BIOLOGICAL ACTIVITIES OF ADIANTUM CAPILLUS-VENERIS COLLECTED FROM DUHOK PROVINCE (IRAQ)

FALAH SALEH MOHAMMED, MUSTAFA SEVİNİDK, CELAL BAL, HASAN AKGÜL and ZELİHA SELAMOĞLU

ABSTRACT. This study determined the DPPH free-radical scavenging activity, total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI), DNA-protective activity, antiproliferative activity, antimicrobial activity and phenolic contents of methanol (MeOH) and dichloromethane (DCM) extracts of A. capillus-veneris leaves collected from the province of Duhok (Iraq). As a result of the studies, it was determined that the MeOH extract of A. capillus-veneris had a 49.74% free-radical scavenging activity at 2 mg/mL concentration. It was found that the extracts were effective against the test microorganisms at a concentration level of 200-400 µg/mL. TAS, TOS and OSI values were 3.086±0.066, 21.532±0.525 and 0.698±0.002, respectively. The DNA-protective activity of the extracts was found to be weak compared to the positive control. It was found that, depending on the increase in concentration, the extracts showed antiproliferative activity on A549 cells. Furthermore, the HPLC analyses found Catechin, Cinnamic acid, Chlorogenic acid, Caffeic acid, p-Coumaric acid, Rosmarinic acid and 4-Hydroxybenzoic acid with various ppm values. Consequently, it was determined that A. capillus-veneris could be a potential natural source pharmacologically.

1. INTRODUCTION

Plants have always been used by people to improve medical conditions or to lessen their impact [1]. Plants have drawn the attention of many research teams due to their role in fighting various medical conditions such as atherosclerosis, cerebral cardiovascular events, diabetes, hypertension and Alzheimer’s disease [1,2].

Previous studies by different research groups have found that different plants have different antibacterial, antiviral, antifungal, antiproliferative, anti-inflammatory, antioxidant, antimutagenic, anticarcinogenic, antidepressant, and antitumor properties [3-8].
A. capillus-veneris commonly grows in warm, tropical climates with a high moisture content. It has creeping rhizome roots and an aromatic fragrance. The leaves of the plant are generally double-rowed, tender, glabrous, and can grow up to 50 cm (Figure 1). As a cosmopolitan species, the plant is widely distributed in suitable climates [9].

FIGURE 1. Adiantum capillus-veneris L.

This study used MeOH and DCM extracts from the leaves of A. capillus-veneris. Phenolic content, antioxidant potential, oxidant potential, antimicrobial potential, DNA-protective activity and antiproliferative action of the extracts were determined. Within this scope, it was found that the plant could potentially be used as a natural source for pharmacological applications.

2. Material And Method

2.1. Collection of plants and laboratory studies

The study material A. capillus-veneris was collected from the Duhok province of Iraq. Herbarium samples are preserved in the herbarium of Zakho University, Science Faulty, Biology Department. The plant parts were cleaned with distilled water and dried under favorable conditions. Later, components of the plant were pulverized using a mechanical grinder. After the grinding process, 15 g of the plant components were extracted with methanol (MeOH) and dichloromethane (DCM) in
a Soxhlet extractor at 50 °C for approximately six hours. Then, crude extracts were obtained by evaporating the solvents of the extracts using a Rotary Evaporator.

2.2. Antioxidant Activity Tests

Stock solutions were prepared at concentrations of 0.25, 0.5, 1 and 2 mg/mL using DMSO (Dimethyl sulfoxide). 50 µL of the prepared solutions was added to 160 µL 0.039% DPPH. The prepared solutions were incubated for 30 minutes. Following the incubation process, a reading for absorbance at 517 nm was obtained. These processes were repeated for all specified concentrations [10]. Rosmarinic acid (RA) and ascorbic acid (AA) were used as reference antioxidants. DPPH free radical scavenging percentages were determined using the following formula: inhibition% = [(Abs control - Abs sample)/Abs control]x100.

