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RESEARCH ARTICLE

Phytochemical investigation of *Cousinia pterocaulos* (C.A. Mey.) Rech. f. (Asteraceae)

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Abstract

Cousinia pterocaulos (C.A. Mey.) Rech. f., is an endemic species of the Asteraceae family in Iran and Transcaucasia, which is naturally distributed in northern regions of Iran. In the current study, we evaluated the composition of essential oil, total flavonoids and phenolic compounds, and free radical scavenging activity of *C. pterocaulos* extract. Plant materials were collected from a natural population of *C. pterocaulos* in Mazandaran, and identified based on description provided in Flora Iranica. Essential oil from aerial parts of this plant was extracted using Cleavinger type apparatus and analyzed its composition using GC and GC/MS apparatus. Moreover, the methanolic extracts from aerial parts were used to determine its total flavonoid and phenolic compounds and also capacity in scavenging the stable DPPH free radicals. The results indicated that total phenolic and flavonoids contents in this species is relatively small compared with other *Cousinia* species. Meanwhile, its capacity in scavenging free radicals is relatively higher than other species that were reported in previous studies. Furthermore, the main essential oil composition was 4,6-Heptadiyn-3-one (43.8%), which has not been detected in other *Cousinia* species. These findings revealed that each *Cousinia* species has a specific phytochemical property, leading to a unique biological activity.

Keywords
Antioxidant activity, *C. pterocaulos*, essential oil, flavonoids, phenolic compounds

Introduction

Medicinal herbs have played an important role in the history of human civilization. These plants have been used as a source of medication, and several new drugs are produced using medicinal plants (Petrovska 2012; Calixto 2019).

The genus *Cousinia* Cass. is one of the largest genera of Asteraceae family with about 600 species (Paşayeva et al. 2020). However, more than 400 species of the genus are naturally distributed in south-west Asia, especially Iran that with more than 200 species poses four biodiversity centers for the genus (López-Vinyallonga et al. 2009). Meanwhile, these species have a restricted distribution pattern compared to other genera of Asteraceae (Rechinger 1986).

C. pterocaulos (C.A. Mey.) Rech. f., is a species of section *Serratuloideae* Bung. that is defined as an endemic species for Iran and Talish. This species is a perennial caespitose plant, with several, thick and branched stems up to 20–45 cm high. The aerial organs of these plants are covered by a tomentose indumentum. Leaves leathery with sparsely tomentose above and densely grey tomentose beneath. Basal leaves are lanceolate up to 6 cm long and 5 cm in width. The head is single and terminal (Rechinger 1986).

Various evaluations are available about the employment of *Cousinia* taxa in traditional medicine. For example, Singh (2012) reported that leaves and roots of *C. thomsonii* C. B. Clarke, are used to treat body pains and swelling in Indian traditional medicine. In traditional Iranian medicine, the aerial parts of *C. microcarpa* Boiss. were used to cure respiratory diseases, toothache, mouth ulcers, and rheumatism (Amiri et al. 2014). Moreover, according to Tuzlac (2011), fresh shoots of *C. eriocephala* Boiss. & Hausskn., are consumed after peeled in Turkey.

Previous evaluations (e.g. Paşayeva et al. 2020) revealed that natural antioxidants can prevent different oxidative stresses related to diseases, such as cancer, rheumatoid arthritis, cardiovascular disease and also neurodegenerative disorders. In addition, because of the wide application, therapeutic efficacy, and low toxicity, increasing attention has been paid to natural compounds to study their antioxidant activities.

The biological activity of each species is directly related to its phytochemical properties, and detection of the chemical composition of secondary metabolites (such as essential oil) or their amounts (total phenolics and flavonoids) is very essential (Noori et al. 2015; Talebi et al. 2019).

Several investigations have been conducted on phytochemical compounds (Marco et al. 1993; Paşayeva et al. 2020), biological activities, including: antimicrobial and cytotoxic (Shahverdi et al. 2007; Iranshahy et al. 2016; Paşayeva et al. 2020), and antioxidant characteristics of different species of *Cousinia*. Meanwhile, there is

no study on the phytochemical properties of *C. pterocaulos*. Therefore, in the current investigation, we studied the phytochemical characteristics of this species in terms of the composition of essential oils and antioxidant activities including total flavonoid and phenolic compounds. This is the first case study of this species.

Material and methods

Plant material

We harvested aerial parts of *C. pterocaulos* from a natural population in Iran (Mazandaran-Lasem) in 2018 (July). Plant samples were identified based on descriptions provided in Flora Iranica (Rechinger 1986), and voucher samples are deposited in the herbarium of Tehran University (no. 48403). The harvested materials were air dried, cut into small parts and ground to coarse powder by a blender.

