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Bioactivity of Methanolic Leaves and Stem Extracts of *Adiantum capillus-veneris* L. From Southeast of Marivan

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ABSTRACT

Introduction: *Adiantum capillus-veneris* Linn is a medicinal plant belonging to Polypodiales order. *Pare-siavashan* is the name of *Adiantum capillus-veneris* Linn in pharmaceutical textbooks of Iranian Traditional Medicine. *A. capillus-veneris* L. as a medicinal plant was utilized to treat various infectious diseases. In this study, the extract of *A. capillus-veneris* L. from Kurdistan province is investigated for the first time.

Materials and Methods: The plant is collected from southeast of Marivan in October 2017. The aim of the current study was to investigate cytotoxicity, antimicrobial, antioxidant properties and phytochemical screening of methanol extract and polar and nonpolar subfractions of the leaves and stem of *A. capillus-veneris* L. separately. The antioxidant activity, total phenolic content (TPC) and total flavonoid content (TFC) of the samples were determined using inhibition of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu, and aluminum chloride colorimetric methods, respectively. Gentamicin, rifampin and nystatin were used as positive controls in an antimicrobial method. Both 50% lethal concentrations (LC_{50}) and 50% inhibitory concentration (IC_{50}) of the samples were identified using regression analysis.

Results and Discussion: The leaves and stem extracts indicated the highest antioxidative activities in DPPH test that IC_{50} values =56.23 ± 0.85 and 44.66 ± 1.22 µg/ml respectively, which was higher than the synthetic antioxidant butylated hydroxyl toluene (BHT: IC_{50}= 19 ± 1.03 µg/ml). The total phenolic contents of leaves and stem extracts, as Gallic acid equivalents, were 83.62 ± 1.87 and 147.39 ± 2.35 mg/g, respectively. The amounts of total flavonoids compounds of leaves and stem extracts were also determined 58.50 ± 0.78 and 35.63 ± 0.39 mg, respectively. Alkaloids, triterpenes, flavonoids, saponins, tannins were also identified in all the extracts and glycosides were detected only in water and hexane fraction extract of the stem, also hexane extraction fraction of stem was devoid of triterpenes and the stem and leaves which contain no tannins. The leaves water extract of *A. capillus veneris* L. showed maximum zone of inhibition against bactrial strains. The hexane extracts of leaves and stem of *A. capillus-veneris* L. showed the weakest antimicrobial activity. The brine shrimp lethality bioassay was applied for the isolation of anitumour and cytotoxic agents. The leaves and stem extracts of *A. capillus-veneris* L. showed significant cytotoxic activity with LC_{50} values of 125.893 and 97.7237 (µg/ml), respectively. Vincristine sulfate (LC_{50} 0.751 µg/ml) was used as the reference standard of brine shrimp lethality bioassay.

Conclusion: This study suggested that the extracts of the *A. capillus-veneris* L. may be a promising source for novel anticancer agents. The extracts showed moderate to acceptable antibacterial activities.

