

Polyphenols and antioxidant capacity of selected Compositae species

Original Article

Abstract:

Species belonging to the Compositae family are known for their rich content of phenolic compounds and flavonoids with notable antioxidant properties. Given the increasing demand for natural antioxidants in various industries, this study aims to evaluate the total phenolic and flavonoid content alongside antioxidant activities of ethanolic extracts of six Compositae species; to identify interspecific differences and highlight their potential for use in pharmaceutical, cosmetic, and food sectors. The investigated species include *Centaurea salonitana*, *C. atropurpurea*, *Achillea atrata*, *A. clavennae*, *Filago arvensis*, and *Xeranthemum annuum*. Phenolic content was quantified utilizing the Folin–Ciocalteu method (GAE), while flavonoid content was measured as rutin equivalent (RuE). The antioxidant activity was determined via the DPPH assay (IC₅₀ values). Significant interspecific variation was observed; notably, *Achillea atrata* exhibited the highest phenolic content, whereas *Centaurea salonitana* was distinguished by its elevated flavonoid content.

Key words:

antioxidant activity, Asteraceae, flavonoids, phenols, secondary metabolites

Apstrakt:

Polifenoli i antioksidativni kapacitet odabranih vrsta iz familije Compositae

Vrste iz familije Compositae poznate su po visokom sadržaju fenolnih jedinjenja i flavonoida, koji poseduju izražena antioksidativna svojstva. Imajući u vidu sve veću potražnju za prirodnim antioksidansima u raznim industrijama, cilj ovog istraživanja je bio da se proceni ukupan sadržaj fenola i flavonoida, kao i antioksidativna aktivnost etanolnih ekstrakata šest vrsta familije Compositae; da se identifikuju interspecijske razlike i istakne njihov potencijal za upotrebu u farmaceutskom, kozmetičkom i prehrambenom sektoru. U okviru istraživanja ispitivani su etanolni ekstrakti sledećih vrsta: *Centaurea salonitana*, *C. atropurpurea*, *Achillea atrata*, *A. clavennae*, *Filago arvensis* i *Xeranthemum annuum*. Ukupan sadržaj fenola određen je primenom Folin–Ciocalteu metode i izražen kao ekvivalent galne kiseline (GAE), dok je sadržaj flavonoida izražen kao ekvivalent rutinu (RuE). Antioksidativna aktivnost određena je DPPH metodom putem IC₅₀ vrednosti. Rezultati su pokazali značajne interspecijske varijacije, pri čemu je *Achillea atrata* imala najviši sadržaj fenolnih jedinjenja, dok se *Centaurea salonitana* istakla visokim sadržajem flavonoida.

Ključne reči:

antioksidativna aktivnost, Asteraceae, flavonoidi, fenoli, sekundarni metaboliti

Introduction

The secondary metabolites produced by plants exhibit a diverse array of biological and pharmacological effects. Among these, phenolic compounds are particularly notable due to their pronounced antioxidant properties, which play a vital role in the prevention of various oxidative stress-related diseases, including cancer (Dai & Mumper, 2010). A highly diverse subset of polyphenolic compounds are the flavonoids, critical in processes

ranging from plant–pathogen interaction, pollination and light screening. Their accumulation is often enhanced in response to biotic and abiotic stressors associated with the increase in reactive oxygen species, emphasizing their protective, antioxidant function. In addition, flavonoids have demonstrated superior antioxidant capabilities *in vitro* compared to well-established antioxidants such as vitamin C and vitamin E, highlighting their potential for applications in products related to health preservation and enhancement (Hernández et al. 2009).

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Research has confirmed the presence of antioxidant polyphenols across various higher plants groups (Lewis, 2017), and a correlation between phenolic content and antioxidant properties across different taxa (Quettier et al. 2000; Piluzza & Bullitta, 2011). Among the representatives of the highly diverse and numerous Compositae family, the presence of phenolic compounds and antioxidant activity has been studied in several *Achillea* species. These studies have shown a correlation between the high content of flavonoids detected in these species and their strong free radical scavenging activity (Tuberoso et al. 2009; Salomon et al. 2021). According to Salomon et al. (2021), the ethyl acetate fraction of the extract from the aerial parts of *A. atrata* L. exhibited higher phenolic content and consequently greater antioxidant activity compared to *A. millefolium* L.

