ARTICLE IN PRESS

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
x (x): x-x, 2026
DOI: 10.9775/kvfd.2026.36536

RESEARCH ARTICLE

Biochemical and Pharmacological Evaluation of Selected *Artemisia* Species with Antioxidant and Antimicrobial Activities

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How to cite this article?

Rahimova S, Boyraci GM, Yildiz O, Asadov E, Seyidzada Z, Türkan F: Biochemical and Pharmacological Evaluation of Selected *Artemisia* Species with Antioxidant and Antimicrobial Activities. *Kafkas Univ Vet Fak Derg*, 2026 (Article in Press).
DOI: 10.9775/kvfd.2026.36536

Article ID: KVFD-2026-36536
Received: 05.03.2026
Accepted: 07.08.2026
Published Online: 11.08.2026

Abstract

The genus *Artemisia* is recognized for its diverse secondary metabolites and therapeutic potential; however, evaluating species from the Nakhchivan region is significant for mapping their regional bioactive profiles. This study investigated the chemical characteristics, antioxidant activity, and antimicrobial effects of three *Artemisia* species collected from the Nakhchivan Autonomous Republic to reveal their potential pharmacological benefits. Ethanolic extracts of *Artemisia lerchiana*, *Artemisia absinthium*, and *Artemisia dracunculus* were prepared and total phenolic content (TPC) and total flavonoid content (TFC) were determined. Antimicrobial activity was assessed against *Bacillus cereus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. The TPC value of the *Artemisia* species ranged from 19.620 to 21.875 mg GAE/g plant, with *A. dracunculus* L. exhibited the highest TFC (6.642±0.076 mg QE/g plant). According to the FRAP assay, *A. absinthium* L. showed the highest antioxidant activity (3.551±0.220 µmol TE/g plant), whereas *A. lerchiana* and *A. dracunculus* exhibited the lowest SC_{50} values in the DPPH assay. The extracts displayed varying degrees of antimicrobial activity, with *A. dracunculus* being the most effective, particularly against *B. cereus* and *C. albicans*, yielding inhibition zones of 12.05±0.05 mm and 14.48±0.03 mm, respectively. Gram-negative bacteria exhibited higher resistance to the tested extracts than Gram-positive species. These findings suggest that *Artemisia* species from the Nakhchivan region are promising sources of natural antioxidant and antimicrobial agents.

Keywords: *Artemisia*, Phenolic Compounds, Antimicrobial, Antioxidant, Environment

Introduction

Plants are one of the richest natural components for human health and well-being. Therefore, it is very necessary to cultivate, use and study valuable plants in detail on a scientific basis. In modern times, the comprehensive study of the gene pool of the world, identification and protection of its resources is one of the priority directions. Currently, because of active and intensive use of natural resources, significant reductions in vegetation are observed [1]. If vegetable plants are widely used properly, various diseases spreading among the population will be significantly reduced, some diseases caused by vitamin deficiency will be eradicated, and several diseases that cannot be cured now will be prevented.

In this regard, the widespread consumption of vegetables and leafy greens plays a crucial role in promoting and maintaining human health. Identifying the sources of biologically active substances for the creation of new therapeutic preparations is crucial. Among naturally occurring medicinal compounds, terpenoids, particularly sesquiterpene lactones and coumarins, have attracted considerable scientific interest in the last 40-50 years [2]. These compounds are commonly found in the plant world, and as natural substances, they have great pharmacological

effects [3]. Species belonging to the *Artemisia* genus (wormwood) are considered important natural reservoirs of these bioactive constituents.

A. dracunculus L. is a small, shrub-shaped, perennial plant [4]. It typically grows on dry slopes, grasslands, and open fields is widely distributed in Japan, Europe, India, North America, Iran, and China [5]. In the literature, *A. dracunculus* L. is known by the common name "Tarragon" [2] and is also known as "Tarkhun"; it is a valuable medicinal plant in Iranian culture [6]. Other *Artemisia* species, including *A. absinthium* L. and *A. lerchiana*, are widely distributed in Azerbaijan, particularly in the Greater and Lesser Caucasus regions, the Samur-Shabran Plain and the Kura-Aras Plain [7]. This genus contains chemical compounds such as monoterpenes, sesquiterpenes, sesquiterpenes, coumarins, sesquiterpene lactones, flavonoids, polyacetylenes and sterols [4].