Introduction

Adiantum capillus-vener L. belongs to the *Adiantaceae* family. It is one of the most common and widely distributed species (Singh et al., 2008). *Adiantum* is a large genus of ferns which are widely distributed throughout the world. Ethno medicinally, the genus is important and popularly known as "Hansraj" in the Ayurvedic System of Medicine. About nine species of *Adiantum* are found in India (Mohini et al., 1990). *A. capillus veneris Linn.* has been shown to exhibit antimicrobial activity against different bacterial and fungal strains (Singh et al., 2008). This plant is used medicinally by indigenous Americans. The Mahuna people utilize the plant interior for rheumatism. It is utilized as a lotion for bumblebee and centipede stings by the Navajo people of Kayenta. The Navajo people also smoke it or take it internally to treat mental illness (Wyman and Harris, 1951). Natural sources have been used to cure many diseases since several centuries ago. Nowadays, synthetic drugs are available with fast-reaching effects in curing various diseases, but there are people who still prefer traditional medicines because of their fewer side effects (Cai et al., 2004). Plants contain phytochemical molecules such as vitamins, lignins, flavonoids terpenoids, phenolic acids, stilbenes, tannins, quinones, coumarins, alkaloids, amines, betalains, and other metabolites, which are wealthy in antioxidant activity. The results of Farokhzad et al. (2016) research showed that spray of apple fruit foliar with ascorbic acid probably tends to increase the total acidity, vitamin C, total antioxidant content, total flavonoid and PAL enzyme activity. Phytochemical screening of extracts was performed to determine the composition of secondary metabolites. The secondary metabolites of plants with unknown pharmacological activities have been extensively investigated as a source of medicinal agents (Gracelin et al., 2013). The antioxidant effects are considerable for reducing oxidative stress, which affecting damaged biological molecules (Farhat et al., 2013). The antiasthma and anti dyspnea activities of the plants can be depended on their antioxidant activity. Oxidative stress is the dissymmetry between oxidants and antioxidants in the protection of oxidants, potentially leading to damage. Reactive oxygen species (ROS) are a class of compounds that are formed from oxygen metabolism. These highly reactive molecules such as, hydroxyl radical, peroxide and superoxide radicals, can cause severe damages to cells and tissues during various diseases, which are linked to heart disease, carcinogenesis and many other health themes. Synthetic antioxidants such as butylated hydroxyl anisole (BHA), propyl gallate (PG), and butylated hydroxyl toluene (BHT) have been used to decline oxidation (Lee et al., 2003; Borneo et al., 2009). The studies have determined that different plant extracts have antimicrobial activities. Different extracts of *A.capillus-veneris* L. had demonstrated potential antibacterial activities against various microorganisms, including gram negative, gram-positive bacteria and yeast (Ishaq et al., 2014). In recent years, bioactive compounds and natural product extracts have been investigated by brine shrimp lethality bioassay (BSLA) as an indicator for cytotoxicity (Ghosh et al., 2012). Researchers have tried to use plant extract for the green synthesis of nanoparticles and the evaluation of bioactivities (Shams and Pourseyedi, 2015). The purpose of this study was to investigate cytotoxicity, antimicrobial and antioxidant properties. The present research carried out the in vitro bioactivities of all fractions, i.e., hexane, ethyl acetate and water collected methanol extracts from the leaves and stem of *A. capillus-veneris* L. separately. Therefore, the plant was collected from Iran. Hence the rationale of this study is to establish the bioactivities of *A. capillus-veneris* L. in Marivan town.

Materials and Methods

Chemicals and reagents

All analytical grade solvents such as methanol, dimethyl sulphoxide (DMSO), standard Folin-Ciocalteu and phenol reagent, sodium acetate, sodium carbonate and all cultures media were obtained from Merck (Darmstadt, Germany). Ultra-pure water was used for the purpose of experiments. Microbial strains were provided by Iranian Research Organization for Science and

Technology (IROST). Brine Shrimp (*Artemia salina*) eggs were also obtained from Advanced Hatchery Technology, INC, Salt Lake City, UTAH 84126, USA.

Preparation of samples

Leaves and stem parts of *A. capillus-veneris* L. were collected from Sarkal highlands of Marivan region in October 2017. The plant was deposited as a voucher sample in Kashan University, Kashan, Iran. The whole leaves and stem samples of *A. capillus-veneris* L. were washed with natural water twice and dried for 3 days in the shade at room temperature in the laboratory, Natural Essential Oil Research Institute, University of Kashan. The dried leaves and stem samples were ground by a grinder for 30 seconds. The whole powder samples of *A. capillus-veneris* L. were packed in a sealed plastic bottle until extraction.

