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A Comparative Evaluation of Potential Bioactive Properties and Phenolic Profiles of Five Mediterranean Asteraceae Species

Asuman KARADENİZ-PEKGÖZ[1]✍
[1]Mehmet Akif Ersoy University, Faculty of Science and Art, Department of Biology Burdur/Türkiye
[1]https://orcid.org/0000-0002-8141-7849
✉: asumankaradeniz@gmail.com

ABSTRACT

In recent years, plants with bioactive properties as well as nutritional value have been densely researched. Asteraceae, the most species-rich family of flowering plants, includes numerous wild species most of which are consumed as bioactive compound-rich vegetables and herbal teas. In this study, radical scavenging, antibacterial, and phytotoxic activity as well as phenolic content of some Mediterranean Asteraceae species, *Calendula arvensis, Cichorium intybus* subsp. *intybus, Glebionis coronaria, Scolymus hispanicus*, and *Tragopogon porrifolius* subsp. *longirostris,* were investigated. As a result, *C. intybus, G. coronaria,* and *S. hispanicus* extracts have higher 2,2-diphenyl-1-picril-hydrazyl radical scavenging activity than that of the others ($P<0.05$). The highest superoxide radical scavenging activity and the highest gallic acid equivalent total phenolic content were detected in *G. coronaria* extract. The highest catechin equivalent total flavonoid content was found in *C. intybus* and *S. hispanicus* extracts, respectively. High-performance liquid chromatography analyses showed that the extracts contained various levels of phenolic acids and quercetin. *C. intybus*, *S. hispanicus*, and *T. porrifolius* extracts inhibited the growth of both gram-positive bacteria: *Listeria monocytogenes* and *Staphylococcus aureus* and gram-negative bacteria: *Salmonella enterica* subp. *enterica* and *Escherichia coli*. (Minimal inhibitory concentration = 4 mg mL^{-1}). All the concentrations of gallic acid (as a positive control) and 4 g L^{-1} concentrations of *C. arvensis*, *S. hispanicus* and *T. porrifolius* extracts showed severe inhibition on garden cress (*Lepidium sativum* L.) seedlings.

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Akdeniz Bölgesi'nden Beş Asteraceae Türünün Biyoaktif Potansiyeli ve Fenolik Profili Üzerine Karşılaştırmalı Bir Değerlendirme

ÖZET

Besin değerinin yanısıra biyoaktif özellikleri de olan bitkiler son yıllarda oldukça yoğun araştırılmaktadır. Tür çeşitliliği bakımından çiçekli bitkilerin en zengin familyası olan Asteraceae çoğu sebze ve bitki çayı olarak tüketilen ve biyoaktif bileşiklerce zengin olan birçok yabani tür içerir. Bu çalışmada, *Calendula arvensis, Cichorium intybus* subsp. *intybus, Glebionis coronaria, Scolymus hispanicus* ve *Tragopogon porrifolius* subsp. *longirostris* türlerinin radikal süpürücü, antibakteriyel ve fitotoksik aktivitesi ile fenolik içerikleri araştırıldı. Sonuç olarak *C. intybus, G. coronaria* ve *S. hispanicus* ekstraktları diğerlerinden daha yüksek 2,2-difenil-1-pikril-hidrazil radikali süpürücü aktivite gösterdi ($P<0.05$). En yüksek süperoksit radikali süpürücü aktivite ve gallik aside eşdeğer toplam fenolik madde *G. coronaria* ekstraktında gözlendi. En yüksek kateşine eşdeğer toplam flavonoid miktarı sırasıyla *C. intybus* ve *S. hispanicus* ekstraktlarında gözlendi. Yüksek Basınçlı Sıvı Kromatografi analizleri ekstraktların çeşitli düzeylerde fenolik asitler ve kuersetin içerdiğini gösterdi. *C. intybus*, *S. hispanicus* ve *T. porrifolius* ekstraktları hem gram-pozitif (*Listeria monocytogenes* ve *Staphylococcus aureus*) hem de gram-negatif (*Salmonella enterica* subp. *enterica* ve *Escherichia coli*) bakterilerin büyümesini inhibe etti (Minimal İnhibitör

Botanik

Araştırma Makalesi

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Geliş Tarihi : 05.07.2022
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Anahtar Kelimeler
Antioksidan
Antibakteriyel
Fitotoksik
Asteraceae
Fenolik içeriği

Konsantrasyon = 4 mg mL^{-1}). Gallik asidin (pozitif kontrol) bütün konsantrasyonları ile *C. arvensis*, *S. hispanicus* ve *T. porrifolius*'un 4 g L^{-1} konsantrasyonları tere (*Lepidium sativum* L.) fideleri üzerinde şiddetli inhibisyon gösterdi.

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INTRODUCTION

The last few decades have been the era that plant active metabolites not only shed light on research for new medicinal, agricultural, and industrially important active compounds but also paved the way for many functional food candidates for public health (Shaikh et al., 2016). Plant extracts have been used since ancient times in curing a wide range of diseases such as diabetes, microbial infections, and stress-related disorders, and are evaluated as sources of antioxidants (Rashed et al., 2018; Petropoulos et al., 2019; Rayan et al., 2020). Infectious diseases are leading causes of death in low-income countries, also during the course of the pandemic, COVID-19 has become a significant cause of death in many countries (WHO, 2022). The unceasing production and usage of antibiotics and the rapid spread of new infections worldwide trigger the emergence of multidrug-resistant pathogens (Rashed et al., 2018). These factors have pulsed the science environment to generate new and capable antimicrobial solutions. Return to nature/naturals is the new trend to avoid different disorders among people. The need to control disease-causing microorganisms with new antimicrobials has pushed researchers towards natural products and, over the last few years, herbal sources have emerged as potential candidates (D'Amato et al., 2018).

