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CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIBACTERIAL ACTIVITY OF *Adiantum capillus-veneris* L. EXTRACT FROM ALGERIA

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ABSTRACT. The present study evaluates the antioxidant and antibacterial activities as well as the chemical composition of the aerial part of *Adiantum capillus-veneris* L., which are endemic to the flora of Algeria. The total phenolic amount of the hydromethanolic extract, as measured by the Folin-Ciocalteu reagent procedure, was found to be about 23.44 ± 0.14 mg GAE/g DW, while the flavonoid content value was 4.66 ± 0.01 mg CE/g DW, as measured by aluminum chloride colorimetric method. The antioxidant power of the extract was analyzed using the DPPH method. DPPH radicals were scavenged with an IC_{50} value of 65.85 µg/mL. The antibacterial activity of hydromethanolic extract against five bacterial strains of *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 25853), *Staphylococcus aureus* (ATCC 25923), *Streptococcus pneumoniae* and *Bacillus cereus* (ATCC 10876) were carried out using the disc diffusion test and the microdilution method. *Streptococcus pneumoniae* is the most sensitive bacterium with inhibition zone of 9.0 ± 1.1 cm and MIC=10 mg/mL. Based on HPLC analysis, we find that quercetin 3-O-glucoside was the most abundant compound in the plant extract. This study demonstrates that *Adiantum capillus-veneris* could be a potential natural source of antibacterial and antioxidant compounds.

Keywords: antibacterial activity, antioxidant activity, flavonoids, polyphenols, HPLC.

INTRODUCTION

Reactive oxygen species, including singlet oxygen, superoxide ion and hydrogen peroxide, are extremely reactive toxic molecules that are produced by cells during their metabolism. They are responsible for serious oxidative damage to proteins, lipids, enzymes and DNA due to covalent bonding and lipid peroxidation, with consequent tissue damage (BENABDERRAHIM *et al*., 2019). Natural antioxidants have been of great interest owing to their ability to scavenge free radicals. Free radicals have been associated with the development of a series of diseases, including cancer, neurodegeneration, and inflammation (BABA and MALIK, 2015). The antioxidants in plants, including phenolics and flavonoids may offer a protective

effect against a wide range of diseases (GULCIN, 2012). Due to the growing need for natural antioxidants, especially in dietary preservation, plants with such potential properties should be studied more intensively.

The selection of active compounds in medicinal plants is now of great interest as these plants may serve as promising sources of new prototype antibiotics and have enormous therapeutic potential against many infectious diseases. Unlike synthetic drugs, the antibacterial activities of phytochemicals have fewer side effects (TORRES *et al*., 2007). The development of an antibacterial agent from plants seems interesting, as it will allow the development of a phytomedicine to fight microbes. At present, many clinically active antibiotics are becoming less effective due to the development of resistance, causing serious clinical problems in the treatment of infectious diseases. Therefore, biomolecules from plant sources represent one of the alternatives in the fight against antibiotic resistance (BASTOS *et al*., 2009).

Adiantum capillus-veneris L. (Maidenhair fern) is a tufted fern belonging to the family Pteridaceae. This species is widespread in regions with warm or tropical temperatures and high humidity (KASHKOOE *et al*., 2021). Maidenhair fern is a hardy plant, up to 35 cm high, with an aromatic scent and a creeping rhizome. The medicinal parts are the fronds, rhizomes and roots. The plant is largely found in various regions such as southern Europe, the Atlantic coast of Ireland and northern Africa (DEHDARI *et al*., 2018).

The aims of this study were to: (i) determine the total phenol and flavonoid contents, (ii) assess the total antioxidant and antibacterial capacity; (iii) identify and quantify the predominant phenolic compounds present in the extract by HPLC-DAD-MS analysis of *Adiantum capillus-veneris* from Algeria.

MATERIALS AND METHODS

Plant material and preparation of extract

The plant was collected from Mascara, Algeria. It was cleaned and air-dried at 45°C to a constant weight and crushed to a powder. According to the conventional maceration process, which is carried out at room temperature, 100 g of plant powder was extracted by 1L of methanol/water mixture, with a ratio (85:15). The mixture was stirred overnight at 4°C and left to stand for a few hours. The floating fraction was then filtered to obtain filtrate 1, which is stored at 4°C. The extraction was repeated once, and the precipitate was made up to 1liter with 50% methanol to give filtrate 2 which was added to filtrate 1. Thereafter, the hydromethanolic extract was condensed until dryness and stored at 4°C in dark, sealed tubes for analysis (MERGHEM *et al*., 1995). Solutions were prepared by dilution with DMSO (dimethyl sulfoxide) for the antibacterial activity and methanol for the antioxidant potential.