Preparations of the extracts

100 g of aerial powdered plant materials was extracted with 300 ml of 70% methanol at room temperature for 20 h using maceration. We repeated the process 3 times and the mixed extractions were filtered and evaporated under reduced pressure at 40 °C using a HEIDOLPH rotary evaporator. We dissolved a small part of the methanol extract in distilled water, which was subsequently partitioned with various amounts of water and ethanol to obtain the water sub-extract and the ethanol sub-extract, respectively. Totally four sub-extracts were obtained.

Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity

We measured the capacity of extracts to scavenge stable DPPH free radicals based on the Clarke et al. (2013) method. Three concentrations of the extract were mixed with 60 ml of DPPH solution (Merck) and the plates were kept for 30 min at room temperature in the dark. The absorbance was measured by a Shimadzu UV-Vis spectrophotometer at 517 nm. We used butylhydroxytoluene (BHT) as the standard.

Content of total phenolic

We determined the total phenol content based on the Folin-Ciocalteu method (Clarke et al. 2013). In this regard, we diluted small amounts of each extract in methanol, then mixed them with 5 ml of Folin-Ciocalteu reagent, which was diluted 1/10 with distilled water. After 10 min, this solution was mixed with a 4 ml mg / ml Na_2CO_3 solution, then the entire solution was left for 30 min. We used acid gallic as

the standard and total phenol content was calculated as mg gallic acid equivalents per g of dry extract (mg GAE/g extract).

Total flavonoid content

According to the Yang et al. (2011) method, the total flavonoid content of this extract was calculated by $AlCl_3$. We mixed 100 ml 0.2 mg/ml of ethanol solution of extract with 100 mL of 2% $AlCl_3$ in the plates. After 15 min of incubation at room temperature, we measured the absorbance at 415 nm using a UV-vis Shimadzu spectrometer. We expressed the total flavonoid content as milligrams of equivalent rutin per g dry extract (mg Ru/g extract).

Essential oil extraction

Plant samples in the flowering stage were harvested for phytochemical analysis. We dried the aerial parts of plants at room temperature and subsequently used them for the extraction of essential oils using the hydro-distillation method using a Clevenger-type apparatus. We used a GC and GC–MS apparatus for detecting the composition of essential oil. An Agilent 6890 N GC system was used for GC analysis, which is equipped with a 5975 MSD and an FID, using an HP5 MS column (30 m × 0.5 µm, 0.33 m). The injection volume was 2 µl, and the injector temperature was 200 °C with a 10:1 split ratio. Helium was used as a carrier gas and its column flow rate was 1.5 ml min^{-1} in a constant flow mode. The oven temperature was linearly programmed in the range of 50-100 °C with the step of 5 °C min^{-1}, then increased 100 to 200 °C for 4 min at a rate of 5 °C min^{-1}, then increased 230 to 260 °C for 2 min at a rate of 10 °C min^{-1} and held for 2 min. The retention index of essential oil compounds was detected by two series of n-alkanes: C8–C20 and C21–C40 under the same chromatographic conditions. The constituents of oils were determined by comparing the mass spectra and retention indices with those obtained from authentic samples, the NIST MS Search, Automated Mass Spectral Deconvolution and Identification System (AMDIS) software. The relative percentages of detected components were calculated from the GC peak area.

Statistical analyses

We used SPSS ver.15 for statistical analyzes to determine the mean and standard deviation and compare the differences in values between the standard and experimental group.

Results

Antioxidant activity of plant extract

We used the DPPH test to evaluate the ability of the extract to trap free radicals and antioxidant activity. In this regard, we determined the $IC50$ amount (concentration that a sample is capable of trapping or inhibiting 50% of free radicals), using the logarithmic concentration / response curve. Additionally, the total contents of flavonoids and phenolic compounds were calculated as the main secondary metabolites, which present antioxidant activity.

Total phenolic content

We determined the total phenolic content, according to the concentration of gallic acid. The total phenolic compounds was 29.07± 0.2 µm, which was 0.0105 % of the total extract. The standard curve is presented in Fig. 1.

Total Flavonoid Content

We expressed the content of flavonoids as rutin equivalents (Fig. 2). Total flavonoid/ rutin contents were 26.04 ± 0.6 µm, which were calculated as 0.0047 % of total extract.