Extraction procedure and fraction

The dried powder samples of *A. capillus-veneris* L. were extracted with methanol (500 ml) by soxhlet extractor for 12 h until complete extraction. After extraction, it was filtered and evaporated by rotary evaporator (hiedolf, RotaryEvaporator, model-RE 801, Germany) to give amophorous solid masses. The methanolic extracts (3 g) were dissolved in water (300 ml), then the fractions were divided with 150 ml of hexane, ethylacetate, and water, respectively. All collected fractions were concentrated with a vacuum evaporator and further dried with a vacuum oven (Lee et al., 2009).

Total phenolic content (TPC)

Total phenolics constituents of the plant extracts of *A. capillus-veneris* L. were assessed by Folin– Ciocalteu colorimetric method (Velioglu et al., 1989). 0.02 ml of leaves extract (5mg/ml) was mixed with 0.1 ml of Folin–Ciocalteuphenol reagent and 3 ml distilled water. After 3min, 0.3 ml of sodium carbonate (2%) was appended, and the mixture was determined by UV-visible spectrophotometry at 760 nm. These results were expressed as mg gallic acid equivalent (GAE) per gram of extract.

Total flavonoid content (TFC)

Total flavonoid content was determined by the modified aluminum colorimetric method and quercetin used as the standard (Chang et al., 2002). 0.5 ml of extract (1mg/ml) and standard (0.5 ml) were placed in different test tubes after 0.1 ml of aluminum chloride (10%), 0.1ml of Sodium acetate (1 M), 1.5 ml of methanol and 2.8 ml distilled water were appended to each tube and then mixed, as well as, 0.5 ml of methanol was used as a negative control. All tubes were incubated at room temperature for 30min. The absorbance was measured by UV-visible spectrophotometry at 415 nm. The concentration of flavonoid was expressed as mg quercetin equivalent (QE) per gram of extract.

DPPH Radical scavenging activity

The antioxidant assay was determined with DPPH method. Radical-scavenging activity of the plant samples was examined based on the previous method, and BHT (synthetic standard antioxidant) was used as a positive control (Ebrahimzadeh et al., 2010). At first, different concentrations of the sample (0.5, 5, 50, 100, 250, 500, 800 and 1000 µg /ml) were prepared, then each of the concentrations was mixed with 3 ml of a methanol solution of DPPH (0.1 mMol/l). The mixtures were incubated under constant agitation at room temperature for 30 min. The absorbance of the resulting solution was measured at 517 nm UV-visible Spectrophotometer. Percentage of DPPH scavenging activity was calculated according to the formula:

$$\text{Antiradical activity (\%)} = (A_{control}-A_{sample}) / A_{control} \times 100$$

Here $A_{control}$ is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. IC_{50} value was calculated. The decrease in absorbance of the reaction mixture indicates stronger DPPH radical scavenging activity. Each sample was assayed in triplicate, and mean values were calculated.

Preliminary phytochemicals screening

At first, 1g of the plant samples dissolved in 100 ml distilled water. The obtained solution was used for phytochemical screening.

Determination of total alkaloids

One-gram powder samples of *A.capillus-veneris* L. were dissolved in 3 ml of ammonia solution. After a few minutes, 10 ml of

chloroform was appended to the conical flask under agitation and then filtered. The chloroform was evaporated from the crude extract by water bath and added Mayer's reagent. A cream colour precipitation was obtained immediately that showed the presence of alkaloids (Soforowa, 1982).

Test for flavonoids

At first, 1ml of the stock solution was taken in a test tube, and a few drops of dilute NaOH solution was appended. An intense yellow colour appeared in the test tube. After that, a few drops of dilute acid were added. It became colourless, which indicated the presence of flavonoids (Tongco et al., 2014).

Test for saponins

2g powder samples of *A. capillus-veneris* L. were taken in a test tube and diluted with 20 ml distilled water. It was shaken by hand for 30 min. A foam layer was obtained on the top of the test tube. This foam layer indicated the presence of saponins (Tongco et al., 2014).