Total phenolic content

Polyphenols were quantitated by spectrophotometric analysis, according to the protocol assumed by MILIAUSKAS *et al*. (2004). We proceed by mixing 1 mL of extract, 5 mL of Folin Ciocalteu (2 M) diluted 10 times and 4 mL of sodium carbonate (Na_2CO_3) at a concentration of 75 g/L. We measure the absorbance at 765 nm, after 1 hour of incubation at room temperature. The calibration curve is established with gallic acid, applying the same assay steps. All the measurements are repeated 3 times.

Total flavonoid content

The determination of flavonoids was performed using the colorimetric method described by ARDESTANI and YAZDANPARAST (2007). 500 µL of the studied extract was mixed with 2 mL of distilled water, then 150 µL of a 15% sodium nitrite solution ($NaNO_2$) was added. After 6 minutes, 150 µL of 10% aluminium chloride ($AlCl_3(H_2O)_6$) was added to the mixture. Then 2 mL of 4% sodium hydroxide (NaOH) was introduced into the tube and the final volume was immediately made up to 5 mL. Against the blank (containing all solutions except the extract), the reading is taken by a spectrophotometer after 15 minutes at 510 nm. Simultaneously, catechin was used as a positive control, and the calibration curve was performed under the same experimental conditions.

HPLC analysis

A Shimadzu-Prominence-I-HPLC system with LC-2030 pump and photodiode array (PDA: LC-2030 / LC-2040) equipped with a surveyor UV-VIS diode array detection (DAD) and LCQ advantage max ion trap mass spectrometer equipped with an electrospray ionization (ESI) system (from Thermo fisher scientific, Waltham, MA, USA) was used for the intended HPLC analysis. The HPLC column (Supelco, 150 mm x 4.6 mm, particle size 5 µm) and the gradient were implemented for the flavonoids separation. Eluent A was aqueous acetic acid (1%, v/v) and pure methanol acetic acid was a B eluent. The separation was an isocratic stage of 2% B for 2 minutes accompanied by a linearity gradient of 2% to 20% B for 10 minutes and then 100% B for 65 min, and 100% B for 68 minutes, with a flow rate of 0.8 mL/min.

The absorption spectra were measured in the 190 to 400 nm range. The effluent chromatographic stream was directed into the mass spectrometer (MS) interface, electrospray ionization (ESI). Mass spectrometry data were acquired in the negative ionization mode at 280 nm scanning from 100 to 800 m/z under the parameters as follows: other mass spectrometer conditions were as follows: drying gas (N2) flow: 1.5 L/min; source temperature: 350°C; temperature: 300°C, MS fragmentation energy: 1.4 V; nebulizing gas pressure: 30 psi. The phytochemical identification in the extracts was highlighted by the retention times and spectra (UV, MS) compared with literature data.

Antioxidant activity evaluation using DPPH assay

To analyze the solutions for their antiradical properties, they were firstly dissolved in methanol at different concentrations. 50 µL of these solutions are mixed with 1950 µL of a methanolic solution of DPPH (6.10^{-5} M). This mixture is stirred for 30 seconds, then incubated for 30 minutes at room temperature in the dark. The absorbance of the reaction mixture is then measured at 517 nm against a pure methanol blank (SHIMADA *et al.*, 1992). Ascorbic acid and catechin were used for comparison. The percentage of DPPH radical inhibition was calculated by the following equation:

$$I_{DPPH}\ \% = (A_0\text{-}A_1/A_0) \times 100$$

where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. The results were estimated in terms of IC_{50} (the concentration of extract which inhibits 50 % of free radicals present in the reaction medium) and it was calculated from the linear regression graph.

Antibacterial activity

Bacterial strains

The different bacterial strains used in this study, from Gram-positive or Gram-negative group, are *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC25853), *Staphylococcus aureus* (ATCC25923), *Streptococcus pneumoniae* (clinical isolated) and *Bacillus cereus* (ATCC10876). The microorganisms were supplied from the Laboratory of Bioconversion, Engineering Microbiology and Health Safety (University of Mascara, Algeria).

