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Evaluation of phytochemicals, antioxidant activity and elemental content of *Adiantum capillus veneris* leaves

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ABSTRACT

The Adiantum capillus veneris was screened for phytochemicals and metal content. The antioxidant activity was also determined by different methods. It was observed that it contains 8.3 % moisture, 11.44 % ethanol extractable matter and 24.00 % water extractable matter. The soxhlet extraction of Adiantum capillus veneris showed the presence of phenolics and terpenoids (2.73 %), fats and waxes (0.20 %), alkaloids (0.53 %), quaternary and N-oxides (26.33 %) and fiber (67.23 %). Ten elements Mg, Ca, K, Mn, Fe, Co, Na, Ni, Cu, and Zn were determined from plant sample using inductively coupled plasma atomic emission spectroscopy (ICP-AES) technique among which Ca and K were found to be at major level. Adiantum capillus veneris leaves contain 224.76 mg FA/gm dw of phenolics and 49.62 mg equi Rut/gm dw of flavonoids in aqueous extract. The ethanol extract showed good antioxidant activity as compared to standard i.e. ascorbic acid, it exhibits low IC_{50} value 0.3986 mg/gm for DPPH assay and 0.695 mg/gm for ABTS assay. Results obtained indicated that Adiantum capillus veneris leaves are endowed with free radical scavenging molecules and it can be used as a potential source of natural antioxidants and nutrients.

Key words: *Adiantum capillus veneris*, phytochemicals, antioxidant activity, Elemental content, ICP-AES.

Abbreviations: *FA: Ferulic acid, Rut: Rutin, DPPH: 1, 1-diphenyl, 2-picrylhydrazyl, ABTS: 2, 2- azino-bis-3-ethylbenzothiazoline-6-sulfonic acid, IC_{50}: effective concentration at which the antioxidant activity was 50%, TPTZ: Tripydyltriazin*

INTRODUCTION

Phytochemicals are naturally occurring, biologically active chemical compounds in plants. They act as a natural defense system for host plants and provide colour, aroma and flavour. These are non-nutritive plant chemicals that have protective or disease preventive properties [1]. Many antioxidant compounds naturally occurring in plant source have been identified as free radicals or active oxygen scavengers [2]. Plants are endowed with phytochemicals like carotenoids, flavonoids and polyphenols, they posses antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. The human body requires both metallic and nonmetallic elements within certain permissible limits for growth and good health [3]. Therefore, determination of element compositions in foods and related products is essential for understanding their nutritive importance [4, 5]. Several attempts have been made to determine the element contents of herbs, medicinal or aromatic plants, and tea leaves from many parts of the world by using different instruments.

Reactive oxygen species (ROS) are generated by many redox processes that normally occur in the metabolism of aerobic cells. These species are highly reactive and harmful to the cells. If not eliminated, ROS can damage important molecules such as proteins, DNA and lipids. Cells express several defense mechanisms, including antioxidant enzymes and non-enzymatic compounds that help to prevent the damaging effects of ROS. However, these endogenous systems are often insufficient for complete scavenging of ROS. Their excess has been implicated in the development of chronic diseases, such as cancer, arteriosclerosis and rheumatism. A study shows that antioxidant substance with free radicals plays important role in prevention of free radical- induced diseases.

Adiantum capillus veneris (Family: Adiantaceae**)** is one on the most common species with potential importance for medicinal and nutritive purpose. *Adiantum* species were used for chest complaints, cough, expectorant, increase lactation, colds, aid kidney function, antiparasitic and dandruff. The fresh or dried leafy fronds are antidandruff, antitussive, astringent, demulcent, depurative, emetic, weakly emmenagogue, emollient, weakly expectorant, febrifuge, galactogogue, laxative, pectoral, refrigerant, stimulant, sudorific and tonic [6]. The dried fronds of the plants are used to make a tea [7, 8] and also used as a garnish on sweet dishes (Maybe R). Syrup is made from the plant - it makes a refreshing summer drink.

The objective of the research was to investigate the phytochemicals present in the sample qualitatively and quantitatively, determination of mineral content and to asses the antioxidant activity of *Adiantum capillus veneris* leaves in different solvents.

EXPERIMENTAL SECTION

2.1 Apparatus:

Absorption spectra's were recorded on UV-visible spectrophotometer (UV-1800, Shimadzu). The Karl Fischer Titration assembly (Model: VEEGO / MATIC-I, C-3828) with VEEGO/MATIC-I main unit solenoid valve assembly and One pair of platinum sensors was used for determination of moisture content. An ICP-AES instrument was used for determination of mineral content from the sample. Instrumental conditions used in the ICP -AES equipment are given in table 1.