Test for glycosides

The extracts were washed with hot petroleum ether to remove color and fat. Additionally 2 ml of ferric chloride reagent was appended to the obtained deposition. The black solution was transferred to a test tube, then 2 ml of concentrated sulfuric acid was added with 45 degrees angle until it created tangles. Amethystine ring between two layers indicates the presence of cardiac glycosides (Hossain et al., 2013).

Test for tannins

The stock solution (3 ml) was taken in a test tube and diluted with chloroform and appended 1ml of acetic anhydride. Finally, sulphuric acid (1 ml) was appended carefully by the side of the test tube, to the solution. A green colour was formed which showed the presence of tannins (Soforowa, 1982).

Test for triterpenoids

The plant extracts (5 mg) were dissolved in 2 ml of chloroform and then acetic anhydride (1 ml) was appended to it. Concentrated sulphuric acid (1 ml) was appended to the solution. Formation of a reddish colour shows the presence of triterpenoids (Soforowa, 1982).

Microorganisms

The panel of microorganisms, including *Staphylococcus aureus* (ATCC 29737), *Proteus vulgaris* (PTCC 1182), *Escherichia coli* (ATCC 10536), *Bacillus subtilis* (ATCC 6633), *Klebsiella pneumonia* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 27853), *Staphylococcus epidermidis* (ATCC 12228), *Shigella dysenteriae* (PTCC 1188), *Salmonella paratyphi-A serotype* (ATCC 5702) of bacteria, *Candida albicans* (ATCC 10231) of yeast and *Aspergillus brasiliensis*(PTCC 5011), and *Aspergillus niger* (ATCC 16404) of mould were used in this study. The bacterial strains were cultured overnight at 37^{o}C in nutrient agar (NA) and fungi were cultured overnight at 30^{o}C sabouraud dextrose agar (SDA).

Antimicrobial assays

Disc diffusion method

The antimicrobial activities of the samples of *A. capillus-veneris* L. were determined by the disc diffusion method (Watts et al., 2017). The extracts were dissolved in DMSO (30 mg/ml) and filtered by 0.45 µm millipore filter for sterilization. The suspension of bacteria (100 µl of 10^8 CFU/ml) was spread on müller-hinton agar (MHA) medium. The discs (6 mm in diameter) impregnated with 10 µl of the extract solution (300 µg/disc), DMSO (as negative control) and gentamicin, rifampin for bacteria and nystatin for fungi (as positive controls) were placed on the inoculated agar (24 h at 37 °C). The antimicrobial activity was used as a measure by diameters of inhibition zones, and each assay was repeated three times.

Micro-well dilution assay

Microbial strains sensitive to the plant extracts in disc diffusion assay were studied for their minimal inhibitory concentration (MIC) values using the published micro-well dilution assay method (Gulluce et al. 2004). The suspension of each microorganism was adjusted to 0.5 McFarland standard turbidity. The plant samples were serially diluted two folds with 10 % DMSO in a concentration range from 7.8 to 500 µg/ml in 10 ml sterile test tubes containing brain-heart infusion (BHI) broth for bacterial strains and sabouraud dextrose (SD) broth for yeast. Then 95 µl of the cultures media was appended to each well

and 5 µl of the inoculums into each well. After, 100 µl of serial dilutions were added to each well. The final volume in each well was 200 µl. The last well containing 195 µl of the cultures media without the samples and 5 µl of the inoculums was the negative control. The antibiotics mentioned above were utilized as standard positive controls in conditions identical to the test materials. The MIC values were defined as the lowest concentration of the plant extracts required for inhibiting the growth of microorganisms. All tests were repeated three times.