Disc diffusion method

The antibacterial potency was achieved by the disc diffusion method. First, the tested extract was dissolved in DMSO and filtered through a 0.45 µm sterile filter membrane. Then, 100 µL of bacterial inoculums containing 10^8 CFU/mL were spread on plates containing Mueller Hinton agar, and discs (6 mm diameter) soaked with 10 µL of extract solution (10 mg/disc) were positioned on the media surface. Two control discs were used which contained DMSO and gentamicin (10 µg/disc) as negative and positive controls, respectively. The plates were incubated for 24 h at 37°C, and experiments were done in triplicate. The diameters of the inhibition zones were measured (RIOS *et al*., 1988).

Determination of the minimum inhibitory concentration (MIC)

This test is performed using sterile 96-well microplates according to the protocol described by AKOMO *et al*. (2009). A series of twofold dilutions from 200 mg/ml to 0.39 mg/ml was prepared. 100 µL of each extract dilution was incubated with 100 µL of strain suspensions containing 10^6 CFU. The first well was used as a negative control; it was inoculated with broth only. However, the final well was used as a positive control and was inoculated with strain suspensions. The microplate was then incubated at 37°C for 24 hours. The MIC was taken as the lowest concentration for which there was no turbidity.

RESULTS AND DISCUSSION

Total polyphenol and flavonoid contents

The phenolic content is expressed as milligram equivalents of gallic acid per gram of dry extract (mgGAE/g DW). The polyphenol's result level, as presented in Tab. 1, showed that the hydromethanolic extract exhibited moderate phenolic content (23.44 ± 0.14 mg GAE/g DW). YAZDANI and KASHI (2021) reported that the total phenolic content of the methanolic extract *of A. capillus-veneris* L. was 83.62 ± 1.87 mg GAE/g DW which is much higher in comparison to our results.

The flavonoid content is expressed as milligrams of catechin equivalent per gram of dry extract (mg EC/g E). The experimental results showed that the total flavonoid content of hydromethanolic extract was (4.66 ± 0.01mg EC/g DW).

The polyphenolic content is qualitatively and quantitatively variable from one plant to another, which can be attributed to the specificity of the chemical method of quantification of phenolic compounds, but also the nature of the standard used (EZZOUBI *et al*., 2016). Indeed, some chemical substances used as standards tend to overestimate the concentration of the examined compounds. This is notably the case for quebracho tannins (SCHOFIELD *et al*., 2001).

Table 1. The total phenolic and flavonoids contents and antioxidant activity of hydromethanolic extract of *Adiantum capillus-veneris.*

Phenols (mg GAE/g DW)	**Flavonoids (mg CE/g DW)**	**IC_{50}DPPH (µg/mL)**	**IC_{50}DPPH (µg/mL) (Catechin)**	**IC_{50}DPPH (µg/mL) (Ascorbic acid)**
23.44 ± 0,14	4.66 ± 0.01	65.85± 3.25	14.26± 1.45	17.21± 2.01

HPLC analysis

The chemical analysis by high-performance liquid chromatography (Fig. 1, Tab. 2), revealed the presence of gentisic acid, chlorogenic acid, *p*-coumaric acid, caffeic acid derivative, ferulic acid, kaempferol, quercetin, 3-p-coumaroylquinic acid, apigenin-7-O-glucoside, 3,5-Di-O-caffeoylquinic acid, isorhamnetin-3-O-di-glucoside, quercetin 3-O-glucoside, epicatechin 7-O-rutinoside, kaempferol 3-O-glucoside, ferulic acid derivatives and hydroxycinnamic derivative. Quercetin 3-O-glucoside (949.5 µg/g) was the major identified compound followed by 3,5-Di-O-caffeoylquinic acid (769.0 µg/g), hydroxycinnamic derivative (404.3 µg/g) and kaempferol 3-O-glucoside (359.6 µg/g).

