QUALITATIVE AND QUANTITATIVE PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT POTENTIAL OF AMARANTHUS VIRIDIS L. FROM PAKISTAN

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Abstract- Purpose of this study was to conduct qualitative and quantitative phytochemical analysis of Amaranthus viridis and to assess its antioxidant activity by using different assays. Ethnolic extract was prepared from A. viridis leaf powder and its qualitative phytochemical screening was done. Then quantitative phytochemical screening was carried out for the detected phytochemicals. Antioxidant activity analysis was carried out by using DPPH Radical Scavenging Activity assay, ABTS Radical Cation Assay, Phosphomolybdenum Assay and Hydrogen Peroxide Scavenging Activity assay. Qualitative Phytochemical screening resulted in the presence of alkaloids, saponins, flavonoids, tannins, total phenolics and absence of terpinoids and resins in A. viridis leaf extract. Quantitative phytochemical screening showed the amount of alkaloids was 93mg/g followed by flavonoids (8mg/g), saponins (4.4mg/g) and tannins (2.7mg/g). TP contents of A. viridis were 490mg/100g. All the four assays used for radical scavenging activity showed the presence of high oxidative potential of A. viridis. IC50 values were in this order; 12.60µg/ml (Phosphomolybdenum assay), 13.22 µg/ml (ABTS assay), 27.97 µg/ml (DPPH assay), 52.77 µg/ml (Hydrogen peroxide assay). Results showed the high antioxidant activity of A. viridis which is due to presence of high phenolic contents revealed by phytochemical analysis.

Index Terms- Phytochemical; Antioxidant; Amaranthus Viridis; Scavenging Activity; Radical

I. INTRODUCTION

Eighty percent of world population, especially developing countries use traditional medicines obtained from plant extracts. Antioxidant and antimicrobial activity of these plants extracts is the basic reason to use them in pharmaceuticals, natural therapy and alternative to medicines [1]. Demand for herbal products has been growing and import and export of plant materials is also increasing in recent years. The phytochemicals having unknown pharmacological activities are extensively evaluated for use in medicines [2]. Phytochemicals have two groups primary and secondary, primary metabolites are amino acid and suger etc, secondary metabolites are phenolics, alkaloids, tannin and anthraquinones etc [3].

Amaranthus viridis belongs to *Amaranthaceae* family and contains variety of phytochemicals such as flavonoids quercetin and rutin. Flavonoids have a biochemical effect which inhibits activity of enzymes, hormone regulation and have antimicrobial, antioxidant and anticancer activities. Medicinally it is used to treat boils and abscesses poultice of leaves applied on inflammations. Use for skin cleansing and acne problem [4]. *Amaranthus viridis* was reported as a potent source of protein. Amino acid composition of this plant meets with the protein standard of World Health Organization (WHO). It also has a number of minerals like magnesium, calcium, iron and zinc and significant amount of the two fatty acids (linoleic and linolenic) that are essential in humans.

A. viridis is widely distributed plant in Asia. In rural areas of Pakistan it is used as leafy vegetable but unfortunately there is very less information collected about the pharmacognostic, phytochemical and nutritional investigation of this plant. *A. viridis* was evaluated to check its nutritional value for war affected internal displaced persons (I.D.Ps) in the province of Khyber Pakhtunkhwa, Pakistan [5]. But still there is need to evaluate this plant with respect to its phytochemical analysis and antioxidant activity. This study aimed to conduct phytochemical evaluation of *A. viridis* and to assess its antioxidant potential.

II. MATERIALS ANS METHODS

A. Chemicals used

Dragendoff's reagent, Mayer's reagent, $FeCl_3$, dilute NaOH, chloroform, con. Sulphuric acid, 1% ferric chloride solution, 10% acetic acid, con. Ammonium hydroxide, dil. ammonium hydroxide, potassium ferro-cyanide, 20% ethanol, diethyl ether, n-butanol, Gallic acid, Folin ciocalteau reagent, sodium carbonate, DPPH, Ascorbic acid, Solution of ABTS, potassium persulfate, ammonium molybdate, sodium phosphate, phosphate buffer, hydrogen peroxide.