On the other hand, phytochemicals can be utilized for biopesticide production as a replacement for synthetic chemicals in agricultural production. The term phytotoxicity is usually used for the same meaning as the term allelopathy, as a group of interactive reactions containing both stimulatory and inhibitory influences, among plants or sometimes between higher plants and microbes (Weston & Duke, 2003). Allelopathy majorly takes place in agricultural management like crop re-establishment, crop protection, and weed control. Efforts to attain eco-friendly compounds for weed control in the agroecosystems are the new trend (Scavo et al., 2020).

There is a wide range of wild species in the Mediterranean basin which have been utilized in food and medicine throughout centuries. Asteraceae family, the most species-rich family of flowering plants, includes numerous species yet to rehabilitate and are native to the Mediterranean basin. Most of these wild species are consumed for their fresh and tender leaves in salads (Petropoulos et al., 2019).

In this study, phenolic compounds in the plant extracts which have lots of bioactive properties (Zhang et al., 2019) were considered probable radical scavengers, bacterial growth inhibitors, and phytotoxic chemicals. *Calendula arvensis* (Vaill) L., *Glebionis coronaria* (L.) Spach, *Cichorium intybus* subsp. *intybus* L., *Scolymus hispanicus* L., and *Tragopogon porrifolius* subsp. *longirostris* (Sch. Bip.) Greuter, belongs to the Asteraceae family and the last three are edible and in the same tribe (Cichorieae or Lactuceae). These taxa have some bioactive properties such as antioxidative and antimicrobial. Nevertheless, current literature on their phytotoxicity or potent herbicidal activity is very limited. This study was conducted in order to contribute to the scientific knowledge about the antioxidant, antibacterial, and phytotoxic activities of the edible or traditionally used parts of *C. arvensis, C. intybus, G. coronaria, S. hispanicus,* and *T. porrifolius* grown in the specific Mediterranean regions. As far as we know, this study reports SO RSAs of these plants, also phytotoxicity of current plant extracts on cress (*Lepidium sativum* L.) seedlings and antibacterial effects of *T. porrifolius* on an organism for the first.

MATERIALS AND METHODS

Chemicals and Reagents

Methanol (extra pure), Folin-Ciocalteu phenol reagent, dimethyl sulphoxide (DMSO), and nitroblue tetrazolium (NBT) were supplied from Merck (Germany). Aluminium chloride ($AlCl_3$), sodium nitrite ($NaNO_3$), catechin, sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), L-ascorbic acid, gallic acid and DPPH (2,2-diphenyl-1-picril hydrazyl) from Sigma-Aldrich (Sigma-Aldrich Co. St. Louis). Mueller Hinton II Broth was purchased from Oxoid (Massachusetts-USA).

Plant Material

Traditionally consumed aerial parts of the plants were collected from rocky places in *Quercus coccifera* maquis at Akdeniz University Campus (Antalya/Turkey) area (altitude 10 m) on 5 May 2019. All the plant parts but *S. hispanicus* were stems with

leaves and flowers. *S. hispanicus* was stem with leaves (Figure 1, Table 1). A part of all the plant samples were dried in a cool and shady place and used for the bioactivity assays and other parts were saved as herbarium material. Voucher specimens were coded as MPL201911 (to 15) and deposited in the Medicinal Plants Laboratory of Mehmet Akif Ersoy University. Plant names were confirmed with the sources such as Flora of Turkey and the East Aegean Islands (Davis, 1985) and Plants of the World Online Powo (2022). (Table 1).

Figure 1. Plants used in the study: *Calendula arvensis, Glebionis coronaria, Cichorium intybus* subsp. *intybus*, *Scolymus hispanicus* and *Tragopogon porrifolius* subsp. *longirostris*, from left to right.
Şekil 1. Çalışmada kullanılan bitkiler: Soldan sağa, Calendula arvensis, Glebionis coronaria, Cichorium intybus subsp. intybus, Scolymus hispanicus ve Tragopogon porrifolius subsp. longirostris.

Table 1. Plants used in the study
Çizelge 1. Çalışmada kullanılan bitkiler

Plant *Bitki*	Abbreviation used in the study *Çalışmada kullanılan kısaltması*	English name *İngilizce adı*	Local name *Yerel adı*
Calendula arvensis (Vaill) L.	CA	Field marigold	Aynısafâ
Cichorium intybus subsp. *intybus* L.	CI	Chicory	Hindibâ
Glebionis coronaria (Syn. *Chrysanthemum coronarium*) (L.) Spach	GC	Garland	Sarı papatya
Scolymus hispanicus L.	SH	Golden thistle	Şevketibostan
Tragopogon porrifolius subsp. *longirostris* (Sch. Bip.) Greuter	TL	Purple salsify	Yemlik

Extraction

Air-dried and powdered plant parts were extracted three times with methanol at 40°C by using a magnetic stirrer, for three days. Afterward, the obtained methanolic extracts were filtered and evaporated to obtain crude extracts. Extract yields were calculated by the following formula:

$$Yield(\%) = \frac{\text{weight of crude extract}}{\text{weight of plant powder}} \times 100$$

Distilled water was applied to crude extracts to dissolve. Equal volumes of petroleum ether were used for partitioning each to remove chlorophylls and other lipophilic compounds. Finally, the remaining aqueous extracts were lyophilized (Harput et al., 2012). Water extracts for early seedling growth bioassay were prepared from dried and powdered plants by mixing continuously at room temperature (22°C) for 24 h. Extracts were filtered (Whatman No.1) and used as 0.5, 2, and 4 g dry matter L^{-1} concentrations for the phytotoxicity assay. Methanol extracts were prepared for HPLC analysis, DPPH and SO radical scavenging activity (RSA) tests, total flavonoid and total phenolic contents, and antibacterial activity.

