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ESTIMATING PHYTOCHEMICAL CONTENT, ANTIOXIDANT AND ANTIBACTERIAL EFFICACY OF WILD ADIANTUM CAPILLUS-VENERIS

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Abstract

This study evaluated some chemical compounds, and the antioxidant and antibacterial activities of *Adiantum capillus-veneris*. The samples were collected from Penjwen in Sulaymaniyah city, Iraq. The methanolic extract had an overall phenolic value of 45.89 milligrams GAE/gram, evaluated through the Folin-Ciocalteu reagent. The measured total flavonoid content was 24.58 mg RE/g, determined using the aluminum chloride method. Moreover, the methanolic extract contained total glycoside (121 mg securidaside/g) and total saponin (16 mg/g). The HPLC analysis found that the sample contained ferulic acid, p-coumaric acid, apigenin, catechin, and luteolin. Of these compounds, p-coumaric acid and catechin were the most abundant in concentration. The extract's antioxidant activity was assessed using the DPPH method, with the *A. capillus-veneris* extract exhibiting scavenging of DPPH radicals at an IC50 value of 106 µg/mL, compared to 250 µg/mL for ascorbic acid. The antibacterial activity of methanolic extract against both gram positive (*Enterococcus faecalis* and *Staphylococcus aureus*) and gram negative (*Escherichia coli* and *Pseudomonas aeruginosa*) bacteria was evaluated using the disc diffusion method. The diameters of inhibition zone values for all tested bacteria increased at higher *A. capillus-veneris* extract

concentrations, with *S. aureus* and *E. coli* being the most sensitive bacteria. The minimum inhibitory concentration of the extract was 100 µg/mL. This study highlights the potential of *A. capillus-veneris* as a natural source of antioxidant and antibacterial compounds.

Keywords: Adiantum capillus-veneris, Chemical compound, HPLC, Antioxidant, antibacterial.

تقدير المحتوى الكيميائي والفعالية المضادة للأكسدة وللبكتريا لنبات كزبرة البئر البري Adiantum capillus-veneris

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الخلاصة

تهدف هذه الدراسة الى تقدير محتوى النبات كزبرة البئر *Adiantum capillus-veneris* من بعض المركبات الكيميائية اضافة الى دراسة فعاليتها المضادة للأكسدة وللبكتيريا، تم جمع هذه النباتات من مايس 2023 من منطقة بنجوين في مدينة السليمانية والتي تقع في شمال العراق وعلى خطوط عرض 36°20'46.2"N وعلى خطوط الطول 44°42'39.4"E وعلى ارتفاع 1083م عن مستوى سطح البحر. وقد اظهرت النتائج ان محتوى المستخلص الميثانولي لهذا النبات من الفينولات الكلية والتي تقديرها بطريقة كاشف فولن كان 45.89 ملغم حامض الغاليك/غم، في حين كان المحتوى من الفلافونيدات الكلية والتي قدرت بطريقة لكلوريد الألمنيوم كان 24.58 ملغم روتين/غم. وقد تم دراسة المحتوى من الكلايكوسيدات الكلية والتي بلغت 121 ملغم سيكيورياساييد/غم والصابونينات الكلية 16 ملغم/غم. وقد وجد عند تحليل المستخلص بواسطة HPLC احتوائه على حامض الفريوليك وحامض الكوماريك والابجين والكاتكين والليتينول وكان كل من حامض الكوماريك والكاتكين هما الأكثر تركيزا قياسا بالمركبات الأخرى. وقد اظهرت نتائج الفعالية المضادة للأكسدة بواسطة طريقة DPPH، ان قيمة IC50للمستخلص كانت 106 ميكروغرام/مل مقارنة ب 250 ميكروغرام/مل لحامض الأسكوربيك، كما بينت نتائج فعالية المستخلص ضد البكتيريا موجبة الغرام (*Staphylococcus aureus* *Enterococcus faecalis*) وسالبة الغرام (*Escherichia coli* and *Pseudomonas aeruginosa*) (and بطريقة الانتشار القرصي ان يتم منطقة التثبيط لجميع أنواع البكتيريا المدروسة ازدادت بزيادة تركيز المستخلص. وقد لوحظ ان كلا من *S. aureus* و*E. coli* هما الأكثر حساسية قياسا بأنواع البكتريا الأخرى قيد الدراسة. وقد وجد ان الحد الأدنى لتركيز التثبيط (MIC) لهذا المستخلص هو 100 ميكروغرام/مل. ومن خلال هذه النتائج تم التوصل الى أن هذا

النبات يحتوي على تراكيز عالية من المركبات الفعالة والتي ممكن ان تعد مصدرًا طبيعيًا واعدا كمركبات مضادة للأكسدة وللبكتيريا.

كلمات مفتاحية: Adiantum capillus–veneris، المركبات الكيميائية، HPLC، مضادة الأكسدة ومضادة البكتيريا.

Introduction

Plants are among of the most significant sources of medicines. The oldest forms of medicine were traditionally derived from medicinal plants. Every community has utilized plants throughout history, and in poor countries, most people receive their primary medical care from plant-based traditional medicine (24). Culture and philosophy impact traditional medical practices, which differ from country to country. According to World Health Organization (WHO) estimates, about 80% of the global population depend on natural products for their health as herbal medicines are inexpensive, readily available, and free of side effects (40). The use of medicinal plants to replace chemical and synthetic drugs is increasing, contrary to some people's belief that traditional medicine is worthless and unusable in modern science (36).