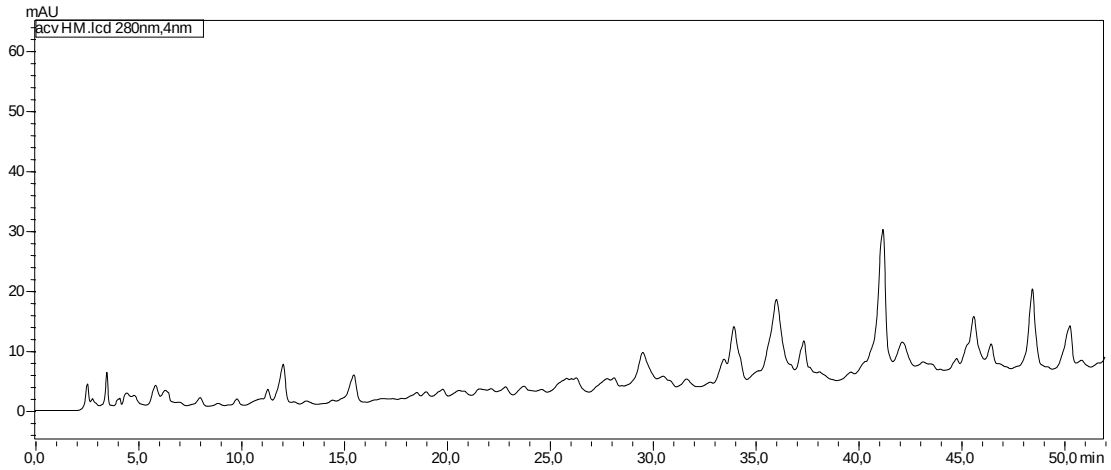

Figure 1. Representative high performance liquid chromatography profile of *Adiantum capillus-veneris* hydromethanolic extract.

YUAN *et al*. (2012) reported that the leaves of *Adiantum capillus-veneris* contain kaempferol-3-glucosides in high amounts. Similarly, ZEB and ULLAH (2017) identified kaempferol-3-sophorotrioside with the highest concentration (58.7 mg/g). In the study of AL-HALLAQ *et al*. (2015), the main polyphenolic compounds in the aqueous extract prepared by refluxing the dried coarsely powdered plant material for 15 min, were found to be ellagic acid (5.48 mg/g) and quercetin-3-O-glucoside (3.96 mg/g). These data are not consistent with our findings, where ellagic acid was not identified in the methanolic extract.

Extraction is an essential step in the isolation and identification of phenolic compounds. The extraction of phenolic compounds is influenced by several factors, such as the chemical nature of the phenolic compound, the extraction method, the particle size of the sample, the extraction solvent, pH and temperature, etc. (IGNAT *et al*., 2011; HASBAY *et al*., 2018). The choice of solvent is also vital for optimal extraction. Indeed, BAJKACZ *et al*. (2018) observed how the polyphenol content varied according to the solvents and extraction times used. As reported by LÓPEZ-FERNÁNDEZ *et al*. (2020), the most common extraction

solvents for the extraction of polyphenols are methanol, acidified methanol or combinations of methanol and water.

Furthermore, the chemical composition of plant extracts is influenced by the cultivation method, the phenological stage, the nature of the soil, the climate at the time of sampling (temperature and rainfall), as well as the constitution of the sample (stem-leaf-flower ratio) (LEE *et al*., 2003; MILIAUSKAS *et al*., 2004; EBRAHIMI *et al*., 2008).

These results provide a better understanding of the composition of bioactive compounds, which may be responsible for the antioxidant and antibacterial properties of the aerial part of *A. capillus-veneris*. Indeed, quercetin and its glycosides, the majority compound, are considered a valuable functional food ingredient, and with a wide range of therapeutic applications such as anticancer, antioxidant, antimicrobial and anti-inflammatory uses.

Table 2. HPLC-DAD-MS identification of phenolic compounds in *Adiantum capillus-veneris* hydromethanolic extract.

Rt	Extract (µg/g)	λ max	MH-	Compounds
3.52	72.8	272	153	gentisic acid
6.43	60.9	262	353	chlorogenic acid
9.92	19.9	307	163	*p*-coumaric acid
11.07	51.8	267	489	caffeic acid derivatives
12.14	183.8	332, 283	193	ferulic acid
15.49	149.7	356, 255	284	kaempferol
25.73	108.1	271 332	301	quercetin
29.54	325.8	306, 313	337	3-p-coumaroylquinic acid
33.97	353.4	255 353	431	apigenin-7-O-glucoside
36.03	769.0	204 345	515	3,5-Di-O-caffeoylquinic acid
37.37	257.3	231 277	639	isorhamnetin-3-O-di-glucoside
41.21	949.5	255 353	463	quercetin 3-O-glucoside
42.15	255.5	265, 348	597	epicatechin 7-O-rutinoside
45.62	359.6	271 285	447	kaempferol 3-O-glucoside
46.46	110.1	288 414	389	ferulic acid derivatives
48.47	404.3	291 309	349	hydroxycinnamic derivative
50.30	219.6	276 435	389	ferulic acid derivatives

Antioxidant activity

The extracts showed a high DPPH radical neutralization effect, which is demonstrated by the graphical plot which traces an exponential curve with the presence of a stationary phase, which defines the almost complete reduction of DPPH to its non-radical form. The percentage of inhibition exceeds 90% at a concentration of 1000 µg/mL (Fig. 2). The minimum concentration that inhibits 50% of the DPPH radical was 65.85 ± 3.25 µg/mL. This value remains lower than those of the reference antioxidants, catechin (14.26 ± 1.45 µg/mL) and ascorbic acid (17.21 ± 2.01 µg/mL) (Tab.1).