Among the medicinal products currently in use, preparations obtained from plants are predominant. Thus, in the second half of the 20th century, 40% of the medicines used in clinical practice in the region were herbal medicinal preparations, and several *Artemisia* species *(A. absinthium* L., *A. annua* L., *A. scoparia* Waldst. et Kit., *A. vulgaris* L.) were included in the pharmacopoeia as official medicinal plants because of their bioactive components (phenolic acids, flavonoids, essential oils, etc.) [8].

Based on these considerations, this study aims to provide a comprehensive evaluation of the chemical composition, antioxidant capacity, and antimicrobial potential of three *Artemisia* species collected from the Nakhchivan Autonomous Republic, thereby elucidating their suitability for medicinal and functional applications.

Material and Methods

Ethical Statement

This study did not involve any procedures requiring ethical approval.

Plant Material and Extraction

Wormwood *(Artemisia absinthium* L.), Lerchian wormwood *(Artemisia lerchiana* weber ex stechm.) and tarragon *(Artemisia dracunculus* L.) were collected during June 2024 from different locations within the Nakhchivan Autonomous Republic and prepared for subsequent analyses. *Artemisia absinthium* and *Artemisia lerchiana* were collected from Babek District of Nakhchivan Autonomous Republic, whereas tarragon species were collected from backyards in Nakhchivan city. Information regarding the collection localities, geographic coordinates, and sampling dates of the species is summarized in *Table 1*.

Voucher specimens are stored in the herbarium collection of the Department of Biology at Nakhchivan State University, Nakhchivan, Azerbaijan.

The sample preparation and extraction procedure is illustrated in *Fig. 1*. Briefly, the plants were dried at room temperature, ground, and prepared for analysis. The samples were extracted with 50% ethanol-water at a concentration of 0.23 g/mL and agitated at 150 rpm for 24 h in an incubator (Microtest MCI 55) at 25°C. All chemicals and reagents used in the study were purchased from Sigma and Merck companies.

Determination of Total Phenolic Content (TPC)

The Folin-Ciocalteu method was used to determine the TPC values of the samples [9] as gallic acid equivalents (GAE). To summarize the analysis, 20 µL of the sample, 680 µL of pure water, and 400 µL of the 0.2 N Folin Ciocalteau reagent were mixed. After, the mixture was kept in the dark for 5 min and then 400 µL of sodium carbonate solution (10%) was added. Absorbance values versus concentration were measured at 760 nm using a UV-Vis spectrophotometer (Biochrom Libra) after 2

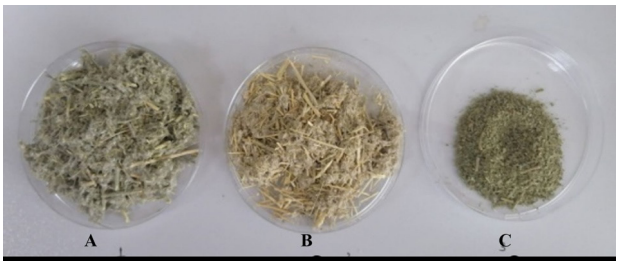

Fig 1. Schematic sample preparation and extraction procedure for *Artemisia* species

Table 1. *Information about the locality, coordinates and collections date of selected Artemisia species collected from Babek district and Nakhchivan city of Nakhchivan AR, Azerbaijan*

***Artemisia* Samples/ Herbarium Number**	**Collections Locality**	**Coordinate**	**Collection Date**
Artemisia dracunculus L./1024	Nakhchivan AR, Nakhchivan City	N39.203'517"EO45.426057	02.06.2024
Artemisia absinthium L./ 1025	Nakhchivan AR Babek District, Gahab Village	N38015'15"EO 45030'01"	08.06.2024
Artemisia lerchiana weber ex stechm./1026	Nakhchivan AR Babek District, Gahab Village	N39016'16"EO45030'28"	08.06.2024

h. The results were reported as milligrams of gallic acid equivalents per gram of dry sample (mg GAE/g plant) [10].

