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Research Article

Biological activities of different plant species belonging to the Asteraceae family

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Abstract: *Achillea biebersteinii* and *Anthemis tinctoria*, which are widely distributed species of the Asteraceae family, are used in folk medicine in the form of herbal tea or extract in the treatment of many diseases. The aim of this study was to investigate the chemical content, antimicrobial, antioxidant, enzyme inhibitor activities and cytotoxic effects of 80% ethanol extract of these two species and make a comparative analysis. In accordance with the data obtained, the major component of *A. biebersteinii* was determined as Cyclododecane (14.47%), while that of *A. tinctoria* was determined as Phytol (23.15%). *A. biebersteinii*, which showed moderate activity in terms of antimicrobial activity, produced more active inhibition than *A. tinctoria* did. Both plants showed high levels of antioxidant activity. The total phenol and total flavonoid contents of *A. tinctoria* were higher than those of *A. biebersteinii*. It was determined that there was no significant activity when the extracts were compared with galanthamine, which is the reference drug in terms of enzyme inhibitory activity. When the *in vitro* anticancer activity of human breast cancer cell line was examined, it was determined that *A. tinctoria* had a cytotoxic effect at high concentrations (IC_{50};0.82mg/mL), and *A. biebersteinii* showed strong cytotoxicity at all concentrations (IC_{50};<0.0625mg/mL). These two plants of the same family were evaluated in terms of many different biological parameters and it was revealed that *A. biebersteinii* was more active than *A. tinctoria*. However, in vivo studies are needed to determine whether these plants can be used as phytotherapeutic agents.

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1. INTRODUCTION

The Asteraceae family has a wide distribution, especially in the Caucasus, Central Asia and Europe, with 1100 genus and approximately 25000 species worldwide. In Turkey, although most are endemic, there are 152 genus and 1230 species of this family (Yıldırımlı, 1999). Many species of this family are both edible and can be used as medicinal plants, as they generally

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have some content rich in secondary metabolites such as essential oil compounds, sesquiterpenes and flavonoids. Due to this richness of content, it is used in medicinal herbal teas and standardized extracts as an antitussive, anti-inflammatory, and sedative, as well as in skin care and cosmetic products (Li *et al*., 2013; Butala *et al*., 2021). Furthermore, in vitro studies have reported that species belonging to this family are potential therapeutic agents for various tumorigenesis (El Omari *et al*., 2021).

The antimicrobial activity of edible plants may play an active role in reducing antibiotic use or inhibiting specific pathogens (Dzoyem *et al*.,2013; Panda *et al*.,2019). Oxidative stress has a complex effect on metabolism, and there can be said to be multiple variables that affect physiological reactions in this process. The type of oxidant, density, and interaction with antioxidant compounds may cause different results on metabolic activities (Baroni *et al*., 2021). Antioxidants acting in the defense mechanism of cells may not be sufficient to suppress free radicals, and when a pathological result occurs, exogenous antioxidants may have a positive effect on the healing process. In such a case, antioxidant-rich medicinal plants can be used to strengthen metabolic defense (Li *et al*., 2014; Xu *et al*., 2017; Yu *et al*., 2021).

The two best-known genera of the Asteraceae family are *Achillea* and *Anthemis*. Many species in these two genera are actively used in folk medicine (Nemeth & Bernath, 2008). In particular, the antioxidant and antimicrobial effects of different species belonging to these genera have been shown in many studies (Candan *et al*., 2003; Kotan *et al*., 2010; Varasteh-Kojourian *et al*., 2017; Elshamy *et al*., 2021; Hoi *et al*.,2021).) In addition, phytochemical studies have reported that these plants have antioxidant, antiproliferative, antidiabetic, antiprotozoal, and antispasmodic potential (De Mieri *et al*., 2017; Şabanoğlu *et al*., 2019).

In this study, chemical compound analysis, antioxidant, antimicrobial activities and antiproliferative effects of *Achillea biebersteinii* and *Anthemis tinctoria* from the Asteraceae family growing in the flora of Turkey were investigated. It is expected that the obtained data will add positive value to phytotherapeutic studies.

