Evaluation of Antioxidant and Cytotoxic Activities of Aerial Parts of *Adiantum capillus-veneris* L. Growing in Bangladesh

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ABSTRACT: The present study evaluated the antioxidant and cytotoxic activities of methanolic extract of aerial parts of *Adiantum capillus-veneris* L. and its different solvent fractions. The *in vitro* antioxidant activity was assessed by using 2,2'-diphenyl-1-picrylhydrazyl (DPPH) radicals. The analysis revealed that ethyl acetate soluble fraction had the highest DPPH radicals scavenging property with IC_{50} value of 1.05 µg/ml as compared to positive control ascorbic acid (IC_{50} = 1.34 µg/ml). In addition, *ex vivo* cytotoxicity assay of *A. capillus-veneris* L. extract and its different fractions were performed against HELA cells line where 5-Fluorouracil was used as positive control. The result demonstrated that ethyl acetate and n-hexane soluble fractions showed prominent cytotoxicity with IC_{50} value of 5.68 µg/ml and 17.15 µg/ml, respectively. The study affirmed that superior antioxidant and cytotoxic activities were shown by ethyl acetate soluble fraction of methanolic extract of aerial parts of *A. capillus-veneris* L. growing in Bangladesh which indicate the presence of bioactive phytoconstituents in the extractives.

Key words: Antioxidant, cytotoxicity, *Adiantum capillus-veneris* L., fractionation, bioactivity.

INTRODUCTION

Adiantum capillus-veneris L. (*A. capillus-veneris* L.) belongs to the family Pteridaceae, is a perennial fern located worldwide especially in Mexico, warmer part of America, western Himalaya, southern part of India, and other tropical or subtropical regions.[1,2] The genus *Adiantum* has 250 species and most of them are used as traditional medicines to treat cold, fever, bronchial disorder, jaundice, hepatitis, rheumatic fever, skin rashes, tumors of spleen, liver, and other viscera by the people of India, China, Pakistan, and Bangladesh.[3-6] In Indian and Bangladeshi folk medicine, it has a diverse role in healing bronchitis, jaundice, kidney dysfunction, dandruff, increase lactation, parasitic infection, and general cure all.[7-9]

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Previous phytochemical analyses reported that *A. capillus-veneris* L. is a prominent source of triterpenoid and flavonoids derivatives like quercetin, quercetin-3-o-glucoside, quercetin-3-o-rutinoside, isoadiantone, isoadiantol-B, 3-methoxy-4-hydroxyfilicane, and 3,4-dihydroxyfilicane, etc.[9] In addition, other bioactive phytoconstituents like phenolics, sterols, quinic acids, shikimic acids, ketones, diols, esters of hydroxycinnamic acid, etc. have been separated from *A. capillus-veneris* L.[10] Because of these bioactive compounds, *A. capillus-veneris* L. performs anti-inflammatory, antidiarrheal, antispasmodic, antimicrobial, analgesic, antinociceptive, antidiabetic, agglutinating, antiviral, hypoglycemic, antifungal, and detoxifying effects.[11-14] A study revealed that methanolic extract of *A. capillus-veneris* L. leaf had pronounced analgesic,

anti-inflammatory and hypoglycemic effects in Swiss Albino mice.[14,15] Ethnopharmacological investigation among tribal people specially chakma, marma, and tanchangya of Khagrachchari district, Bangladesh found that local traditional practitioners use aerial parts of *A. capillus-veneris* L. for healing skin problems like rashes, psoriasis, dermatitis, and different skin strains.[16] The traditional practitioners dispensed different liquid or semisolid preparations of *A. capillus-veneris* L. to tribal people for getting cure of skin lesion and rashes. In addition, Nilforoushzadeh *et al*. revealed that methanolic extract of *A. capillus-veneris* L. had wound healing effects of human umbilical vein endothelial cells and normal human dermal fibroblast line.[17]

The recent study evaluates the *in vitro* antioxidant and *ex vivo* cytotoxic activities of methanolic extract of aerial parts of *A. capillus-veneris* L. and its different solvent fractions. According to Wahab *et al*., antioxidant activity of any bioactive phytoconstituent is conducted with cytotoxic effects through reactive oxygen species (ROS) inhibition.[18] Therefore, a qualitative correlation was made between antioxidant and cytotoxic activities of methanolic extract of *A. capillus-veneris* L. and its solvent fractions.