Ferric Reducing Antioxidant Power (FRAP)

FRAP assay is based on reducing the Fe^{+3} tripyridyltriazine complex to a blue-colored Fe^{2+} chelate in the presence of antioxidant compounds. The FRAP reagent was prepared by mixing 0.3 M acetate buffer solution (pH 3.6), 10 mM TPTZ (2,4,6-tripyridyl-s-triazine) (prepared with 40 mM HCl), and 20 mM $FeCl_3$ solutions in a 10:1:1 ratio, respectively. Briefly, 50 µL of sample and 1.5 mL of FRAP reagent were added to the test tube and incubated in the dark at 37°C for 4 min. The absorbance of reaction mixture was measured at 593 nm using a spectrophotometer. Trolox was used as the standard, and values are given as µmoL Trolox equivalent (TE) per gram sample [11].

Scavenging Activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) Radical

The DPPH free radical scavenging activity of the samples was determined according to Değirmenci et al. [12]. Briefly, 750 µL of sample and 750 µL of DPPH (prepared in 100 µM methanol) were mixed and incubated at room temperature in the dark for 50 min. The absorbances were measured at 517 nm at the conclusion of the period. The result was determined by calculating the SC_{50} (mg/mL), which is defined as the concentration of the sample that causes a 50% decrease in the concentration of DPPH radicals.

Determination of Total Flavonoid Content (TFC)

The TFC was determined using the aluminum chloride colorimetric method. A 60 µL of extract was diluted with 540 µL of methanol and subsequently mixed with 600 µL of 2% (w/v) $AlCl_3$ solution. Following a 30 min of incubation at room temperature, the absorbance values were read at 415 nm. The results are given in mg quercetin equivalent (QAE) per gram sample [13].

Determination of Phenolic Compositions via HPLC-PDA

The phenolic profiles of the plant samples were assessed according to the methodology described by for 25 phenolic compounds [14]. A 10 mL of extract was taken to adjust pH values of 2 and then it was submitted to liquid-liquid extraction using diethyl ether twice, with volumes of 10 mL and 5 mL, respectively. The extraction was carried out at a stirring speed of 200 rpm for 15 min at room temperature. The identical procedure was replicated using ethyl acetate, and the resulting organic phases from each extraction step were pooled into a pre-weighed flask. The solvent in the organic phase was removed using a rotary evaporator at 40°C. The remaining substance was dissolved in 2 mL of methanol, filtered through a 0.45 µm membrane filter, and then injected into the RP-HPLC-PDA system.

The phenolic substances profile was determined using Shimadzu Corporation LC 20AT HPLC device, which can be analyzed simultaneously in the wavelength range of 200-800 nm, equipped with a photo diode array (PDA) detector (M30A) and a C18 column (250 mm × 4.6 mm, 5 µm; GL Sciences). The mobile phase was carried out with a gradient program consisting of acetonitrile, water and acetic acid. The injection volume was 20 µL, the flow rate was maintained at 1.0 mL/min, and the column temperature was set to 30°C. The concentrations of the phenolic compounds were determined using standard calibration curves, and the results were represented in µg/g.

Determination of Antimicrobial Activity

The antimicrobial activity of plant extracts was investigated using the agar diffusion method [15]. The antimicrobial effects of plants were tested against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 35218, *Bacillus cereus* RSKK 709, *Staphylococcus epidermidis* ATCC 12228, and *Candida albicans* ATCC 10231. All microbial suspensions were adjusted to 0.5 McFarland standard for bacteria and 2 McFarland standard for yeast and then inoculated onto Mueller-Hinton agar using sterile swabs. Wells were opened on the medium with a sterile mushroom borer (6 mm diameter), and 75 µL of each extract (0.23 g/mL) was added to the wells.