2. MATERIAL and METHODS

2.1. Plant Material and Extract Preparation

The aerial parts of the plants in full flowering periods were collected. *A. tinctoria* was collected from Yozgat-Akdağmadeni (39°39`N; 35°,52` E, 866 m) and *A. biebersteinii* was collected from Yozgat-Gedikhasanlı (39°,35` N; 35°,9` E, 1128 m) in June 2017. The collected fresh aerial parts were dried at room temperature. The plants were identified in Yozgat Bozok University Biology Department.

After the collected plant samples (leaves) were dried and ground, 10 grams of samples were taken and dissolved in 80% ethanol. GC-MS was utilized to determine the ingredients and relative percentages of the extracts that were filter-dried in a rotary evaporator (Sacchetti *et al*., 2005). Helium gas was used as the carrier gas at a constant flow rate of 1.5 ml per minute. In splitless mode, the injection volume of 1 µl was designed to be 5 per minute between 80-300 and was set at 300°C for 2 minutes after the run. Total running time was 1 hour (Eruygur & Dural, 2019). The chemical content of the extract acquired from the dried leaves was researched through different libraries (W9N11.L, NIST05a.L and wiley7n.I).

2.2. Specification of Total Phenolic Content

The total phenol content of the plant extracts was determined through reaction with Folin-Ciocalteu (F-C) reagent. Extracts diluted first with DMSO were mixed with distilled water and diluted F-C reagent. After waiting for 5 minutes, 7.5% Na_2CO_3 was suffixed and incubated for 1 hour and finally absorbance was measured at 650 nm. DMSO (blank) and Gallic acid in DMSO (reference) were also run in parallel.

2.3. Evaluation of Total Flavonoid Content

The total flavonoid content in the plant extracts was defined through the aluminum chloride colorimetric assay. It was calibrated by preparing serial dilution solutions. The reagent (150 µL, 0.3 mg/mL) prepared with ethanol was mixed with 2% $AlCl_3$ on a microplate. The absorbance value of the solution, which was kept at 22°C for 15 min, was measured at 435 nm. Then, the total flavonoid contents of the extracts were expressed as mg of quercetin equivalent on their dry weight.

2.4. DPPH Assay

The antioxidant activity of the extracts was expressed as a percentage and the procedure was performed in accordance with the DPPH free radical assay as stated by Eruygur *et al.* (2019). The DPPH solution was freshly prepared by dissolving in ethanol. 20 µL of the plant extracts dissolved in DMSO were mixed with 180 µL of DPPH solution (40 µg/mL) in a 96-well plate. After the well plates were left in the dark for fifteen minutes, absorbance levels were measured at 540 nm on a spectrophotometer. DMSO and gallic acid were accepted as control and standard, respectively. The experiments were repeated three times and the results were evaluated by calculating the standard deviation (SD).

2.5. ABTS Radical Scavenging Assay

Test samples and ABTS radical stock solution were prepared in accordance with the DPPH method and diluted (7 mM ABTS, 140 mM potassium persulfate) just before analysis, then stored at room temperature. The ABTS working solution was prepared fresh by diluting with ethanol, the absorbance value was measured at 734 nm and the value was taken as 0.70 ± 0.02. On a microplate, 50 µL of sample solution at a concentration of 0-1 mg/mL was mixed with 100 µL of ABTS working solution, the mixture was incubated for 10 minutes at room temperature, and then the absorbance was read at 734 nm. ABTS scavenging activity was evaluated using gallic acid as antioxidant standards.

2.6. Antineurodegenerative Activities

Acetylcholinesterase (AChE), butyrylcholinesterase (BChE) and also tyrosinase inhibitory activity assays were performed according to the protocol of Eruygur *et al.* (2019) using a microtiter plate according to the spectrophotometric method. The applied extract concentration was 2 mg/ml and galantamine and kojic acid were accepted as positive controls. The absorbance was determined with an Epoch microplate reader. The results are shown as percentage inhibition of the samples compared to the controls.