Table 1: Instrumental conditions used in the ICP AES equipment

Nebulizer gas flow	0.8 L min−1
Auxiliary gas flow	1.0 L min−1
Coolant flow	12.0 L min−1
ICP RF power	1.6 KW
Sample uptake rate	1.5 mL min−1
Plasma power	1400 W
Reading time	Auto

2.2 Chemicals:

All chemicals used for experiments were of analytical grade. The DPPH [1, 1-diphenyl, 2-picrylhydrazyl] and ABTS [2,2- azino-bis-3-ethylbenzothiazoline-6-sulfonic acid di-ammonium salt] were procured from Sigma chemicals (Bangalore, India), while methanol AR grade, ethanol, L-Ascorbic acid, F.C reagent (Folin-Ciocalteau reagent), Ferulic acid (FA), trichloroacetic acid (TCA), TPTZ (tripydyltriazine), potassium ferricyanide ($K_3Fe(CN)_6$), ferric chloride ($FeCl_3$), sodium nitrite, aluminum chloride, sodium hydroxide and sodium acetate were procured from Sisco Research Laboratories, Mumbai, India. The K. F. grade ethanol, Karl- Fisher reagent, chloroform and ethyl acetate were purchased from s d fine chemicals, India.

2.3 Sample preparation and extraction:

The leaves used for investigation were purchased from local retailer. Sample was identified by botanist and voucher specimen (WP 81) has been deposited at the museum of the Agharkar Research Institute, Pune. The leaves were shade dried at room temperature (23 ^{0}C). The dried leaves were crushed and ground to fine powder in mortar and pastel. The powder was sieved through a mesh and was stored in an air-tight container for further use. For extract preparation 1 g of the sample was mixed in different solvents like water, methanol and ethanol by stirring it for 3 hrs. It was then filtered and centrifuged (R-8C DX lab. centrifuge) at 2500 rpm for 15 min. The extract was filtered and dried over a hot water-bath to get dry powder. The extract was then re-suspended in solvent to make a solution

with different concentration (0.3 – 2.00 mg/mL). The extract prepared was stored in refrigerator at 4 ^{0}C and further used for different assays to measure the antioxidant activity.

2.4 Phytochemical screening:

Phytochemical screening of *Adiantum capillus veneris* leaves were performed according to the standard procedure suggested by Ayoola et al. [9]. The small amount of extracted sample was screened qualitatively for presence of terpenoids, flavonoids, saponins, tannins, anthraquinones, reducing sugar and cardiac glycoside. Further, the sample was extracted with soxhlet assembly for quantitative determination of alkaloids, phenolics, terpenoids, fibers, fats and waxes. The scheme for phytochemical screening by soxhlet extraction is shown in figure 1. Moisture content from the sample was then determined by using Karl-Fischer titrimetric method. Percentage of water and ethanol extractable matter was also determined.