Radical Scavenging Activity Assays

DPPH RSA potentials of the methanolic extracts of the plants were detected according to the method of Blois (1958). Each methanol sample or control of 200 µL was added DPPH, a stable free radical, (50 µL, 1 mM) at certain concentrations and then mixed well. The remaining DPPH had an absorbance value of 517 nm after 30 minutes. The RSA of a negative control well that contained only DPPH and solvent was taken as a comparison to the RSA of the extract. Ascorbic acid was the positive control. All assays were conducted in triplicates. RSA was asserted as percent inhibition and calculated by the following equation:

$$Inhibition\ percentage = \frac{\text{Abs (control)} - \text{Abs (sample)}}{\text{Abs (control)}} \times 100 \quad (1)$$

Inhibitory concentration (IC_{50}) values were also calculated.

Superoxide (SO) RSA was detected via the method of Kunchandy & Rao (1990). In sum, a non-enzymatic system was established to generate superoxide radical. The mixture which included 10 µL of nitroblue tetrazolium (NBT) (1 mg mL^{-1} solution in dimethyl sulphoxide, DMSO) and 30 µL of each sample was dissolved in DMSO. 100 µL of alkaline DMSO (1 mL of which contained 5 mM NaOH in 0.1 mL of water) was put to make a final volume of 140 µL and the absorbance was read at 560 nm. Ascorbic acid was the positive control. All assays were done in triplicates. RSA was asserted as percent inhibition and calculated by the following equation:

$$Inhibition\ percentage = \frac{\text{Abs (sample)} - \text{Abs (control)}}{\text{Abs (sample)}} \times 100 \quad (2)$$

Inhibitory concentration (IC_{50}) values were also calculated.

Antibacterial Activity

The Minimum Inhibitory Concentration (MIC) method using a 96-well plate by Klančnik et al., (2010) was applied to examine antibacterial activities of the extracts on the following gram (+) bacteria: *Listeria monocytogenes* (ATCC 02028) and *Staphylococcus aureus* (ATCC 25923) along with two gram (–) bacteria: *Salmonella enterica* subp. *enterica* (ATCC 700408) and *Escherichia coli* (ATCC 35150). The concentrations that entirely inhibited bacterial growth (MIC) were determined by spectrophotometric absorbance read at 600 nm in a microplate reader to detect microbial growth. Bacterial strain starters were grown overnight, and shaken at 37°C. The inoculum density was regulated to catch 0.5 on the McFarland scale with an interval of 0.080-0.100 optical density at 600 nm. 20 mg mL^{-1} of extract was prepared with DMSO and each well was added 160 µL extract which is serially diluted with Mueller Hinton II Broth. The highest final concentration of the extract was 4 mg mL^{-1}. MIC values were determined via incubation of the bacteria in 96-well microplates for 24 and 48 h at 37°C.

Phytotoxicity Assay: Early Seedling Growth Bioassay

Tests were conducted using 70 mm glass Petri dishes and two layers of filter paper. All materials but seeds were sterilized. Undamaged and almost identically sized cress seeds, (Paşa Seed Company, Balıkesir/Turkey, Cultivar name: Zeybek©) a crop that is sometimes considered a weed (Kadioğlu & Yanar, 2004), were pregerminated in distilled water for 24 h at 25°C at an incubator. 25 pregerminated seeds were laid on the filter paper in each dish which contained 4 mL of filter-sterilized plant extract and gallic acid at 0.5, 2, and 4 g L^{-1} concentrations. Each condition was tested in triplicates. All dishes were incubated in the dark, at 25°C, for 72 h. Blank (distilled water) experiments accompanied every treatment. Seeds were treated with one of the phenolic compounds, responsible for the phytotoxic activity, with gallic acid, also, in this experiment. Seedling (root and shoot) lengths were measured by using a ruler. In each case, the mean number of germinated seeds (N) and mean lengths of roots and shoots (L) were taken.

$$\text{Relative seed germination: } RSG = \frac{N}{\text{Nb}} \times 100 \quad (3)$$

where Nb is the mean number of germinated seeds in the blank.

$$\text{Relative root and shoot growth: } RRG = \frac{L}{\text{Lb}} \times 100 \quad (4)$$

where Lb is the mean length of the seedlings in blank and percent germination indices are calculated as follows:

$$GI = \frac{RSG \times RRG}{100} \quad (5)$$

Phytotoxicity ratings of tested extracts were determined according to the germination index scale proposed by Trautmann & Krasny, (1998) as; GI<40%: severe inhibition, 40%<GI<60%: strong inhibition, 60%<GI<80%: mild inhibition, GI>80%: no inhibition (Pinho et al., 2017).