The plates were incubated at 37°C for bacteria and 25°C for yeast for 24 h. After incubation, the inhibition zone diameters (mm) formed around the cavities were measured [16]. In addition, ofloxacin (1 mg/mL) and chlorhexidine (1 mg/mL) were employed as positive controls to evaluate and compare the antimicrobial activities of the tested agents.

Statistical Analysis

The experimental data were analyzed using the SPSS version 20.00 (SPSS Inc., Chicago, IL, USA) program. All measurements were performed in triplicate, and the results are reported as mean±standard deviation. Group comparisons were evaluated using One-way analysis of variance (ANOVA) and Duncan's multiple comparison test was subsequently applied to determine significant differences between the mean values ($P \leq 0.05$).

Results

Total Phenolic Content, Total Flavonoid Content and Antioxidant Activity

TPC, TFC and antioxidant activity (via FRAP and DPPH) analyzes were performed to determine the potential of these *Artemisia* species are natural antioxidant sources *(Fig. 2)*. As indicated in *Fig 3-A*, the TPC of the plant extracts ranged from 19.620±0.389 to 21.875±1.659 mg GAE/g plant. Comparatively, *A. dracunculus* L. showed

Fig 2. A) *Artemisia absinthium* L., **B)** *Artemisia dracunculus*, **C)** *Artemisia lerchiana* species

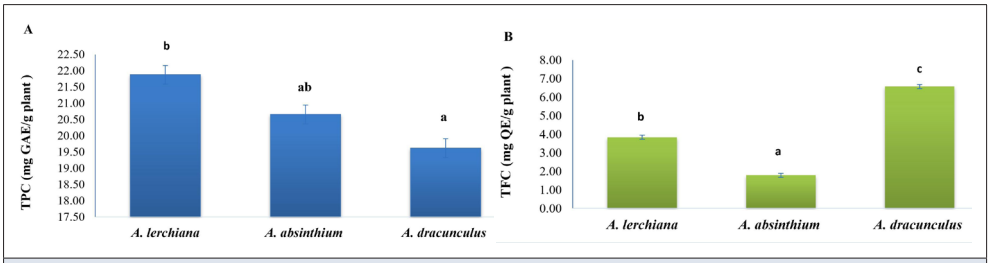

Fig 3. A) Total phenolic content (TPC) in different plants. **B)** Total flavonoid content (TFC) in different plants. Data represents as mean±S.D. (n=3). Different letters [(a-c)] indicate significant differences (P≤0.05)

statistically lowest TPC value with 19.620±0.389 mg GAE/g plant (P≤0.05). All extracts exhibited significant variations in their total flavonoid levels *(Fig. 3-B)*, and the *A.dracunculus* L. plant displayed the highest total flavonoid value of 6.642±0.076 mg QE/g plant.

The FRAP values of the extracts ranged from 1.929±0.13 to 3.551±0.220 µmoL TE/g. Among the three evaluated species, *A. absinthium* L. extract exhibited the statistically highest ferric reducing capacity, reaching 3.551±0.220 µmoL TE/g plant *(Fig. 4-A)*. In the DPPH radical scavenging assay, the SC_{50} values for extracts ranged from 0.373±0.015 to 0.453±0.009 mg/mL *(Fig. 4-B)*. A higher level of activity in scavenging radicals is indicated by lower SC_{50} values. This revealed that *Artemisia lerchiana weber ex stechm* and *Artemisia dracunculus* L. had statistically the highest antioxidant activity versus free radical scavenging activity.

Quantification of Phenolic Compounds in Plant Samples

Phenolic compounds in the *Artemisia* species were analyzed by HPLC-PDA using 25 phenolic standards. The plant extracts investigated were found to contain the following phenolic acids: *p*-hydroxybenzoic acid, protocatechuic acid, syringic acid, chlorogenic acid, rutin, ellagic acid, ferulic acid, apigenin, chrysin, pinocembrin and vanillic acid, all of which were quantified. The remaining standards, namely epicatechin, caffeic acid, *p*-coumaric acid, catechin hydrate, myricetin, daidzein, luteolin, quercetin, *t*-cinnamic acid, hesperetin, ramnetin, caffeic acid phenethyl ester, galangin and naringenin were not detected in any of the analyzed samples.