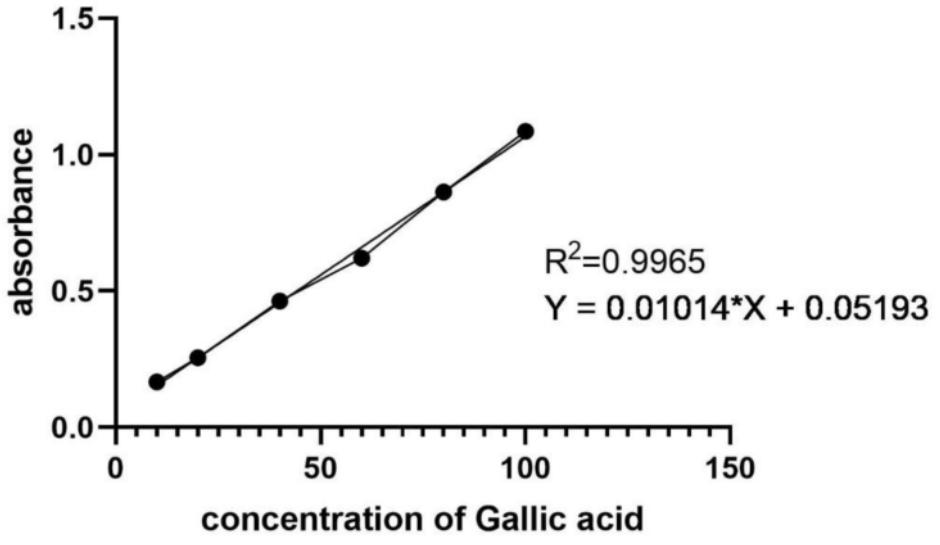

Figure 1. Standard gallic acid standard curve for determination of the total phenolic content.

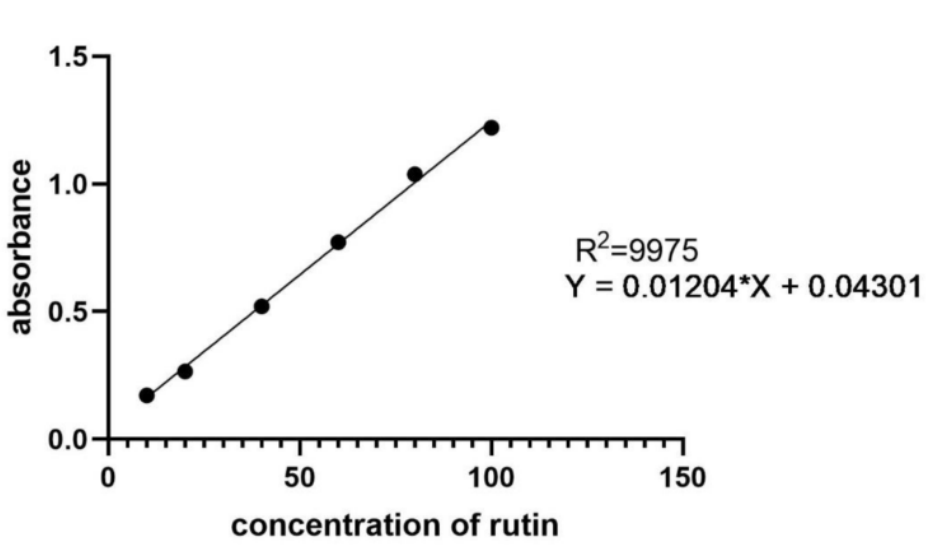

Figure 2. The rutin curve used to determine total flavonoid content in the *C. pterocaulos* extract.

DPPH assay

The logarithmic concentration response curve of the *C. pterocaulos* extract revealed its favorable and dose-dependently effect in the collection and scavenging of free radicals. Each increase in extract concentration increases the percentage of free radical scavenging (Fig. 3). The IC_{50} of this extract was calculated as 47.52.

Essential oil composition

We analyze the extracted essential oil using a GC/MS apparatus and determined the major oil content. In this regard, 4,6-Heptadiyn-3-one was the main content (43.8%). Additionally, oxalic acid and 4-(benzoyloxy)-2H-pyran-3-one were the second (6.2%) and third (5.1%) components, respectively (Table 1).

