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Assessment of antioxidant, antimicrobial, antibiofilm, carbonic anhydrase, and α-glucosidase inhibitory activities of *Alchemilla vulgaris* L. extracts

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Abstract

This study was to investigate the biological properties (antioxidant, enzyme inhibitory, and antimicrobial activity) of water extract (WE), 50:50% water:ethanol extract (WEE), and ethanol extract (EE) of aerial parts of *Alchemilla vulgaris* L. which was traditionally used to alleviate and treat many diseases. The WEE extract exhibited the highest total phenolic content (TPC), with a value of 612 µg GAE/mL, while the EE extract demonstrated the highest total flavonoid content (TFC), with a value of 86.3 µg QE/mL. The data showed that the WEE extract exhibited the highest radical scavenging capacities with SC_{50} values of 0.0056 and 0.0028 mg/mL as determined by the DPPH and ABTS assays, respectively. The WEE extract also showed the highest antioxidant activities with 267 and 0.081 µM TEAC as determined by FRAP and CUPRAC assays, respectively. Furthermore, with IC_{50} values of 0.0628 and 0.0535 mg/mL, WEE extract was found to be an effective inhibitor of bovine carbonic anhydrase (BCA) and α-glucosidase. The EE extract showed activity against both Gram-negative and Gram-positive bacteria used in the study. EE extract has the highest activity against *Yersinia pseudotuberculosis* and the lowest activity against *Streptococcus pyogenes*. Moreover, the extracts significantly reduced the biofilm ability of the *Acinetobacter baumannii* isolate. EE and WE reduced the biofilm formation capacity of the strain to a weak level, while WEE extract reduced it to a moderate level. The results suggest that EE has a significant effect in combating antibiotic resistance, while WEE has high antioxidant activity and a good inhibitory effect against BCA and α-glucosidase enzymes. In addition, the results revealed that solvent extracts with different solvent compositions and polarities may have different effects on diverse bioactivity tests.

Keywords: Antibiofilm, antioxidant activity, *Alchemilla vulgaris* L., carbonic anhydrase, α-glucosidase

1. Introduction

Throughout history, the utilization of medicinal plants has been documented as an alternative therapeutic tool for the treatment of numerous diseases. [1]. The medicinal plants have been known to contain a variety of secondary metabolites, including phenolic acids, flavonoids, anthocyanins, lignans, and coumarins [2]. These secondary metabolites have been demonstrated to exhibit various biological activities, including antioxidant, antimicrobial, antidiabetic, and anticancer properties [3]. A considerable number of plant species have been found to possess the ability to scavenge and hinder the process of free radical formation, which is known to induce oxidative damage in biomolecules. The presence of antioxidant activity in these secondary metabolites has been identified as a key factor in their function [4]. Moreover, plant based natural antioxidants are favored over synthetic ones due to their safety profiles [5]. Therefore, there is considerable interest in scientific research that focuses on the bioactivity of natural products.

Alchemilla is commonly referred to as "Lady's Mantle" or "Lion's Foot", and species belonging to this genus are predominantly distributed across Europe and Asia, being found in northeastern Anatolia (Türkiye), northern Iraq, and northwestern Iran [6]. A wide range of biological activities has been attributed to various species of *Alchemilla*, including antioxidant, anticancer, antidiabetic, and antimicrobial properties [1,7,8].

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The genus *Alchemilla vulgaris* L., an herbaceous perennial plant in the family Rosaceae, is widely used in folk medicine throughout the world, and in traditional medicine, the plant is commonly used for the treatment of ulcers, wounds, eczema, and digestive problems [7]. *A. vulgaris* has been characterized by strong antioxidant activity due to the presence of phenolic constituents and large quantities of tannins, flavonoids, and phenolic carboxylic acids [9]. The aerial parts of the plant are used in the treatment of gastrointestinal diseases and for the acceleration of wound healing, a consequence of their potent antimicrobial and anti-inflammatory properties [1].

Reactive oxygen species (ROS), which have been identified as agents of a range of diseases, including oxidative stress, cancer, Alzheimer's, diabetes, and aging, have been shown to induce oxidative damage to biomolecules [10]. Antioxidants have been shown to inhibit or delay the oxidation process by obstructing the initiation or propagation of oxidizing chain reactions [11].