Total Phenolic and Total Flavonoid Contents

The method of Singleton & Rossi, (1965), and the method of Zhishen et al., (1999)— with minor modifications— were used to detect total phenolic content (TPC) and total flavonoid content (TFC) of the extracts, respectively. For TPC, 10 µL of sample or standard (10-500 µg mL^{-1} gallic acid) plus 150 µL of diluted Folin-Ciocalteu reagent (1:4 reagent/water) and 50 µL of saturated sodium carbonate (7.5%) were added to each well of a 96-well plate and incubated for 2 h at room temperature. The absorbance was read at 725 nm. TPC was expressed as gallic acid equivalent (GAE). For TFC, 10 µL 5% sodium nitrite was added to the 10 µL sample or standard. After 5 min., 10 µL 10% aluminum chloride, 150 µL 1 M sodium hydroxide, and 50 µL water were added. The plate was mixed well. Then the absorbance was read at 510 nm. Methanol was used as the control. TFC was expressed as catechin equivalent (2-250 µg CE mL^{-1}).

HPLC Analysis

HPLC analysis of phenolic compounds (benzoic acid, caffeic acid, ferulic acid, p-coumaric acid, vanillic acid, and quercetin) were done at Scientific and Technology Application and Research Center of Mehmet Akif Ersoy University. System: Shimadzu Prominence, CBM: 20ACBM, Detector: DAD (SPD-M20A), Pomp: LC20 AT, Column Oven: CTO-10ASVp, Autosampler: SIL 20ACHT, Computer Programme: LC Solution, Mobile Phase A: 3% Formic acid, Mobile Phase B: Methanol. The elution gradient was applied at a flow rate of 1 mL min^{-1}. was: 95%A/5%B for 3 min., 80%A/20%B for 2 min., 60%A/40%B for 10 min.,

50%A/50%B for 10 min., 100%B for 10 min. until the end of the run. 10 µL methanol samples were injected into the column (Caponio et al., 1999). Benzoic acid LOD: 0.03 ppm, 270 nm, RT: 34 min., caffeic acid LOD: 0.01 ppm, 280 nm, RT: 22.7 min., p-coumaric acid LOD: 0.01 ppm, 320 nm, RT: 26.1 min., ferulic acid LOD: 0.01 ppm, 320 nm, RT: 30.1 min., quercetin LOD: 0.01 ppm, 360 nm, RT: 70.4 min., vanillic acid LOD: 0.11 ppm, 320 nm, RT: 19.2 min.

Statistical Analysis

One-way analysis of variance followed by Tukey HSD test was used to evaluate differences among groups in DPPH and SO RSA, TPC, and TFC tests. Non-normal data which also weren't suitable for transformation were treated with the Kruskal-Wallis test. The significance level was set at $P<0.05$. IBM SPSS Statistics software version 22 was used for statistical analysis.

RESULTS AND DISCUSSION

Radical Scavenging Activity and Phenolic Contents

Radical scavenging activities of the plant extracts (yields as %; 18.33, 10.25, 31.51, 16.28 and 17.10 for CA, GC, CI, SH and TL, respectively) were evaluated by DPPH and SO RSA. DPPH radical is a rare type of stable organic nitrogen radical that has a deep purple color. DPPH RSA assay is based on the reducing ability of antioxidants towards DPPH. Antioxidant ability can be seen in the decrease of its absorbance (Brand-Williams et al., 1995). On the other hand, the nitroblue tetrazolium (NBT) assay tests whether the extracts scavenge SO anions. Alkaline DMSO, used as an SO generating system, reacts with NBT to give colored diformazan. Following the DPPH RSA of ascorbic acid which was the positive control, activities of SH, GC, and CI extracts (IC_{50} values; 124.28, 136.87, and 140.20 µg mL^{-1}, respectively) were higher than the others ($P<0.05$). The lowest IC_{50} means the highest RSA. It shows the extract is effective in lower concentrations (a concentration in which the extract can scavenge 50% of the radical). The highest SO RSA was detected in GC extract. The IC_{50} values of DPPH and SO RSA were shown in Table 2. The highest gallic acid equivalent TPC was determined in GC extract (114.25 µg GAE mL^{-1}) followed by CI and TL extracts (82.92 and 76.51 GAE mL^{-1}, respectively).

Table 2. DPPH and SO radical scavenging activity (IC_{50} values) and phenolic profiles of the plant extracts
Çizelge 2. Bitki ekstraktlarının DPPH ve SO radikali süpürücü aktiviteleri (IC_{50} değerleri) ve fenolik profili

	Antioxidant activity[m] *Antioksidan aktivite[m]*				HPLC analysis (µg mL^{-1}) *YBSK analizi (µg mL^{-1})*					
PE BE	DPPH RSA (µg mL^{-1})	SO RSA (µg mL^{-1})	TPC (µg GAE mL^{-1}) *TFİ(µg GAE mL^{-1})*	TFC (µgCE mL^{-1}) *TFLİ (µg KE mL^{-1})*	Vanillic acid	Caffeic acid	p-Coumaric acid	Ferulic acid	Benzoic acid	Quercetin
CA	274.97±3.94a	59.60±7.65abc	66.97±2.44c	22.16±5.01b	2.221	27.00	0.016	0.058	21.30	4.044
GC	136.85±2.41c	36.91±13.10a	114.25±8.24a	30.50±4.66b	0.314	3.787	0.467	0.020	0.49	0.515
CI	140.23±4.66c	70.94±2.13bc	82.92±4.88b	54.16±8.47a	2.138	9.238	0.004	0.107	0.22	10.08
SH	124.28±0.06c	63.46±5.05abc	30.97±0.79d	48.50±8.78a	0.914	11.36	0.372	0.011	nd	1.237
TL	182.17±14.72b	90.35±25.01c	76.51±4.98b	27.50±3.43b	0.702	4.950	0.099	0.059	0.006	7.142
AA	15.89±2.10d	49.08±5.30ab								