Adiantum capillus-veneris, a fern belonging to the Pteridaceae family, is found worldwide, including in Iraq and neighboring countries (5), and thrives in areas characterized by high humidity and warm temperatures (25). Various active compounds such as phenylpropanoids, flavonoids, terpenoids, carotenoids, steroids, and other chemicals have been extracted from different *Adiantum* species (5). *A. capillus-veneris* has been shown to have properties that include analgesic, anti-diabetic, anti-thyroidal, anticonvulsant, hypocholesterolemic, antidiarrheal, antimicrobial, wound healing, goitrogenic, anti-inflammatory, antispasmodic, antioxidant, and anti-asthmatic effects (14). Extracts from this fern have exhibited promising microbiological activities (11). The protective effects against various diseases may be attributed to the presence of antioxidants like phenolic and flavonoid compounds found in plant (21).

The active compounds found in medicinal plants have garnered significant interest, since they hold promise as potential sources of new found prototype antibiotics and exhibit vast therapeutic possibilities against numerous contagious diseases. The antibacterial properties of phytochemicals are associated with fewer side effects (39).

The prospect of developing antibacterial agents from plants is intriguing, as it could pave the way for the creation of phytomedicines to combat microbes. With many clinically active antibiotics losing effectiveness due to their increasing resistance, the current landscape presents significant clinical challenges in infectious disease treatment. Therefore, biomolecules derived from plants have emerged as viable alternatives in the battle against antibiotic-resistant pathogens (8).

Although medicinal herbs, including ferns, are extensively utilized in Iraq, there is a scarcity of information regarding their active phytochemicals and biological properties (3). Therefore, this study sought to estimate the phytochemical composition and some biological activities of the extract in the plants under examination.

Materials and Methods

Plant collection and identification: Arial parts of *A. capillus-veneris* were collected at an altitude of 1083 m in May 2023 from Penjwen in Sulaymaniyah city in the north of Iraq. The latitude of the sampling place was 36°20'46.2"N and the longitude is 44°42'39.4"E. Table 1 shows meteorological data of Penjwen during March, April, and May. The plant was identified at the Iraqi National Herbarium by the taxonomist Ali Haloob Kadhim and also accepted by (20).

Table 1: Meteorological data of Penjwen for March, April, and May 2023.

Month	Sum	Average					
	Rainfall (mm)	Temperature (°c)	Humidity (%)	Pressure	Sun duration (hr.)	Wind speed (m/sec.)	Wind direction
March	241	9.7	74	872.9	5.3	1.8	200
April	136.6	12.8	63	873.5	7.1	1.8	190
May	47	17.9	52	874.3	7.3	1.7	190

Plant extract preparation: The plant parts were cleaned and dried at a room temperature of 25°C. The samples were ground well using an electric blender and then stored at 4°C. The extraction was carried out according to (38) with minor modifications. Plant powder (100 g) was soaked in 1000 mL of methanol overnight on an automatic shaker at 30°C. The extraction was filtered through Whatman filter paper No. 1. Thereafter, the methanolic extract was dehydrated until reaching a stable weight at 38°C, then preserved at 4°C.

Phytochemical contents: Total phenolic content was determined in the methanolic extract with Folin-Ciocalteu reagent described by (1) with minor modifications. 100 µl of extract (1 mg/mL methanol) was mixed with 4 mL of 10% Folin-Ciocalteu reagent and allowed to react for 5 min. at room temperature (RT). After that, 2 mL of 20% sodium carbonate solution was added and left for 60 min. at RT. Concerning blanks, the same steps above were repeated except for the extract. The measurement was done by using a spectrophotometer at 765 nm. The total amount of phenolic compounds was represented in milligrams of gallic acid equivalent (GAE) (Sigma-Aldrich, Germany) per gram of dried extract.

Total flavonoid content was evaluated using the aluminum chloride colorimetric method (26) with slight modifications. Initially, 0.5 mL of extract (1 mg/mL methanol) was diluted to 1 mL with methanol, then mixed with 4 mL of dH2O. Subsequently, 0.3 mL of 5% NaNO2 was added, followed by 0.3 mL of 10% AlCl3 after a 5-minute incubation. After standing for 6 minutes, 2 mL of 4% NaOH was added, and the final volume of the mixture was made up to 10 mL with double dH2O. Following a 15-minute incubation at RT, the absorbance was measured at 510 nm. The total flavonoid was evaluated using a calibration curve and expressed as milligrams of rutin equivalent (RE) (Sigma-Aldrich, Germany) per gram of dried extract.

For determination of glycosides, 1 mL of the extract (1 mg/mL) was mixed with 1 mL of freshly prepared Baljet's reagent [1% picric acid (95 mL) +10% NaOH (5 mL)]. Following an hour, the solution was diluted with 20 mL of dH2O, and the absorbance was measured by spectrophotometer at 495 nm. The results of the total glycoside

content were calculated based on a standard curve and expressed as mg of securidaside per gram of dried extract (22).

The total saponin content of the plant powder was measured according to the method described by (18). A measured weight (5 g) of the plant powder was mixed with 50 mL of 20% methanol. The solution was heated at 55ºC for 90 minutes in a water bath. After that, it was filtered via Whatman filter paper No. 42. The residue left after filtration was re-extracted with 50 mL of 20% methanol and both extracts were mixed and then condensed to approximately 40 mL at 90ºC. The combined extract was then moved to a separating funnel and mixed with 40 mL of diethyl ether. This process was repeated multiple times until the color of the aqueous layer became transparent. Normal butanol (60 mL) was used to extract the saponins. In a pre-weighed dish, the combined extracts were evaporated to dryness after washing with a 5% aqueous NaCl. An average was obtained by repeating the procedure twice more.