The concentrations of the identified phenolic compounds are presented in *Table 2*. Ferulic acid and chrysin were detected and quantified in all the examined plant samples. Rutin and ellagic acid were detected only in *A. absinthium* L., with concentrations of 2244.667±11.66 and 46.681±1.01 µg/g plants, respectively. Syringic acid was found only in the *A. lerchiana* plant at a concentration of 218.909±6.54 µg/g plant. Similarly, vanillic acid was exclusively found in the *A. dracunculus* L. plant, with a concentration of 97.153±4.58 µg/g plant.

Antimicrobial Activity

The antimicrobial activity of the ethanolic extracts of three *Artemisia* species was evaluated using the agar

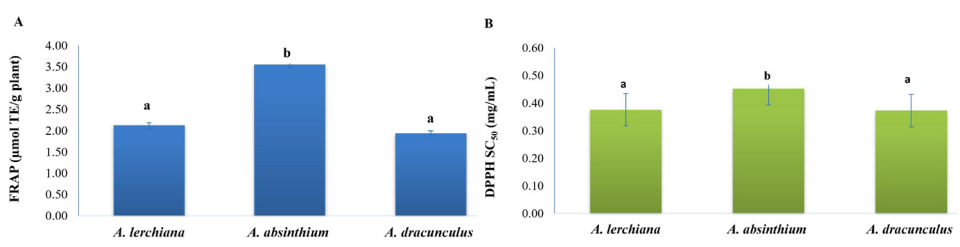

Fig 4. A) Values of FRAP in different plants, **B)** SC_{50} values for DPPH analyses in different plants. Data represents as mean±S.D. (n=3). Different letters [(a-b)] indicate significant differences (P≤0.05)

Table 2. *The phenolic profiles of the samples using the HPLC-PDA*

Content of Phenolic Acids (µg/g plants)	Types of Plant		
	A. lerchiana	***A. absinthium* L.**	***A. dracunculus* L.**
Protocatechuic acid	1.715±0.01	n.d.	200.662±4.22
Chlorogenic acid	56.471±0.21	n.d.	694.017±1.41
p-OH benzoic acid	39.489±1.98	n.d.	54.308±7.09
Syringic acid	218.909±6.54	n.d.	n.d.
Rutin	n.d.	2244.667±11.66	n.d.
Ellagic acid	n.d.	46.681±1.01	n.d.
Ferulic acid	117.656±3.41	113.899±6.32	76.478±3.55
Apigenin	21.029±1.61	n.d.	44.695±2.42
Krisin	22.293±0.95	69.259±1.13	28.703±3.11
Pinocembrin	n.d.	37.066±2.18	367.817±8.76
Vanillic acid	n.d.	n.d.	97.153±4.58

Mean values from three repetitions ± standard deviations. n.d, not detected

well diffusion method at a concentration of 0.23 g/mL. The inhibition zone diameters obtained against five test microorganisms, namely *B. cereus, S. epidermidis, S. aureus, E. coli,* and *C. albicans* were provided in *Table 3*. Among the tested extracts, *A. lerchiana s*howed an inhibition area of 10.10±0.10 mm against *B. cereus;* 12.07±0.06 mm against *S. aureus;* 15.5±0.10 mm against *S. epidermidis*, and 11.10±0.10 mm against *C. albicans*. Notably, *A. lerchiana* was the only species that demonstrated activity against *S. aureus* and showed the strongest inhibitory effect against *S. epidermidis*.