The carbonic anhydrase (CA) enzyme is of pivotal importance in a multitude of physiological processes [12]. Carbonic anhydrase inhibitors (CAIs) are employed in clinical settings as diuretics, anti-glaucoma, anti-obesity, and antineoplastic agents [13]. Consequently, there is a scientific imperative to explore novel natural CA inhibitors.

Diabetes mellitus (DM) is a prevalent metabolic disorder, characterized by elevated blood glucose levels, triggered by the development of either insulin deficiency or resistance. The α-glucosidase enzyme plays a crucial role in regulating the process of carbohydrate digestion [14]. Inhibition of the activity of the α-glucosidase enzyme can delay the absorption of glucose by the body. Thus, there is a significant need to identify novel natural α-glucosidase inhibitors for the management of diabetes.

A major global public health challenge is represented by the alarming prevalence of Gram-negative bacteria that exhibit high levels of antibiotic resistance. The morbidity and mortality rates associated with these infections are high, largely due to a paucity of effective treatment options [15]. Research and development are vital to determine more effective natural antibacterials to combat bacterial infections.

The literature contains various studies investigating such as the phenolic composition, antimicrobial, and antioxidant properties of *A. vulgaris*, with a particular focus on the biological activities [1,6,7,8,16]. A review of the literature revealed that no study had been conducted to evaluate the antibiofilm and carbonic anhydrase inhibition activities of *A. vulgaris*. The objective of the present study was to evaluate the antibiofilm, antioxidant, and antimicrobial activity and carbonic anhydrase and α-glucosidase inhibition abilities of aerial parts of *A. vulgaris* extracts.

2. Materials and methods

2.1. Chemicals and reagents

Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), methanol, acetic acid, gallic acid, quercetin, ammonium acetate, ammonium nitrate, ethanol, NaOH, $CuCl_2$, NaCl, HCl, Na_2CO_3, CH_3COONH_4, neocuproine (2,9-Dimethyl-1,10-phenanthroline), DPPH• (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), $FeCl_3.6H_2O$, , α-glucosidase (*Saccharomyces cerevisiae*, lyophilized powder, ≥ 10 units/mg protein), 4-nitrophenyl-α-D-glucopyranoside, sulfanilamide (4-aminobenzenesulfonamide), Folin-Ciocalteu's, phenol reagent, and yeast extract were purchased from Sigma Aldrich (St. Louis, MO, USA). TPTZ (2,4,6-tris(2-pyridyl)-s-triazine), bovine carbonic anhydrase (BCA, lyophilized powder, ≥ 2000 W-A units/mg protein), tryptone, and crystal violet were purchased from Merck (Darmstadt, Germany). 4-nitrophenil acetate was purchased from BLDpharm (Shanghai, China).

2.2. Plant material and sample preparation

The dried aerial parts of *A. vulgaris* were obtained from an herbalist in Trabzon in February 2025. The dried aerial parts were pulverized using a grinder. Three different solvents, water, 50:50% water:ethanol, and ethanol were utilized for the extraction process. The water, 50:50% water:ethanol, and ethanol extracts are coded WE, WEE, and EE, respectively.

The dried and powdered aerial parts (10 g) of *A. vulgaris* were subjected to solvent (100 mL) extraction for a period of 2 hours, employing continuous stirring at ambient temperature. The quantity of the extracts was determined subsequent to filtration through 0.45 µm syringe filters (Whatman) and concentrating under reduced pressure. The process of dissolution was carried out for each extract in its own solvent, with the objective of achieving the desired concentration. The extracts were then maintained at a temperature of 4°C until further utilization in subsequent experiments.