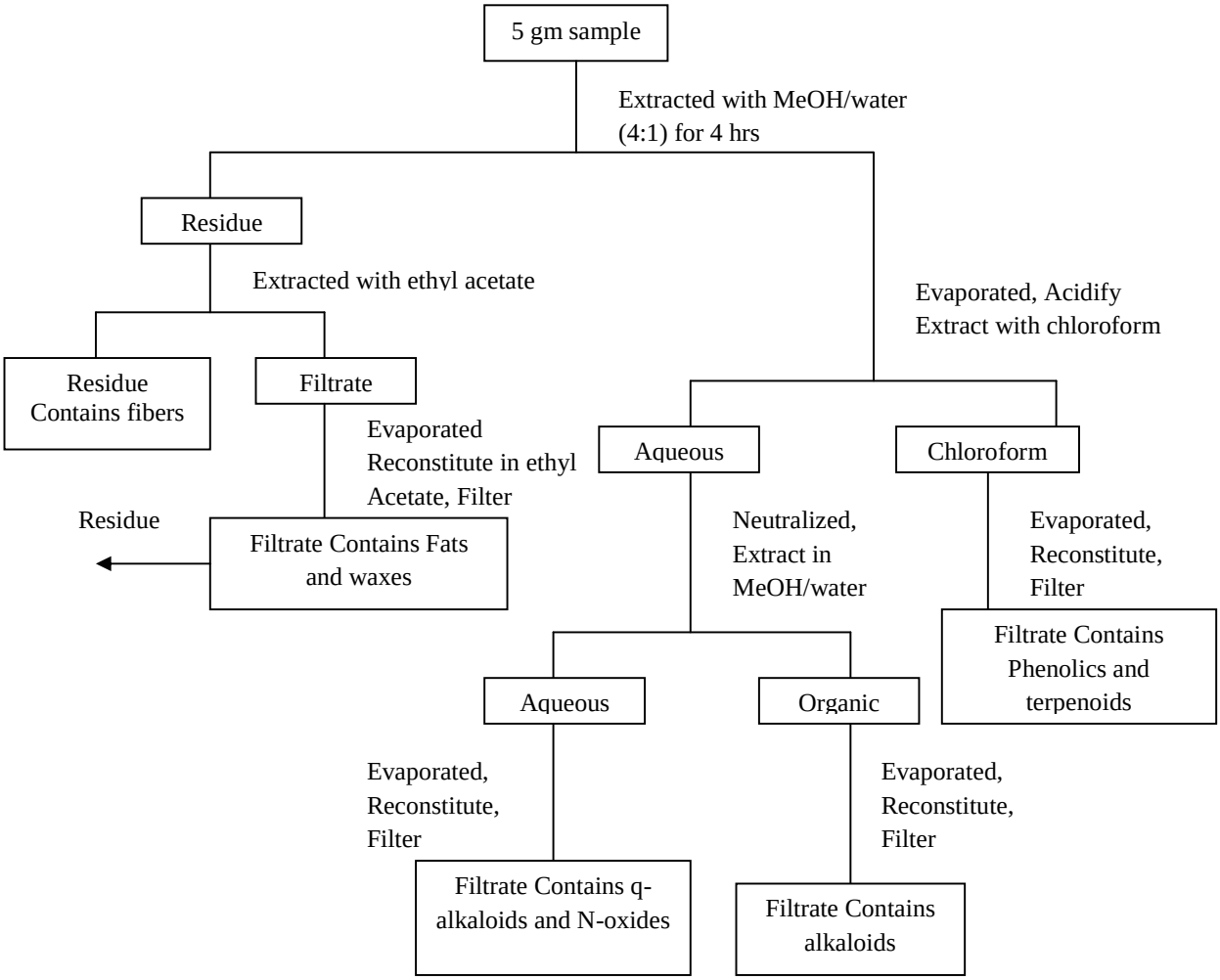

Figure 1: Schematic representation of soxhlet extraction for quantitative estimation of phytochemicals from *Adiantum capillus veneris* leaves

2.5 Total Phenolic:

Total phenolic content was measured by Folin-Ciocalteau method [10] with some modification. 1mL of extract was added to 1 ml distilled water and 0.2 ml of 1 N Folin-Ciocalteau reagent, incubated for 1 min at 23 ^{0}C and then 5 % Na_2CO_3 was added. Absorbance was measured at 765 nm on UV- visible spectrophotometer (PC-1600, Shimadzu Co,) after incubation of 2 hrs at room temperature. The phenolic content was expressed as milligram equivalent of ferulic acid per gram dry weight of sample.

2.6 Total flavonoids:
The determination of flavonoids was performed according to the colorimetric assay [11]. Distilled water (4 ml) was added to 1 ml of sample extract. Then, 5% sodium nitrite solution (0.3 ml) was added followed by 10% aluminum chloride solution (0.3 ml). Test tubes were incubated at ambient temperature for 5 min, and then 2 ml of 1 M sodium hydroxide were added to the mixture. Immediately, the volume of reaction mixture was made to 10 ml with distilled water. The absorbance of the pink colour developed was determined at 510 nm. A calibration curve was prepared with rutin and the results were expressed as mg rutin equivalents per gram dry weight of sample.

2.7 Antioxidant assay:
2.7.1 ABTS radical cation scavenging assay:
The ability of the test sample to scavenge $ABTS^{\cdot+}$ radical cation was compared to acetic acid standard. The assay was performed according to an improved method as described by Re et al, [12] with some minor modification. 3 ml of ABTS radical cation solution was mixed with 20 µl of the test sample (0.20–1.00 mg/ml) and the absorbance was measured at 734 nm after 1 min. The percentage inhibition of absorbance was calculated and plotted as a function of the concentration of standard. The inhibition percentage of ABTS was calculated using following equation,

ABTS cation radical scavenging activity (%) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$

Where, A_{blank} is the absorbance without extracts at 734 nm, and A_{sample} is the absorbance of the test solution.