2.7. Antimicrobial Activity

Using the microdilution method of Eloff (1998), the Minimum Inhibitory Concentration (MIC) values of the ethanol extracts obtained from the plants used in the study on predetermined bacteria and fungi were determined. *Candida albicans* (ATCC 10231), *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 29213), *Pseudomonas aeruginosa* (ATCC 27853) and *Enterococcus faecalis* (ATCC 29212) strains were used as microbial agents in this study. The extract was dissolved with 8% DMSO. Then, 10 µl of sample was added to the first line of the microtiter plate diluted with 90 µl of broth. In the second line, 50 µL of sample was added and two-fold serial dilution was made with the broth. The concentration of the plant extracts in the well after the application was 5.00-0.002 mg/ml. The final inoculum size was 0.5-2.5×103 CFU/mL in Candida and 5×105 CFU/mL in bacteria per well. Candida culture was diluted with Mueller Hinton Broth (Accumix®) and bacterial culture was diluted with Sabouraud Dextrose Broth (Himedia ME033). 50 µL of fungal and bacterial suspension were added to the prepared samples and incubated for 16-24 hours at 35°C and 37°C, respectively. To indicate growth, 50 µL (2 mg/mL) of 2,3,5-Triphenyltetrazolium chloride (TTC) (Merck, Germany) was added to

each well. Microtiter plates were also incubated for 2 hours at 37°C. The decrease in the intensity of the red color of formazan at the end of this period was accepted as the MIC value. The experiment was repeated in duplicate and the standard deviation was recorded as zero.

2.8. Cell Viability Assay

Human breast cancer cell line, MDA-MB-231 and mouse fibroblast cell line, L929 were obtained from the ATCC (USA) and maintained in DMEM supplemented with 100 U/mL penicillin, 10% fetal bovine serum and 100 µg/mL streptomycin. The cell viability of the 80% ethanol extracts of *A. bieberstenii* and *A. tinctoria* was assessed using the XTT assay. In short, exponentially growing cells were seeded in 96-well plates and cultured until 50% confluence. Various concentrations of the plant extracts were then added and the final concentration in each well was 0.0625, 0.125, 0.25, 0.5, 1 mg/mL, respectively for 24 hours. Untreated and 0.5%-DMSO-treated cells were designated as negative and solvent control, respectively. At the end of exposure, the detection of viable cells was performed by adding 50 µL of XTT labeling mix to each well and re-incubating at 37°C for 4 hours. The absorbance of each well was measured by a microplate reader (Thermo, Germany) at 450 nm versus the control. The cell proliferation was calculated as % relative to the control (100% viability).

2.9. Statistical Analysis

The results of the biological activity analysis repeated three times were expressed as mean ± standard deviation values. Statistical evaluation of the obtained data was performed with Graphpad 6.0 software.

3. RESULTS and DISCUSSION

3.1. GC-MS Analysis of the Extracts

In this study, the chemical components of 80% ethanol extracts of *A. biebersteinii* and *A.tinctoria* were evaluated using GS-MS (Table 1).

Table 1. Chemical components of 80% ethanol extracts of *A. biebersteinii and A. tinctoria.*

Chemical Components	RT	Relative Content (%)	
		A.biebersteinii	*A. tinctoria*
Pyrrolidine, N-(3-methyl-3-butenyl)	12.917	1.27	
1,4:3,6-Dianhydro-.alpha.-d-glucopyranose	18.673		1.31
1-Dodecanol (CAS)	28.258	3.87	2.34
Phenol, 2,4-bis(1,1-dimethylethyl)	29.379		1.44
(-)-Caryophyllene oxide	31.222	0.96	
Maltoxazine	32.618	1.72	
Cyclododecane	33.465	14.47	7.08
(-)-Loliolide	35.130	2.29	1.25
Quinoline, 3-(methylthio)-	36.228		1.40
Hexadecanoic acid (CAS)	38.397		2.00
Hexadecanoic acid, ethyl ester (CA	38.844	6.12	4.43
Phytol (CAS)	40.732	4.72	23.15
9-Octadecenoic acid, (E)-	41.224		11.38
Ethyl linoleate	41.447	3.49	
Ethyl Oleate	41.544		3.82
Isosteviol methyl ester	45.945	3.30	2.90
Methyl steviol	46.048	11.86	3.17
TOTAL		54.07	65.67

According to the data obtained, "Cyclododecane" was the main component for *A.biebersteinii* at 14.47%, followed by "Methyl steviol" at 11.86%. The main component of *A. tinctoria* was determined as "Phytol" at 23.15%, followed by "9-Octadecenoic acid" at 11.38%. It was observed that the number of components determined at the beginning of flowering was higher than the value obtained from the plants harvested during the full flowering period. Sevindik *et al*. (2018) determined the main components of the same plant to be 1,8-cineole (20.36%) and cyclohexanone (8.39%). Emir & Emir (2020) identified morin and quercetin (1598.4 µg/g, 1416.0 µg/g, respectively) as the main components of flavonoids from *A. tinctoria*. As the phenolic contents may vary when the plants are collected from different localities, the seasonal and growing conditions should be evaluated.