B. Plant material

The leaves of *Amaranthus viridis* were collected from Rawalpindi, Pakistan, in the month of August-September 2012. These were identified and confirmed from Herbarium of Pakistan, Quid-i-Azam University Islamabad then the voucher specimens were submitted at the Herbarium of Pakistan for the future references.

C. Extract formation

The leaves were cut into pieces, dried under shade then ground it into fine powder and stored in zipper bags for use in future. The ethanolic extracts was obtained by weighing 20g of powder soaked into 100ml of 60% ethanol and put at room temperature for 7 days. After that it was filtered by using filter paper, solvent was removed by using rotator evaporator. Then dried extract was weighed and saved in plastic vials and labeled.

D. Qualitative phytochemical screening

Qualitative phytochemical screening was performed by following the method of [6]. Plants filtrates were prepared by boiling 20g of the plant powder in distilled water by using mechanical shaker. Then the solutions were filtered by using filter paper No. 1, these filtrates were used for the phytochemical screening of Alkaloids, Tannins, Saponins and Flavonoid. Plant powder of *Amaranthus viridis* was also analyzed to find out the quantity of certain phytochemicals [7].

E. Determination of total Phenolics

Total phenolic contents were determined by previously reported method [8].

F. Antioxidant activity analysis

Antioxidant activity of plant material was analyzed by using following four assays;

1. DPPH Radical Scavenging Activity

The DPPH assay was done by previously documented protocol with some modifications [9]. The scavenging activity was calculated from percentage of DPPH radical scavenged by using following equation:

Scavenging activity (%) = [(control absorbance-sample absorbance) / (control absorbance)] ×100.

IC_{50} value was calculated by drawing correlation curve graph between scavenging activity and the concentrations of the samples. Ascorbic acid having concentrations in the range of 250-15.62µl/ml was taken as the positive control.

2. ABTS Radical Cation Assay

ABTS radical cation scavenging activity was also determined by a previously used method with some changes [10]. Following formula was used to calculate the % scavenging activity of sample and standard:

Scavenging activity (%) = [(control absorbance-sample absorbance) / (control absorbance)] ×100.

IC_{50} value was calculated by similar method used in 1st assay.

1. **3.** Phosphomolybdenum Assay

Antioxidant activity of plant extract and standard (ascorbic acid) in phosphomolybdenum assay was also assessed by standard method [11].

IC_{50} value was calculated by using similar method used in 1st and 2nd assay.

2. 4. Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity assay was used to investigate the antioxidant activity of plant extracts [12]. Antioxidant activity was calculated from following formula:

% scavenging of hydrogen peroxide = (1-absorbance of sample / absorbance of control) × 100.

IC_{50} value was calculated similarly as in 1st, 2nd and 3rd assay.

III. RESULTS

A. Phytochemical Screening

Plant powdered filtrate of *Amaranthus viridis* was analyzed qualitatively for seven phytochemicals, results revealed the presence of alkaloids, saponins, flavonoids, tannins and total phenolics in *A. viridis* and absence of terpinoids and resins. Results of qualitative phytochemical analysis of *A. viridis* are shown in Table 1. Then quantity of present phytochemicals was investigated. Results for alkaloids, saponins, flavonoids and tannins are displayed in Table 2. In *A. viridis* alkaloids was 93mg followed by flavonoids (8mg), saponins (4.4mg) and tannins (2.7mg). Result for total phenolic contents was calculated by drawing calibration curve of gallic acid and expressed in mg G.A/g of extract. TP contents of *A. viridis* were 490mg/100g (Fig 1).