TAS, TOS and OSI values of the plant extracts were determined using Rel Assay branded commercial kits (Rel Assay Diagnostics Kits, Turkey). Trolox and hydrogen peroxide were used as the TAS calibrator and TOS calibrator, respectively. TAS results were expressed in mmol Trolox equiv./L. TOS results were expressed in μmol H₂O₂ equiv./L [11,12]. The OSI (AU: Arbitrary unit) value was calculated based on the following formula (1):

OSI (AU) = \[\frac{\text{TOS, } \mu\text{mol } \text{H}_2\text{O}_2 \text{ equiv.}/\text{L}}{\text{TAS, mmol Trolox equiv.}/\text{L \times 10}}\]

2.3. Determination of Phenolic Contents

The phenolic contents of the plant were scanned using an HPLC device. A DAD detector was used as detector. The injection volume was set to 20 µL. A: 3% acetic acid and B: methanol was used in the mobile phase. The flow rate was set to 0.8 mL per minute. Chromatographic separation was carried out with an Agilent Eclipse XDB-C18 column (250x4.6 mm; id 5 µm) at 30 °C [13].

2.4. Antimicrobial Activity Tests

Tests for the antimicrobial activity of MeOH and DCM extracts of the plant components were conducted using the agar dilution method recommended by the
Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). The minimum inhibitory concentration (MIC) for MeOH and DCM extracts was determined against standard bacterium and fungus strains. *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606 were used as bacterium strains. *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 ATCC 13803, *C. glabrata* ATCC 90030 were used as fungus strains. All extracts were tested at 800-12.5 µg/mL concentrations and all dilutions were performed using distilled water. Bacterium and fungus strains were obtained from the American culture collections. Colony formation was interpreted as the presence of growth and the absence of colony was interpreted as inhibition. Additionally, control plates were used for each study series. The lowest dilution that prevented bacteria and fungi reproduction was the minimum inhibitory concentration (MIC) [14-18].

### 2.5. DNA-Protective Activity Test

Standard solutions were prepared from MeOH and DCM extracts of the plant parts at concentrations of 25, 50, 100 and 200 µg/mL, and their DNA-protective activity was determined using pBR 322 supercoiled DNA. 0.5 µg plasmid pBR 322 supercoiled DNA was put into Eppendorf tubes and 10 µL of the standard solutions of the extracts was added. 10 µL Fenton agent (30 mM H₂O₂, 50 µM ascorbic acid and 80 µM FeCl₃) was added to them and incubation was performed at room temperature for 10 minutes. The mixture was prepared with a final volume of 20 mL and set aside to rest at 37 °C for 30 minutes. Subsequently, it was analyzed by electrophoresis on 1% agarose gel containing DNA ethidium bromide [19].

### 2.6. Antiproliferative Activity Test

An MTT test (3- [4, 5-dimethylthiazol-2-il] -2, 5-diphenyl-tetrazolium bromide) was performed in order to find the cell habitability on A549 cells of MeOH and DCM extracts of the plant components. After 70-80% unification was achieved, cells were separated using a 3.0 mL Trypsin-EDTA solution (Sigma-Aldrich, MO, ABD). Following the separation process, they were planted on the plates. After the planting process, they were incubated for 24 hours. Following the incubation process, the extracts were subjected to a dilution process at different concentrations (25, 50, 100 and 200 µg/mL) and the cells were incubated for 24 hours. Controls were applied
with a growth medium that was not supplemented with FCS. After an incubation period of 48 hours, the supernatants were dissolved in the growth medium and replaced with 1 mg/mL MTT (Sigma). Then incubation was performed at 37 °C until a purple precipitation was formed. The supernatants were collected and dissolved by adding dimethyl sulfoxide (DMSO) (Sigma-Aldrich, MO, ABD) to MTT which was absorbed by the cells. Then the plates were measured at 570 nm using the Epoch spectrophotometer (BioTek Instruments, Winooska, VT) [20].