3.2. Antioxidant Activity

The studied extracts exhibited ABTS scavenging activity near to that of the positive control gallic acid and it was dose-dependent in all species (Figure 1). The IC_{50} value calculated for the extracts was 18.06 ± 0.01 µg/mL for *A. biebersteinii* and 6.37 ± 0.01 µg/mL for *A. tinctoria.* As seen in Figure 1, ABTS radical scavenging activity of the extracts was close to that of the reference drug gallic acid (IC_{50}: 0.39 ± 0.01 µg/mL). The DPPH radical scavenging activity IC_{50} value calculated for the extracts was 502.9 ± 0.009 µg/mL for *A. biebersteinii* and 399.04 ± 0.02 µg/mL for *A. Tinctoria* which was lower than that of gallic acid (IC_{50}: 29.11 ± 1.76 µg/mL) (Figure 2). In a previous study, the leaf and plant methanol extracts of *Achillea sivasica* showed strong activity with IC_{50} values of 0.12 µg/mL and 0.22 µg/mL when tested for DPPH free radical scavenging activity (Haliloglu *et al*., 2017). In another study, the *Achillea millefolium* extracts represented moderate DPPH radical scavenging activities with SC_{50} values of 0.266 ± 0.003 mg/mL for methanol extract and 0.495±0.005 mg/mL for ethanol extract (Barut *et al*., 2017).

The antioxidant activity values of 80% ethanol extracts of *A. biebersteinii, A. tinctoria* were analyzed and evaluated using DPPH and ABTS methods. According to the data obtained, it can be said that *A.biebersteinii* and *A.tinctoria* (IC_{50} value: 18.06 ± 0.008 µg/mL, IC_{50} value: 6.37 ± 0.009 µg/mL, respectively) have a high level of antioxidant activity, especially for ABTS radical scavenging activity. According to the DPPH test, when the extract values and gallic acid values were compared, the antioxidant activity values were seen to be moderate. The antioxidant activity of *A. tinctoria* with DPPH and CUPRAC methods was investigated by Emir & Emir (2020) and IC_{50} values were found to be 302.18 ± 2.74 and 514.62 ± 3.82 mg TE/g, respectively. Varasteh-kojourian *et al*. (2017) investigated the antioxidant activity of *A. biebersteinii* by preparing ethanol and methanol extracts from different parts (inflorescence, stem, leaves and aerial parts) of the plant, and reported that the methanol leaf extract of the plant was significantly higher (0.0276 ± 0.003 mg/ml for DPPH, 0.16 ± 0.016 mg/ml for BCB and 13.96 ± 0.26 mg/ml for TBARS IC_{50}s) than that of the other extracts. Sabanoğlu *et al*. (2019) compared two different *Achillea* species and found that the radical scavenging activity of *A. biebersteinii* was considerably higher than that of *A.wilhelmsii*. The results of the different studies demonstrated that both species, especially the methanol extract, show high antioxidant activity. The current study with ethanol extract of the same species showed similar results to previous studies and showed high radical scavenging activity.

Figure 1. ABTS radical scavenging activity of *A. biebersteinii* and *A. tinctoria* ethanol extracts.

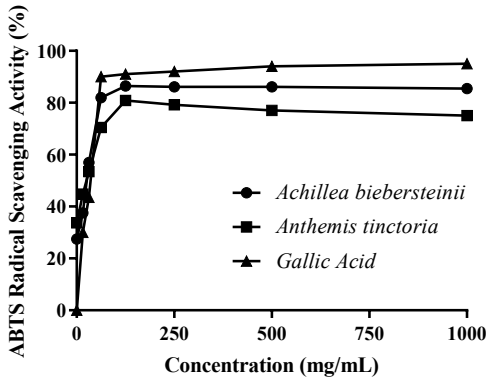


Figure 2. DPPH radical scavenging activity of *A. biebersteinii* and *A. tinctoria* ethanol extracts and positive drug gallic acid.