Table 1. The main compounds of essential oil of *C. pterocaulos*

Compounds	RT (min)	Percentage
4,6-Heptadiyn-3-one	4.0	43.8
Oxalic acid	4.4	6.2
4-(benzoyloxy)-2H-pyran-3-one	5.1	5.1
2-(4α-hydroxybenzyl)-2-methyl-1,3-dithiane	8.3	1.00
3-Methylhomoadamantane	37.1	2.1
Benzyl o-tolyl ether	44.8	3.3

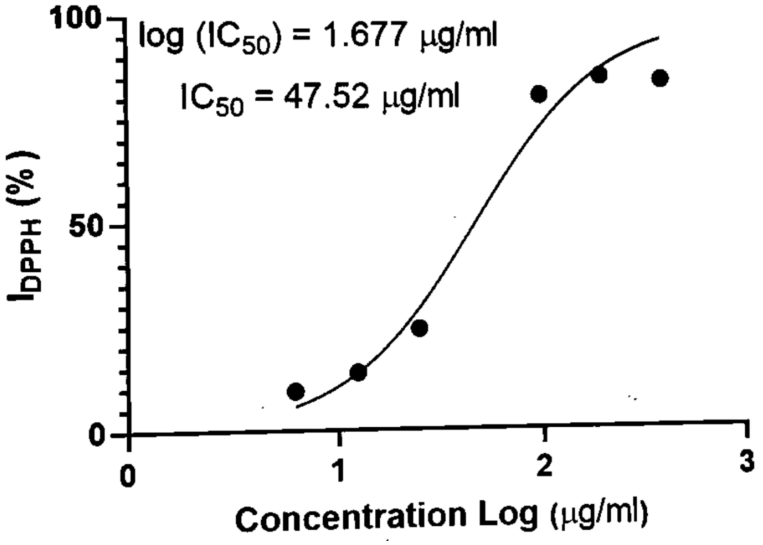

Figure 3. The logarithm-concentration response curve of the extract in the elimination or capture of free radicals.

Discussion

In the current study, we investigated some phytochemical characteristics of *C. pterocaulos* including total flavonoids and phenolic compounds and also essential oil composition. According to previous investigations (Ahmed et al. 2016; Noori and Talebi 2017), flavonoids and phenolic compounds are considered secondary metabolites of plants that have an aromatic ring bearing at least one hydroxyl group. Until now, more than 8000 phenolic compounds were extracted from different plant species. However, the mentioned secondary metabolites are found in medicinal herbs and pose several biological activities such as antioxidant, anti-inflammatory, and also skin protection against UV radiation (Andreu et al. 2018; Meng et al. 2018; Talebi et al. 2017, 2020).

Our findings revealed that the total contents of phenolic and flavonoids in *C. pterocaulos* are relatively lower than those of some other *Cousinia* species. For example, Thirugnanasambandan et al. (2020) determined the total phenolic (133.39) and flavonoids (68.69) contents of *C. azmarensis* that were more than 4 and 2 times higher than our evaluated species. Additionally, the total phenolic and flavonoids content of *C. iconica* were determined by Paşayeva et al. (2020). They observed that the total phenolic content of this species is 9 times higher than *C. pterocaulos*, meanwhile its total flavonoids content is 5 times lower than our examined species.

We used the DPPH method to determine the antioxidant capacity of *C. pterocaulos* extract. Baliyan et al. (2022) suggested that it is a well-known, simple, popular

antioxidant, and rapid and inexpensive method to determine antioxidant capabilities. This method relies on the elimination of DPPH, a stabilized free radical. The antioxidant activity based on DPPH was *IC*50 = 47.52, which was less than those calculated for *C. iconica* (IC_{50}=90.39, see Paşayeva et al. 2020) and *C. azmarensis* (IC_{50}=99.76, see Thirugnanasambandan et al. 2020).

Essential oil is a highly volatile biochemical secondary metabolite that is biosynthesized of different products in cytoplasmic fluid. Essential oils are secreted by glandular trichomes located in aerial parts of plants including leaves, stems, flowers or fruits (Yarmoohammadi et al. 2017; Naeem et al. 2018; Sadgrove et al. 2022).

The essential oil composition of *C. pterocaulos* was determined by GC / MS and 4, 6-Heptadiyn-3-one (43.8%) was detected as the main compound. No similar study is available for this species to compare these results. However, we found some research on essential oil composition of other *Cousinia* species. For example, Tekin et al. (2018) evaluated the essential oil composition of *C. sivasica* Hub.-Mor. and detected hexadecanoic acid (42.8%) as the main compound. Furthermore, the main compositions of essential oil in *C. harzensis* and *C. calocephala* were m-benzyl benzyl alcohol (46.7%) and 3-methyl-tetrahydrofuran (24.6%), respectively (Salimi-Sabour et al. 2021). However, no major nor trace compounds of the essential oil composition of *C. pterocaulos* were found in other *Cousinia* species. These findings revealed that the chemical composition of essential oil in each *Cousinia* species is unique and has a specific biological activity. However, environmental factors highly affect the composition of essential oil (Mahdieh et al. 2018).

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