Brine Shrimp Lethality Assay (BSLA)

Cytotoxic activity was determined by brine shrimp lethality bioassay (BSLB). The brine shrimp lethality test was performed according to Meyer et al. (1982) method with minor adaptations. Brine shrimp lethality bioassay is widely applied in the bioassay for the bioactive compounds (Zhao et al., 1992). In this study, the brine shrimp, *Artemia salina*, was utilized as a convenient monitor for the screening. The eggs of the brine shrimp were hatched in artificial seawater (3.8% NaCl solution) for 48 h to mature shrimp called nauplii. The samples were dissolved in DMSO (3.2 %) plus sea water (3.8% NaCl in water) to attain concentrations of 10 µg/ml, 100 µg/ml, 300 µg/ml, 500 µg/ml, 700 µg/ml, 1000 µg/ml. Vincristine sulfate (VS) and DMSO were utilized as positive and negative controls, respectively. The lethality percent was determined by comparing the mean number of dead larva in the test and the control tubes. Then matured shrimps were utilized by each of the experimental vials and the control vial. After 24 hours, the number of surviving nauplii in each vial was counted. The lethal concentrations of plant extract, resulting in 50% mortality of the brine shrimp (LC_{50}) from the 24 h counts, and the dose-response data were transformed into a straight line using a trendline fit linear regression analysis; LC_{50} was derived from the best-fit line obtained (Patil et al., 2016).

Results and Discussion

The extract of *A. capillus veneris* L. was obtained by Soxhlet apparatus. The percent yield of the leaves and stem plant were 19.81 and 18.09 % w/w, respectively. The yields of extracts were under the study of Ishaq et al. (2014).

Total phenolic and flavonoid content

The total phenolic contents of the extracts were identified using Folin-Ciocalteu phenol reagent in a colorimetric test. The results showed that the leaves and stem extracts had the highest total phenolics of 83.62 ± 1.87 and 147.39 ± 2.35 mg Gallic acid equivalents per g extract, respectively. The total flavonoid content of extracts was determined by the aluminium chloride colorimetric assay using quercetin as standard. The TFC of leaves and stem extract of *A. capillus-veneris* L. was 58.50 ± 0.78 and 35.63 ± 0.39 mg, respectively. Aluminium chloride forms acid stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxide group of flavones and flavonols; also, it can form liable complexes containing ortho dihydroxide groups in A/B rings of flavonoids (Kamtekar et al., 2014). The most common polyphenolic compounds are flavonoids that are detected ubiquitously in plants (Khatiwora et al., 2010). More than 6000 flavonoids have been determined in plants (Bag et al., 2015). Pourmorad et al. (2006) reported that the total phenolic and flavonoid content of methanolic extract of *A. capillus-veneris* L. was 22.3 ± 3 and 78.3 ± 4.5, respectively. The difference in our results (83.62 ± 1.87 and 58.50 ± 0.78, respectively) was climate change and soil fertility (Pourmorad et al., 2006). The results of TFC of metanolic extract (58.50 ± 0.78) indicated a better amount compared to the study of (Molan and Mahdy, 2014) from Iraq (12.9±0.17 mg quercetin quivalen/g). Phenolic compounds have redox properties, which allow them to act as an antioxidant. It was found that flavonoids show antioxidant activity, and their effects on human nutrition and health are considerable. These extracts were utilized for further antioxidant and antimicrobial studies

Antioxidant activity

The free radical scavenging ability of *A. capillus-veneris* L. methanolic extracts was measured by bleaching of the purple-coloured solution of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), according to the method described by Willfiams (Brand et al., 1995).

IC_{50} values were 56.23±0.85 (leaves) and 44.66±1.22 (stem) in extracts for their antioxidant activity by DPPH assay. The IC_{50} for BHT as a positive control was 19 ± 1.03 µg/ml. The findings of this study showed that the leaves and stem extracts have considerable amounts of phenols and flavonoids because their free radical scavenging power is compatible with gallic acid. The radical scavenging effect of *A.capillus-veneris* L. in Pourmorad et al. (2006), reported from Iran, was much weaker (4mg/ml, 44±1 Scavenging (%)) than the present study. The current study represented the importance of plants as a beneficial source of antioxidants that prevent the occurrence of non-communicable diseases like cancer, diabetes, dementia and myocardial infarction, for which free radicals are considered one of the major contributing factors (Shirazi et al., 2014).