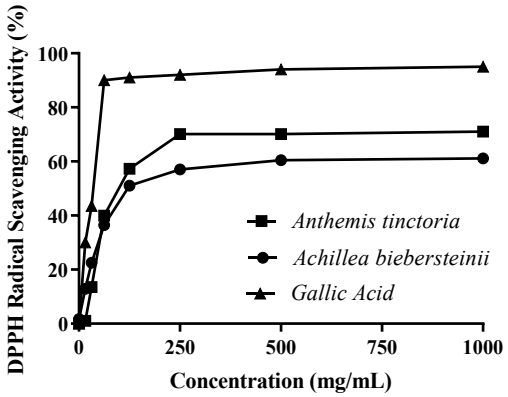
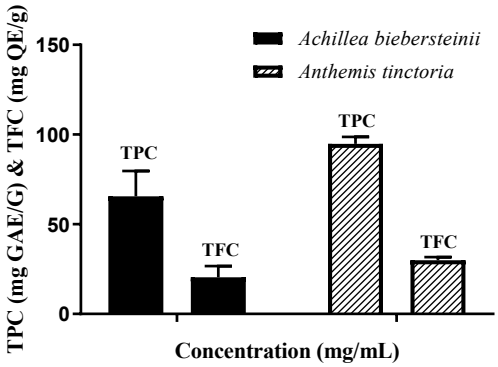


Figure 3. TPC and TFC of *A. biebersteinii* and *A. tinctoria* ethanol extracts.



When the data obtained were evaluated in terms of total phenol and total flavonoid contents, it was determined that both the total phenol and total flavonoid contents of *A.tinctoria* were higher than *A. biebersteinii* (94.9 ± 3.8 mg GAE/g, 65.5 ± 14.2 mg GAE/g for phenol and 29.9 ± 1.8 mg QE/g 20.5±6.3 mg QE/g for flavonoid, respectively) (Figure 3). According to other researchers studying this plant, the total phenol and flavonoid values of methanol extract of *A. tinctoria* L. var. *tinctoria* were determined as 21.4 ± 4.2 (mg GAE/g) and 9.7 ± 2.56 (mg QE/g), respectively (Emir & Emir 2020). Orlando *et al.* (2019) investigated the total phenol and flavonoid components of two species of the genus Anthemis with three different extracts (EA, MeOH, and aqueous) and found that the total phenol and flavonoid content of the MeOH extract of *A. tinctoria* species was higher than that of *A. cretica*. Total phenol and flavonoid ratios in the EA extract of *A. tinctoria* were reported as 26.46 ± 1.11mg GAE/g for phenol, and 45.82 ±

0.40mg GAE/g for the flavonoid. In the current study, conducted with different varieties of the same species, the total phenol content of EA extract was found to be higher. In a previous study investigating the total phenol and flavonoid content of different extracts of *A. biebersteinii* in 2017, it was reported that methanol leaf extract had the highest phenol and flavonoid component (Varasteh-kojourian *et al.*, 2017). In the light of these studies, it can be said that the phenol and flavonoid content of the plants belonging to these species give different results in different parts of the plant, while the solvents in the extracts prepared from the plant can cause a change in the phenol and flavonoid content.

3.3. Enzyme Inhibitory Activity

A. biberstinii and *A. tinctoria* ethanol extracts were tested for the inhibition of AChE and BChE, α-amylase, tyrosinase and α-glucosidase, and the results are given in Table 2. All the enzyme inhibitory activity results are stated as mean ± standard deviation values of three parallel measurements of each group.

Table 2. Enzyme inhibitory activity of 80% ethanol extracts of *A. biebersteinii* and *A. tinctoria.*

Extracts (with 80% ethanol)	Anticholinesterase activity		Antidiabetic activity		Anti-tyrosinase activity
	AChE	BChE	α – glucosidase	α -amylase	tyrosinase
A. biebersteinii	9.51 ± 0.31	24.0 ± 3.42	17.13 ± 1.44	11.29 ± 0.14	70.49±3.34
A. tinctoria	7.88 ± 2.07	8.21 ± 2.03	N.A.	61.58 ± 1.34	72.91±4.69
Referance Drug	58.33 ± 0.44[a]	49.65 ± 0.81[a]	36.48 ± 0.33[b]	53.89 ± 0.15[b]	26.19 ± 0.62[c]

The data are expressed as mean values of three independent experiments ± standard deviation.
a: Galanthamine hydrobromide; b: Acarbose; c: Kojic acid; N.A: Not active