HPLC analysis: About 100 mL of chloroform was added to 20 grams of powder plant and placed on the electric vibrator for 3 hours to remove the fat from the sample. The chloroform layer was then removed using a separation funnel, and the sample dried at 38°C to ensure that no residues of chloroform remained. Then, 10 g of the dried sample was taken to extract using 100 mL of 70% methanol as the solvent. The extraction process was carried out using an Ultrasonic Bath at RT for 1 hour. The solvent was evaporated using rotary evaporator under vacuum, and dried at 38°C. Individual phenolic compounds were quantified using reversed phase HPLC analysis, employing a chromatographic system comprising a chemstation software, UV detector, binary pump, autosampler, online vacuum degasser, and a Zorbax Eclipse Plus-C18-ODS column. The gradient elution method used methanol (eluent A) and 1% formic acid in water (eluent B), with a sequence of 40% B (0-4 min) and 50% B (4-10 min). Operating conditions included a column temperature of 30ºC, a flow-rate of 0.7 mL/min, and an injected 100 µl volume of both samples and standards, automatically done via an autosampler. Spectra were acquired at 280 nm (32).

Antioxidant activity: The DPPH assay was assessed according to the method described by (4) with some modifications. To analyze the ascorbic acid and the extract for their antioxidant activities, they were dissolved in methanol (95%) at various concentrations (30, 60, 120, 250, 500 ppm). Then 50 µL of these solutions were combined with 1950 µL of a DPPH (6 x 10-5 M). The mixture was shaken vigorously, then incubated at RT in the dark for 30 min. A control was prepared by mixing 50 µL of methanol (95%) with 1950 µL of methanolic DPPH. The absorbance of the solution was then measured at 515 nm. The percentage of antioxidant activity was determined by the following equation:

$$\text{Antioxidant activity (\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of the sample}}{\text{Absorbance of control}} \times 100$$

The IC 50 value of the samples, representing the concentration of samples needed to inhibit 50% of the DPPH free radical, was measured by the relative correlation between antioxidant concentration and inhibition capacity.

Antibacterial activity: Gram positive bacteria (*Enterococcus faecalis* and *Staphylococcus aureus*) and gram negative bacteria (*Escherichia coli* and

Pseudomonas aeruginosa) were used. The isolates were confirmed using the vitek2 compact system at the microbiology lab/Central laboratory/College of Agricultural Engineering Science/University of Duhok.

Disc diffusion assay was used to evaluate the antimicrobial activity. This method was used as it is approved by CLSI standards (12). The plant extract underwent dissolution of sterile distilled water before passing via a 0.25 um sterile filter. After that, four concentrations of the *A. capillus-veneris* extract (25, 50, 75, and 100 µg/mL) were prepared through dissolving in sterile distilled water. Fresh overnighted bacterial suspensions were adjusted to 0.5 McFarland turbidity standard which is equal to 1.5×10^8 CFU/mL (27). Then, Nutrient Agar base was inoculated with bacteria suspensions (1.5×10^8 CFU/mL) using sterile swabs that were spread homogeneously on the petri dishes. Subsequently, discs (6 mm diameter) soaked with 10 µL of different concentrations of the plant extract were placed on the media surface. The two control discs included were: one with sterile distilled water as a negative control and another with amikacin (AK) (10 µg/disc) as a positive control. The inhibition zone was measured (mm) using a vernier tool after the plates were incubated at 37°C for 24 hours, with each experiment conducted in four replicates. The diameters of the disc and the halo of inhibition produced for each material were used to calculate the size of the inhibition zones, as described below (15 and 30).

Size of inhibition zone = diameter of halo - diameter of the disc

Minimum Inhibitory Concentration (MIC): For the preparation of the nutrient broth, suspend 13.0 g in 1000 mL dH_2O, heat to dissolve the medium, and sterilize using autoclave at 121°C for 20 min. The fresh overnight bacteria culture was adjusted to 0.5 McFarland to determine the MIC of *A. capillus-veneris* extract. A bacterial culture of 5 mL was dispensed into the tubes, followed by 10 mL of various concentrations of the plant extract (25, 50, 75, and 100 µg /mL). In addition, the tube containing Amikacin was used as negative control, while the tube that contained only bacterial culture served as a positive control. The tubes were left to incubate overnight at 37°C. After 24 hours, the tubes were visually checked for turbidity (bacterial growth). The MIC assay was performed in four replicates (6).

Statistical analysis: The collected data was submitted to SPSS program (35) for statistical analysis. One-way ANOVA (for the effect of treatment on the bacterial species, separately) and Two-way ANOVA (for the effect of the interaction between the treatment and bacterial species, together) were applied. However, the means within ANOVA were separated using Duncan's multiple range test at $P \leq 0.01$ (17).

Results and Discussion

Phytochemical contents: Figure 1 illustrates the concentration of total phenol, flavonoid, glycoside, and saponin in the dry extract of *A. capillus-veneris*. The total phenol is quantified as milligram of gallic acid per gram of dry extract (mg GAE/g). The results revealed that the dry plant extract displayed a moderate phenolic content, measuring 45.89 mg GAE/g. While (33) found lower levels (21.17 mg GAE/g) of phenolic compounds in the methanolic extracts of *A. capillus-veneris*. In contrast, a previous study (41) indicated a significantly higher total phenolic content of 83.62 mg

GAE/g in methanolic extract of the plant. The flavonoid content is represented as milligrams of rutin per gram of dry extract (mg RE/g). According to the analysis findings, the total amount of flavonoid was 24.58 mg RE/g.

Concerning the concentration of total glycoside, the glycoside content is represented as milligram of securidaside per gram of dry extract (mg securidaside/g). The total glycoside content of the methanolic extract was 121 mg securidaside/g. With regard to the saponin percentage, it was noted that the plant powder extract contained 16 mg/g. These results corroborate those of (2), who found that all parts of *A. capillus-veneris* contained phenol, flavonoid, glycoside, and, saponin. Polyphenolic composition varies both in quality and quantity among different plants, a variability that may be ascribed to the specificity of the chemical quantification methods for phenolic compounds and the type of standard employed (19).