2.3. Determination of total phenolic and flavonoid contents

2.3.1. Total phenolic content (TPC)

The total phenolic content of the aerial parts of *A. vulgaris* extracts was determined using the Folin-Ciocalteu reagent method, as described by Slinkard and Singleton [17]. 50 µL of the sample solution was mixed with 250 µL of 0.2 N Folin-Ciocalteu reagent. Then 750 µL of Na_2CO_3

(7.5%) was added to the mixtures, and the reaction solutions were incubated for 2 hours. Following the conclusion of the incubation period, the absorbances were determined spectrophotometrically at a wavelength of 765 nm. The standard calibration graph of gallic acid was prepared and the amount of phenolic compound in the samples was calculated as gallic acid equivalent (μg GAE/mL).

2.3.2. Total flavonoid content (TFC)

The total flavonoid content of the extracts obtained from the aerial parts of *A. vulgaris* was determined in accordance with the method established by Fukumoto and Mazza [18]. Following the addition of the sample solutions to the test tubes, 1 M ammonium acetate (CH_3COONH_4) and 10% aluminum nitrate ($Al(NO_3)_3.9H_2O$) were introduced to the tubes in the prescribed manner. Following the conclusion of the incubation period (40-minutes), the absorbances were measured at a wavelength of 415 nm. The standard calibration graph of quercetin was prepared and the amount of flavonoid compound in the samples was calculated as quercetin equivalent (mg QE/mL).

2.4. Determination of antioxidant activities

2.4.1. DPPH • radical scavenging activity

The DPPH radical scavenging activities of extracts obtained from the aerial parts of *A. vulgaris* were examined by employing the method that had been described by Brand-Williams et al. [19]. The testing concentrations of extracts from the aerial parts of *A. vulgaris* were adjusted with the objective of yielding results indicative of scavenging activity. The methodology comprised the mixing of the extracts with a DPPH solution, with the mixture then being maintained at ambient temperature and in the absence of light for a period of 50 minutes. The absorbance of the solution treatment with standard and extracts was measured at a wavelength of 517 nanometers. A graph was generated based on the concentrations that corresponded to the values of the absorbances that had been determined. The quantity of sample necessary to reduce the DPPH concentration by 50% was determined in mg/mL, and this is represented in the graph as the SC_{50} (half of the maximal scavenging concentration) value. A comparison was made between the scavenging capacity of the extracts and that of the standard antioxidant, Trolox.

2.4.2. ABTS $^{\bullet+}$ radical scavenging activity

The ABTS radical scavenging activities of extracts obtained from the aerial parts of *A. vulgaris* were examined by employing the method that had been described by Re et al. [20]. The preparation of the ABTS stock solution involved the dissolution of ABTS, followed by its mixture with a potassium persulfate solution. Subsequently, the mixture was left at ambient temperature and in darkness for a period of 18 hours in order to obtain the ABTS radical cation ($ABTS^{\bullet+}$). At the end of this period, it was diluted to approximately 1/50 of its original concentration, and its absorbance was adjusted to 0.07 at 734 nm. Trolox was utilized as the antioxidant standard, and was studied in triplicate at six distinct concentrations. Following a 20-minute interval, the absorbances of each sample were measured at a wavelength of 734 nm. The quantity of sample required to reduce $ABTS^{\bullet+}$ concentration by 50% was calculated as mg/mL, with the results expressed as SC_{50}.

2.4.3. Ferric reducing antioxidant power (FRAP)

The FRAP effects of the extracts obtained from the aerial parts of *A. vulgaris* were evaluated in vitro according to the method described by Benzie and Strain [21]. Following a series of preliminary trials, it was established that all extracts should be diluted to a concentration of 0.1 mg/mL. Subsequently, 50 μL of each extract and standard solution were combined with 1.5 μL of freshly prepared FRAP reagent. Subsequent to a 20-minute incubation period, the absorption values were measured at a wavelength of 595 nm. The results were calculated in μM TEAC (Trolox Equivalent Antioxidant Capacity) by employing a standard curve that had been prepared from Trolox solutions.

2.4.4. Cupric reducing antioxidant capacity (CUPRAC)

The original description of the CUPRAC assay was provided by Apak and colleagues [22]. This assay was modified and utilized for the analysis of the aerial parts of *A. vulgaris* extracts in the present study. Preliminary trials indicated that all extracts should be studied by dilution to a concentration of 0.1 mg/mL. In the initial phase of the experimental procedure, equal volumes of a Cu (II) chloride solution, a neocuproin solution, and an ammonium acetate buffer were added to test tubes, respectively. Then, the spectrophotometric measurement of the absorbance values was conducted at a wavelength of 450 nm. The antioxidant capacity of the extracts was calculated in μM TEAC, utilizing a standard antioxidant Trolox graph that had been studied at various concentrations.