Several studies have been conducted on the antioxidant activity of the aerial part of *Adiantum capillus veneris*. BOUDJEMA *et al*. (2020) reported that the methanolic extract of the aerial part of *Adiantum capillus veneris* harvested from Boumerdes (Algeria) has a lower antioxidant effect corresponding to an IC_{50} value of 280 µg/mL, which is even four times higher than the value obtained in our study.

The scavenger effect of the extract on free radicals is depending on the existence of free OH groups, in particular 3-OH, with a 3',4'-rthodihydroxy configuration (HEIM *et al*., 2002).

In our current work, the largest amount of phenolic compound was quercetin 3-O-glucoside. It is determined by multiple studies that quercetin act as a potent main antioxidant as its capacity to directly reduce, scavenging and bleaching free radicals (OZGEN *et al*., 2016; XU *et al*., 2019). Therefore, the existence of quercetin in the hydromethanol extract might play a significant role in increasing its primary antioxidant activity.

3,5-Di-O-caffeoylquinic acid was the second most abundant compound of the plant sample. Owing to an ortho-dihydroxy phenyl ring, caffeoylquinic acid derivatives are known as both efficient free radical scavengers and metal ion chelators (HUNG *et al*., 2006).

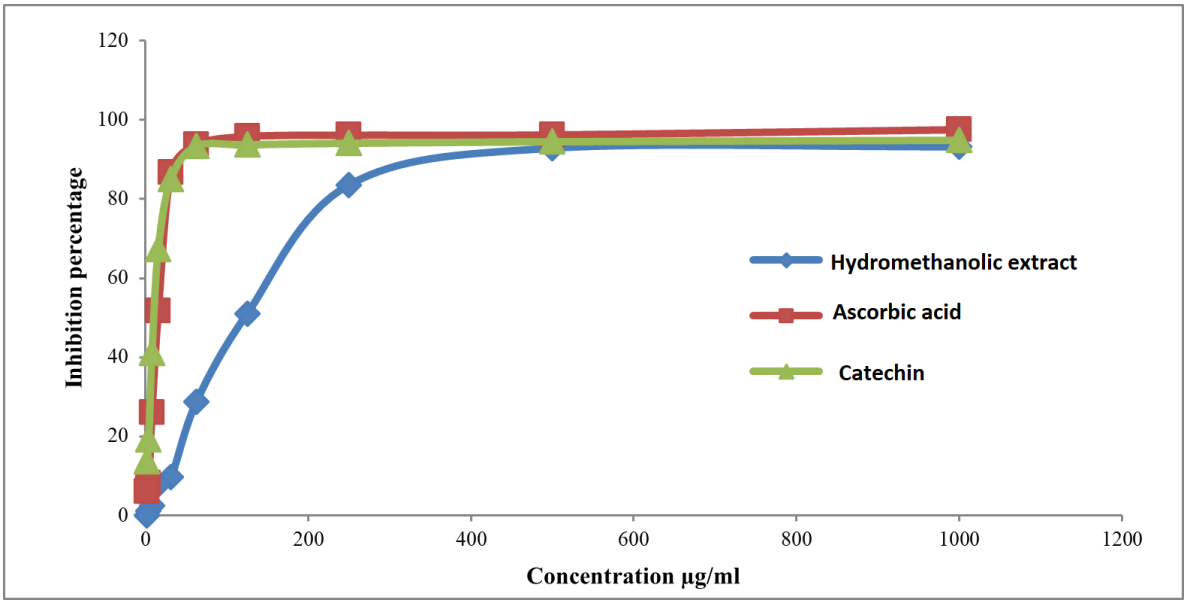

Figure 2. Antioxidant power tested by the DPPH radical scavenging method of hydromethanolic extract from *Adiantum capilus-veneris.*

Antibacterial activity

According to the results presented in Table 3, the hydromethanolic extract revealed zones of inhibition against *E. coli*, *S. pneumoniae* and *S. aureus* (10.0 ± 0.9, 9.0 ± 1.1, 6.0 ± 1.7 respectively). It is obvious from the result presented here that *A. capilus-veneris* extracts have no antimicrobial efficacy against *P. aeruginosa* and *B. cereus*. The minimum inhibitory concentration was 10 mg/mL^{-1}. Our results confirmed the results of BESHARAT *et al*. (2009), who showed that the ethanolic extract of the aerial parts of *A. capillus-veneris* does not have antimicrobial capabilities against *P. aeruginosa*.