Recently, Buzhala et al. (2022) have examined the total phenolic and flavonoid contents, along with antioxidant activities of several *Centaurea* species from Kosovo. Specifically, *C. atropurpurea* Waldst. & Kit. (synonym for *C. calocephala* Willd.) was found to possess the highest concentrations of these metabolites and exhibited the strongest antioxidant properties. According to Carev et al. (2023), many species of the large and taxonomically complex genus *Centaurea* are used in traditional medicine in Croatia for the treatment of various conditions, including *C. salnitana* Vis. which is known for its anti-inflammatory and wound-healing properties. However, this phytochemical study did not investigate the phenolic and flavonoid content of this species.

Prior research of immortelle (*Xeranthemum annuum* L.) from southern Serbia by Stanković et al. (2011) identified it as a valuable natural source of biologically active compounds due to significant concentrations of polyphenols and flavonoids in extracts from the dried aerial parts, along with notable antioxidant potential. Yet similar investigations into *Filago arvensis* L. remain absent.

All these findings incited this study which endeavors to conduct a comparative analysis of the total phenolic and flavonoid content and antioxidant activity of ethanol extracts from six species representing four genera within the Compositae family that grow wild in Serbia: *Centaurea salnitana* Vis., *C. atropurpurea* Waldst. & Kit., *Achillea atrata* L., *A. clavennae* L., *Filago arvensis* L. and *Xeranthemum annuum* L.

Materials and Methods

Chemicals

Ethanol was purchased from "Zorka Pharma"

(Šabac, Serbia). Standards of phenolic acids (gallic acid) and flavonoids (rutin hydrate), as well as chlorogenic acid and 2,2-diphenyl-1-picrylhydrazyl (DPPH), were obtained from Sigma Chemicals Co. (St. Louis, MO, USA). The Folin-Ciocalteu reagent, 3-tert-butyl-4-hydroxyanisole (BHA), and aluminum chloride (AlCl₃) were sourced from Fluka Chemie AG (Buchs, Switzerland). All other solvents and reagents used in the study were of analytical grade.

Plant material

The plant material was collected from central, southern, and southeastern Serbia, as well as Montenegro. Aerial parts from ten representative individuals per species were selected as plant material. To preserve chemical integrity, these specimens were air-dried in darkness at room temperature and subsequently stored in sealed containers for further analysis. Species identification was verified. Voucher specimens were deposited in the Herbarium of the Department of Biology and Ecology, Faculty of Science, University of Kragujevac, under the following numbers: *Centaurea salnitana* (116/014-1), *C. atropurpurea* (116/014-2), *Achillea atrata* (116/014-3), *A. clavennae* (116/014-4), *Filago arvensis* (116/014-5), and *Xeranthemum annuum* (116/014-6).

Preparation of plant extracts

The air-dried plant material (10 g) was finely ground to obtain powdered material and subsequently extracted using 100 mL of 96% ethanol (plant material to solvent ratio 1:10, w/v) by maceration at room temperature for 24 hours. The mixture was filtered through Whatman No. 1 filter paper, and the residue was re-extracted with an additional 100 mL of the same solvent under the same conditions. This step was repeated after 48 hours to ensure maximal extraction of bioactive compounds. The combined extracts were concentrated to dryness under reduced pressure at 40 °C using a rotary evaporator. Dried extracts were stored in sterile sample tubes at 4 °C until further analysis. For spectrophotometric analysis, the dry extracts were dissolved in methanol to a final concentration of 1 mg/mL.

Determination of total phenolics in the plant extracts

The total phenolic content was determined spectrophotometrically following the method outlined by Singleton et al. (1999). A methanolic extract solution at a content of 1 mg/mL was used for the analysis. The reaction mixture was prepared by combining 0.5 mL of the methanolic plant extract solution, 2 mL of 7.5% sodium bicarbonate (NaHCO₃), and 2.5 mL of 10% Folin-Ciocalteu

reagent dissolved in water. A blank sample was prepared using 0.5 mL of ethanol, 2 mL of 7.5% NaHCO₃, and 2.5 mL of 10% Folin-Ciocalteu reagent. The prepared test tubes were incubated at 45 °C for 45 minutes. Absorbance was measured at 765 nm using a spectrophotometer. All samples were analyzed in triplicate, and the mean absorbance value was recorded. A standard calibration curve was generated using gallic acid solutions, and the total phenolic content of the extracts was quantified based on the measured absorbance. Results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GA/g).