2.5. Determination of enzyme inhibition

2.5.1. Carbonic anhydrase inhibitory activity

The aerial parts of *A. vulgaris* extracts were tested for carbonic anhydrase inhibitory (CAI) activity by using bovine carbonic anhydrase enzyme (BCA). The CA enzyme catalysis the hydrolysis of *p*-nitrophenyl acetate (PNPA) to *p*-nitrophenol and p-nitrophenolate ions. The

measurement of the absorbency at 348 nm, resulting from the formation of *p*-nitrophenol and *p*-nitrophenolate, is conducted at the conclusion of the reaction. In order to determine the inhibition activity of extracts, a series of reactions were conducted in test tubes. The reactions, comprised 150 µL of enzyme, 50 µL of inhibitor, 550 µL of 0.05 M phosphate buffer (pH: 7.4) and 750 µL of 3 mM substrate (*p*-nitrophenyl acetate). Inhibition activity is expressed in terms of IC_{50} values, representing the concentration of the sample that yields 50% inhibition of enzyme activity [23]. Sulfanilamide was studied as a standard inhibitor. The IC_{50} values of sulfanilamide and the samples were calculated in mg/mL.

2.5.2. α-Glucosidase inhibitory activity

The α-glucosidase enzyme activity of the aerial parts of *A. vulgaris* extracts was investigated through a modified approach [24]. A volume of 20 µL of sample and 30 µL of α-glucosidase enzyme (Saccharomyces cerevisiae, lyophilized powder, ≥ 10 units/mg protein) were added to 650 µL of phosphate buffer (pH: 6.8 and 0.1 M). The mixture was then maintained at 37 °C for a period of 10 minutes. Subsequently, 75 µL of substrate (4-nitrophenyl-α-D-glucopyranoside) was added to the tubes. The mixture was then maintained at the initial temperature for a further 20 minutes. Finally, 650 µL of 1 M Na_2CO_3 was added to the tubes. Absorbance values were measured at 405 nm in a UV/VIS spectrophotometer. Acarbose (positive control) was studied as a standard inhibitor. The IC_{50} values of acarbose and the samples were calculated in mg/mL.

2.6. Determination of antimicrobial activities

2.6.1. Agar diffusion assay

For the agar diffusion test, 6 different microorganisms were used (SA: *Staphylococcus aureus* ATCC 25923 and SP: *Streptococcus pyogenes* ATCC 19615, EC: *Escherichia coli* ATCC 25922, PA: *Pseudomonas aeruginosa* ATCC 43288, PV: *Proteus vulgaris* ATCC 13315, YP: *Yersinia pseudotuberculosis* ATCC 911). Holes were made in the agar using sterile hole punchers (diameter 6 mm), and extracts were added to each hole (50 µL). Ampicillin was used as a positive control, and 50 µL was loaded into the central hole. The prepared petri dishes were kept at room temperature for 2 hours and then incubated at 37°C for 24 hours, and the inhibition zone diameters were measured with a scale [25].

2.6.2. Minimum inhibitory concentrations (MIC)

Minimum inhibitory concentrations (MIC) of the aerial parts of *A. vulgaris* extracts were determined using the broth microdilution method. Initial concentrations of the extracts were used as 10 mg/mL. MIC values were investigated against previously determined clinical antibiotic-resistant biofilm forming capacity of *A. baumannii* strains. The assays have been performed in 96 plates and in triplicate [25,26].