Table 1: Qualitative Phytochemical Analysis of *Amaranthus viridis*

Phytochemicals	Presence/Absence
Alkaloids	+ve
Tannins	+ve
Saponins	+ve
Flavonoids	+ve
Total phenolics	+ve
Terpenoids	-ve
Resins	-ve

Key: +ve= Presence, -ve= Absence

Table 2: Quantitative Phytochemical Analysis of *Amaranthus viridis*

Phytochemicals	Quantitay(mg/g)
Alkaloids	93
Flavonoids	8
Saponins	4.4
Tannins	2.7

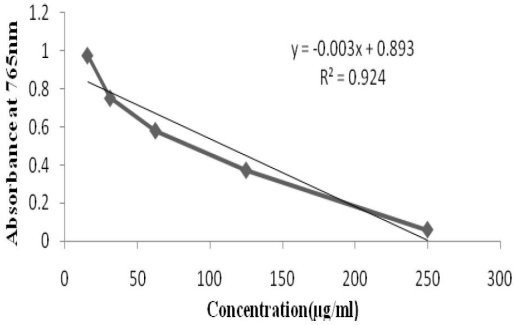

Fig1. Calibration curve of standard Gallic acid for determination of total phenolics contents.

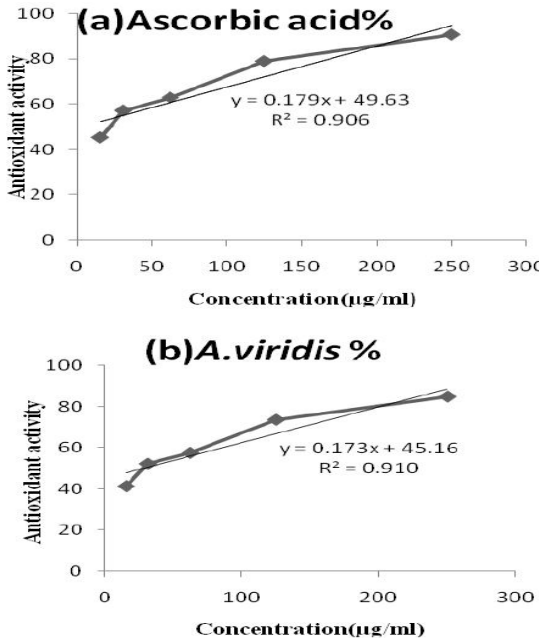

Fig2. Correlation curve of antioxidant activity% of (a) Ascorbic acid (b) *Amaranthus viridis* by DPPH assay.

B. Antioxidant analysis

Antioxidant activity of *Amaranthus viridis* was investigated by using following four methods.

1. DPPH-Assay

Ethanolic extract of *Amaranthus viridis* showed potent antioxidant activity.IC_{50} value of standard and plants is shown in Fig 2. IC_{50} of Ascorbic acid was 1.79 and of *Amaranthus viridis* was 27.97. Low IC_{50} value means scavenging activity of sample was high and vice versa.

% scavenging activity is shown in Table 3. It is clear from the results that scavenging activity was dose dependent; it increased as the concentration increased. Concentrations used were 250µg/ml, 125µg/ml, 62.5µg/ml, 31.25µg/ml, and 15.62µg/ml while the scavenging activity of Ascorbic acid was 91.81%, 79.05%, and 62.92%, 57.2% and 45.30% respectively, same case was with scavenging activity of *Amaranthus viridis* which was also increased with the concentration such as 84.90%, 73.71%, 57.59%, 52.28%, 41.37% was the scavenging activity of *Amaranthus viridis* respectively.

Table 3: Percentage Scavenging Activity of *Amaranthus viridis* and Ascorbic acid by DPPH Assay at Different Concentrations.

Conc. (µg/ml)	% scavenging activity	
	Ascorbic acid	*A. viridis*
250	90.81	84.90
125	79.05	73.71
62.5	62.92	57.59
31.25	57.2	52.28
15.62	45.3	41.37

2. ABTs Radical Cation Assay

Standard curve of ABTs Radical cation Assay showed that ethanolic extract of *Amaranthus viridis* have very good antioxidant activity. Ascorbic acid has 3.522 IC_{50} value while *Amaranthus viridis* has 13.218 (Fig 3). It was observed that lower IC_{50} value would reflect high scavenging activity.