3. Results And Discussion

3.1. Antioxidant Activity

Compounds with antioxidant properties play an essential role in the body’s defense system against reactive oxygen species (ROS). ROSs may cause early ageing, cancer and cardiovascular diseases. The antioxidant defense system plays a role in suppressing or dampening the effects of ROSs. When elements of the antioxidant defense system produced by the body cannot adequately reduce the effects of ROSs, supplementary natural antioxidants may be taken. Natural antioxidants increase the antioxidant capacity of the plasma and reduce the risk of many diseases. The increased intake of dietary antioxidants may help support the process of limiting antioxidant concentrates and also may support the normal functioning of various physiological systems [21,22]. Therefore, the natural antioxidant potential of *A. capillus-veneris* leaves was assessed in this study. In the present study, DCM extracts of *A. capillus-veneris* leaves did not demonstrate any antioxidant properties while MeOH extracts were found to have antioxidant properties in parallel with increases in the concentration levels. The results are shown in Table 1.

<table>
<thead>
<tr>
<th>Concentration (mg/mL)</th>
<th>Ascorbic acid (%)</th>
<th>Rosmarinic acid (%)</th>
<th>MeOH</th>
<th>DCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>27.717</td>
<td>25.501</td>
<td>5.750</td>
<td>0.095</td>
</tr>
<tr>
<td>0.5</td>
<td>69.398</td>
<td>43.744</td>
<td>12.283</td>
<td>0.439</td>
</tr>
<tr>
<td>1</td>
<td>95.721</td>
<td>79.694</td>
<td>23.018</td>
<td>1.356</td>
</tr>
<tr>
<td>2</td>
<td>95.740</td>
<td>93.849</td>
<td>49.742</td>
<td>7.660</td>
</tr>
</tbody>
</table>
Previous studies reported that Ether and MeOH extracts of *A. capillus-veneris* demonstrated normal DPPH free radical scavenging activities [23,24]. Another study on Ethanol, Butanol, Ether and MeOH extracts of *A. capillus-veneris* found that the extracts demonstrated DPPH free radical scavenging activities at normal levels [25]. This study found that the DCM extract of *A. capillus-veneris* demonstrated no DPPH free radical scavenging activities while the MeOH extract demonstrated DPPH free radical scavenging activities by 49.74% at 2 mg/mL concentration. According to the obtained data, it was determined that the leaves of *A. capillus-veneris* had antioxidant potential and could be used as a natural antioxidant source.

Oxidative stress is a condition where the ROS concentration is temporarily or continuously increased and, consequently, cellular metabolism and regulation as well as cellular components are damaged. [26]. Oxidative stress index (OSI) shows the extent to which endogenous oxidant compounds are suppressed by endogenous antioxidant compounds. In the current study, the TAS and TOS values of *A. capillus-veneris* were determined. Also, OSI values were determined on the basis of the TAS and TOS values. According to the results of the study, TAS, TOS and OSI values of *A. capillus-veneris* were 3.086±0.066 mmol/L, 21.532±0.525 μmol/L and 0.698±0.002, respectively. This is the first study that has examined the TAS, TOS and OSI values of *A. capillus-veneris*. In previous studies conducted on plants, the TAS, TOS and OSI values of *Mentha longifolia* subsp. *longifolia* were reported to be 3.628 ± 0.234 mmol/L, 4.046 ± 0.615 μmol/L and 0.112 ± 0.025, respectively [27]. Additionally, the TAS, TOS and OSI values of *Rhus coriaria* var. *zebaria* were reported to be 7.342±0.189 mmol/L, 5.170±0.525 μmol/L and 0.071±0.009, respectively [28]. In another study, the TAS values of ethanolic extracts of *Calendula officinalis* were reported to be 5.55±0.41 mmol/L [29]. It can be seen that *A. capillus-veneris* used in our study has lower TAS values than *M. longifolia* subsp. *longifolia*, *R. coriaria* var. *zebaria* and *C. officinalis*. Also, it was found that *A. capillus-veneris* had higher TOS and OSI values than *M. longifolia* subsp. *longifolia* and *R. coriaria* var. *zebaria*. Consequently, it was determined that *A. capillus-veneris* produced more oxidant compounds than these plants; and that the antioxidants responsible for suppressing these were in inadequate amounts, and therefore the plant had higher OSI values.