2.6.3. Antibiofilm properties

The 1/2 MIC values of the aerial parts of *A. vulgaris* extracts were used as a reference for the antibiofilm activity test against antibiotic-resistant *A. baumannii*, which has a strong biofilm formation capacity. After overnight incubation of A. baumannii in 3 mL LB medium, 1/100 dilutions were added to 96-well plates together with 1/2 MIC extracts. Plates were incubated at 37 °C for 24 hours. The suspension in the 96-well plate was then decanted, and the plate was washed three times with distilled water. 200 µL of 1% crystal violet dye was added to each well and incubated for 20 minutes at ambient temperature. The crystal violet was removed from the plates, washed with distilled water, and allowed to dry for 15 minutes at ambient temperature. 200 µL of 95% ethanol was added into the wells. Optical absorbance (A) was measured at 620 nm on a spectrometer. The experiment was carried out in triplicate. The evaluation was based on four different criteria [26].

2.7. Statistical evaluation

The measurements were made in triplicate, and the mean values are reported. Standard deviations were calculated in Microsoft Excel. The percentage relative standard deviations were in the range of 2-5%. The regression analyses were done by using Microsoft Excel with R^2 values over 0.98.

3. Results and discussions

3.1. Evaluation of total phenolic and flavonoid contents

Phenolic and flavonoid compounds represent a noteworthy group of antioxidants, which are present in significant concentrations within the plants.

In the present work, WEE had the highest total phenolic content, with 612 ± 1.92 µg GAE/mL (Table 1). According to the literature, Vlaisavljević et al. [1] reported that the total phenolic contents of methanolic, ethanolic, ethyl acetate, and water extracts of *A. vulgaris* were 7.71, 7.40, 9.65, and 6.89 mg GAE per g of extract, respectively. Tasić-Kostov et al. [27] reported that the total polyphenol contents of ethanolic and water extracts of *A. vulgaris* were 110.80 and 82.16 µg GA/mg, respectively. Jelača et al. [16] reported that the ethanolic extract of *A. vulgaris* L. represents a valuable source of bioactive compounds. They also reported that the total phenolic content of *A. vulgaris* extract was 7.55 ferulic acid equivalents per gram of dry weight.

The total flavonoid content of the WE, WEE, and EE extracts is shown in Table 1. The EE had the highest total flavonoid content, with a value of 86.3 ± 2.5 μg QE/mL, while the WE extract demonstrated the lowest total phenolic content, with a value of 43.8 ± 1.8 μg QE/mL. In the literature, Kanak et al. [6] reported that twenty flavonoids were detected in aqueous ethanol (80% v/v) obtained from the leaves of *A. vulgaris*. Jelača et al. [16] reported that the total flavonoids content of the *A. vulgaris* extract was 6.99 quercetin equivalents per gram of dry weight. Tasić-Kostov et al. [27] reported that the total flavonoid contents of ethanolic and water extracts of *A. vulgaris* were 128.09 and 52.29 μg rutin/mg, respectively.

Table 1. Total phenolic and flavonoid contents of the aerial parts of *A. vulgaris* extracts

Samples	Total phenolic content (μg GAE/mL)	Total flavonoid content (μg QE/mL)
WE	462 ± 1.67	43.8 ± 1.8
WEE	612 ± 1.92	54.5 ± 4.1
EE	423 ± 2.89	86.3 ± 2.5

3.2. Evaluation of antioxidant activity

The present study investigated the antioxidant activities of the WE, WEE, and EE extracts, as determined by the DPPH, ABTS, FRAP, and CUPRAC methods.

In the present work, the SC_{50} values of DPPH scavenging activities of standard and extracts are presented in Table 2. The DPPH radical scavenging assay showed that WEE had the highest antioxidant activity, with an SC_{50} value of 0.0056 ± 0.0002 mg/mL, whereas EE had the lowest activities, with an SC_{50} value of 0.0099 ± 0.0004 mg/mL. According to the literature, Boroja et al. [7] reported that the SC_{50} values of DPPH radical scavenging in methanolic extract of above ground parts of *A. vulgaris* was 0.0059 mg/mL. In another study, Tasić-Kostov et al. [27] reported that the SC_{50} values of DPPH radical scavenging activity in the aerial parts of ethanolic and water extracts of *A. vulgaris* with, 0.11 and 27.22 μg/mL, respectively.