MATERIALS AND METHODS

Plant material. The aerial parts of *A. capillus-veneris* L. were collected from Matiranga and Panchchari upzilas of Khagrachchari district, Bangladesh in the month of June and November 2017. A voucher specimen of *A. capillus-veneris* L. with accession number of DACB-45943 has been deposited in Bangladesh National Herbarium, Dhaka, Bangladesh. The green aerial parts were then dried under shade, blended to powder and stored in air tight container.

Chemicals and instruments. Ascorbic acid (Merck, India), 2,2'-diphenyl-1-picrylhydrazyl (Merck, Germany), gentamicin, trypsin, HELA cell, DME medium, 10 % fetal bovine serum were purchased from authorized suppliers. 5-Fluorouracil was gifted by Beximco Pharmaceuticals Limited, Bangladesh. The cytotoxic activity was performed with the help of biological biosafety cabinet (NU-400E, Nuaire, USA), CO_2 incubator (Nuaire, USA), trinocular microscope with camera (Olympus, Japan), and hemocytometer (Nexcelom, USA). All instruments and chemicals were handled with care under safety environment.

Extraction and fractionation of *A. capillus-veneris* L.

Air dried and powdered aerial parts of *A. capillus-veneris* L. (1000 g) were macerated with methanol at ambient temperature for 48 h with intermittent shaking and this process was repeated thrice. The combined methanolic extract was filtered and concentrated using rotary evaporator to obtain oily residue of 88.08 g. This oily residue (88.08 g) was mixed with 250 ml of aqueous methanol (90:10). Then the preparation was subjected to fractionation with 250 ml of each n-hexane, chloroform, and ethyl acetate, respectively to yield n-hexane (21.47 g), chloroform (19.60 g), ethyl acetate (15.33 g), and aqueous methanol (18.86 g) soluble fractions. The methanolic extract along with all fractions were subjected to antioxidant and cytotoxicity analyses.

Determination of *in vitro* antioxidant activity of *A. capillus-veneris* L.

The antioxidant or free radicals scavenging activity of *A. capillus-veneris* L. was carried out with 2,2'-diphenyl-1-picrylhydrazyl (DPPH) free radicals by using the method described by Brand *et al*.[19] During this analysis, ascorbic acid (positive control) of 5, 20, 50, 100, 250, 500, and 1000 µg/ml along with DPPH of 20 µg/ml were prepared in methanol and were kept in light proof box. Different concentrations (5, 20, 50, 100, 250, and 500 µg/ml) of each of methanolic extract and its soluble fractions were obtained in methanol. Then, 2 ml of each of prepared sample (methanolic extract, soluble fractions or ascorbic acid) from different concentrations was mixed with 2 ml of DPPH solution (20 µg/ml). The mixture was incubated in a dark place for 30 minutes and was analyzed by

UV/Vis spectrophotometer (Shimadzu, Japan) at λmax of 517 nm.

Inhibition of free DPPH radicals in percent (I %) was calculated by using the following equation:

$$\text{Percent of inhibition (I \%)} = \left[\frac{\text{Absorbance (control)} - \text{Absorbance (sample)}}{\text{Absorbance (control)}}\right] \times 100\ \%$$

The free radicals scavenging activity was expressed as IC_{50} (µg/ml) which is defined as the concentration of sample necessary to scavenge 50 % of free DPPH radicals. The IC_{50} was calculated by a regression equation generated by plotting percentage of inhibition (I %) against the respective concentrations of the samples.

Ex vivo cytotoxic activity of *A. capillus-veneris* L.

Cytotoxicity of methanolic extract of aerial parts of *A. capillus-veneris* L. and its different soluble fractions were examined in Centre for Advanced Research in Sciences (CARS), Dhaka, Bangladesh against HELA cell line where 5-Fluorouracil was used as positive control. HELA, a human cervical carcinoma cell line was maintained in DMEM (Dulbecco's modified eagles' medium) containing 1 % penicillin-streptomycin (1:1), 0.2 % gentamycin, and 10 % fetal bovine serum (FBS). HELA cells were seeded onto 96-well plate with 2×10^4 cells per well (100 µl) and incubated at 37 ^oC under a humidified atmosphere of 5 % of CO_2 for 24 h. Next day, the serum free medium containing 25 µl of autoclaved crude extract and fractions were added to cell medium in test wells. In addition, the cell medium in control wells was changed to serum free medium containing an equivalent volume of dimethyl sulfoxide (DMSO). Cytotoxic activity was examined after 48 h of incubation using cell counting kit-8 (CCK-8), a nonradioactive colorimetric cell proliferation and cytotoxic assay kit (Sigma Aldrich, USA). Duplicated wells were used for each sample.