### 3.2. Phenolic Contents

In the current study, the phenolic contents of *A. capillus-veneris* were scanned using an HPLC device. The results obtained in the study are shown in Table 2.
TABLE 2. Phenolic content of A. capillus-veneris

<table>
<thead>
<tr>
<th>Phenolic Compound</th>
<th>Concentration (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catechin</td>
<td>56.21</td>
</tr>
<tr>
<td>Cinnamic acid</td>
<td>19.87</td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>56.76</td>
</tr>
<tr>
<td>Caffeic acid</td>
<td>1.59</td>
</tr>
<tr>
<td>p-Coumaric acid</td>
<td>4.47</td>
</tr>
<tr>
<td>Rosmarinic acid</td>
<td>130.8</td>
</tr>
<tr>
<td>4-Hydroxybenzoic acid</td>
<td>16.32</td>
</tr>
</tbody>
</table>

The plants produce a wide range of biologically active chemicals, secondary metabolites, playing a role in the fight against pests and diseases [30]. Catechin is reported to have antioxidant, antimutagenic, antimicrobial and anticarcinogenic properties [31-34]. Chlorogenic acid is reported to have antioxidant, antibacterial, anticarcinogenic, anti-inflammatory and DNA-protective properties [35-39]. It has been reported that caffeic acid has antioxidant, antibacterial, anti-inflammatory, anticarcinogenic and hepatoprotective properties [40-44]. Rosmarinic acid is reported to have antiviral, antibacterial, anti-inflammatory, anticarcinogenic and antioxidant properties [45]. Cinnamic acid is a natural organic acid with several biological activities. Cinnamic acid and its derivatives are significant compounds due to their antibacterial, antiviral and antifungal properties [46]. Coumaric acid has been reported to have antioxidant, anticancer, antimicrobial, antiviral, anti-inflammatory, anti-thrombocyte aggregation, anxiolytic, antipyretic, analgesic properties as well as many other biological activities including mitigating effects against diabetes, obesity, hyperlipemia and gout [47]. It has been reported that hydroxybenzoic acid has antioxidant properties and is beneficial for preventing and reducing the risk of diabetes, coronary heart disease, cancer, Alzheimer’s disease and cataracts [48]. The HPLC scans in our study found varying degrees of Catechin, Cinnamic acid, Chlorogenic acid, Caffeic acid, p-Coumaric acid, Rosmarinic acid and 4-Hydroxybenzoic acid in A. capillus-veneris. The therapeutic potential of medicinal plants depends on the presence of bioactive components that create specific physiological and pharmacological activities [49]. Within this scope, it was found that A. capillus-veneris could be a natural source in terms of the compounds determined in this study.
3.3. Antimicrobial activity

Since pathogens have recently developed resistance against currently available antibiotics, the search for new alternative sources for the treatment of communicable diseases has become inevitable. Plants are rich sources of secondary metabolites with antimicrobial effects [50]. Within this scope, the antibacterial and antifungal potential of *A. capillus-veneris* were evaluated in this study. The results are shown in Table 3.

**TABLE 3. Antimicrobial Activity of A. capillus-veneris**

<table>
<thead>
<tr>
<th></th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E</th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>J</th>
</tr>
</thead>
<tbody>
<tr>
<td>MeOH</td>
<td>800</td>
<td>800</td>
<td>800</td>
<td>800</td>
<td>400</td>
<td>400</td>
<td>800</td>
<td>400</td>
<td>400</td>
</tr>
<tr>
<td>DCM</td>
<td>400</td>
<td>400</td>
<td>800</td>
<td>800</td>
<td>200</td>
<td>200</td>
<td>800</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>1.56</td>
<td>3.12</td>
<td>1.56</td>
<td>3.12</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Amikacin</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1.56</td>
<td>3.12</td>
<td>3.12</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ciprofloksasin</td>
<td>1.56</td>
<td>3.12</td>
<td>1.56</td>
<td>1.56</td>
<td>3.12</td>
<td>3.12</td>
<td>-</td>
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<tr>
<td>Flukanazol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.12</td>
<td>3.12</td>
<td>-</td>
</tr>
<tr>
<td>Amfoterisin B</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.12</td>
<td>3.12</td>
<td>3.12</td>
</tr>
</tbody>
</table>