Galanthamine is a reversible inhibitor of cholinesterase and the plant derived drug is used clinically for the palliative treatment of Alzheimer's disease. Therefore, the current study data were compared with galanthamine as a reference drug. The percentage inhibition of AChE was determined to be 9.51 ± 0.31 and 7.88 ± 2.07 for *A.biebersteinii* and *A. tinctoria,* respectively. The percentage inhibition of BChE was 24.0 ± 3.42 and 8.21 ± 2.03, respectively, which was lower than galanthamine (the reference drug) (58.33 ± 0.44 and 49.65 ± 0.81 for AChE and BChE, respectively) at the concentration of 0.1 mg/mL. According to Emir & Emir (2020) methanol extract of *A. tinctoria* L. var. *tinctoria* L. showed 50% inhibition against AChE, BChE and tyrosinase enzymes at the concentration of 254.7 ± 4.86 µg/mL, 166.2 ± 3.74 µg/mL and 415.7 ± 1.85 µg/mL, respectively). In another study, 19.3 and 15.4 mg/ml concentrations demonstrated 50% inhibition on AChE and BChE for *Achillea schischkinii* (Türkan *et al*., 2020). The α-glucosidase inhibitory activity of *Achillea tenorii* has been reported as IC_{50} =32 µg/ml and it has been stated that the strong enzyme inhibitory activity may be attributed to the compound luteolin, which is present in the extract (Venditi *et al.*, 2015). Compared to the previously reported findings, the results of the current study are low, but this may be due to the fact that the solvent and extraction method used will greatly change the amount of phytochemical components contained in the extract.

3.4. Antimicrobial Activity

In the evaluation of the antimicrobial activity of the extracts of the plants used in this study, it was considered significant if MIC ≤ 0.1 mg/ml, moderate if 0.1 < MIC ≤ 0.625 mg/ml, and weak if MIC > 0.625 mg/ml (Kuete, 2010; Awouafack *et al*., 2013).

Table 3. The Antimicrobial Activity Values of 80% Ethanol Extracts of *A. biebersteinii, A. tinctoria.*

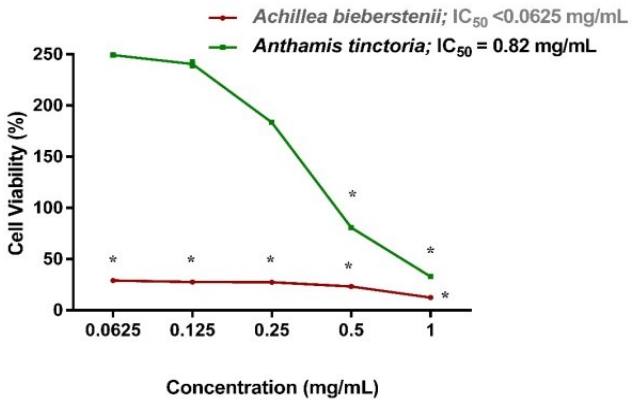
	E.coli	*S.aureus*	*P.aeruginosa*	*B.cereus*	*C.albicans*	*C.tropicalis*
	ATCC 25922	ATCC 29213	ATCC 27853	ATCC11778	ATCC10231	DSM11953
A. biebersteinii	1.25	0.156	1.25	2.5	>2.5	1.25
A. tinctoria	1.25	1.25	2.5	>2.5	>2.5	2.5

According to the results obtained in the study, *A.biebersteinii* extract (0.156 mg/ml) showed a moderate effect on *S.aureus*, while *A. tinctoria* extract did not show antimicrobial activity (Table 3). Kilic *et al*. (2018) used two different methods to evaluate the antimicrobial activity of methanol extracts of *A. tinctoria* and *A. biebersteinii* plants collected from the Amasya region. According to the results of the disc diffusion method, *A. tinctoria* showed higher zone inhibition compared to ceftriaxone antibiotic and generally had antimicrobial properties on *E. coli, P. aeruginosa*, *S. aureus, and K. pneumoniae,* However, in the same study, *A. biebersteinii* species showed antimicrobial activity only on *S. aureus* and *K. pneumoniae*. It was observed that the MIC results, which was the other method applied, provided similar results to the disc diffusion method. Sevindik *et al*. (2018) evaluated the antimicrobial activity of *A. biebersteinii* collected from the Erzincan region. and *A. biebersteinii* was found to have an inhibitory effect on pathogenic microorganisms in the method used, although this effect was lower than that of antibiotics (tetracycline and gentamicin). Uysal *et al*. (2005) reported that *A. tinctoria var. Pallida* did not have significant antimicrobial activity. In the data obtained in the current study, the antimicrobial activity of *A. biebersteinii* supports the findings of studies in the literature, while a difference was determined in respect of the antimicrobial activity of *A. tinctoria* species. The great variability in the results can be attributed to studies having been conducted with different plant extracts and with plants collected from different localities, so there is a clear need for more comprehensive studies.