Determination of total flavonoids in the plant extracts

The total flavonoid content in the analyzed herbal extracts was determined spectrophotometrically according to the method of Quettier et al. (2000). The test sample was prepared by mixing 1 mL of a methanolic extract solution (1 mg/mL) with 1 mL of a 2% aluminum chloride (AlCl₃) solution in methanol. The reaction mixture was incubated at room temperature (20°C) for 60 minutes. Absorbance was measured at 415 nm using a spectrophotometer. Each sample was analyzed in triplicate, and the mean absorbance value was recorded. A standard calibration curve was generated using rutin solutions, and the total flavonoid content of the extracts was quantified based on the measured absorbance. Results were expressed as milligrams of rutin equivalents per gram of extract (mg RU/g).

Evaluation of antioxidant activity

The ability of the plant extract to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radicals was assessed using a standard method (Takao et al., 1994). DPPH was dissolved in methanol to a content of 80 µg/mL. The plant extract solution was prepared in methanol at a content of 1 µg/mL, followed by serial dilutions to obtain concentrations of 500, 250,

125, 62.5, 31.25, 15.62, 7.81, 3.90, 1.99, and 0.97 µg/mL. Each diluted solution (1 mL) was mixed with 1 mL of DPPH solution. After incubating the mixtures in the dark at room temperature (20 °C) for 30 minutes, absorbance was measured at 517 nm. The inhibition percentage was calculated using Equation (1), and IC₅₀ values were determined from the % inhibition versus content plot, using nonlinear regression analysis. Data are presented as mean ± standard deviation (n=3).

$$\text{Equation (1): DPPH Scavenging Activity (\%)} = [(A_0 - A_1) / A_0] * 100$$

A₀ is the absorbance of the control (blank) solution, and A₁ is the absorbance of the sample solution, which contains both the antioxidant and DPPH.

Statistical analysis

Experimental measurements were performed in triplicate and presented as the arithmetic mean of the three analyses ± standard deviation. To assess differences in total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AOA) among the six Compositae species, one-way analysis of variance (ANOVA) was conducted. When significant differences were detected (p<0.05), Tukey’s HSD post-hoc test was applied to determine pairwise differences between species. Statistical analysis was performed using the SPSS software package (SPSS for Windows, version XII, 2008, Chicago, IL).

Results and discussion

Total phenolic content

The total phenolic content of ethanolic extracts from the six Compositae species was quantified using a spectrophotometric method with the Folin–Ciocalteu reagent. This method is based on the measurement of the redox potential of phenolic compounds in solution. Upon dissolution, the phenols produce a proton and a phenoxide anion, which subsequently

Table 1. Total amount of phenolic compounds (TPC), total amount of flavonoids (TFC) and total antioxidant activity (AOA) of Compositae species

	TPC	TFC	AOA
<i>Centaurea salonitana</i> Vis.	119.05 ± 9.71 ^{ab}	81.28 ± 4.98 ^b	107.00 ± 9.56 ^b
<i>Centaurea atropurpurea</i> Waldst. & Kit.	97.11 ± 6.52 ^{bc}	75.36 ± 4.55 ^b	101.74 ± 8.52 ^b
<i>Achillea atrata</i> L.	122.32 ± 10.06 ^a	87.95 ± 5.62 ^a	92.66 ± 7.27 ^a
<i>Achillea clavennae</i> L.	100.91 ± 8.21 ^{bc}	55.14 ± 3.21 ^c	131.07 ± 10.34 ^c
<i>Filago arvensis</i> L.	83.56 ± 4.23 ^d	48.23 ± 2.11 ^c	139.21 ± 10.89 ^c
<i>Xeranthemum annuum</i> L.	94.60 ± 5.87 ^c	62.44 ± 2.89 ^c	143.12 ± 12.10 ^c

Values sharing the same letter within a column are not significantly different according to Tukey’s test (p≥0.05)

reduce the Folin–Ciocalteu reagent, resulting in the generation of a blue color. The intensity of this color is proportional to the content of phenolic compounds in the sample. The total phenolic content varied between the tested extracts, with notable differences between the species (**Tab. 1**). The total phenolic content in the ethanolic extracts of the six Compositae species ranged from 83.56 to 122.32 mg of gallic acid equivalents per gram (mg of GA/g). Among the tested species, *Achillea atrata* exhibited the highest content of total phenolics (122.32 mg of GA/g), while *Filago arvensis* displayed the lowest content (83.56 mg of GA/g). The observed variability in the phenolic content across the studied species may be a function of genetic differences, environmental conditions, or developmental stages of the plants.