Statistical analysis revealed that *A. dracunculus L.* showed the highest activity against *B. cereus* and *C. albicans,* with zone diameters of 12.05±0.05 and 14.48±0.03 mm, respectively. In contrast, none of the extracts showed any inhibitory effect on *E. coli*. *A. dracunculus* L. exhibited the highest antimicrobial activity against *B. cereus* and *C. albicans,* while *A. lerchiana* showed the highest activity against *S. epidermidis* and was the only species effective against *S. aureus*. As expected, the positive controls exhibited substantially greater antimicrobial activity than the plant extracts. Ofloxacin produced inhibition zones of 33.83 mm against *B. cereus,* 35.50 mm against *S. aureus,* and 36.67 mm against *S. epidermidis* while chlorhexidine generated 21.67 mm zone of inhibition against *C. albicans.*

Discussion

The present study demonstrated that the investigated *Artemisia* species possess considerable antioxidant and antimicrobial potential, despite exhibiting relatively similar total phenolic contents. This finding suggests that the biological activities of these species cannot be explained solely by the overall quantity of phenolic compounds. Rather, the observed differences are more likely associated with species phytochemical composition, including the diversity, relative abundance, and possible synergistic interactions of individual phenolics. Therefore, the biological properties of *Artemisia* species appear to depend not only on total phenolic content but also on qualitative differences in their phytochemical profiles [17].

Consistent with this interpretation, all investigated *Artemisia* species exhibited comparable total phenolic contents, indicating that they represent valuable natural

Table 3. *Antimicrobial activity of the ethanol extracts of A. lerchiana, A. absinthium L., A. dracunculus L.*

Samples	Inhibition Zone (mm)				
	B. cereus	***S. epidermidis***	***S. aureus***	***E. coli***	***C. albicans***
A. lerchiana	10.10±0.10^{b}	15.5±0.10^{c}	12.07±0.06^{a}	-	11.10±0.10^{b}
A. absinthium L.	9.07±0.06^{a}	9.57±0.06^{a}	-	-	10.05±0.05^{a}
A. dracunculus L.	12.05±0.05^{c}	14.03±0.06^{b}	-	-	14.48±0.03^{c}
Ofloxacin	33.83±0.29^{d}	36.67±0.29 ^{d}	35.5±0.50^{b}	36.5±0.50	-
Chlorhexidine					21.67±0.29 ^{d}

$^{a-c}$ *Different letters in the same column indicate statistical difference (P≤0.05)*

sources of phenolic antioxidants. Previous studies have also demonstrated that *Artemisia* species possess considerable phenolic content, although the reported levels vary substantially among studies. Such variations are most likely attributable to differences in plant species, geographical origin, environmental conditions, harvesting period, plant organ, extraction solvent, and extraction methodology [18]. Therefore, direct comparisons of absolute phenolic values should be interpreted with caution, while the overall evidence consistently supports the rich phenolic composition and antioxidant potential of *Artemisia* species [19]. Accordingly, the relatively high phenolic content observed in the present study provides a biochemical basis for the antioxidant properties of the extracts; however, the subsequent HPLC-PDA analysis clearly indicates that biological activity is more closely associated with qualitative phytochemical composition than with total phenolic concentration alone [20].

A similar trend was observed for total flavonoid content (TFC), with significant differences detected among the investigated *Artemisia* species *(Fig. 3-B).* These findings indicate species-specific variation in flavonoid accumulation, with *A. dracunculus* exhibiting the greatest flavonoid enrichment among the studied taxa [21]. Previous studies have likewise reported substantial variability in flavonoid content across *Artemisia* species, which has been attributed to differences in species, environmental conditions, plant developmental stage, geographical origin, and extraction procedures. Consequently, the observed differences are consistent with the well established phytochemical diversity of the genus and further support the importance of species selection when evaluating the biological potential of *Artemisia* plants [22]. Although absolute values differ among studies, these findings collectively indicate that flavonoid accumulation is strongly influenced by species characteristics and extraction conditions. Accordingly, the relatively high flavonoid contents observed in the present study further support the antioxidant potential of the investigated *Artemisia* species. Flavonoids are known to donate hydrogen atoms or electrons to reactive oxygen species, stabilize free radicals through resonance, and modulate oxidative stress-related signaling pathways. Therefore, the relatively high flavonoid levels detected, particularly in *A. dracunculus,* may partially explain its pronounced antioxidant performance.