According to LIMA *et al*. (2019) phenolic compounds have interesting antibacterial power against pathogenic bacteria. These compounds can act in several ways to destroy bacteria, including inhibiting the nucleic acid synthesis and the plasma membrane and disrupting enzymatic activity or energy metabolism of bacteria (CUSHNIE and LAMB, 2005).

Two flavonoids (quercetin 3-O-glucoside and kaempferol 3-O-glucoside) and two phenolic acids (3,5-Di-O-caffeoylquinic acid and hydroxycinnamic derivative) were the most abundant compounds of the plant sample (Tab. 2).

The antibacterial activities of phenolic acids have been demonstrated in various studies on different pathogens (LOW and DONACHIE, 1997; STOJKOVIĆ *et al*., 2013). It was found that phenolic acids had antimicrobial activity against *P. aeruginosa*, *E. coli* and *S. aureus*. Phenolic acids have been shown to have potent antibacterial and antibiofilm properties against

emerging nosocomial pathogens (KARUNANIDHI *et al*., 2013). LI *et al*. (2014) have investigated the influence of subinhibitory concentrations of phenolic acids on factors associated with the virulence of enterotoxigenic *S. aureus*. At subinhibitory concentrations (1.25 mg/mL), phenolic acids significantly inhibited hemolysis and coagulase titter. Reduced fibrinogen binding and decreased production of enterotoxins were observed at concentrations ranging from 1/16 MIC to 1/2MIC (LI *et al*., 2014).

Flavonols, such as quercetin and its derivatives and kaempferol and its derivatives, are among the most important class of flavonoids that show potent antibacterial activities. Quercetin and its derivatives showed significant antibacterial activity against some strains of bacteria, including *S. aureus*, methicillin-resistant *S. aureus* (MRSA), and *Staphylococcus epidermidis* (GEOGHEGAN *et al*., 2010). In another study, among the nine flavonoids (from the leaves of *Scutellaria oblonga* Benth), quercitin-3Oglucoside could successfully kill *S. aureus* and a reduction in biofilms (90-95%) was observed (RAJENDRAN *et al*., 2016).

Table 3. Antibacterial activity of the hydromethanolic extract of *Adiantum capillus-veneris.*

	S. aureus	***P. aeruginosa***	***E. coli***	***B. cereus***	***S. pneumoniae***
Inhibition zone diameter (mm)	6.0 ± 1.7	0.00	10.0 ± 0.9	0.00	9.0 ± 1,1
MIC (mg/mL)	10	ND	10	ND	10

CONCLUSION

This research provides data on the chemical and biological characterization of *Adiantum capillus-veneris*. In the hydromethanolic extract, nine phenolic acids and five flavonoids were detected by HPLC-MS. The antioxidant activity, estimated using the DPPH assay, revealed that the *A. capillus-veneris* extract had high antioxidant potential, in agreement with the total polyphenolic content. The *A. capillus-veneris* extract showed moderate antimicrobial activity against the tested bacterial strains. These results reinforce the importance of this species and widen new research perspectives as well as the design of new plant-based drugs sourced from indigenous plant material.

References:

[1] AKOMO, E.F.O., ZONGO, C., KAROU, S.D., OBAME, L.C., SAVADOGO, A., ATTEK, C., TRAORE, A.S. (2009): *In vitro* antiplasmodial and antibacterial activities of *Canthium multiflorum* Schum and Thonn (Rubiacea) extracts. *Pakistan Journal of Biological Sciences* **12** (12): 919–923. doi: 10.3923/pjbs.2009.919.923.

[2] AL-HALLAQ, E.K., LITESCU, S.C., KASABRI, V., ABDUL-RAZZAK, K.K., ABAZA, I.M., AFIFI, F.U. (2015): Hypocholesterolemic effects of *Adiantum capillus-veneris* L. aqueous extract in high cholesterol diet-fed rats and HPLC-MS determination of its polyphenolics. *Revue Roumaine de Chimie* **60** (4): 357–365.