2.7.2 FRAP assay:
The antioxidant activity of extracts was determined using assay of ferric reducing/antioxidant power (FRAP). For that purpose, freshly prepared FRAP reagent (2.5 ml of a 10 mM tripydyltriazine (TPTZ) solution in 40 mM HCl add 2.5 ml of 20 mM $FeCl_3.6H_2O$ and 25 ml of 0.3 M acetate buffer at pH 3.6.) was heated at 37 ^{0}C and mixed with 40 µL of sample extract and the reaction mixture was incubated at 37 ^{0}C for 15 min. Absorbance was measured at 593 nm on UV-Visible spectrophotometer detailed procedure explain elsewhere [13]. Aqueous solutions of known Fe (II) concentrations in the range of 100–2000 µM ($FeSO_4.7H_2O$) were used for calibration.

2.7.3 Scavenging effect of 1,1Diphenyl, 2-picrylhydrazyl (DPPH) radical:
The method given by Rajurkar and Gaikwad (2010) [13] was used to test for DPPH radical scavenging activity. 100 µL extracts of the sample at different concentration were mixed with 2 ml of freshly prepared DPPH solutions. The mixture was shaken and kept for 30 min in dark at room temperature. The absorbance was measured at 517 nm against blank on UV-Visible spectrophotometer. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and calculated using the similar equation as that of ABTS scavenging assay. The calibration curve was prepared by using ascorbic acid as standard. The DPPH radical scavenging capacity was estimated from the difference in absorbance for the sample and blank and expressed as percentage of DPPH scavenging.

2.7.4 Reducing power assay:
The reducing power of extract was quantified by the method described by Rajurkar and Gaikwad (2010) [13]. 0.1 ml of reaction mixture with the conc. 0.3- 2.0 mg/ml in all extracts in phosphate buffer was incubated with potassium ferricyanide (2 ml, 1 % solution) for 20 min. The reaction was terminated by adding trichloroacetic acid solution (10 mg/100 ml) in reaction mixture. The mixture was centrifuged at 2500 rpm for 15 min. then 1.5 ml of above supernatant solution was mixed with 2 ml distilled water and 0.1 % ferric chloride solution and absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

2.8 Elemental content:
For the determination of mineral content, 1 g of each sample was accurately weighed and digested with 15 ml of a 5:1 mixture of nitric acid and perchloric acid. Then 10 ml water was added and the solution was filtered through Whatman No. 41 filter paper to remove any turbidity or suspended matter; 2–3 drops of HCl were added and the solution was made up to 50 ml. All the solutions were stored in tightly capped polythene bottles and further analyzed by ICP-AES instrument [5].

RESULTS AND DISCUSSION

3.1 Phytochemical screening:

The ethanol extract of leaves of *Adiantum capillus veneris* showed the presence of flavonoids, terpenoids, saponins, tannins and reducing sugar. Whereas, the anthraquinones and cardiac glycosides were absent in ethanol extract of sample. A variety of herbs and herbal extracts contain different phytochemicals with biological activity that can be of valuable therapeutic index. Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds [14]. Different phytochemicals have been found to possess a wide range of activities, which may help in protection against chronic diseases. For example, Phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids and alkaloids have anti-inflammatory effects [15, 16, 17]. Rupasinghe et al., [18] have reported that saponins possess hypocholesterolemic and antidiabetic properties. The terpenoids have also been shown to decrease blood sugar level in animal studies [19]. Steroids and triterpenoids showed the analgesic properties [20]. The steroids and saponins are responsible for central nervous system activities.

The results for soxhlet extraction of *Adiantum capillus veneris* leaves showed in Figure 2. It was also observed that, it contain 8.3 % moisture, 11.44 % ethanol extractable matter and 24.00 % water extractable matter.