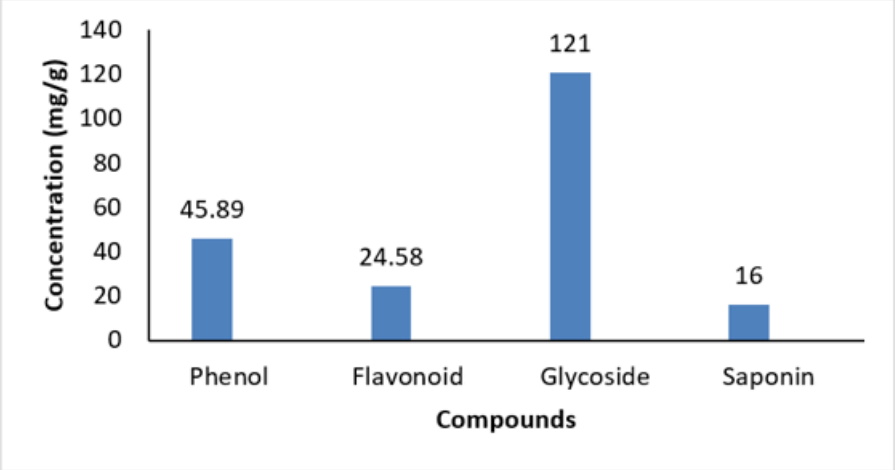

Figure 1: Total phenol, flavonoid, glycoside, and saponin contents of methanolic extract of A. capillus-veneris.

HPLC analysis: Figure 2 shows the chromatogram of various phenolic compounds of *A. capillus-veneris* methanolic extracts using HPLC, which resulted in 5 peaks. The quantitative analysis results for each identified compound revealed the presence of ferulic acid, p-coumaric acid, apigenin, catechin, and luteolin (Table 2). The major identified compounds were p-coumaric acid (152.2 µg/g) and catechin (152 µg/g), followed by luteolin (125.2 µg/g) and apigenin (99 µg/g), while ferulic acid had the lowest value 39.1 µg/g. These findings agree with (11) that the hydromethanolic extract of *A. capillus-veneris* contains ferulic acid, p-coumaric acid, apigenin, and catechin.

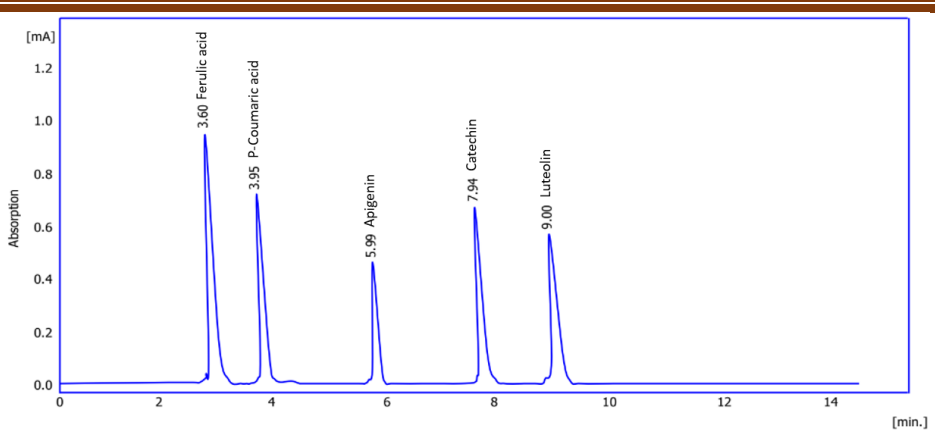

Figure 2: HPLC chromatogram showing different phenolic contents of methanolic extract of A. capillus-veneris.

Extraction has a crucial role in isolating and identifying phenolic compounds. Several factors influence the extraction process, including the attributes of phenol, along with the selected extraction method, extraction solvent, sample particle size, temperature, and pH (23). In fact, (7) observed variations in polyphenol content based on the choice of solvents and extraction durations. According to (29), commonly used extraction solvents for polyphenols include acidified methanol and methanol. Additionally, the chemical content of plant extracts is affected by climatic conditions, geographical origin, cultivation techniques, harvest timing, and sample composition (16).

Table 2: HPLC analysis of the phenolic contents of methanolic extract of A. capillus-veneris.

Compounds	Retention times (min.)	Concentration (µg/g)
Ferulic acid	3.60	39.1
P-Coumaric acid	3.95	152.2
Apigenin	5.99	99.0
Catechin	7.94	152.0
Luteolin	9.00	125.2

Antioxidant activity: The methanolic extract exhibited a significant DPPH radical neutralization effect (Figure 3). The percentage of antioxidant activity increased along with the increase in *A. capillus-veneris* methanolic extract and ascorbic acid concentration. The highest percentage of antioxidant activity was recorded at a concentration of 500 µg/mL in the plant methanolic extract (88.9%) and ascorbic acid (71.59%). The minimum concentration of *A. capillus-veneris* extract that inhibits 50% of the DPPH radical was 106 µg/mL. In comparison, the IC50 was 250 µg/mL recorded by ascorbic acid.

Several studies investigated the antioxidant properties of *A. capillus-veneris*. According to (10), the methanolic extract of this plant exhibited a weaker antioxidant effect, with an IC50 value of 280 µg/mL.

Phenolic compounds, which are widely found in plants and have high antioxidant activity, can be extracted mostly through their plant extracts (34). Moreover, phenolic and flavonoid contents play antioxidant roles among the various biological properties identified in *A. capillus-veneris* extracts (11). The structure of phenolic compounds, particularly the benzene ring and the number and position of the OH groups, determines

their ability or potential to have an antioxidant function. Antioxidant molecules are stabilized as a result of their reaction with free radicals (30). Due to resonance stabilization, the aromatic ring that donates the hydrogen atom transforms into a less reactive phenoxy radical. Phenolic compounds exhibit various functions as hydrogen donors, reducing agents, metal chelators, and singlet oxygen quenchers (37).