[3] ARDESTANI, A., YAZDANPARAST, R., (2007): Inhibitory effects of ethyl acetate extract of *Teucrium polium* on *in vitro* protein glycoxidation. *Food and Chemical Toxicology* **45** (12): 2402–2411. doi: 10.1016/j.fct.2007.06.020

[4] BABA S.A., MALIK S.A. (2015): Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of *Arisaema jacquemontii* Blume. *Journal of Taibah University for Science* **9** (4): 449–454. doi: 10.1016/j.jtusci.2014.11.001

[5] BAJKACZ, S., BARANOWSKA, I., BUSZEWSKI, B., KOWALSKI, B., LIGOR, M. (2018): Determination of flavonoids and phenolic acids in plant materials using SLE-SPE-UHPLC-MS/MS method. *Food Analytical Methods* **11**: 3563–3575. doi: 10.1007/s12161-018-1332-9

[6] BASTOS, A.M., LIMA, F.M., CONSERVA, L.M., ANDRADE, V.S., ROCHA, E.M., 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**, 16. doi: 10.1186/1476-0711-8-16

[7] BENABDERRAHIM, M.A., YAHIA, Y., BETTAIEB, I., ELFALLEH, W., NAGAZ, K. (2019): Antioxidant activity and phenolic profile of a collection of medicinal plants from Tunisian arid and Saharan regions. *Industrial Crops and Products* **138**: 111427. doi: 10.1016/j.indcrop.2019.05.076.

[8] BESHARAT, M., RAHIMIAN, M., GHAEMI, E., 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.

[9] BOUDJEMA, K., BENMANSOUR, F., GHEZALI, S., OUAMAR, L., HALI, L., 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 Developmentn* **5** (2): 70–85.

[10] CUSHNIE, T.T., LAMB, A.J., (2005): Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents* **26** (5): 343–356. doi: 10.1016/j.ijantimicag.2005.09.002

[11] DEHDARI, S., 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.

[12] EBRAHIMI, N.S., HADIAN, J., MIRJALILI, M.H., SONBOLI, A., YOUSEFZADI, M. (2008): Essential oil composition and antimibacterial activity of *Thymus caramanicus* at different phonological stages. *Food chemistry* **110** (4): 927–931. doi: 10.1016/j.foodchem.2008.02.083

[13] EZZOUBI, Y., BOUSTA, D., EL MANSOURI, L., BOUKHIRA, S., SIHAM, L., ACHOUR, S., 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): 31–7.

[14] GEOGHEGAN, F., WONG, R., RABIE, A. (2010): Inhibitory effect of quercetin on periodontal pathogens *in vitro*. *Phytotherapy Research* **24** (6): 817–820.

[15] GULCIN, I. (2012): Antioxidant activity of food constituents: an overview. *Archives of Toxicology* **86**: 345–391.

[16] HASBAY, I., GALANAKIS, C.M. (2018): Recovery technologies and encapsulation techniques. *In*: Galanakis, C.M. (eds) *Polyphenols: Properties, Recovery, and Applications*. Elsevier: Amsterdam, The Netherlands: 233–264.

[17] HEIM, K.E., TAGLIAFERRO, A.R., BOBILYA, D.J. (2002): Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *Journal of Nutritional Biochemistry* **13:** 572–584. doi: 10.1016/s0955-2863(02)00208-5

[18] HUNG, T.M., NA, M.K., THUONG, P.T., SU, N.D., SOK, D.E., SONG, K.S., SEONG, Y.H., BAE, K.H. (2006): Antioxidant activity of caffeoyl quinic acid derivatives from the roots of *Dipsacus asper* Wall. *Journal of Ethnopharmacology* **108** (2): 188–192. doi: 10.1016/j.jep.2006.04.029.

[19] IGNAT, I., VOLF, I., 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

[20] KARUNANIDHI, A., THOMAS, R., VAN BELKUM, A., NEELA, V. (2013): *In vitro* antibacterial and antibiofilm activities of chlorogenic acid against clinical isolates of *Stenotrophomonas maltophilia*including the trimethoprim/sulfamethoxazole resistant strain. *BioMed Research International*: 7 pages, Article ID 392058. doi: 10.1155/2013/392058.

[21] KASHKOOE, A., SARDARI, F. A., MEHRABADI, M.M., 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. doi: 10.2174/1570163817666200316111445

[22] LEE, K.W., KIM, Y.J., LEE, H.J., LEE, C.Y. (2003): Cocoa has more phenolic phytochemicals and a higher antioxidant capacity than teas and red wine. *Food chemistry* **51:** 7292–7295. doi: 10.1021/jf0344385

[23] LI, G., QIAO, M., GUO, Y., WANG, X., XU, Y., XIA, X. (2014): Effect of subinhibitory concentrations of chlorogenic acid on reducing the virulence factor production by *Staphylococcus aureus*. *Foodborne Pathogens and Disease* **11** (9): 677–683. doi: 10.1089/fpd.2013.1731