Preliminary phytochemicals screening:

Qualitative phytochemical methods identified the presence of a particular phytochemical in the leaves and stem extract. The estimated amount of phytochemicals in the samples was determined based on the intensity of the color change observed during screening. The plant extracts have consisted of saponins, triterpenes, alkaloids, glycosides, tannins and flavonoids. Table 1 shows the results of phytochemical screening of the leaves, stem and their fraction of *A. capillus-veneris* L. As expected, saponins are very hydrophilic, which dissolve in water readily; therefore, the saponin test afforded a more visible detection of this group of natural products in the mathanol extracts. Tannins and flavonoids, due to their hydroxyl groups, are detected in the methanolic extracts. Cardiac glycosides are known to possess serious toxicity because they could affect the heart and atrial fibrillation. Cyanogenic glycosides are toxic because of the neurological effects (Tropical Ataxic Neuropathy) of these compounds. Cardiac glycosides do not exist in this plant. Triterpenes are less polar than diterpenes, but they are also detected in normal amounts in methanolic extracts. This is due to some polar functional groups in their structure, such as hydroxyl and carbonyl groups. Preliminary phytochemical investigation of the aqueous, ethylacetate, methanolic and hexane extracts of the leaves and stem were compared. Saponins, alkaloids, and flavonoids were present in all the solvents extracts of leaves and stems, whereas triterpenes were evident in all the solvents' extracts except hexane extract the stem. The comparison showed that glycosides were present in aqueous and methanolic extract of the stem only. Tannins were present in all extracts except hexane stem and leave extracts. As a result, it is concluded that the findings of the present study demonstrated the presence of saponins, alkaloids and flavonoids, which is in line with studies conducted in Pakestan and India (Rajurkar and Gaikwad, 2012) and (Ishaq et al., 2014). However, there are reports which do not converge with our findings. For example, the phytochemical investigation revealed the presence of sugars, flavonoids, triterpenoids, and steroids for *A. capillus-veneris* L. collected locally in Cape Coast, Ghana, but the rest of the compounds were absent. It suggests that the phytochemicals identified in the plant extracts may be due to the variation in methods and the liquor which extracts the active plant components.

Antimicrobial activity

The extracts of *A. capillus veneris* L. were tested against twelve microorganisms. The samples showed moderate to good antimicrobial activities with inhibition zone diameter from 10 to 15 mm, which inhibited the growth of all tested microorganisms at MIC values between 125-500 µg/ml on the tested microorganisms (Table 2).

Assessment of drug resistance pattern of the test bacterial strains is shown in Table 3. The leaves water extract of *A. capillus veneris* L. showed maximum zone of inhibition against *B. subtilis* (13 mm), *S. epidermidis* (14 mm), *S. aureus* (11 mm), *E. coli* (11 mm), *S. dysenteriae* (10 mm), *P. vulgaris* (13 mm), *P. aeruginosa* (15 mm), and *C. albicans* (12 mm). The methanolic extract of leaves showed good antimicrobial activity against *B. subtilis* (12 mm), *S. epidermidis* (11 mm), *S. aureus* (10 mm), *E. coli* (10 mm), *S. dysenteriae* (10 mm), *P. vulgaris* (12 mm), *P. aeruginosa* (14 mm), and *C. albicans* (11 mm) strains.