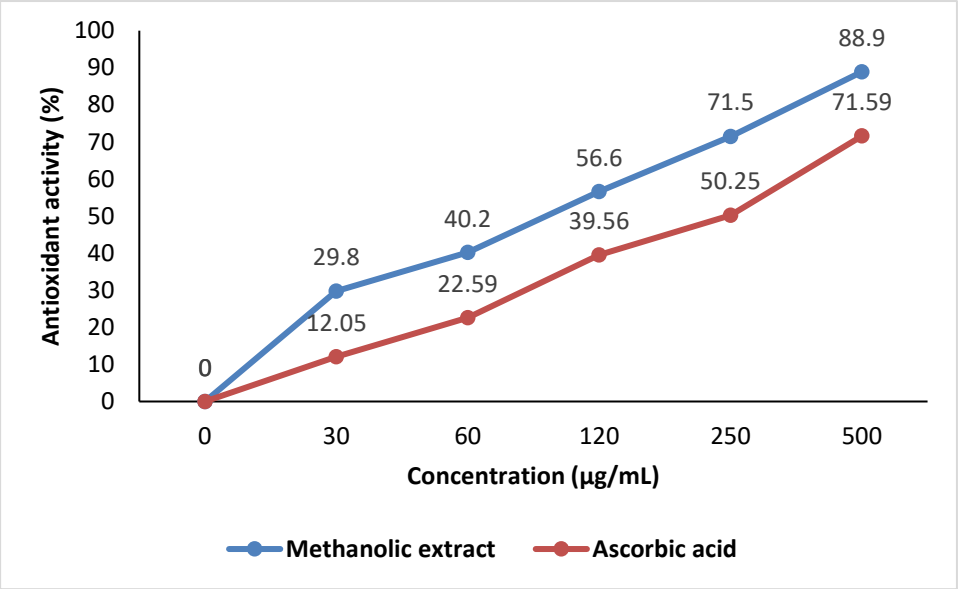

Figure 3: Antioxidant activity (%) of methanolic extract from A. capillus-veneris.

Antibacterial activity: The antibacterial activity of various concentrations of the plant methanolic extract (25, 50, 75, and 100 µg/mL) against gram positive (*E. faecalis* and *S. aureus*) and gram negative (*E. coli* and *P. aeruginosa*) bacteria was determined by the presence of inhibition zones. The highest antibacterial activity was observed against *S. aureus* (9.53 mm), *E. coli* (8.14 mm), *E. faecalis* (4.71 mm), and *P. aeruginosa* (4.13 mm) at a concentration of 100 µg/mL (Figure 4).

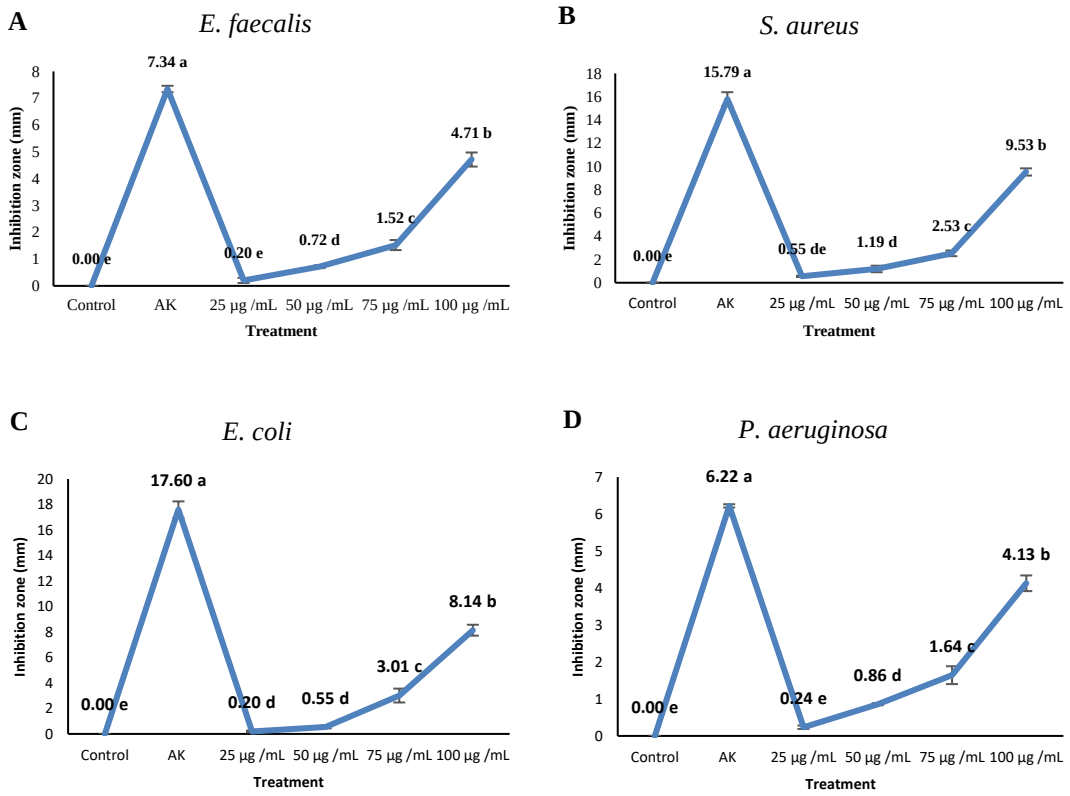

Figure 4: Bacterial inhibition zone assay of four bacteria by A. capillus-veneris extract and amikacin. (A) E. faecalis. (B) S. aureus. (C) E. coli. (D) P. aeruginosa.