m: Means of three replicates±SD, different letters show significant differences ($P<0.05$), PE: Plant extract, RSA: radical scavenging activity, TPC: total phenolic content, TFC: Total flavonoid content, GAE: gallic acid equivalent, CE: catechin equivalent, nd: not determined, AA:Ascorbic acid

m: Üç tekrarın ortalaması±SS, farklı harfler istatistiksel olarak önemli farklılıkları gösterir ($P<0.05$), BE: Bitki ekstraktı, RSA: radikal süpürücü aktivite, TFİ: toplam fenolik içeriği, TFLİ: toplam flavonoid içeriği, GAE: gallik aside eşdeğer, KE: kateşine eşdeğer, nd: belirlenemedi, AA: askorbik asit

The highest catechin equivalent TFC was found in CI and SH extracts, (54.16 and 48.50 µg CE mL^{-1}, respectively) (Table 2). The highest quercetin content was also found besides vanillic and ferulic acids in CI extract. Higher both TFC (catechin equivalent) and quercetin show that *C. intybus* is rich in flavonoids. Moreover, flavonoids are chemosystematic markers in the Cichorieae tribe (Sareedenchai & Zidorn, 2010). *C. intybus* contains several important metabolites such as 3-o-caffeoylquinic acid (Shilpa and Lakshmi 2019), hydroxycinnamic acids, including chlorogenic and cichoric acid (Sinkovič et al., 2015), and flavonoids (Dalar & Konczak, 2014; Papetti et al., 2017) that are responsible for bioactivity.

Antibacterial Activity

CI, SH, and TL extracts inhibited growth of both gram (-) (*E. coli* and *S. enterica*) and gram (+) (*S. aureus* and *L. monocytogenes*) bacteria (Minimal inhibitory concentration (MIC) = 4 mg mL^{-1}) in this study (Figures 2, 3 and 4). CA and GC extracts were weak to inhibit bacterial growth in 4 mg mL^{-1} concentrations, although they are rich in phenolic substances (Table 2). There are some reports on the antibacterial activity of both methanol and other solvent extracts such as chloroform or petroleum ether of *C. arvensis* and *G. coronaria*. For example, Tosun et al., (2012) found that essential oil and methanolic extract of *C. arvensis* showed moderate antibacterial activities against *S. aureus*. It contains sesquiterpene and flavonol glycosides, triterpene saponins and alcohols, flavonoids, carotenoids, xanthophylls, phenolic acids and is antibacterial on *Bacillus subtilis*, *E. coli*, and *S. aureus* (Kemper, 1999). The minimum concentration of inhibition for leaf extract of *C. arvensis* was determined as 2 µg mL^{-1} in chloroform against *K.*

pneumoniae and *E. coli* and in petroleum ether against *E.* coli (Jamal et al., 2014). *G. coronaria* has also an inhibitory effect on bacterial growth. For example, non-polar extract of *G. coronaria* has an antibacterial effect on *Staphylococcus mutans* at IC_{50} of less than 20 ppm concentration, besides a moderate value of DPPH RSA (EC_{50}=587 ppm) (Rayan et al., 2020). Antibacterial effects of *G. coronaria* essential oil were demonstrated against the gram-positive strains while it failed to inhibit gram-negative bacterial growth (Bardaweel et al., 2015). Caffeoylquinic acids are reported as the major component of *G.* coronaria (Lai et al., 2007; Wan et al., 2017).

As seen in the literature, *C. arvensis*, *G. coronaria*, *C. intybus* and *S. hispanicus* have certain DPPH RSAs. Besides its RSA activity, *C. intybus* has antibacterial activity on both our tested gram (+) and gram (-) bacteria, as well. There are some studies exhibiting the antibacterial activity of chicory. Its root and aerial extracts showed antibacterial activity with the disc diffusion method on both gram (-) and gram (+) bacteria. The ethyl acetate extract was the most active (Petrovic et al., 2004). Shaikh et al., (2016) determined potential activities of *C. intybus* on *Pseudomonas aeruginosa*, *B. subtilis, S. aureus, S. epidermidis*. Its seed extracts showed to have MIC values below 0.1 mg mL^{-1} against the pathogenic microorganisms tested, including *S. aureus*, *P. aeruginosa*, and *E.* coli. Ethyl acetate and ethanol extract were found considerably responsive to *S. aureus* and *P. aeruginosa*. In another study, *C. intybus* MIC was 16 to 256 µg mL^{-1} against *E. coli* CCM 3988, *S. enterica* CCM 3807, *Yersinia enterocolitica* CCM 5671, and three gram (+)