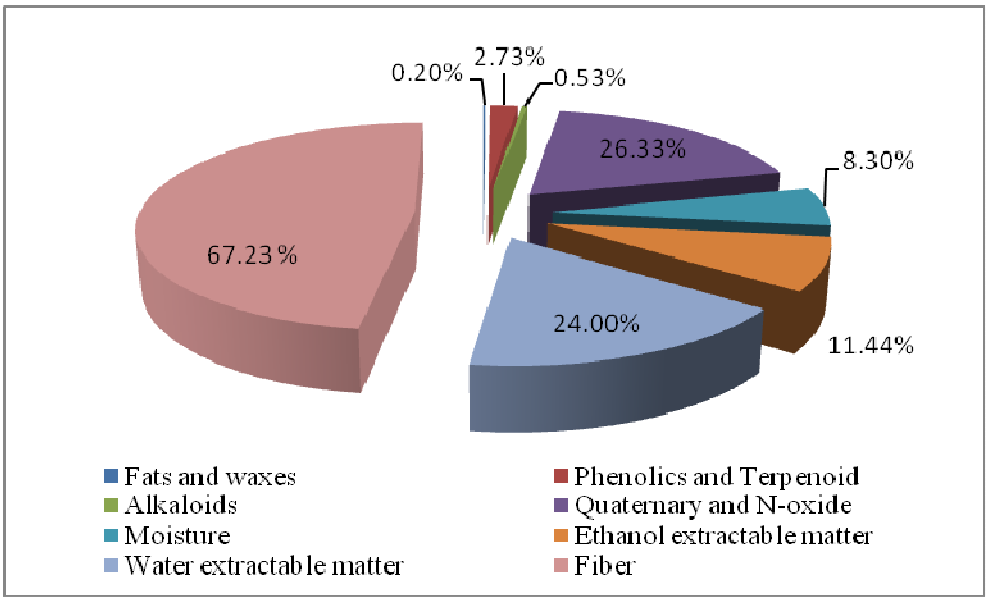

Figure 2: Quantitative estimation of *Adiantum capillus veneris* leaves extract. All results were expressed as mean ± standard deviation (N=2)

3.2 Total phenolics:

Phenolic compounds are ubiquitous bioactive compounds and a diverse group of secondary metabolites universally present in higher plants [15]. The phenolic compounds may contribute directly to antioxidative action. Folin–Ciocalteu reagent was used to determine total polyphenol in sample extracts. This reagent oxidises phenolates, resulting in the production of complex molybdenum-tungsten blue which can be detected spectrophotometrically at 765 nm [10]. F.C. reagent is employed routinely in studying phenolic antioxidants [21]. For the *Adiantum capillus veneris* leaves extract, the phenolic content was found to be in the order of the aqueous > methanol > ethanol. The aqueous extract showed highest phenolic content i.e. 224.76 mg FA/gm dw and that of ethanol was least 36.53 mg FA/gm dw. The results were depicted in Table 2. The water is good solvent for *Adiantum capillus veneris* as large amount of phenolics compounds are soluble in water. The Phenolic compounds are known as powerful chain breaking antioxidants [22].

Table 2: Total phenolics and flavonoids for *Adiantum capillus veneris* [a]

Solvent	Total phenolics [b]	Total flavonoids [c]
Aqueous	224.76 ± 9.75	49.62 ± 0.875
Methanol	156.34 ± 9.70	78.18 ± 1.741
Ethanol	36.53 ± 3.65	50.15 ± 4.79

[a] = All results were expressed as mean ± standard deviation (N=2)
[b] = Total phenolics were express as mg equi FA/gm dry weight of sample
[c] = Total flavonoids were express as mg equi Rut/gm dry weight of samp

3.3 Total flavonoids:

Flavonoids are natural phenolic compounds and well known antioxidants. Total flavonoids can be determined in the sample extracts by reaction with sodium nitrite, followed by the development of coloured flavonoid-aluminum complex formation using aluminum chloride which can be monitored spectrophotometrically at 510 nm. The results showed that *Adiantum capillus veneris* leaves extract contain 49.62, 78.18 and 50.15 mg equi Rut/gm dw of sample in aqueous, methanol and ethanol extract respectively. The results are shown in table 2. In various studies, antioxidant activity of the plant extracts was found to be fairly high which are rich in flavonoids [23]. Some flavonoids were reported to exhibit potential for anti–human immunodeficiency virus functions [24].

3.4 Antioxidant assay:

3.4.1 ABTS cation radical scavenging assay:

The ability of the test sample to scavenge $ABTS^{\cdot+}$ radical cations was compared with acetic acid standard. The ABTS cation radical scavenging activity (%) of the water, methanol and ethanol extracts of *Adiantum capillus veneris*, compared to Ascorbic acid and rutin are shown in figure 3. The antioxidant activity was expressed as an effective concentration at 50%. The effective concentration (IC_{50}) values of the extracts and standard are shown in Table 3.