Table 3 shows the effect of various concentrations of *A. capillus-veneris* extract and amikacin against the tested bacteria. Regarding the interaction between treatment and the type of bacteria, the highest value of the inhibition zone appeared against *E. coli* (17.60 mm) and *S. aureus* (15.79 mm) when using amikacin followed by *A. capillus-veneris* extract at a concentration of 100 µg/mL against *S. aureus* (9.53 mm) and *E. coli* (8.14 mm). While, the lowest diameter of inhibition zone values was shown against *E. faecalis* (0.20 mm) and *E. Coli* (0.20 mm) at a concentration of 25 µg/mL. Furthermore, the diameter of inhibition zone values for all tested bacteria increased along with the increase in *A. capillus-veneris* methanolic extract concentration. The antibacterial activity of the extract at 100 µg/mL (6.63 mm) was higher than the other concentrations. Concerning the type of bacteria, *S. aureus* (4.93 mm) and *E. coli* (4.91 mm) seemed to be more susceptible to the plant extract than other tested bacteria. Overall, the highest antibacterial activity was observed against all tested bacteria when using amikacin.

Table 3: Effect of different concentrations of A. capillus-veneris extract and amikacin against the tested bacteria.

	E. faecalis	*S. aureus*	*E. Coli*	*P. aeruginosa*	MSE	Overall mean
Control	0.00 h	0.00 h	0.00 h	0.00 h	0.14	0.00 E
AK	7.34 bc	15.79 a	17.60 a	6.22 c	1.31	11.74 A
25 µg/mL	0.20 g	0.55 g	0.20 g	0.24 g	0.05	0.30 E
50 µg/mL	0.72 g	1.19 f	0.55 g	0.86 g	0.09	0.83 D
75 µg/mL	1.52 f	2.53 e	3.01 de	1.64 f	0.22	2.18 C
100 µg/mL	4.71 d	9.53 b	8.14 b	4.13 d	0.60	6.63 B
MSE	0.57	1.22	1.33	0.47		
Overall mean	2.42 B	4.93 A	4.91 A	2.18 B		

Means having different superscript letters are differed significantly, with the small letters denoting the effect of interaction and the capital letters denoting the effect of main factors.

According to the results presented in Table 4, the treatment that inhibited the growth of bacteria was taken as the MIC. Regarding all types of bacteria, the concentration treatment of 100 µg/mL of plant extract recorded absolute inhibition of observed bacterial growth (no turbidity), while the other concentrations (50 and 75µg/mL) showed inhibition of bacterial growth but still exhibited light turbidity. On the other hand, the 25µg/mL concentration showed heavy turbidity indicating the growth of bacteria according to (9). Greater antimicrobial activity of the plant extracts was indicated by wider zones of inhibition (31). Our results confirm the findings of (11), who found that the extract of *A. capillus-veneris* has antibacterial capabilities against *E. Coli* and *S. aureus*. While (9) reported that the extract does not possess antibacterial effects against *P. aeruginosa* our findings indicate otherwise. Climatic factors, geographical location, and timing of harvest may contribute to the variations in the proportions of chemical contents. It is important to note that these fluctuations in chemical composition are responsible for their biological activities or medicinal effects, which differ from one region to another (16). (28) stated that phenolic compounds exhibit intriguing antibacterial properties against pathogenic bacteria. These substances have the ability to impact bacteria in various ways, such as hindering nucleic acid synthesis and plasma membrane function, as well as interfering with bacterial enzymatic processes or energy metabolism (13).

Table 4: Observations of MIC test for A. capillus-veneris extract and amikacin.

Bacterial strain	Concentrations of plant extract (µg/mL)				AK (µg/mL)
	25	50	75	100	
E. faecalis	+	+	+	-	-
S. aureus	+	+	+	-	-
E. Coli	+	+	+	-	-
P. aeruginosa	+	+	+	-	-

"–" = No bacterial growth; "+" = bacterial growth (turbidity).

Conclusions

This paper presents information on some phytochemical and biological activity of *A. capillus-veneris*, which was found to contain phenolic, flavonoid, glycoside, and saponin compounds. In the methanolic extract of the plant, five phenolic compounds (ferulic acid, p-coumaric acid, apigenin, catechin, and luteolin) were detected by

HPLC. According to the DPPH assay, the methanolic extract of the plant demonstrated significant antioxidant activity. The plant extract exhibited a higher level of antioxidant activity compared to the ascorbic acid, as well as showed antibacterial activity against *E. faecalis, S. aureus, E. coli* and *P. aeruginosa*. The MIC of the plant extract against the tested bacterial strains in this study was 100µg/mL. Even though the obtained *A. capillus-veneris* exhibits a promising antibacterial agent, additional research is suggested to develop and investigate the safe use of this species as a potential drug.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

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References

1. Ahmad, Z. M., Hamzah, H. M., and Lazim, Z. S. (2023). Analysis of chemical profiles of different *Pistacia atlantica* parts at Sulaymaniyah and Halabja region in IRAQ. Applied Ecology and Environmental Research, 21(1): 561-574. DOI: 10.15666/aeer/2101_561574.
2. Akbar, S., and Akbar, S. (2020). Adiantum venustum G. Don./*Adiantun capillus-veneris* L. (Pteridaceae). Handbook of 200 Medicinal Plants: A comprehensive review of their traditional medical uses and scientific justifications. Springer Nature Switizerland, PP: 103-107.