Based on the observed total phenolic contents, the six species can be classified into three distinct groups. The first group comprised two species with the highest phenolic contents: *Achillea atrata* (122.32 mg of GA/g) and *Centaurea salonitana* (119.05 mg of GA/g), both showing notably high levels of phenolic compounds, indicative of substantial antioxidant potential. The second group includes *Achillea clavennae* (100.91 mg of GA/g) and *Centaurea atropurpurea* (97.11 mg of GA/g) which demonstrated intermediate phenolic levels, which may suggest a moderate potential for antioxidant activity. Finally, the third group includes *Xeranthemum annuum* (94.60 mg of GA/g) and *Filago arvensis* (83.56 mg of GA/g) representing species with the lowest phenolic contents, potentially linked

to a reduced capacity for scavenging free radicals compared to the other species. Phenolic compounds are secondary metabolites found in plants, serving various biological roles, such as protecting against oxidative damage (Dai & Mumper, 2010). These findings emphasize the significant role of phenolic content in the antioxidant potential of different species within the *Compositae* family (**Fig. 1**).

The results of this study indicate that species from both *Achillea* and *Centaurea* genera exhibit a notably high phenolic content consistent with previous research findings. Studies have consistently reported the richness of these genera in phenolic compounds (Tuberoso et al., 2009). Specifically, *Centaurea salonitana* leaves contain 34.4 mg CAE/g dw, while its inflorescences contain 21.9 mg CAE/g dw (Buzhala et al., 2022). A significantly higher content of phenolic compounds was observed in *Centaurea atropurpurea* leaves (218.5 mg CAE/g dw), whereas its inflorescences contained 21.4 mg CAE/g dw (Buzhala et al., 2022). Based on the present study, both *Centaurea salonitana* and *C. atropurpurea* exhibited a notably high phenolic content (119.05 mg GAE/g and 97.11 mg GAE/g respectively), which indicates a significant bioactive potential of these species and place them among the more phenolic-rich members of the genus *Centaurea*. When compared to our other tested species, such as *C. orientalis* (19.38 mg GAE/g), *C. nigra* (12.00 mg GAE/g), and *C. phrygia* (8.08 mg GAE/g), the differences are indicating strong interspecific variation in secondary metabolite content. Previous studies have reported a wide range of phenolic concentrations within