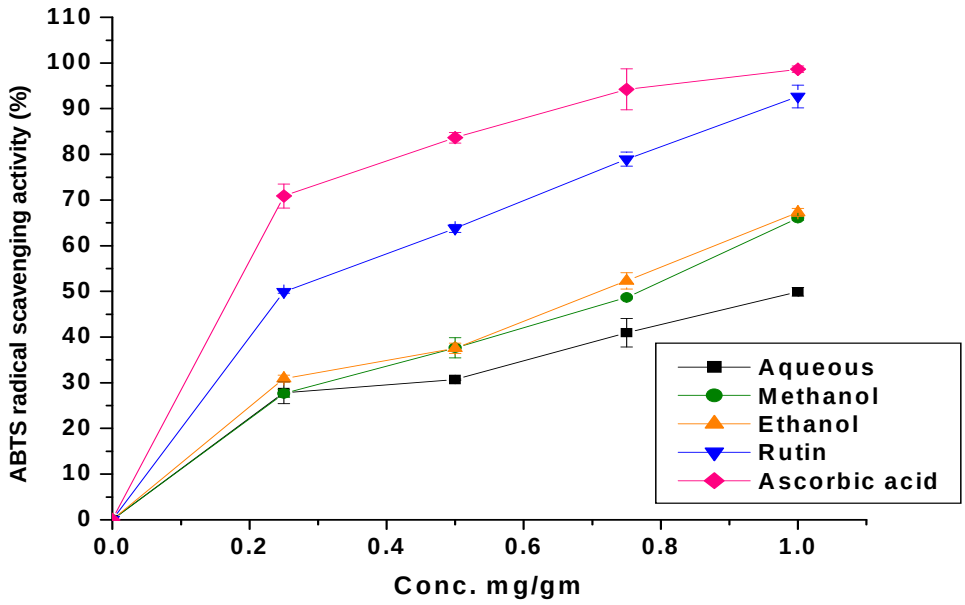

Figure 3: **ABTS cation radical scavenging activity of the extract from *Adiantum capillus veneris* in different solvents. Ascorbic acid and rutin used as control. All results were expressed as mean ± standard deviation (N=2). The vertical bars represent the standard deviation for each data**

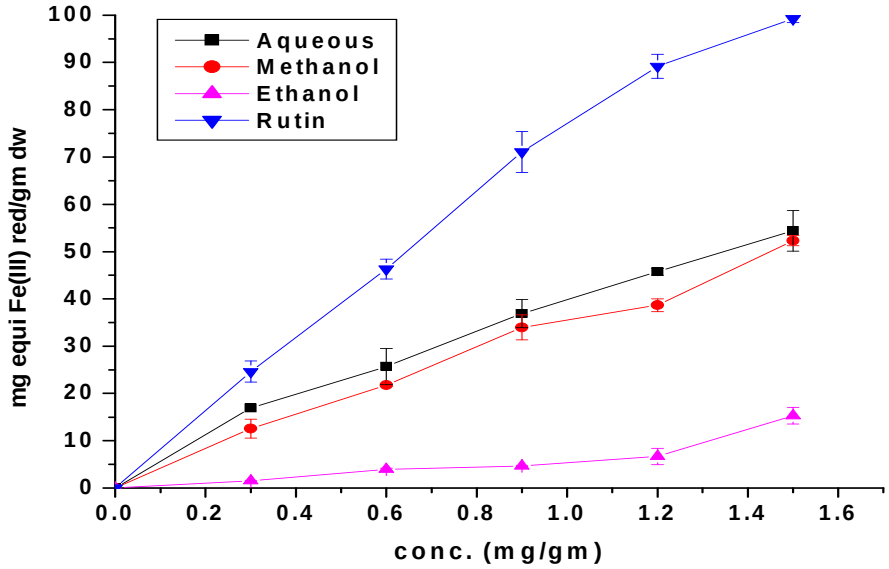

Figure 4: FRAP values of the extract from *Adiantum capillus veneris* in different solvents express in mg equi. of Fe (III) reduction per gram dry weight of sample. Rutin was used as control. All results were expressed as mean ± standard deviation (N=2). The vertical bars represent the standard deviation for each data.

Table 3: The IC_{50} values for *Adiantum capillus veneris* leaves in various extracts [a]

Solvent	IC_{50} value	
	DPPH assay	ABTS assay
Aqueous	1.448	0.973
Methanol	1.154	0.767
Ethanol	0.3986	0.695
Rutin	0.3253	0.25
Ascorbic acid	0.2969	0.174

[a] = IC_{50} values was expressed as mg/gm

From the IC_{50} values of the extracts, it is seen that the ethanol extract (0.695 mg/gm) had the highest ABTS cation radical scavenging activity as shown by the lowest value of IC_{50}. However, when compared with standard i.e. ascorbic acid (0.174 mg/gm) and control i.e. rutin (0.250 mg/gm), the extracts shows slightly less radical scavenging activity. For each sample, five concentrations (mg/ml) were tested. The highest ABTS radical scavenging rate was found to be 67.28 % for ethanol extract while, the lowest was found for water extract (49.91 %). The antioxidant activity values decreased in the order of ethanol > methanol > water extracts.