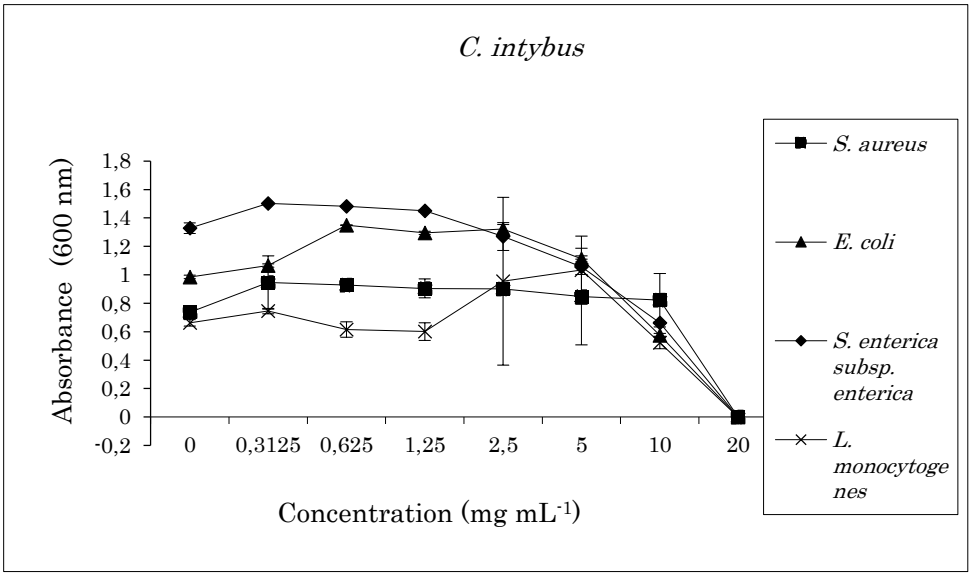

Figure 2. MIC graphic of *Cichorium intybus* subsp. *intybus* extract.
Şekil 2. Cichorium intybus subsp. intybus ekstraktının MİK grafiği

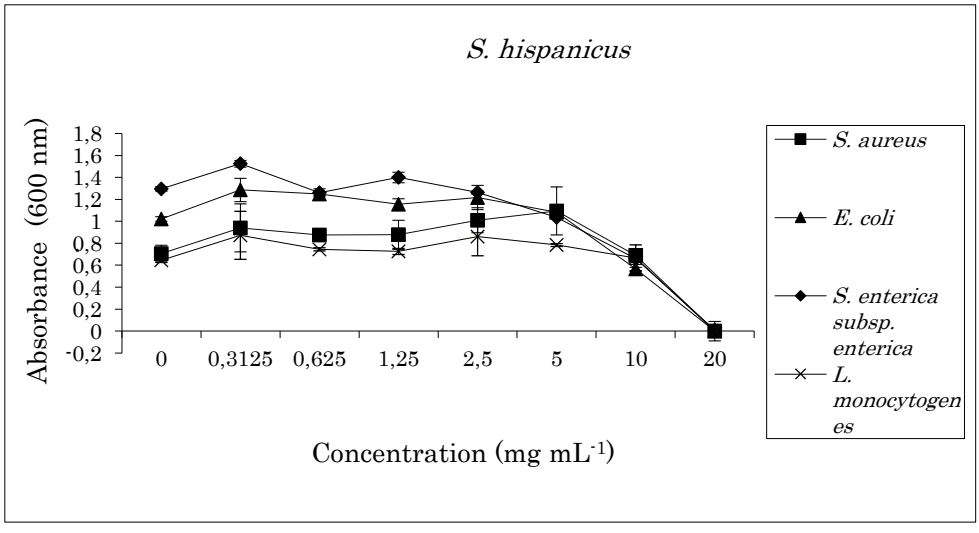

Figure 3. MIC graphic of *Scolymus hispanicus* extract
Şekil 3. Scolymus hispanicus ekstraktının MİK grafiği

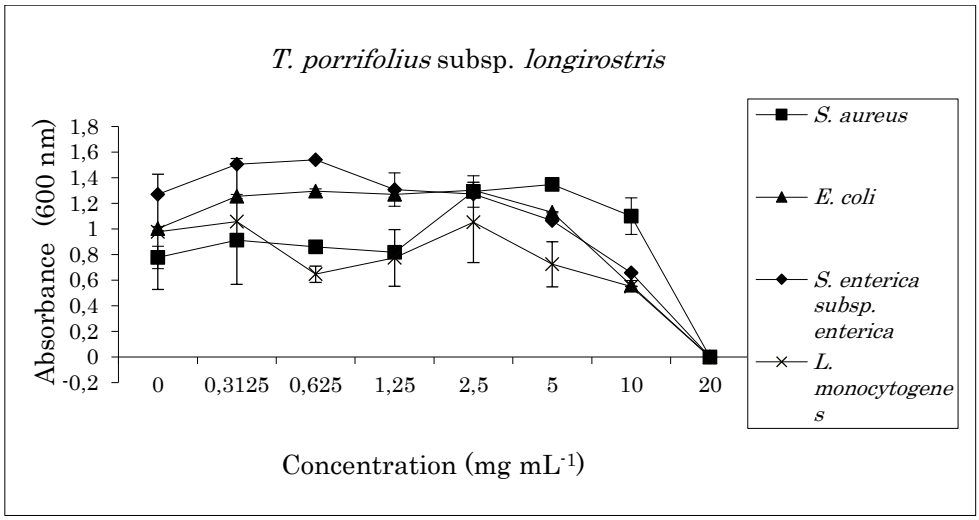

Figure 4. MIC graphic of *Tragopogon porrifolius* subsp. *longirostris* extract
Şekil 4. Tragopogon porrifolius subsp. longirostris ekstraktının MİK grafiği

bacteria: *Bacillus thuringiensis* CCM 19, *L. monocytogenes* CCM 4699, *S. aureus* CCM 2461 (Rashed et al., 2018). The whole plant of *C. intybus* is active against *Enterococcus faecalis*, with MIC values of 13.01 µg mL^{-1} and 6.50 µg mL^{-1}, respectively (Gür et al., 2017). Antifungal activities of *C. intybus* have also been reported (Abbas et al., 2014; Rehman et al., 2014; Shaikh et al., 2016; Gür et al., 2017). HPLC/DAD/MS analysis identified some hydroxycinnamic acids and flavonoids (quercetin, kaempferol, luteolin, and apigenin glycosides) in chicory (Heimler et al., 2009) and chicoric acid is its main component (Heimler et al., 2009; Tardugno et al., 2018). Although folkloric use of chicory has a rich historical background just a few of its constituents have been studied in terms of pharmacological potential. Data on the toxicity of *C. intybus* is currently limited (Mahadeva Rao et al., 2020).