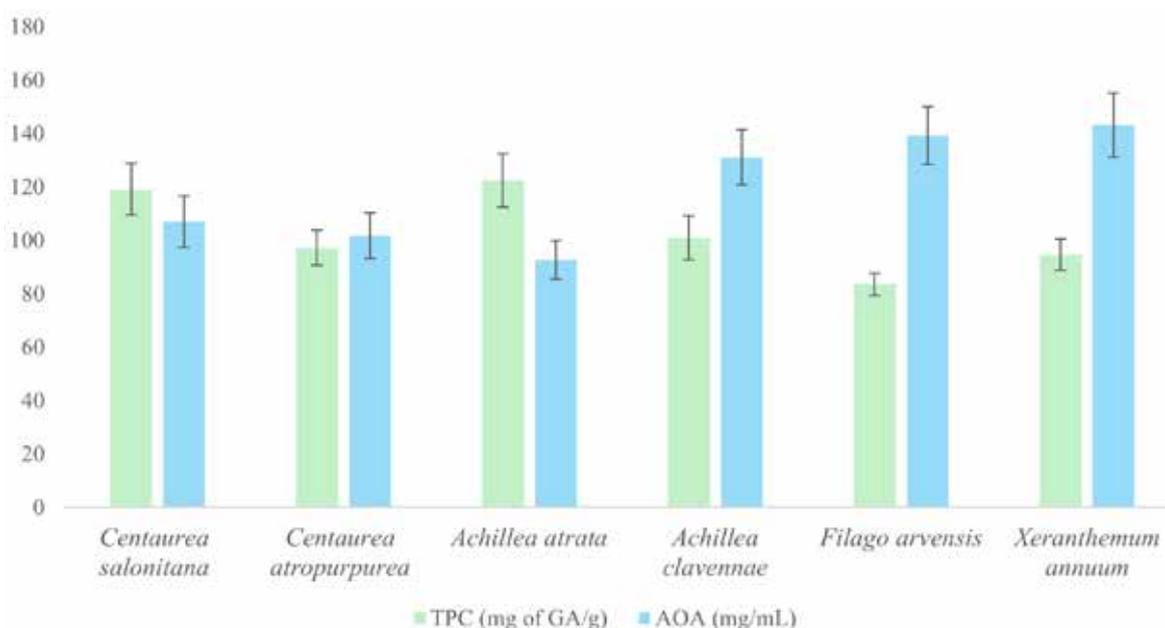


Fig. 1. Comparative bar chart of phenolic content and antioxidant capacity

the genus *Centaurea*, depending on species, plant parts, and extraction methods. Kenny et al. (2014) observed total polyphenol content ranging from 61.7 to 173.2 mg GAE/g in aqueous and ethanolic extracts of the aerial parts of *C. nigra*. Similarly, Albayrak et al. (2017) reported values between 82.27 and 175.40 mg GAE/g for species such as *C. kurdica*, *C. rigida*, *C. amanicola*, *C. cheirolopha*, and *C. ptosimopappoides*. In contrast, lower concentrations were noted in *C. aksoyi* and *C. amaena* (3.28–12.92 mg GAE/g) (Aktumsek et al., 2011). These findings align with the current study, where both species were placed among those with moderate to high phenolic levels. However, differences in absolute values may be attributed to variations in extraction methods, plant part analyzed, or environmental conditions at the time of collection.

Likewise, the high phenolic content observed in *Achillea atrata* in our study (122.32 mg GA/g) confirms earlier findings indicating the presence of bioactive flavonoids such as luteolin, apigenin, centaureidin, and nevadensin in its polar fractions (Salomon et al., 2021). *Achillea clavennae*, with a moderate phenolic level (100.91 mg GA/g), also showed the presence of pharmacologically active sesquiterpenes and monoterpenes, supporting its potential medicinal value. These findings support the significant pharmacological potential of *Achillea* species. In contrast, present research identified lower phenolic contents in *Xeranthemum annuum* and *Filago arvensis*. This finding aligns only partially with the limited existing data: Stanković et al. (2011) reported higher total phenolic contents in *X. annuum* (ranging from 101.33 to 159.48 mg GA/g), which may suggest that extraction methods and analyzed plant parts significantly influence the final phenolic yield.

The differences in phenolic content between these species may be influenced by ecological factors as well, such as habitat, environmental conditions, and seasonal variations, all of which can affect the biosynthesis of secondary metabolites, including phenolic compounds. Gaining insight into the distribution of phenolic compounds in these species is crucial for assessing their pharmacological potential and for their application in herbal medicine and other industries.

Flavonoids content

The content of flavonoids in the ethanolic extracts of the observed six different Compositae species was determined using a spectrophotometric method with AlCl_3 , a process resulting in the formation of metal-flavonoid complexes, which can be measured spectrophotometrically. The total flavonoid content varied among the tested extracts, showing significant

interspecific differences (Tab. 1), and ranged from 48.23 to 87.95 mg of rutin equivalents per gram (mg of Ru/g). The highest content of flavonoids was detected in the extract of *Achillea atrata* (87.95 mg of Ru/g), while *Filago arvensis* showed the lowest content (48.23 mg of Ru/g). These results indicate considerable variation in flavonoid levels across the studied species, which may reflect differences in their biochemical pathways, environmental conditions, or adaptive response strategies.

Based on the observed flavonoid contents, the six species can be classified into three distinct groups. The first group consists of species with the highest contents of flavonoids, notably *Achillea atrata* (87.95 mg of Ru/g). This species stands out due to its relatively high flavonoid content, which may suggest enhanced antioxidant properties. The second group includes species with moderate flavonoid contents: *Centaurea salonitana* (81.28 mg of Ru/g) and *Centaurea atropurpurea* (75.36 mg of Ru/g). These species exhibit intermediate flavonoid levels, which may contribute to moderate antioxidant activities, providing them with some degree of protection against oxidative stress. The third group includes species with the lowest flavonoid contents among the studied species: *Xeranthemum annuum* (62.44 mg of Ru/g), *Achillea clavennae* (55.14 mg of Ru/g), and *Filago arvensis* (48.23 mg of Ru/g). These species show lower levels of flavonoids, which could be associated with a reduced capacity for scavenging free radicals compared to the other species.

