti-tyrosinase, anti-amylase, and anti-glucosidase assays were tested for detecting enzyme inhibitory effects. The enzyme inhibitory effects were evaluated as standard compound equivalents. Briefly, galantamine was used for AChE and BChE, kojic acid was used for tyrosinase, while acarbose was used for α-amylase and α-glucosidase inhibition assay). Antimicrobial evaluation was also performed with some bacteria and fungi strains and all experimental details are as per previous publication [10].

2.4. LC–MS analysis

Protocatechuic (**1**), neochlorogenic = 3-CQA (**2**), chlorogenic = 5-CQA (**3**), caffeic (**5**) acids, orientin (**7**), vitexin (**8**), and apigenin (**12**) were obtained from Extrasynthese (Genay, France). LC–MS analysis was performed using a Thermo Scientific Dionex Ultimate 3000 RSLC (Germering, Germany) consisting of 6-channel degasser SRD-3600, high pressure gradient pump HPG-3400RS, autosampler WPS-3000TRS and column compartment TCC-3000RS coupled to Thermo Scientific Q Exactive Plus (Bremen, Germany). LC–MS conditions were reported in our previous paper [10].

2.5. Statistical analysis

Statistical evaluation was performed using SPPS v. 14.0 program (one-way ANOVA with Tukey's assay) and $p < 0.05$ was considered as significant.

3. Results and discussion

3.1. LC–MS phenolic profiles

LC–MS profile of *Centaurea* extracts revealed the presence of protocatechuic, caffeic, monocaffeoylquinic acids (CQA), monoferuylquinic acid (FQA), and flavonoids (Table 1). Protocatechuic (**1**), 3-CQA (**2**), 5-CQA (**3**), caffeic acids (**5**), orientin (**7**), vitexin (**8**), and apigenin (**12**) were identified by comparison of their retention time (t_R), and LC–MS-MS-fragmentation with standard compounds. All other derivatives were readily distinguished by their MS^2 fragmentation pattern and chromatographic behavior on reverse phase column. Acylquinic acids were identified according to the guide for identification of phenolic acids [11,12] supported by UV λ_{max} and retention time relative to commercially available 3-caffeoylquinic acid (neochlorogenic acid) and 5-caffeoylquinic acid (chlorogenic acid). Patuletin-O-hexoside and hispidulin were identified by comparison of their LC–MS/MS-fragmentation with literature data [13,14]. Both *Centaurea* extracts showed similar phenolic LC–MS profile. However, 1,3-diCQA was found only in *C. drabifolia*, while 1-CQA and vitexin were presented only in *C. lycopifolia* (Fig. 1).

3.2. Total bioactive components and antioxidant capacity

Total phenolic content in the studied *Centaurea* extracts varied from 18.83 to 32.84 mgGAE/g extract (Table 2). The water and methanol extracts contained higher concentration of phenolics compared to ethyl acetate. The highest concentration was detected in CL-Water (32.84 mgGAE/g), followed by CL-MeOH (28.82nmgGAE/g), and CD-MeOH (24.70 mgGAE/g). The methanol extracts contained highest total flavonoids with the following order; CD-MeOH (22.39 mgRE/g) > CL-MeOH (14.10 mgRE/g) >nCL-EA (11.90 mgRE/g) > CD-EA (10.38 mgRE/g). Apparently, flavonoids accounted for more than 60% of total phenolics in the studied methanol extracts. Our findings are in agreement with earlier studies, which also reported higher concentration of total bioactive components in the methanol extracts [15]. In addition, methanol extracts from several *Centaurea* species showed higher concentration of these bioactive components as reported previously [16].

The use of multiple assays has been argued to be useful to highlight the antioxidant propensities of herbal extracts. In this context, the antioxidant profiles of the studied *Centaurea* extracts were