3. Al-dulimi, Y. A., Sh., & H. Al-Ali, H. (2024). Effect Of Ground Fertilization With Humic Acid And Spraying With Seaweed Extract And Baking Yeast And Their Effect On The Bukthorn Tafahe Seedling. Anbar Journal Of Agricultural Sciences, 22(1), 115–128. https://doi.org/10.32649/ajas.2024.183705.
4. Al-Rawi, A., and Chakravarty, H. L. (2014). Medicinal plants of Iraq. Ministry of Agriculture. Iraq, Baghdad, PP: 109.
5. Alsaloom, A. N. (2023). Testing and Evaluation of Bioactive Compounds in Soybean. Iraqi Journal of Agricultural Sciences, 54(1): 85-92. DOI: 10.36103/ijas.v54i1.1678.
6. Al-Snafi, A. E. (2015). The chemical constituents and pharmacological effects of *Adiantum capillus-veneris*: A Review. Asian Journal of pharmaceutical Science and Technology, 5(2): 106-111.
7. Azeez, S. H., and Gaphor, S. M. (2019). Evaluation of antibacterial effect against Porphyromonas gingivalis and biocompatibility of essential oil extracted from the gum of Pistacia atlantica kurdica. BioMed research international, 2019(1): 9195361. https://doi.org/10.1155/2019/9195361.
8. Bajkacz, S., Baranowska, I., Buszewski, B., Kowalski, B., and Ligor, M. (2018). Determination of flavonoids and phenolic acids in plant materials using SLE-SPEUHPLC-MS/MS method. Food Analytical Methods 11: 3563−3575. https://doi.org/10.1007/s12161-018-1332-9.
9. Bastos, A. M., Lima, F. M., Conserva, L. M., Andrade, V. S., Rocha, E. M., and Lemos, R. P. (2009). Studies on the antimicrobial activity and brine shrimp toxicity of *Zeyheria tuberculosa* (Vell.) Bur. (Bignoniaceae) extracts and their main constituents. Annals of Clinical Microbiology and Antimicrobials, 8: 1-6. https://doi.org/10.1186/1476-0711-8-16.
10. Besharat, M., Rahimian, M., Ghaemi, E., and Besharat, S. (2009). Effect of ethanolic extract of *Adiantum capillus-veneris* in comparison with gentamicine on 3 pathogenic bacteria in vitro. Pharmaceutical Sciences, 15(1): 49-52.
11. Boudjema, K., Benmansour, F., Ghezali, S., Ouamar, L., Hali, L., and Fazouane, F. (2020). Phytochemical screening and evaluation of some biological activities of plant extracts *Adiantum capillus-veneris* L. and *Tamarix gallica* L. African Review of Science, Technology and Development, 5(2): 70-85.
12. Boukada, F., Sitayeb, S., Khadem, H., Meddah, B., and Zohra, S.F. (2022). Chemical composition, antioxidant and antibacterial activity of *Adiantum capillus-veneris* L. extract from Algeria. Kragujevac Journal of Science, 2022(44): 91-101. DOI: 10.5937/KgJSci2244091B.
13. CLSI. (2022). Procedure for optimizing disk contents (potencies) for disk diffusion testing of antimicrobial agents using harmonized CLSI and EUCAST criteria: Clinical and Laboratory Standards Institute.
14. Cushnie, T. T., and Lamb, A. J. (2005). Antimicrobial activity of flavonoids. International Journal of Antimicrobial Agents, 26(5): 343-356. https://doi.org/10.1016/j.ijantimicag.2005.09.002.
15. Dehdari, S., and Hajimehdipoor, H. (2018). Medicinal properties of *Adiantum capillus-veneris* Linn. in traditional medicine and modern phytotherapy: A review article. Iranian Journal of Public Health, 47(2): 188-197.

16. Devi, M. T., Saha, S., Tripathi, A. M., Dhinsa, K., Kalra, S. K., and Ghosha, U. (2019). Evaluation of the antimicrobial efficacy of herbal extracts added to root canal sealers of different bases: An in vitro study. International Journal of Clinical Pediatric Dentistry, 12(5): 398-404. https://doi.org/10.5005%2Fjp-journals-10005-1660.
17. Dhifi, W., Bellili, S., Jazi, S., Bahloul, N., and Mnif, W. (2016). Essential oils' chemical characterization and investigation of some biological activities: a critical review. Medicines, 3(4): 25. https://doi.org/10.3390/medicines3040025.
18. Duncan, D. B. (1955). Multiple range and multiple F tests. biometrics, 11(1): 1-42. https://doi.org/10.2307/3001478.
19. Ezeabara, C. A., Okeke, C. U., Aziagba, B. O., Ilodibia, C. V., and Emeka, A. N. (2013). Determination of saponin content of various parts of six citrus species. International Research Journal of Pure and Applied Chemistry, 4(1): 137-143. https://doi.org/10.9734/IRJPAC/2014/5831.
20. Ezzoubi, Y., Bousta, D., E. L., Mansouri, L., Boukhira, S., Siham, L., Achour, S. and Farah, A. (2016). Phytochemical screening, anti-inflammatory activity and acute toxicity of hydro-ethanolic, flavonoid, tannin and mucilage extracts of *Lavandula stoechas* L. from Morocco. International Journal of Pharmacognosy and Phytochemical Research 8(1): 31-37.
21. Govaerts, R. H. A. (2011). World checklist of selected plant families published update. Facilitated by the Trustees of Royal Botanic Gardens, Kew, London.
22. Gulcin, I. (2012). Antioxidant activity of food constituents: An overview. Archives of Toxicology, 86: 345-391. https://doi.org/10.1007/s00204-011-0774-2.
23. Hassan, A. Y. (2023). The Effect of Spraying with the Hormone Brassinolide on the Production of Oil and Glycosides of Goat Pea (Securigera securidaca). In IOP Conference Series: Earth and Environmental Science, 1214(1): p. 012046. DOI: 10.1088/1755-1315/1214/1/012046.
24. Ignat, I., Volf, I., and Popa, V. I. (2011). A critical review of methods for characterisation of polyphenolic compounds in fruits and vegetables. Food Chemistry, 126(4): 1821-1835. DOI: 10.1016/j.foodchem.2010.12.026
25. Jagtap, S. G., Kajale, V. V., Abhyankar, M. M., Kulkarni, A. S., and Ghante, M. R. (2023). Formulation and evaluation of phytosomes of hydroalcoholic extract of *Adiantum capillus-veneris* for antimicrobial activity. Pharmacognosy Research, 15(3): 468-477. https://doi.org/10.1016/j.foodchem.2010.12.026.
26. Kashkooe, A., Sardari, F. A., Mehrabadi, M. M., and Zarshenas, M. M. A. (2021). Review of pharmacological properties and toxicological effects of *Adiantum capillus-veneris* L. Current Drug Discovery Technologies, 18(2): 186-193. https://doi.org/10.2174/1570163817666200316111445.
27. Latif, K., D., & I. Neamah, S. (2024). Physiological Changes And Growth Parameters Of Phaseolus Vulgaris L. Calli Cultures By Chitosan And Calcium Pantothenate Under Heat Stress. Anbar Journal Of Agricultural Sciences, 22(1), 691–705. https://doi.org/10.32649/ajas.2024.183788.
28. Leber, A. L. (2016). Preparation of routine media and reagents used in antimicrobial susceptibility testing. Clinical Microbiology Procedures Handbook, 1-3.