In addition to the *Achillea* species, the present study confirms significant flavonoid content in *Centaurea salonitana* and *C. atropurpurea*. The total flavonoid content in *C. salonitana* leaves was 7.3 mg CE/g dw, while in its inflorescence, it reached 4.7 mg CE/g dw (Buzhala et al., 2022). Similarly, *C. atropurpurea* exhibited a high flavonoid content in leaves (56.1 mg CE/g dw) and a moderate level in inflorescences (5.7 mg CE/g dw) (Buzhala et al., 2022). The present study showed that both *Centaurea salonitana* and *C. atropurpurea* exhibited distinctly higher flavonoid contents, reaching 81.28 mg RE/g and 75.36 mg RE/g, respectively. When compared with the results obtained for other *Centaurea* species, these values stand out as exceptionally high. For example, *C. orientalis*, the richest in flavonoids among our previously tested species, contained 40.57 mg QE/g, while *C. nigra* and *C. phrygia* showed significantly lower values of 28.57 mg QE/g and 24.92 mg QE/g, respectively (Albayrak et al., 2017). Even lower flavonoid levels were reported by Albayrak et al. (2017) for *C. aksoyi* (5.83 mg QE/g) and *C. amaena* (7.69 mg QE/g). Additionally, in the study conducted by Aktumsek et al. (2013), where flavonoid content was expressed in rutin

equivalents (RE), aqueous and methanolic extracts of *Centaurea antalyense* yielded 30.76 mg RE/g and 46.76 mg RE/g, respectively. Other studied species, such as *C. polpodiiifolia* var. *pseudobehen* and *C. pyrrhoblephara*, showed values ranging from 40.38 to 45.94 mg RE/g and 29.43 to 55.75 mg RE/g, respectively. Compared to these previously published data, the flavonoid concentrations found in *C. salonitana* and *C. atropurpurea* are notably higher, suggesting that these species possess a particularly rich profile of flavonoid compounds. Moreover, the presence of specific flavonoids such as luteolin, apigenin, and centaureidin was confirmed in *Achillea atrata* and *Achillea clavennae* (Salomon et al., 2021) while *Xeranthemum annuum* was also identified as a source of flavonoids. The flavonoid contents in plant extracts analyzed in this study ranged from 22.25 to 62.42 mg RU/g, further demonstrating the variability in flavonoid content among species (Stanković et al., 2011).

These findings emphasize the significant role of flavonoids in the antioxidant potential of different species within the *Compositae* family. However, further studies are needed to explore the specific flavonoid composition of different *Achillea* species and their individual contributions to antioxidant activity, as well as to assess their bioavailability and long-term efficacy in practical applications.

Antioxidant potential

The antioxidant activity (IC_{50}) of the ethanolic extracts from six different *Compositae* species was assessed and varied from 143.12 to 92.66 $\mu\text{g/mL}$ (Tab. 1). The IC_{50} values indicate the content of the extract required to inhibit 50% of free radical activity. The highest antioxidant activity was observed in *Achillea atrata* (92.66 $\mu\text{g/mL}$), while the lowest antioxidant activity was recorded in *Xeranthemum annuum* (143.12 $\mu\text{g/mL}$). These results demonstrate considerable variation in the antioxidant potential of the studied species, likely influenced by the chemical composition of each species, particularly the presence and content of bioactive compounds such as phenolics and flavonoids.

Based on the observed antioxidant activity values, the six species can be categorized into three distinct groups. The first group includes the species with the highest antioxidant activity, *Achillea atrata* (92.66 $\mu\text{g/mL}$), which stands out for its strong free radical scavenging ability. The second group consists of species such as *Centaurea atropurpurea* (101.74 $\mu\text{g/mL}$) and *Centaurea salonita* (107.00 $\mu\text{g/mL}$) with moderate antioxidant activity, which may indicate a balanced ability to neutralize oxidative stress. The third group comprises species with the lowest antioxidant activity among the studied species,

namely *Achillea clavennae* (131.07 $\mu\text{g/mL}$), *Filago arvensis* (139.21 $\mu\text{g/mL}$), and *Xeranthemum annuum* (143.12 $\mu\text{g/mL}$). These species show relatively lower antioxidant potential, which could be linked to their lower content of antioxidant compounds.