The SC_{50} values of ABTS scavenging activities of standard and extracts are presented in Table 2. WEE had the highest reducing activities, with 0.0028 ± 0.0001 mg/mL, while WE and EE were exhibited values of 0.0048 ± 0.0002, 0.0243 ± 0.0006, and 0.0055 ± 0.0003 mg/mL, respectively. In the literature, Boroja et al. [7] reported that the SC_{50} values of ABTS radical scavenging in methanolic extract of aboveground parts of *A. vulgaris* was 0.0148 mg/mL. In another study, Vlaisavljević et al. [1] reported that the SC_{50} values of ABTS radical scavenging activity in the aerial parts of 70% ethanol and water extracts of *A. vulgaris* were 119.62 and 37.50 mg TE / g DE, respectively.

As demonstrated in Table 2, the results of the FRAP activities of the standard and the extracts are expressed in terms of μM TEAC. The WEE extract demonstrated the highest level of reducing activity, with a TEAC of 267 ± 1.39 μM, while the EE extract exhibited the lowest reducing activity, with a TEAC of 179 ± 3.42 μM. In the literature, Vlaisavljević et al. [1] reported that the FRAP values of the aerial parts of 70% ethanol and water extracts of *A. vulgaris* were 6405.75 and 3240.09 mg AAE / g DE, respectively.

The CUPRAC activities of the standard and the extracts are presented in Table 2, expressed as μM TEAC. WEE had the highest reducing activities, with 0.081 ± 0.003 μM TEAC, while WE and EE were exhibited values of 0.067 ± 0.002 and 0.064 ± 0.001 μM TEAC, respectively. Vlaisavljević et al. [1] reported that the CUPRAC values of the aerial parts of 70% ethanol and water extracts of *A. vulgaris* were 203.53 and 78.56 mg TE / g DE, respectively.

In this study, in accordance with the literature, it was determined that the WEE extract has a strong antioxidant capacity. Moreover, the results of the DPPH, ABTS, FRAP, and CUPRAC antioxidant activity tests exhibited positive correlation.

Table 2. DPPH and ABTS radical scavenging capacities, FRAP and CUPRAC antioxidant activities of the aerial parts of *A. vulgaris* extracts

Samples	DPPH (SC_{50} values mg/mL)	ABTS (SC_{50} values mg/mL)	FRAP (μM TEAC)	CUPRAC (μM TEAC)
WE	0.0077 ± 0.0003	0.0048 ± 0.0002	206 ± 3.15	0.067 ± 0.002
WEE	0.0056 ± 0.0002	0.0028 ± 0.0001	267 ± 1.39	0.081 ± 0.003
EE	0.0099 ± 0.0004	0.0055 ± 0.0003	179 ± 3.42	0.064 ± 0.001
Trolox	0.0020 ± 0.0001	0.0026 ± 0.0001	—	—

3.3. Evaluation of enzyme inhibition

CAIs are valuable for their use in many medical conditions including diuretic, anti-glaucoma, anti-epileptic, anti-obesity, and anti-cancer [13]. A considerable number of phenolic compounds with antioxidant properties have been documented to possess CA inhibition capabilities [28]. In addition, newly synthesized compounds are being evaluated for their carbonic anhydrase inhibitory activity [23]. In recent years, there has been an increase in the search for CAIs, whether synthetic or natural. Therefore, the aerial parts of *A. vulgaris* extracts were tested for CAI by using BCA. It was evident that all extracts exhibited significant inhibitory activity against BCA. The WEE extract demonstrated the most effective inhibitory activity, exhibiting low IC_{50} values of 0.0628 mg/mL (Table 3).

Table 3. IC_{50} values of the aerial parts of *A. vulgaris* extracts in carbonic anhydrase inhibitory (CAI) activity test

Sample/Standard	IC_{50} Value (mg/mL)
Sulfanilamide	0.0004
WE	0.1748
WEE	0.0628
EE	0.0754