RESULTS AND DISCUSSION

In the current analysis, antioxidant or free radicals scavenging activity of *A. capillus-veneris* L. was determined by using free DPPH radicals for methanolic extract of aerial parts of *A. capillus-veneris* L. and its different soluble fractions (n-hexane, chloroform, and ethyl acetate). Results obtained from the analysis revealed that different fractions including the methanolic extract exhibited significant inhibition of free DPPH radicals. The free radicals scavenging assay demonstrated that ethyl acetate fraction performed the highest percent of inhibition than ascorbic acid (positive control) (Table 1). In addition, the IC_{50} value of ethyl acetate fraction (IC_{50} = 1.05 µg/ml) was greater than ascorbic acid (IC_{50} = 1.34 µg/ml). The n-hexane and chloroform soluble fractions as well as the methanolic extract demonstrated potential free radicals scavenging activity.

In general, plants having phenolics and flavonoids are capable of scavenging free oxidative radicals because these molecules are able to donate hydrogen atom.[20] Herlina *et al*.[21] stated that semipolar proton donating phenolics and flavonoids are more extracted in semipolar ethyl acetate solvent. Several *Adiantum* L. species including *A. capillus-veneris* L., *A. lunulatum* Burm.f., *A. philippenase* L., *A. flabellulatum* L., *A. pedatum* L., and *A. tenarum* L. contain significant amount of bioactive phytoconstituents like phenolics, flavonoids, triterpenoids, and sterols, etc.[10] Beside this, Naema *et al*. quantified total phenolics and flavonoids in *A. capillus-veneris* L. which evidenced the presence of antioxidant activity in methanolic extract of *A. capillus-veneris* L.[22] These bioactive molecules have drawn more attention because of their relation to prevent cancer, skin rashes, inflammation, and coronary heart disorders.[23]

In addition to free radicals scavenging analysis, cytotoxic activity of methanolic extract of *A. capillus-veneris* L. and its different soluble fractions were performed against HELA cell line. The results (Table 2) demonstrated that, *A. capillus-veneris* L. extract and its different fractions exhibited potential

cytotoxicity against HELA cells. The methanol extract and its n-hexane, chloroform, and ethyl acetate soluble fractions exerted IC_{50} value of 52.53, 17.15, 32.35, and 5.68 µg/ml, respectively as compared to 0.87 µg/ml showed by 5-Fluorouracil (positive control). A recent study conducted by Reshi *et al.* found that crude aqueous extract of *A. capillus-veneris* L. had IC_{50} value of 36.96 µg/ml against HELA cell line.[24] Here, IC_{50} (µg/ml) value indicates the concentration of sample with capability to inhibit 50 % proliferation of cancerous cells.

Table 1. Antioxidant activity of methanolic extract of aerial parts of *A. capillus-veneris* L. and its different soluble fractions.

Sample	Percent of inhibition at different concentrations (µg/ml)						IC_{50} (µg/ml)
	5	20	50	100	250	500	
Ascorbic acid (Positive control)	51.3±1.2	63.8±0.7	77.5±2.3	85.8±1.2	92.1±1.2	92.7±1.3	1.34
Methanolic extract	9.2±0.7	17.4±1.1	39.6±1.9	63.8±2.9	73.2±0.4	74.4±0.9	4.37
n-Hexane fraction	5.5±0.8	23.7±1.6	33.1±1.5	57.3±0.8	64.1±1.1	68.4±1.1	4.69
Chloroform fraction	26.2±1.2	48.4±1.9	64.4±0.7	78.1±0.5	84.3±1.2	86.3±1.2	3.08
Ethyl acetate fraction	54.1±1.9	65.4±2.1	78.4±2.1	87.7±1.2	92.7±1.8	93.1±0.7	1.05

Table 2. Cytotoxic activity of methanolic extract of aerial parts of *A. capillus-veneris* L. and its different soluble fractions.