*(A) S. aureus, (B) S. aureus MRSA, (C) E. faecalis, (D) E. coli, (E) P. aeruginosa, (F) A. baumannii, (G) C. glabrata, (H) C. albicans, (J) C. krusei

*100, 50 and 25 µg/mL extract concentrations

Previous studies have reported that the methanol extracts of *A. capillus-veneris* had antimicrobial effects against *Bacillus, Escherichia coli, Staphylococcus, Proteus, Pseudomonas* and *Candida* at concentrations of 0.5-2 mg/mL [51]. Also, another study found that methanol extracts of *A. capillus-veneris* was effective against *Micrococcus luteus, Bacillus subtilis, B. cereus, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Candida albicans, Cryptococcus albidus, Trichophyton rubrum, Aspergillus niger, A. flavus, A. spinulosus, A. terreus* and *A. nidulans* at different concentrations [52]. In the current study, MeOH and DCM extracts of *A. capillus-veneris* were used and it was determined that the extracts were effective on the tested microorganisms at concentrations of 200-800 µg/mL. In addition, as opposed to other studies in the literature, it was found that the MeOH and DCM extracts of *A. capillus-veneris* were also effective against *E. faecalis, A. baumannii, C. glabrata* and *C. krusei* at different concentrations.
3.4. DNA-Protective Activity

In the current study, the DNA-protective activity of MeOH and DCM extracts obtained from *A. capillus-veneris* leaves at concentrations of 25, 50, 100 and 200 µg/mL were tested using pBR322 supercoiled DNA. The results are shown in Figure 2.

![DNA Protective Activity of A. capillus-veneris](image)

* P: DNA, 6: A. capillus-veneris, M: Methanol, D: Dichloromethane

**FIGURE 2. DNA Protective Activity of A. capillus-veneris**

It was determined that the MeOH and DCM extracts from *A. capillus-veneris* leaves had lower DNA-protective properties compared to the positive control at all concentrations applied. In previous studies, various plant types have been reported as having DNA-protective properties [28, 53, 54]. The protective effect of *A. capillus-veneris* against DNA damage was researched for the first time in this study and found to have a low impact.

3.5. Antiproliferative activity

Various plant components with anticarcinogenic properties have been identified in many regions of the world. Today, approximately 75% of anticancer drugs accepted worldwide are derived from plants or other natural products [55].

Cell viability was tested with the lung cancer cell line A549 by preparing standard solutions of both MeOH and DCM extracts from plant samples at concentrations of 25, 50, 100 and 150 µg/mL. The results are shown in Figure 2. Despite recent developments in the treatment of cancer, there is a need for new alternative drugs. Therefore, plants still constitute a significant source for the discovery of new drugs.
Moreover, less than 10% of flowering plants have been investigated in an analytical and pharmacological manner for their potential medical value [56]. In previous studies, ethanol extracts of *A. capillus-veneris* were reported to have antiproliferative effects on human gastric carcinoma SGC-7901 cells [25]. In the current study, MeOH and DCM extracts from the leaves of *A. capillus-veneris* were examined for their antiproliferative properties against the lung cancer cell line A549. The results are shown in Figure 3.

![Figure 3. Cytotoxic Effects of *A. capillus-veneris*](image)

In the current study, the MeOH and DCM extracts of *A. capillus-veneris* leaves were examined and it was found that their antiproliferative effects on the lung cancer cell line A549 increased depending on the increase in concentration. In this context, it was determined that *A. capillus-veneris* could be a natural cancer-fighting source.

4. Conclusion

In this study, the pharmacological potential of the leaves of *A. capillus-veneris* have been investigated. As a result of the studies conducted, it was determined that the plant components showed antioxidant activity and DNA-protective and anticancer potential. It was also found that they displayed antibacterial and antifungal potential against test microorganisms. Additionally, it was determined that it could be a natural source in terms of the compounds found in its content. Consequently, it was found that *A. capillus-veneris* can be considered a natural source in the manufacture of natural pharmacological drugs due to its biological activities.
References


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