The antioxidant potential of *Achillea atrata* and *A. clavennae*, as assessed using the DPPH assay in our study, revealed IC_{50} values of 92.66 $\mu\text{g/mL}$ and 131.07 $\mu\text{g/mL}$, respectively. These values suggest a relatively moderate capacity for radical scavenging. In comparison to previously published data, significant differences in antioxidant strength become evident. Gharibi et al. (2013) reported much lower IC_{50} values for several *Achillea* species: *A. aucherii* showed the weakest antioxidant activity with an IC_{50} of 844 $\mu\text{g/mL}$, followed by *A. kellalensis* (518 $\mu\text{g/mL}$), and *A. pachycephala*, which demonstrated the strongest activity among them with an IC_{50} of 248 $\mu\text{g/mL}$. These differences may be attributed to variations in extraction methods, the specific plant parts analyzed, ecological factors, or differences in phytochemical composition, particularly in the quantity and type of phenolic compounds and flavonoids. Nevertheless, our results confirm that *Achillea* species exhibit a broad range of antioxidant activities, and the moderate potency observed in *A. atrata* and *A. clavennae* may still hold pharmacological relevance, especially when considered in combination with other bioactive properties.

The antioxidant activity observed in the extracts of *Achillea* and other examined species, reflects the high flavonoid content present in their chemical composition. Flavonoids, a subclass of phenolic compounds, are well-known for their ability to scavenge reactive oxygen species (ROS) due to their phenolic hydroxyl groups. These groups play a key role in the neutralization of free radicals, which contributes to the observed strong antioxidant activity (Nikolić et al., 2024). In particular, the flavonoids in *Achillea* species have been shown to exhibit significant antiradical activity, largely due to their conjugated ring structure and the presence of hydroxyl groups, which facilitate hydrogen donation (Tuberoso et al., 2009). This mechanism of action is further enhanced by the free hydroxyl groups, particularly the 3-OH group, which increases the reactivity of flavonoids with oxidants, stabilizing scavenger molecules and preventing oxidative damage (Hernández et al., 2009).

In *Achillea* species, flavonoids such as apigenin, luteolin, and quercetin, which are frequently found in high contents, are particularly effective in scavenging free radicals. This strong antioxidant activity is a result of their ability to donate hydrogen atoms to reactive oxygen species, thereby stabilizing them and

preventing cellular damage. This dual functionality of flavonoids, both neutralizing free radicals and stabilizing scavenger molecules, contributes to their overall antioxidant efficacy (Tuberoso et al., 2009; Hernández et al., 2009).

The antioxidant capacity assessed by the DPPH assay in our study revealed that *Centaurea salonitana* and *C. atropurpurea* exhibited strong radical scavenging activities, with IC_{50} values of 107.00 $\mu\text{g/mL}$ and 101.74 $\mu\text{g/mL}$, respectively. Although a direct comparison with percentage-based radical scavenging data is limited due to differences in methodology and expression units, the results still highlight a notable contrast in antioxidant strength across species. For example, Salachna et al. (2021) reported that among the analyzed species, *C. orientalis* demonstrated the highest DPPH radical scavenging activity with 39.34%, while *C. nigra* and *C. phrygia* showed substantially lower activities of 10.47% and 9.43%, respectively. Similarly, the ABTS radical scavenging potential was most pronounced in *C. orientalis* (91.69%), followed by *C. nigra* (44.62%) and *C. phrygia* (39.69%). Compared to these values, the antioxidant potential of *C. salonitana* and *C. atropurpurea* appears significantly stronger when interpreted through the lens of their low IC_{50} values (indicating higher activity), which suggests a more efficient free radical neutralization at lower extract concentrations. These findings not only underscore the remarkable antioxidant capacity of species but also correlate with their high flavonoid and polyphenol contents, which are likely key contributors to their bioactivity. Such results highlight their potential pharmacological value and encourage further exploration of their chemical composition and possible therapeutic applications.

Comparatively, species such as *Xeranthemum annuum* and *Filago arvensis*, which have lower phenolic and flavonoid contents, likely exhibit reduced antioxidant potential. The lower flavonoid content in these species suggests that they may not be as effective in neutralizing ROS or stabilizing reactive molecules, thus indicating a potentially lower capacity for antioxidant defense (Nikolić et al., 2024). Stanković et al. (2011) reported that species with lower flavonoid content tend to show reduced antioxidant capacity, supporting our findings that the lower content of antioxidant compounds in these species correlates with a weaker ability to neutralize ROS.