Sample	Concentration (µg/ml)	Percent of inhibition against HELA cell line	IC_{50} value (µg/ml)
5-Fluorouracil	20	75.7	0.87
	50	91.5	
	100	95.7	
Methanolic extract	20	19.3	52.53
	50	52.2	
	100	67.3	
n-Hexane fraction	20	51.3	17.15
	50	77.4	
	100	85.1	
Chloroform fraction	20	33.7	32.35
	50	68.2	
	100	79.5	
Ethyl acetate fraction	20	68.7	5.68
	50	89.2	
	100	94.2	

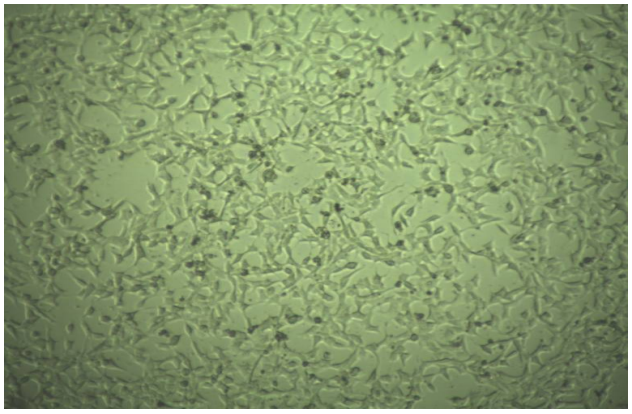

Figure 1. Cytotoxic activity of ethyl acetate soluble fraction of *A. capillus-veneris* L. under an inverted light microscope.

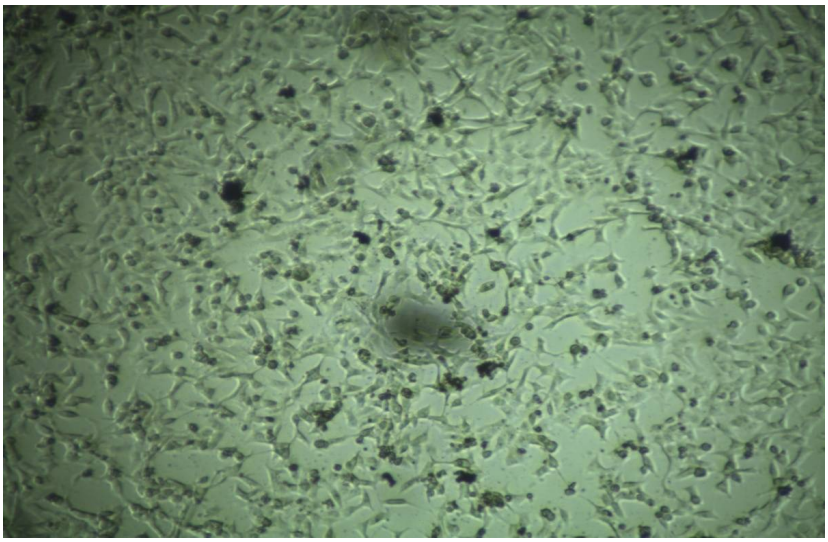

Figure 2. Cytotoxic activity of n-hexane soluble fraction of *A. capillus-veneris* L. under an inverted light microscope.

According to the American National Cancer Institute (ANCI), a promising anticancer product with significant cytotoxic effect should exerts an IC_{50} value less than 30 µg/ml.[25] The ethyl acetate and n-hexane soluble fractions with IC_{50} of 5.68 µg/ml (Figure 1) and 17.15 µg/ml (Figure 2) can be considered as promising cytotoxic products. In agreement with Salae *et al*.[26], bioactive phytoconstituents like triterpinoids and flavonoids are responsible for cytotoxic effects of plant species. These phytoconstituents are highly extracted in ethyl acetate and n-hexane solvent.[26] As a results, ethyl acetate and n-hexane soluble fractions of methanolic extract of *A. capillus-veneris* L. exhibited potential cytotoxicity against HELA cell lines.

CONCLUSION

The present study demonstrated that the aerial parts of *A. capillus-veneris* L. growing in Bangladesh had significant antioxidant and cytotoxic activities. The analyses were performed on methanolic extract of aerial parts of *A. capillus-veneris* L. along with its n-hexane, chloroform, and ethyl acetate soluble fractions. Solubility guided fractionation revealed that ethyl acetate soluble fraction had higher antioxidant (IC_{50} = 1.05 µg/ml) and cytotoxic (IC_{50} = 5.68 µg/ml) activities than the other fractions. The relatively better bioactivity of *A. capillus-veneris* L. growing in Bangladesh as compared to other countries like India and Libya suggested that the quality of soil and environmental conditions of Bangladesh might be facilitated the biosynthesis of active compounds in *A. capillus-veneris* L. The study will be great scientific evidences for ethnomedicinal uses of *A. capillus-veneris* L. by the traditional practitioners in Bangladesh. Additional researches should be conducted to isolate and characterize the bioactive compounds and to elucidate the molecular mechanisms of the pleiotropic activities of *A. capillus-veneris* L.

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