3.4.2 FRAP assay:

FRAP assay is based on the ability of antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ, forming an intense blue Fe^{2+}- TPTZ complex with an absorption maximum at 593 nm. This reaction is pH-dependent (optimum pH 3.6). The absorbance decrease is proportional to the antioxidant content [25]. The FRAP values were expressed as mg equivalent of Fe (III) reduction per gm dry weight of sample. That of for all the extract at various concentrations (0.30 – 1.5 mg/gm) was shown in fig 4. The results showed that the FRAP value of the aqueous extract was higher (54.412 mg equi Fe (III) red / gm dw) than that of methanol (52.307 mg equi Fe (III) red / gm dw) and ethanol (15.292 mg equi Fe (III) red / gm dw) at concentration 1.5 mg/gm. The FRAP value of the extracts increased with increasing concentrations. All results obtain were compared with that of control (Rutin). Hence the aqueous extract was shown the highest antioxidant activity than other extracts.

3.4.3 DPPH radical scavenging assay:

The degree of discoloration of violet colour of DPPH radical, as it gets reduced, indicates the radical scavenging potential of the antioxidant [26]. The radical-scavenging properties of the various extracts of *Adiantum capillus veneris* are given in Fig. 5. All extracts exhibited concentration-dependent DPPH radical scavenging activity. The results obtained for samples were compared with that of standard (Ascorbic acid) and control (Rutin). The antioxidant activity was expressed as an effective concentration at 50% Ethanol extract, with IC_{50} value of 0.3986 mg/gm, showed particulary high free-radical scavenging activity, close to the activity of standard i.e. ascorbic acid (0.2969 mg/gm) and control i.e.rutin (0.3253 mg/gm). The aqueous extract showed approximately four times lower activity with IC_{50} of 1.448 mg/gm (Table 3). The results suggested that the ethanol extract exhibited a good antioxidant activity i.e. 66.61% with less IC_{50} value than other extract.

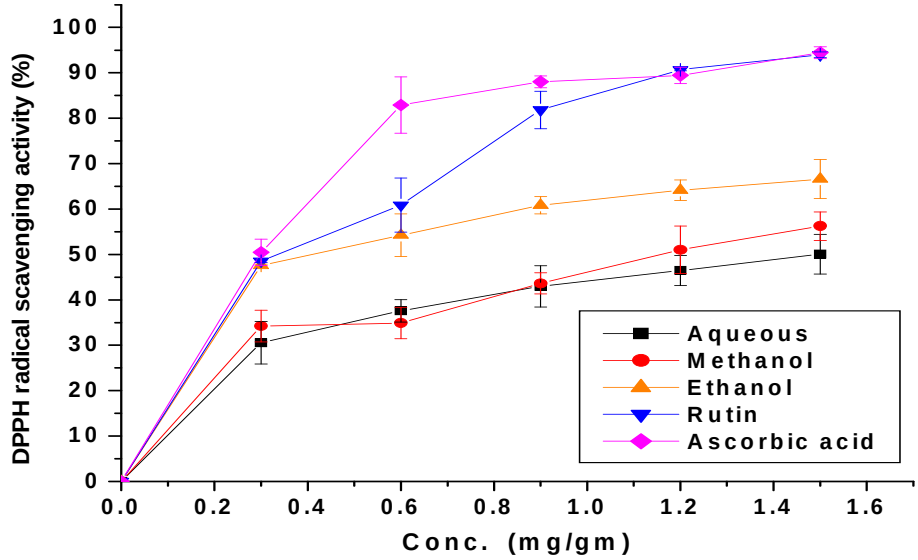

Figure 5: **DPPH radical scavenging activity of the extract from *Adiantum capillus veneris* in different solvents. Ascorbic acid and rutin used as control. All results were expressed as mean ± standard deviation (N=2). The vertical bars represent the standard deviation for each data**

3.4.4 Reducing power assay:

The presence of reductants (antioxidants) in the herbal extracts causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, the concentration of Fe^{2+} was monitored by measuring the formation of Perl's Prussian blue at 700 nm [27] and the results are presented in Figure 6. Ethanol extract in this assay also showed the highest activity than other extracts. The results showed that for *Adiantum capillus veneris* leaves extract the reducing power was in the order of the ethanol > methanol > aqueous with the values of 0.545, 0.33 and 0.322 (absorbance unit) respectively. The reducing power of the ethanol fraction was the highest among all the fractions and it increases linearly with increasing concentration. All extracts at all concentrations exhibited quite low activities than the control. The reducing properties are generally associated with presence of reductones which have been shown to exert antioxidant action by donating hydrogen atom and breaking the free radical chain reaction [28].