Since phenolic and flavonoid compounds are major contributors to antioxidant activity, the antioxidant capacities of the extracts were subsequently evaluated using both FRAP and DPPH assays [23]. Interestingly, the ranking of the investigated species differed between these assays. While *A. absinthium* exhibited the highest ferric reducing power, *A. lerchiana* and *A. dracunculus* demonstrated stronger DPPH radical scavenging activities. This discrepancy indicates that antioxidant activity is mediated through multiple mechanisms rather than a single mode of action. For example, the FRAP assay primarily reflects electron transfer capacity, whereas the DPPH assay mainly evaluates hydrogen atom donation and radical scavenging efficiency. Consequently, phytochemicals possessing different redox characteristics may perform differently depending on the assay employed, explaining why species ranking varied between FRAP and DPPH analyses. Similar assay dependent differences have also been reported for other *Artemisia* species [24,25]. Taken together, these findings suggest that antioxidant capacity is influenced not only by the overall amount of phenolic compounds but also by the composition and relative abundance of individual phytochemicals [26]. This interpretation agrees with previous reports demonstrating that antioxidant activity is not always directly proportional to the total concentration of bioactive compounds [27].

To better explain these differences in antioxidant behavior, the phenolic composition of the investigated extracts was characterized by HPLC-PDA analysis. Although the total phenolic contents of the species were relatively similar, their individual phenolic profiles differed markedly. Each species exhibited a distinct distribution of phenolic acids and flavonoids, suggesting that qualitative rather than quantitative differences may account for the observed biological activities [28]. *A. absinthium* was characterized by a remarkably high rutin content, whereas *A. dracunculus* contained the highest concentrations of chlorogenic acid and pinocembrin and was the only species in which vanillic acid was detected [29]. In contrast, syringic acid was the predominant phenolic compound in *A. lerchiana.* Previous investigations have similarly identified chlorogenic acid, ferulic acid, caffeic acid, pinocembrin, and rutin as major phenolic constituents of *Artemisia* species [30]. Consistent with these reports, chlorogenic acid, ferulic acid, rutin, and pinocembrin were also detected in the present study. However, additional compounds including protocatechuic acid, apigenin, chrysin, pinocembrin, and vanillic acid were identified, revealing a more comprehensive phenolic profile. Furthermore, the chlorogenic acid content of *A. dracunculus* (694.017 µg/g plant) was considerably higher than that reported previously [31]. Chlorogenic acid has been widely reported to exhibit strong antioxidant activity through hydrogen atom donation, transition metal chelation, inhibition of lipid peroxidation, and modulation of cellular oxidative stress pathways. Likewise, rutin possesses remarkable radical scavenging capacity owing to its multiple hydroxyl groups and has additionally been associated with anti-inflammatory and antimicrobial activities. Pinocembrin has been shown to disrupt bacterial membrane integrity,

interfere with microbial energy metabolism, and alleviate oxidative damage, whereas syringic acid contributes to antimicrobial activity through membrane destabilization and oxidative stress induction [32]. Therefore, the distinct biological activities observed among the investigated *Artemisia* species are likely attributable to the combined actions and synergistic interactions of these phenolic constituents rather than to the predominance of a single compound. These findings further support the concept that the biological properties of *Artemisia* extracts depend not only on total phenolic concentration but also on the identity and relative abundance of individual phenolic constituents.

The differences observed in phytochemical composition also appeared to influence antimicrobial activity. Plant secondary metabolites, particularly phenolics, flavonoids, tannins, saponins, and steroids, are known to contribute to antibacterial activity [33]. Therefore, species-specific variations in phenolic composition would be expected to produce corresponding differences in antimicrobial efficacy. In agreement with previous reports [34], the investigated *Artemisia* extracts exhibited species-dependent antimicrobial activities. *A. dracunculus* generally displayed the strongest inhibitory effects against most tested microorganisms, whereas *A. lerchiana* showed the highest activity against *S. aureus* and *S. epidermidis*. Moreover, *A. lerchiana* was the only species exhibiting antibacterial activity against *S. aureus*. These findings are consistent with the HPLC-PDA results and suggest that the elevated chlorogenic acid and pinocembrin contents of *A. dracunculus* may have contributed to its superior antimicrobial performance, whereas the antibacterial activity of *A. lerchiana* may be associated with its high syringic acid content. Conversely, none of the investigated extracts inhibited Gram negative *E. coli*. Although this observation is commonly attributed to the outer membrane acting as an effective permeability barrier, additional factors should also be considered. The antimicrobial efficacy of plant extracts depends on the polarity of active constituents, their ability to diffuse through bacterial envelopes, extract composition, and the concentrations of bioactive molecules reaching intracellular targets. Moreover, the lipopolysaccharide-rich outer membrane of Gram negative bacteria restricts the penetration of many hydrophobic phenolic compounds, thereby substantially reducing their antibacterial effectiveness. Consequently, the absence of activity against *E. coli* probably reflects the combined influence of bacterial structural resistance and the physicochemical characteristics of the extracted phytochemicals rather than a complete lack of antibacterial constituents.