One of our tested plants that has antibacterial activity is *S. hispanicus.* Marmouzi et al., (2017) reported that its roots showed the most promising antibacterial activity on *E. coli* CIP 53126 at a MIC value of 1.56 mg mL^{-1}. Furthermore, flowers exhibited the highest activity on *S. aureus* CIP 483 (3.12 mg mL^{-1}) and *B. subtilis* CIP 5262 (1.56 mg mL^{-1}) while its leaves displayed better activity (MIC=1.56 mg mL^{-1}) against *S. enterica* CIP 8039 and *P. aeruginosa* CIP 82118. On the other hand, lipophilic metabolites (at 100 µM concentration) (Kandil et al., 2020) of pollen extracts of *S. hispanicus* have DPPH RSA as IC_{50}=0.78 mg mL^{-1} (Bakour et al., 2020).

Tested plants which belong to the tribe Cichorieae, have antibacterial effects with MIC at 4 mg mL^{-1} concentration, all of our tested plants have RSA and phenolic content at different levels. All the plants we studied were collected from the same area and at the almost same time on the same day. Most of the tested plants are edible both in Turkey and European countries and have some medicinal properties (Lai et al., 2007; Marmouzi et al., 2017; Tardugno et al., 2018; Abdalla & Zidorn, 2020). In studies conducted on medicinal plants, it's pointed out that the geographical origin of the variety and the harvest season have influences on the chemical composition (Dehkordi et al., 2010). On the other hand, different functional groups of the compounds may cause different bioactive effects.

Phytotoxic Activity

The phytotoxic effects of the extracts on seedling growth of cress seeds were expressed by germination indices. Germination indices as a percentage of cress seeds treated with plant extracts are given in Table 3. Kruskal Wallis test showed that germination indices of plant extracts are significantly different ($P<0.05$). According to the germination index scale of Trautmann & Krasny, (1998) all the concentrations of gallic acid and 4 g L^{-1} concentrations of CA, SH, and, TL extracts showed severe inhibition. Strong inhibition was observed in the 4 g L^{-1} concentrations of CI and GC extracts and 2 g L^{-1} concentrations of CI, SH, and TL. 2 g L^{-1} concentrations of CA and GC extracts and 0.5 g L^{-1} concentrations of CA and SH extracts showed mild inhibition while 0.5 g L^{-1} concentrations of CI, GC, and TL extracts showed no inhibition (Table 3). The germination index can be greater than 100%, in cases that the extract enhances the germination and/or the radicle growth rather than impairing it (Trautmann & Krasny, 1998; Begum et al., 2019). Seedling growth enhanced by GC extract at 0.5 g L^{-1} concentration was observed. Similarly, Chon et al., (2003) found that the root length of alfalfa was scaled up by 13–33% when treated with extracts of *Bidens frondosa*, *Breea segeta, Chrysanthemum indicum,* and *Youngia sonchifolia*, at concentrations below 20 g dry matter L^{-1}.

Table 3. Germination indices of cress seeds treated with the plant extracts.
Çizelge 3. Bitki ekstraktları uygulanan tere tohumlarının çimlenme indisleri.

Plant extract	Germination Index (%)* *Çimlenme İndisi (%)**		
Bitki ekstraktı	0.5 g L^{-1}	2 g L^{-1}	4 g L^{-1}
CA	76.42±3.75efgh	60.04±3.40cdef	6.45±1.05ab
GC	111.38±6.04h	60.50±3.58cdefg	40.38±3.20c
CI	87.52±4.40gh	49.89±3.52cde	43.70±2.70c
SH	64.09±3.54defg	45.76±2.69cde	9.14±1.27b
TL	92.65±4.21fgh	43.69±2.28cd	18.16±2.78b
GA	38.10±3.00c	9.63±1.30b	2.69±0.20a

*: Means±SE, Different letters indicates significant differences (P<0.05) from Kruskal-Wallis test Stepwise Step-down multiple comparisons.
GA:Gallic acid
**:ortalama±SH, Farklı harfler Kruskal-Wallis, ardışık basamaklı çoklu karşılaştırma testine göre istatistiksel olarak önemli farklılıkları gösterir (P<0.05), GA: gallik asit*