Table. 1. Results of phytochemical screening of *A.capillus-veneris* L

Solvent	Plant extract							
	Stem extract				Leaves extract			
	Water	E.acetate	Hexane	Methanol	Water	E.acetate	Hexane	Methanol
Chemical constituents								
Saponins	++	++	+	++	++	+	+	++
Alkaloids	+	+	+	+	+	+	+	+
Tannins	++	+	-	+	+	+	-	+
Triterpenes	+	+	-	++	++	+	+	+++
Flavonoids	+	++	+	++	+	++	+	+++
Glycosides	+	-	-	+	-	-	-	-

Legend: -: Not detected, +: Rare, ++: Abundant, +++: Very abundant.

Table. 2. Antimicrobial activity of methanol extracts of *A. capillus veneris* L.

Microbial strain		Plant extract															
		Leaves								Stem							
		Water		E. acetate		Hexane		Methanol		Water		E. acetate		Hexane		Methanol	
		DD[a]	MIC[b]	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC	DD	MIC
Gram-positive bacteria	*B. subtilis*	13	500	11	500	-	NT	12	250	-	NT	-	NT	-	NT	-	NT
	S. epidermidis	14	125	11	500	-	NT	11	500	10	500	10	500	-	NT	12	500
	S. aureus	11	500	-	NT	-	NT	10	500	10	500	-	NT	-	NT	-	NT
Gram-negative bacteria	*E. coli*	11	500	-	NT	-	NT	10	500	12	500	11	500	-	NT	13	250
	k. pneumonia	-	NT	-	NT	-	NT	-	NT	10	500	-	NT	-	NT	-	NT
	S. dysenteriae	10	500	-	NT	-	NT	10	500	-	NT	-	NT	-	NT	-	NT
	P. vulgaris	13	250	11	500	-	NT	12	250	11	500	10	500	-	NT	10	500
	S. paratyphi-Aserotype	-	NT	-	NT	-	NT	-	NT	-	NT	-	NT	-	NT	-	NT
	P. aeruginosa	15	125	12	500	10	500	14	125	15	250	11	500	-	NT	13	500
Fungi	*C. albicans*	12	500	10	500	-	NT	11	500	12	0.5	-	NT	10	500	-	NT
	A. niger	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA
	A. brasilienis	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA	-	NA

A dash(-) indicate no antimicrobial activity.
[a] Inhibition zone in diameter (mm) around the impregnated discs.
[b] Minimal inhibition concentrations (as µg/ml).
[c] Plant samples were inactive against mould in disc diffusion test so that the MIC agar dilution test for nystatin was not carried out.
NT (not tested), NA (not applicable).

Table. 3. Assessment of drug resistance pattern of the test-bacterial strains

Microbial strain		Antibiotics					
		Rifampin		Gentamicin		Nystatin	
		DD[a]	MIC[b]	DD	MIC	DD	MIC
Gram-positive bacteria	*B. subtilis*	13	125	21	500	NA	NA
	S. epidermidis	40	500	35	500	NA	NA
	S. aureus	10	500	21	500	NA	NA
Gram-negative bacteria	*E. coli*	-	-	23	500	NA	NA
	k. pneumonia	11	500	20	500	NA	NA
	S. dysenteriae	8	500	18	500	NA	NA
	P. vulgaris	10	500	23	500	NA	NA
	S. paratyphi-Aserotype	-	-	21	500	NA	NA
	P. aeruginosa	7	500	22	250	NA	NA
Fungi	*C. albicans*	NA	NA	NA	NA	NA	NA
	A. niger	NA	NA	NA	NA	33	125
	A. brasilienis	NA	NA	NA	NA	NA	NA

The antimicrobial activity of water and methanol fraction of leaves was evaluated, and it was found to be the most active one among other fractions tested. Both extracts of leaves water and leaves methanolic of *A. capillus veneris* L. showed antimicrobial activity against all the gram-positive bacteria strains. The recommendation for future research is to investigate the compound characterization of extracts for more effective antibacterial activity. Antimicrobial activity of methanolic and water extract of *A. capillus veneris* L. has also been reported by Hussain et al. (2014) in India. Their results regarding the leaves methanolic extract against *P. Aeruginosa*, was similar to the leaves water and stem water extract results in our study.