The antioxidant activity of the examined species is closely related to their flavonoid content, which has been well-documented in the literature as a key factor in determining the antioxidant potential of plants (Tuberoso et al., 2009). Our study supports and expands upon this body of work, demonstrating

that flavonoid-rich species, such as *Achillea atrata*, exhibit strong antioxidant activity, while species with lower flavonoid content, like *Filago arvensis* and *Xeranthemum annuum*, have reduced antioxidant capacity. The differences in antioxidant activity between observed species can be attributed to variations in the qualitative and quantitative composition of phenolic compounds and flavonoids, as these are crucial for antioxidant effectiveness. Moreover, environmental factors such as habitat conditions, altitude, soil composition, and exposure to abiotic stressors also play a role in influencing the production of these secondary metabolites. This results in unique antioxidant profiles for each species. However, further studies are necessary to fully understand the phytochemical profiles of these species and their specific roles in mitigating oxidative stress.

Relationship between total phenolic content, total flavonoid content, and antioxidant activity

Pearson correlation analysis was conducted to explore the relationship between total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity (AOA) in the test plants. A strong positive correlation was observed between TPC and TFC (0.869) across all the tested plants, suggesting a significant association between the phenolic and flavonoid content in these species. In contrast, strong negative correlation was found between both TPC and antioxidant activity (AOA) (-0.766) and TFC and antioxidant activity (-0.904) in all the plants. These negative correlations indicate that higher contents of total phenolics and flavonoids do not inherently lead to increased antioxidant activity in this dataset. This outcome is expected, as the IC_{50} values, which represent the content required to inhibit 50% of the antioxidant activity, are inversely proportional to antioxidant potential, implying that a lower IC_{50} indicates higher antioxidant efficiency. Our findings align with previous research, demonstrating a robust correlation between total phenolic content (TPC) and antioxidant activity (Foo et al., 2017). TPC is recognized as a powerful antioxidant, commonly found in a wide range of medicinal plants, as well as fruits and vegetables (Lewis, 2017). The antioxidant potential of phenolic compounds is primarily attributed to their oxidoreduction properties, which allow them to function as hydrogen donors, reducing agents, possible metal chelators, and quenchers of singlet oxygen (Soobrattee et al., 2005). The presence of hydroxyl groups in phenolic compounds enables them to interact with reactive oxygen species (ROS) and reactive nitrogen species, effectively terminating the radical chain reaction and preventing the formation of additional free radicals (Valentão et

al., 2002).

Upon examining the relationship between the content of total phenolic compounds and antioxidant activity, it becomes evident that extracts with higher contents of phenolics tend to exhibit stronger antioxidant activity. This suggests that secondary metabolites, particularly those from the phenolic compound group, play a crucial role in driving the antioxidant properties observed in these plants. Various studies have substantiated the link between the antioxidant activity of plant extracts and their total phenolic content, confirming the significant contribution of phenolic compounds to the overall antioxidant capacity of plants (Piluzza & Bullitta, 2011).

Conclusion

The comprehensive evaluation of total phenolic and flavonoid content, and antioxidant potential of six species from the Compositae family indicates significant variability in the contents of these bioactive compounds among the species, attributable to specific plant adaptations to ecological conditions, suggesting the possibility of chemical diversification depending on the environment. The highest contents of bioactive compounds were recorded in *Achillea atrata*, which stands out as the species with the strongest antioxidant potential, high levels of phenols and flavonoids, making it a potential source for applications in industries dealing with natural products, such as the pharmaceutical, cosmetic, and food industries. On the other hand, species such as *Centaurea salonitana* and *C. atropurpurea* exhibit moderate contents of bioactive compounds, which may also indicate their significance in the prevention of oxidative stress and other potential applications. These results highlight the need for further research that could provide deeper insights into the role of these plants in ecosystems, as well as their potential for the development of new biologically active products. Therefore, the specific adaptations and biochemical profiles of these plants offer invaluable insights for future studies aimed at enhancing the utilization of plant extract in therapeutic and industrial contexts.

This research underscores the potential of the analyzed Compositae species as natural sources of antioxidants, with promising implications for product development in the pharmaceutical, cosmetic, and food sectors.

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