HPLC analysis (Table 2) showed that well ahead the highest caffeic, vanillic and benzoic acids were found in CA extract which has shown the most severe inhibition following gallic acid at 4 g L^{-1} concentration. This suggests causative phytotoxic chemicals could be caffeic, vanillic, and benzoic acids in this plant. Activities of caffeic acid (Batish et al., 2008; Li et al., 2017), and benzoic acid (Zhu et al., 2017) are known. Some studies report the phytotoxicity of *C. arvensis* extracts. For example, Ullah et al., (2012) found that the methanolic extract of *C. arvensis* has toxic potential against *Lemna minor* at 1000 µg mL^{-1} (1 mg mL^{-1}). Not only phenolic compounds but also essential oils can cause phytotoxicity. For example, *C. intybus* oil showed significant phytotoxic activity (61.12% inhibition against *Lemna minor* at a high dose such as 1000 µg mL^{-1}) (Shah et al., 2012). *C. arvensis* extract showed 20%, 50%, and 60% inhibitions on maize, sunflower, and wheat seeds respectively (Khan et al., 2012). Methanol extracts of *C. arvensis* flowers showed 19% DPPH radical inhibition at 250 µg mL^{-1} concentration (Ercetin et al., 2012). On the other hand, about 45% DPPH radical inhibition at 250 µg mL^{-1} concentration of CA extract was found in our study. Possible reasons for this difference can be explained by localities, parts of the plants used, and methodological details. Faustino et al., (2018) identified *C. arvensis* as smart food or natural medicine because of its phenolic acids, flavonoids, and saponins in its aerial parts, detected by UHPLC-MS/MS.

The highest p-coumaric acid was found in GC and SH extracts, respectively. SH extract showed severe inhibition like CA extract at the highest concentration (4 g L^{-1}). Catechin equivalent TFC and the highest DPPH RSA were also found in SH extract (Table 2). *S. hispanicus* is rich in phenolics and flavonoids and has antioxidant (Tabaraki et al., 2013), and phytotoxic (Qasem, 2017) activity. It showed severe toxicity on cress seeds at 4 g L^{-1} concentration in our study. No data was found on the phytotoxic activity of *T. longirostris* despite containing flavonoids, terpenoids, bibenzyl derivatives, benzyl phtalides, stilbenes, dihydro isocoumarin derivatives, phenylmethane derivatives, hydroxy phenylacetic acid derivatives, esters of phenylpropanoic acids, phenylpropane derivatives, spermine derivatives, and coumarin derivatives (Abdalla & Zidorn, 2020). Among the plants we focus on, exhibiting inhibition, there are reports about their phytotoxic impacts on *Lemna minor* (Ullah et al., 2012) or some other seeds or seedlings of plants like sunflower (Ercetin et al., 2012). However, no study has been found about the inhibitory effects of these plant extracts on cress seedlings.

General knowledge about secondary metabolites from commercially interesting Cichorieae genera, including *C. intybus, S. hispanicus,* and *T. porrifolius* taxa, is satisfying. However, most of the other genera of this tribe had not been studied phytochemically at all according to Zidorn (2008). A total of 135 various flavonoid compounds were detected in 354 taxa of 299 species, including many cultivars of common vegetables like chicory and lettuce of the Cichorieae (Lactuceae) tribe (Sareedenchai & Zidorn, 2010). The highest TFC was detected in CI and SH extracts (Table 2), both belonging to the tribe Cichorieae. DPPH RSA of GC extract was not significantly different from that of CI and SH. TPC of GC was also statistically higher than the others (P<0.05). GC extract positively affected the seedling growth of cress seeds contrarily to the others at 0.5 and 2 g L^{-1} concentrations. Probably because of methodological differences or because different compounds are responsible for the phytotoxic activity. *G. coronaria* was reported to have phytotoxic activity, in the literature. Its extract inhibited root and shoot elongations of *Echinochloa crus-galli*, one of the worst weeds, 21%, and 6.3%, respectively at 1000 µg mL^{-1} concentration (Abdelgaleil et al., 2020), and it has a high DPPH RSA and allelopathic activity on the seed germination and seedling growth of two annual weeds (*Sinapis arvensis* and *Phalaris canariensis*) and two crops (*Triticum durum* and *Zea mays*) (Hosni et al., 2013). Caffeoylquinic acids are the major components

of *G. coronaria* (Wan et al., 2017). *C. intybus* root extracts have also a potential for use as bioherbicides (Wang et al., 2011). *S. hispanicus* has phytotoxic activity, too (Zidorn, 2008).

Chon et al., (2005) informed of the most prominent phytotoxic compounds via HPLC as coumarin, *trans*-cinnamic acid, *o*-coumaric acid, *p*-coumaric acid, and chlorogenic acid. Pinho et al., (2017) states that increasing -OH and -OCH_3 groups in the molecule seem to reduce phytotoxicity. Lipophilic phenolics appear to be the most causative chemicals for phytotoxicity. They tested the phytotoxicity of gallic acid, protocatechuic acid, cinnamic acid, syringic acid, 3,4,5-trimethoxybenzoic acid, 4-hydroxybenzoic acid, vanillic acid, p-coumaric acid, caffeic acid, veratric acid, and phenol and they found that cinnamic acid is the most phytotoxic because of its hydrophobicity. Further possible phytotoxic properties of hydrophobic fractions of these plant extracts should be investigated. Phenolic acids are often assumed as phytochemicals in the literature and maybe they are the most widely investigated compound type among the proposed ones (Abdelgaleil et al., 2020).

CONCLUSION

Asteraceae species we studied exhibit phytotoxicity changing from none to severe and including phenolics at various levels. We concluded that these plants have different bioactive properties depending on their phenolic variation. Furthermore, this study is the first to report in the literature on the SO RSA of phytotoxicity of *T. porrifolius* as far as is known. Assaying the bioactivity of polar and apolar fractions of plant extracts will amplify results as well as assaying plant parts separately. The phytotoxicity of these extracts at different concentrations on different weed seeds should be researched as well. Field trials will also contribute to the topic besides laboratory experiments. Briefly, the plants we studied, must be taken into account since they not only bear the potential of phytotoxicity but also are antibacterial agents.

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Contribution of the Authors as Summary

Authors declares the contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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