It has been established that the inhibition of the α-glucosidase enzyme is a pivotal strategy for the prevention of diabetes, which is a major public health concern. For the purpose of this study, the aerial parts of *A. vulgaris* extracts were examined as potential natural inhibitors of the α-glucosidase enzyme. The α-glucosidase enzyme activity of the WE, WEE, and EE extracts was measured as 0.1275, 0.0535, and 0.1623 mg/mL IC_{50}, respectively (Table 4). The highest α-glucosidase enzyme inhibition value of the plant was observed in the WEE, and the lowest in the EE. The present review demonstrated the findings from studies conducted in the literature on the α-glucosidase enzyme activity of different species belonging to the *Alchemilla* family. Methanol and pure water were utilized as solvents in the course of these studies. It was determined that the α-glucosidase enzyme activity of the methanol extract was higher than that of pure water [29,30]. In a study conducted with *A. vulgaris* L. species, different enzyme activities (lipase activity and α-amylase activity) were examined. In the study, leaf and flower parts of the plant were extracted using different solvents (MeOH, MeOH 70%, EtOH, EtOH 70%, Hexane and Chloroform). In the study conducted with these enzymes, it was observed that the ethanol-water mixture showed activity [31].

Table 4T. α-Glucosidase enzyme inhibition activities of the aerial parts of *A. vulgaris* extracts

Sample/Standard	IC_{50} Value (mg/mL)
Acarbose	0.0118
WE	0.1275
WEE	0.0535
EE	0.1623

3.4. Evaluation of antimicrobial activity

The activities of the WE, WEE, and EE extracts against standard bacteria were investigated by the agar diffusion method. The results obtained from the study indicated that the WE and WEE extracts demonstrated no effect against the six bacterial strains employed in the experiment. The investigation revealed that the EE extract exhibited activity against both Gram-negative and Gram-positive bacteria utilized in the study. Ampicillin was used as a control. Accordingly, it was observed that the EE extract exhibited the highest activity against YP and PV, with zone diameters of 18 and 17, respectively. It was also observed that EE extract exhibited the lowest activity against SP, with a zone diameter of 12. In addition, EE extract was established that the same inhibitory effect (15 zone diameter) against SA, PA, and EC (Fig. 1).

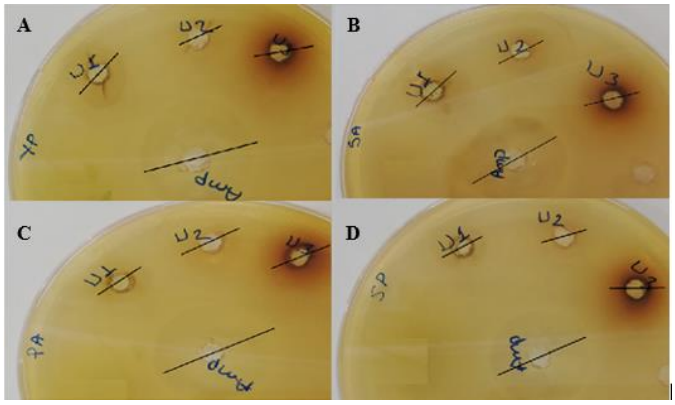

Figure 1. Antimicrobial activity of the aerial parts of *A. vulgaris* extracts

A: YP (*Yersinia pseudotuberculosis* ATCC 911),
B: SA (*Staphylococcus aureus* ATCC 25923),
C: PA (*Pseudomonas aeruginosa* ATCC 43288),
D: SP (*Streptococcus pyogenes* ATCC 19615)
U1: EE (ethanol extract of aerial parts of *Alchemilla vulgaris* L.);
U2: WE (water extract of aerial parts of *Alchemilla vulgaris* L.);
U3: WEE (50:50% water:ethanol extract of aerial parts of *Alchemilla vulgaris* L.);
Amp: Ampicillin (control).

In a study investigating the antimicrobial activity of the lady's claw plant in 2022, it was seen that the extracts had MIC values of 2.5-5-10 mg/mL against different microorganisms [32]. Aerial parts of *Alchemilla alpina* L. were extracted using different solvents (methanol, ethanol, and chloroform), and their antimicrobial activity was determined by disk-disk diffusion method, and it was shown that these extracts inhibited the growth of some bacteria at different rates (8-23 mm) [33]. In a different report investigating the biological activity of the extract of the aerial part of *A. vulgaris*, it has antimicrobial activity against both bacteria and fungi [34]. In 2024, the antimicrobial activity of the plant *Alchemilla holotricha* Juz. was investigated, and the extract had the highest antibacterial activity against *E. coli* with a measurement of 17 mm and the lowest activity against *Bacillus subtilis* with a diameter of 8 mm [35]. In this study, it was observed that the plant has activity against both Gram-negative and Gram-positive bacteria. The findings will contribute to the foundation for more extensive and in-depth research on the potential effects of plant extracts [34].