3.5. Cell Viability Assay

The in vitro cytotoxicity of *A. biebersteinii* and *A. tinctoria* ethanol extracts evaluated using the XTT test are presented in Figure 4. The results from the analysis indicate that *A. tinctoria* extract showed a significant anticancer effect only at its highest concentration (IC_{50}; 0.82 mg/mL) ($p<0.05$). The cell viability results clearly showed that for all concentrations the *A. bieberstenii* extract has a stronger anticancer effect on MDA-MB-231 cells with <0.0625 mg/mL IC_{50} value. Otherwise, the extracts of both plants did not demonstrate significant cytotoxic activity against L929 cells in the applied concentration range (0.0625-1 mg/mL).

Figure 4. The antiproliferative activity of *A. bieberstenii* and *A. tinctoria* extracts.



Similar to the findings obtained in this study, different extracts of *A. bieberstenii* and *A. tinctoria* have been found to have anticancer effects on different cell lines. Baharara *et al.* (2015) reported that silver nanoparticles biosynthesized using *A. bieberstenii* flower extract have an antiapoptotic and inhibitory effect on the MCF-7 cell line. Erdogan *et al*. (2020) reported that the combined form of *A. bieberstenii* with 5-fluorouracil (5-FU) caused inhibition in the human colon cancer cell line.

Studies investigating the cytotoxic effect of *A. tinctoria* are limited. In the research conducted by Raal *et al.* (2022), the methanol extract of this plant was not found to have any cytotoxic effects on the human carcinoma cell lines studied (MKN7, gastric; HepG2, hepatocellular; SW480, colon; KB, mouth; LNCaP, prostate), but the essential oils of the plant had an effective cytotoxic effect on LNCaP and KB cells (IC_{50}: 27.75-29.96 µg/mL). All these data show that the ethanol extract of these two plants provide better results in anticancer studies.

4. DISCUSSION and CONCLUSION

The results of this study demonstrated that 80% ethanol extracts of *A. biebersteinii* and *A. tinctoria* have antioxidant effects (DPPH, ABTS and total phenolic, flavonoid contents), AChE and BChE, tyrosinase inhibitory activity as well as antimicrobial and anticancer activity. Although the antimicrobial activity of *A.bieberstenii* was higher than that of *A.tinctoria*, both showed moderate antimicrobial activity. The antioxidant activity and total phenol-flavonoid content of *A.tinctoria* were found to be higher compared to *A. biebersteinii.* Both extracts showed anticholinesterase inhibitory activity in a concentration-dependent manner. Overall, both extracts showed potent tyrosinase inhibitory activity even from the positive control drug kojic acid at the same concentration. However, more studies are needed to clarify the potential therapeutic effect of *A. biebersteinii* and *A. tinctoria* for tyrosinase-related skin disease or neurodegenerative disease. When the anticancer activities of the plants were evaluated, it was observed that *A. biebersteinii* had a high cytotoxic effect on the breast cancer cell line even at low doses compared to *A. tinctoria*.

The different biological activities of these two plants belonging to the Asteraceae family can be explained by the changes in the phytochemicals in the contents of the plants. In particular, this study has shown that *A. biebersteinii* is a more active in many parameters than *A.tinctoria*. It is expected that the data obtained from these two plants, which have been examined in many ways in terms of biological activity, will shed light on future phytotherapeutic research.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Gulsen Guclu: Investigation, Resources, and Writing - original draft. **Merve Inanir**: Methodology, Supervision, and Validation. **Esra Ucar:** Visualization, Software, Formal Analysis. **Nuraniye Eruygur:** Formal Analysis and Validation. **Mehmet Atas:** Methodology, Supervision, and Validation. **Tansu Uskutoğlu:** Formal Analysis and Validation. **Belgin Coşge Şenkal**: Investigation and Resources

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