3.5 Mineral content:

More than 40 elements have been considered essential to life systems for the survival of both mammals and plants [29]. Trace metals contamination are important due to their potential toxicity to the environment and human health [30]. One important factor for the formation of active constituents in medicinal plants are the trace elements because they are known to play an important role in plant metabolism and active constituents of medicinal plants are metabolic products of plant cells. In the present work concentrations of various elements Mg, Ca, K, Mn, Fe, Co, Na, Ni, Cu, and Zn were determined by ICP-AES technique and the results are shown in Table 4. An examination of table 4 shows that Ca and K are present at major level (11.52 and 17.95 mg/gm, respectively). The abundance of Ca and Mg in the present study was also in agreement with the previous studies, which indicated that this element is the

most abundant element in many medicinal plants and tea leaves [31, 32, 33]. The biological effects of estimated elements (Zn, Fe, Cu, Mn, Ca, Mg, Ni, Co, K and Na) in living systems strongly depend on their concentration [34] and thus should be carefully controlled, especially when herbs and their products are used in human medicine as it is the case with *Adiantum capillus veneris*.

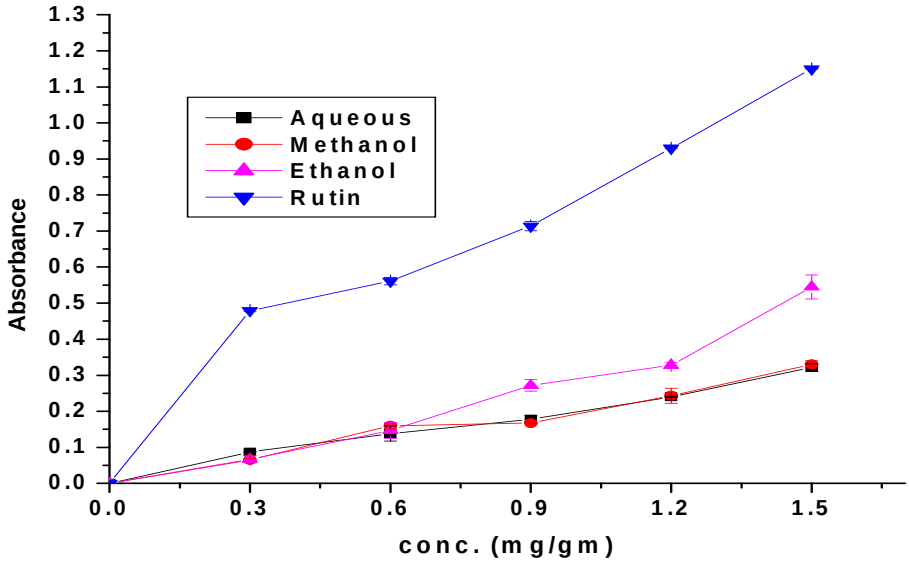

Figure 6: **Reducing power of the extract from *Adiantum capillus veneris* in different solvents. Rutin was used as control. All results were expressed as mean ± standard deviation (N=2). The vertical bars represent the standard deviation for each data.**

Table 4: Elemental concentrations from *Adiantum capillus veneris* leaves determined by ICP-AES instrument.

Elements analyzed	Concentrations
Cu	01.70 [b]
Zn	07.15 [b]
Mn	13.55 [b]
Fe	17.45 [b]
Ca	11.52 [a]
Co	N.D
K	17.95 [a]
Na	12.65 [b]
Ni	00.20 [b]
Mg	02.90 [a]

a= Concentration of element in mg/gm
b= Concentration of element in mg/100 gm
N.D. = Not detected

Concentration of various elements estimated in the present studies decreases in the order, K > Ca > Mg > Fe > Mn > Na > Zn > Cu > Ni. Trace element concentrations in plants vary widely with the soil type, pH, fertilizer and organic content, climate, species, etc. If these plants are used as raw materials for the manufacture of health products, variations of nutrient levels should be closely monitored. The data on trace, minor and major elements in plants is of great importance to understand the pharmacological actions of these medicinal plants.

CONCLUSION

Results indicate that, *Adiantum capillus veneris* leaves are rich in free radical scavenging molecules like terpenoids, flavonoids, saponins, tannins and reducing sugar. Study proves the *in vitro* antioxidant potential of *Adiantum*

capillus veneris leaves extract and found to be comparable with standard (Ascorbic acid) and control (Rutin). This study also provides the information on trace and heavy metal accumulation in plant under study. Some elements (Ca, K and Mg) were found to be significantly at high concentrations. Hence, *Adiantum capillus veneris* leaves are endowed with essential trace elements and free radical scavenging molecules. It can be used as a potential source of natural antioxidant and nutrient.

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