29. Lima, M. C., Paiva de Sousa, C., Fernandez-Prada, C., Harel, J., and Dubreuil, J. D. (2019). A review of the current evidence of fruit phenolic compounds as potential antimicrobials against pathogenic bacteria. Microbial Pathogenesis, 130: 259-270. https://doi.org/10.1016/j.micpath.2019.03.025.
30. López-Fernández, O., Domínguez, R., Pateiro, M., Munekata, P. E. S., Rocchetti, G., and Lorenzo, G. M. (2020). Determination of polyphenols using liquid chromatography tandem mass spectrometry technique (LC–MS/MS): A review. Antioxidants, 9(6): 479. https://doi.org/10.3390/antiox9060479.
31. Phonsatta, N., Deetae, P., Luangpituksa, P., Grajeda-Iglesias, C., Figueroa-Espinoza, M. C., Le Comte, J., Villeneuve, P., Decker, E. A., Visessanguan, W., and Panya, A. (2017). Comparison of antioxidant evaluation assays for investigating antioxidative activity of gallic acid and its alkyl esters in different food matrices. Journal of Agricultural and Food Chemistry, 65(34): 7509-7518. https://doi.org/10.1021/acs.jafc.7b02503.
32. Prasada, K. L., and Anusha, K. (2021). Antimicrobial efficacy of plant extracts combined with endodontic sealer on the growth of oral pathogen - an in vitro study. Journal of Dental Science, 6(2): 1-7. https://doi.org/10.23880/oajds-16000294.
33. Radovanović, B., Mladenović, J., Radovanović, A., Pavlović, R., and Nikolić, V. (2015). Phenolic composition, antioxidant, antimicrobial and cytotoxic activities of *Allium porrum* L. (Serbia) extracts. Journal of Food and Nutrition Research, 3(9): 564-569. DOI: 10.12691/jfnr-3-9-1.
34. Rautray, S., Panikar, S., Sofia, A., and Rajananthini, A. U. (2018). Anti-oxidant and anti-microbial study of Adiantum capillus veneris and Pteris quadriureta L. Journal of Medicinal Plants Research, 12(23): 359-368. https://doi.org/10.5897/JMPR2018.6635.
35. Shi, L., Zhao, W., Yang, Z., Subbiah, V., and Suleria, H. A. R. (2022). Extraction and characterization of phenolic compounds and their potential antioxidant activities. Environmental Science and Pollution Research, 29(54): 81112-81129. https://doi.org/10.1007/s11356-022-23337-6.
36. SPSS. (2019). Statistical Package for Social Sciences, Ver. 26, Use's guide, IBM publications, USA.
37. Talebi, E., Nasrollahi, I., and Bashardoost, Z. (2022). Phytochemical compounds and bioactivity properties of the whole plant of maidenhair fern (*Adiantum capillus-veneris* L.) essential oil. Safe Future and Agricultural Research Journal (SFARJ), 1(1): 1-10. DOI: 10.22034/sfar.2022.156138.
38. Tarhan, L., Urek, R. O., Oner, A., and Nakiboglu, M. (2022). Evaluation of phenolic profiles, antioxidant activities, cytotoxic and apoptotic potentials of *Phlomis angustissima* and *Phlomis fruticosa* medicinal plants from Turkey. European Journal of Integrative Medicine, 55: 102188. https://doi.org/10.1016/j.eujim.2022.102188.
39. Taskin, T., and Bulut, G. (2019). Qualitative and quantitative phytochemical analysis and in-vitro biological activity of *Rheum ribes* L. different parts. Istanbul Journal of Pharmacy, 49(1): 7-13. DOI: 10.26650/IstanbulJPharm.2019.18012.
40. Torres, J. A., Villegas, M. V., and Quinn, J. P. (2007). Current concepts in antibiotic-resistant gram-negative bacteria. Expert review of anti-infective

therapy, 5(5): 833-843. https://doi.org/10.1586/14787210.5.5.833.

41. Vadi, R., Manisha, V., and Swati, K. (2017). Hansraj (Adiantum capillus veneris Linn.): A systematic review on its ethnobotany, phytochemical and pharmacological profile. International Journal of Ayurveda and Pharma Research., 5(6): 5-21. https://ijapr.ijraps.in/index.php/ijapr/article/view/685.
42. Yazdani, M., and Kashi, F. K. (2021). Bioactivity of methanolic leaves and stem extracts of *Adiantum capillus-veneris* L. from southeast of Marivan. Journal of Plant Productions, 43 (4): 487-496. https://doi.org/10.22055/ppd.2020.29309.1757.