A. baumannii is responsible for a variety of medical device-associated infections, urinary tract infections, meningitis, endocarditis, respiratory infections, wound infections, and bacteremia in hospitalized patients. All of these infections are associated with the formation of biofilms [36]. The MIC and antibiofilm effects of extracts

against antibiotic resistant clinical *A. baumannii* isolates were investigated. EE and WE extracts were found to have MIC values of 5 mg/mL, while WEE extract was found to have MIC value of 25 mg/mL against *A. baumannii*. After determining the MIC values, antibiofilm assay was performed using half MIC value and *E. coli* DH5@ isolate as a control strain. According to the data, *A. baumannii* has a strong biofilm formation capacity. When treated with extracts, it was observed that the biofilm formation capacity of the strain decreased. The EE and WE extracts reduced the biofilm formation capacity of the strain to a weak level, while WEE extract reduced it to a moderate level.

In a study conducted by Kardil et al. in 2024 investigating the effect of plant extract on the biofilm formation capacity of clinical antibiotic-resistant *A. baumannii* isolate, it was determined that methanol, water, and 50:50% methanol:water extracts reduced the biofilm formation capacity of *A. baumannii* isolate by approximately 1.7, 1.6, and 1.3 times, respectively [37]. In the same year, Kardil et al., in a study investigating the antibiofilm effect of the *Vaccinium arctostaphylos* L. leaf and fruit extracts against clinical antibiotic-resistant *A. baumannii* isolates, found that the plant's leaf extract significantly reduced the biofilm-forming capacity of the *A. baumannii* isolate compared to the fruit extract [38]. In a study carried out by Ouslimani et al., *A. verticillata* and *C. cassia* were found to possess the ability to inhibit the formation of biofilms, in addition to weakening and dissolving pre-formed biofilms. [39]. In this study, in accordance with the literature, the EE of the plant significantly reduced the biofilm formation capacity of the *A. baumannii* isolate, which has a strong biofilm formation capacity.

4. Conclusions

Medicinal plants represent a significant source of active biological compounds that have the potential for use in the development of new drugs. The choice of solvents and solvent compositions with differing polarities that are utilized in the extraction of plants has affected the type and amount of bioactive components extracted. In this study, the total phenolic and flavonoid contents and the antioxidant, carbonic anhydrase inhibitory, α-glucosidase inhibitory, antimicrobial, and antibiofilm activities of aerial parts of *A. vulgaris* L. were presented. The study showed that the use of diverse solvent compositions resulted in the extraction of different bioactive components, which led to remarkable differences in the biological activity. WEE, together with the highest total phenolic content, showed the highest antioxidant, carbonic anhydrase inhibition, and α-glucosidase inhibition activities. In addition, a good correlation has been found between the antioxidant activity, the total phenolic content, and the enzyme inhibitory effects. In contrast, the findings demonstrate that the EE exhibited the most pronounced total flavonoid, antimicrobial, and antibiofilm activity. In this study it was shown for the first time that the aerial parts of *A. vulgaris* L. have both a carbonic anhydrase inhibitory activity and an antibiofilm effect. The findings of our investigation suggest that the WEE and EE may serve as promising candidates for the prevention and treatment of diseases associated with oxidative damage, cancer, diabetes, and bacterial infections. As a result, further research is needed to confirm these biological activities and to elucidate the underlying mechanisms of action.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Authors' contribution:

UK: Conducted the extraction, antioxidant activity and enzyme activity experiments and wrote the article. ZA: Conducted the antioxidant activity and enzyme activity experiments. AÖD: Performed the antimicrobial, antibiofilm experiments. All authors contributed to the study conception and design. The authors read and approved the final manuscript.

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