Similar antimicrobial activities have previously been reported for *A. absinthium* and *A. lerchiana* [35]. Behbahani et al. [36] observed inhibitory activities of *A. lerchiana* against *S. aureus, B. cereus, E. coli,* and *C. albicans*. Likewise, the ethyl acetate extract of *A. absinthium* inhibited *E. coli* and *S. aureus* [37], whereas Ali et al. [38] reported no antibacterial activity against either microorganism, consistent with the present findings. Such discrepancies are most likely attributable to differences in geographical origin, environmental conditions, plant material, harvesting period, and extraction methodology, all of which influence phytochemical composition. It should also be considered that crude plant extracts represent complex phytochemical mixtures in which synergistic or antagonistic interactions among individual metabolites may substantially influence antimicrobial efficacy. Therefore, biological activity cannot always be directly predicted from the concentration of a single compound. Although the inhibition zones observed in the present study were generally smaller than those produced by the reference antimicrobial agents, the detected activities nevertheless demonstrate biologically relevant antimicrobial potential.

Overall, the present findings demonstrate that relatively similar total phenolic contents do not necessarily translate into similar biological activities. Instead, the observed antioxidant and antimicrobial properties appear to be governed primarily by the qualitative composition and relative abundance of individual phenolic constituents identified by HPLC-PDA analysis. The integration of comprehensive phytochemical profiling with multiple biological assays represents one of the major strengths of the present study, enabling a more robust interpretation of the relationship between chemical composition and biological function.

Nevertheless, several limitations should also be acknowledged. Since biological activities were evaluated using crude extracts, the individual contributions of specific phenolic compounds and their synergistic interactions could not be directly determined. Furthermore, only *in vitro* antioxidant and antimicrobial assays were performed, and the molecular mechanisms responsible for the observed biological effects remain to be elucidated.

The present findings demonstrate that the investigated *Artemisia* species constitute promising natural sources of bioactive compounds with notable antioxidant and antimicrobial properties. The observed biological activities, together with the phytochemical profiles, indicate that these species have considerable potential for further pharmacological and functional applications. Future studies should focus on the isolation and characterization of the compounds responsible for these activities, elucidation of their molecular mechanisms of action, assessment of possible synergistic interactions among phytochemicals, and validation of their efficacy and safety in appropriate *in vivo* models. Such investigations

will contribute to a more comprehensive understanding of the therapeutic potential of *Artemisia* species and support their development as natural bioactive ingredients for food, nutraceutical, and pharmaceutical applications.

Declarations

Availability of Data and Materials: The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements: None

Financial Support: The author received no funding for this study.

Ethical Statement: This study did not involve any procedures requiring ethical approval.

Conflict of Interest: The author declares that there are no competing interests.

Declaration of Generative Artificial Intelligence (AI): The authors declare that the manuscript, including all tables and figures, was prepared by the authors and not generated by AI or AI-assisted technologies. AI-assisted tools were used solely to improve the language and readability of the manuscript.

Author Contributions: SR: investigation, sample harvesting, and writing original draft preparation. GMB: methodology, visualization, writing-original draft preparation, review and editing. OY: methodology, conceptualization and supervision. FT: conceptualization and supervisión. EA and ZS sample harvesting, review and editing. All authors have read and agreed to the published version of the manuscript.

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