[24] LIMA, M.C., PAIVA DE SOUSA, C., FERNANDEZ-PRADA, C., HAREL, J., 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. doi: 10.1016/j.micpath.2019.03.025

[25] LÓPEZ-FERNÁNDEZ, O., DOMÍNGUEZ, R., PATEIRO, M., MUNEKATA, P.E.S., ROCCHETTI, G., LORENZO, G.M. (2020): Determination of polyphenols using liquid Chromatography tandem mass spectrometry technique (LC–MS/MS): A review. *Antioxidants* **9** (6): 479. doi: 10.3390/antiox9060479

[26] LOW, J. C., DONACHIE, W. (1997): A review of *Listeria monocytogenes* and listeriosis. *The Veterinary Journal* **153** (1): 9–29. doi: 10.1016/s1090-0233(97)80005-6

[27] MERGHEM, R., JAY, M., VIRICEL, M.R., BAYET, C., VOIRIN, B., (1995): Five 8-C-benzylated flavonoids from *Thymus hirtus* (Labiatea). *Phytochemistry* **38** (3): 637–640. doi: 10.1016/0031-9422(94)00578-H

[28] MILIAUSKAS, G., VENSKUTONIS, P.R., VAN BEEK, T.A. (2004): Screening of radical scavenging activity of some medicinal and aromatic plant extract. *Food chemistry* **85** (2): 231–237. doi: 10.1016/j.foodchem.2003.05.007

[29] OZGEN, S., KILINC, O.K., SELAMOGLU, Z. (2016): Antioxidant activity of quercetin: a mechanistic review. *Turkish Journal of Agriculture - Food Science and Technology* **4** (12): 1134–1138. doi: 10.24925/turjaf.v4i12.1134-1138.1069

[30] RAJENDRAN, N., SUBRAMANIAM, S., CHRISTENA, L. R., MUTHURAMAN, M. S., SUBRAMANIAN, N. S., PEMIAH, B., SIVASUBRAMANIAN, A. (2016): Antimicrobial flavonoids isolated from Indian medicinal plant *Scutellaria oblonga* inhibit biofilms formed by common food pathogens. *Natural Product Research* **30** (17): 2002–2006. doi: 10.1080/14786419.2015.1104673

[31] RIOS, J.L., RECIO, M.C., VILLAR, A., (1988): Screening methods for natural products with antimicrobial activity: A review of literature. *Journal of Ethnopharmacology* **23** (2–3): 127–149. doi: 10.1016/0378-8741(88)90001-3

[32] SCHOFIELD, P., MBUGUA, D.M., PELL, A.N. (2001): Analysis of condensed tannins: a review. *Animal Feed Science and Technology* **91** (1–2): 21–40. doi: 10.1016/s0377-8401(01)00228-0

[32] SHIMADA, K., FUJIKAWA, K., YAHARA, K., Nakamura, T. (1992): Antioxidative properties of xanthin on autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry* **40** (6): 945–948. doi: 10.1021/jf00018a005

[33] STOJKOVIĆ, D.S., ŽIVKOVIĆ, J. SOKOVIĆ, M., GLAMOČLIJA, J., FERREIRA, I.C.F.R., JANKOVIĆ, T., MAKSIMOVIĆ, Z. (2013): Antibacterial activity of *Veronica montana* L. extract and of protocatechuic acid incorporated in a food system. *Food and Chemical Toxicology* **55**: 209–213. doi: 10.1016/j.fct.2013.01.005

[35] TORRES, J.A., VILLEGAS, M.V., QUINN, J.P. (2007): Current concepts in antibiotic-resistant Gram-negative bacteria. Expert *Review of Anti-Infective Ther*apy **5** (5): 833–43. doi: 10.1586/14787210.5.5.833

[34] XU, D., HU, M-J., WANG, Y-Q., CUI, Y-L. (2019): Antioxidant activities of quercetin and its complexes for medicinal application. *Molecules* **24**: 1123. doi: 10.3390/molecules24061123.

[35] YAZDANI, M., 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. doi: 10.22055/PPD.2020.29309.1757

[36] YUAN, Q., WANG, J., RUAN, J. (2012): Screening for bioactive compounds from *Adiantum capillus-veneris* L. *Journal of the Chemical Society of Pakistan* **34** (1): 207-216.

[37] ZEB, A., ULLAH, F. (2017): Reversed phase HPLC-DAD profiling of carotenoids, chlorophylls and phenolic compounds in *Adiantum capillus-veneris* Leaves. *Frontiers in Chemistry* **5**. doi: 10.3389/fchem.2017.00029.