The previous studies on *A. capillus veneris* L. demonstrated its potency against different microorganisms. For example, *P. vulgaris*, *P. aeruginosa*, and *S. Epidermidis* were sensitive to water, methanolic and E. Acetate of leaves extracts and stem extracts of water, E. acetate and methanolic of *A. capillus veneris* L. in our study which proved to be almost in parallel with the findings of (Mahboubi et al., 2012), (Kumar and Nagarajan, 2012), and (Ishaq et al., 2014). Aquatic, methanolic and acetate extracts of the leaves and aquatic and hexane of stems of *A. capillus veneris* L. showed activity against yeast tested *C. albicans* strain, while hexane extract of leaves and methanolic and acetate extract of stems showed no activity. None of the extracts identified any effects against mould (*A. niger* and *A. brasilienis*). Numerous studies were conducted on *A. capillus veneris* L., collected worldwide, which are comparable with the results of the current study. Ishaq et al. (2014) prepared extracts of *A. capillus veneris* L. from different areas of Swat and Peshawar. The extracts were active against most of the multidrug-resistant (MDR) bacteria strains and fungal strains, isolated from clinical, also, *A. capillus veneris* L. was collected from Mazandaran province, north of Iran by Mahboubi et al. (2012). Their results showed its potency against standard bacterial strains which proved to be almost in accordance with our findings.

Cytotoxic activity

After 24 hours, the surviving brine shrimp larvae were counted, and LC_{50} was assessed. The median lethal concentration of the brine shrimp lethality assay (LC_{50}) for leaves, stem and vincristine sulfate (positive control) are given in Table 4. The methanol extracts of the leaves and stem of *A. capillus-veneris* L. showed good brine shrimp larvicidal activity. The plant extracts have a high amount of bioactive substances and may contain compounds that possess cytotoxicity effects. This study can be considered as the first report on the brine shrimp lethality activity of the methanolic extract of *A. capillus veneris* L. Results of the study showed that brine shrimps cytotoxicity assay could be an easy bioassay to screen medicinal plants. The brine shrimps cytotoxicity assay could be used as an indicator of cytotoxicity and antitumor assay. As such, it is suggested that the plant extracts can be a possible source of cytotoxic compounds that may justify its use as an anticancer drug because of the positive correlation of brine shrimps cytotoxicity assay (Tawaha, 2006) and antioxidant activity, though it requires further investigation. Therefore, the cytotoxic effect may be attributed to the phytochemicals present in the plant; however, this plant is used to treat various infections, cancer prevention, etc. We believe that our results are closer to the fact than the previously cited works. Notably, the indigenous people use sodden *A.capillus-veneris* L. for curing a cough , kidney stone, and urinary tract infection. Therefore, It is suggested by the results of the present research that the plant can be selected as an anticancer drug for further studies.

Table 4. LC50 value of extract and positive control on brine shrimp nauplii

S. No.	Samples	LC_{50}(µg/ml)
1	Leaves	125.893
2	Stem	97.7237
3	VS	0.751

Conclusion

potential source of natural antioxidant and antimicrobial and antitumor. The results of the DPPH, total phenolic and flavonoids for leaves and stem extracts of *A. capillus-veneris* L. show that these extracts have significant antiradical activity. The leaves extract was negative for toxic compounds such as cardiac and cyanogenic glycosides, which means that the leaves are safe for human consumption and possess great promise as herbal tea. The plant extracts illustrated a significant inhibiting activity against the microbial strains. This study shows valuable data on the cytotoxic activity of the leaves. It stems extracts, which should be very useful for any future in vivo or clinical study of these extracts. The diverse antioxidant, cytotoxic and antimicrobial potential of different extracts of *A. capillus-veneris* L. indicates that we can use these data to choose the suitable extracts for a better purification and identification of their active ingredients.

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