Table 4: Percentage Scavenging Activity of *Amaranthus viridis* and Ascorbic acid by ABTs Assay at Different Concentrations.

Conc. (µg/ml)	% Scavenging Activity	
	Ascorbic acid	*A.viridis*
250	88.85	87.14
125	68.66	86.85
62.5	62.04	69.57
31.25	57.85	60.85
15.62	47.00	44.28

Table 4 showed the percentage scavenging activity of Ascorbic acid (88.85%, 68.66%, 62.04%, 57.85% and 47.00%), *Amaranthus viridis* (87.14%, 86.85%, 69.57%, 60.85% and 44.28%) at the concentration of 250µg/ml, 125µg/ml, 62.5µg/ml, 31.25µg/ml and 15.62µg/ml. It was clearly seen that scavenging activity is directly proportion to concentration of antioxidants, when the concentration was increased the scavenging activity of standard and plant automatically increased.

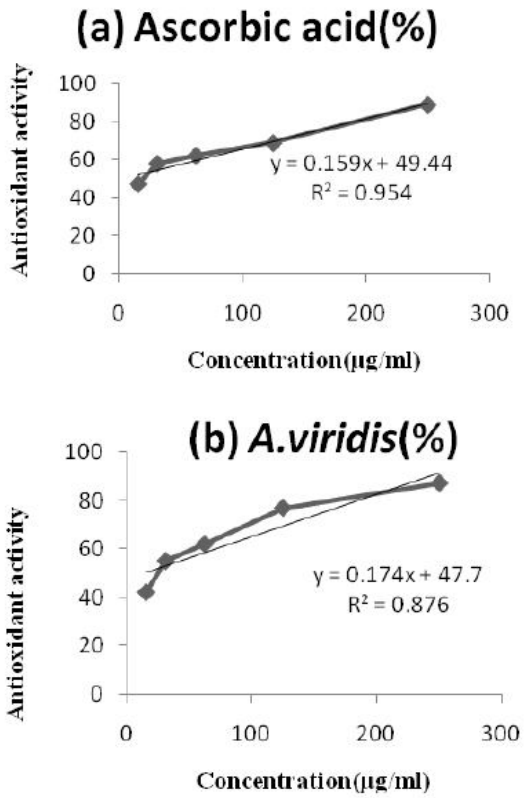

Fig3. Correlation curve of antioxidant activity% of (a) Ascorbic acid (b) *Amaranthus viridis* by ABTs assay.

3. Phosphomolybdenum Assay

Results of this assay also showed that ethanolic extract of *Amaranthus viridis* have significant antioxidant activity. IC_{50} value of *Amaranthus viridis* and Ascorbic acid was 12.60 and 3.026 respectively (Fig 4). Lower IC50 value reflects the high antioxidant activity, it means that *Amaranthus viridis* had greater scavenging activity.

Table 5: Percentage Scavenging Activity of *Amaranthus viridis* and Ascorbic acid by Phosphomolybdenum Assay at Different Concentrations.

Conc. (µg/ml)	% Scavenging Activity	
	Ascorbic acid	*A. viridis*
250	94.63	85.41
125	79.78	83.28
62.5	65.13	78.82
31.25	60.34	68.89
15.62	42.09	34.24

Results for percentage scavenging activity of Ascorbic acid and *Amaranthus viridis* by phosphomolybdenum assay are shown in Table 5. Different concentrations of plant extract and ascorbic acid were made in methanol such as 250µg/ml, 125µg/ml, 62.5µg/ml, 31.25µg/ml and15.62µg/ml. Scavenging activity of Ascorbic acid at these concentrations was 94.63%, 79.78%, 65.13%, 60.s34% and 42.09% and *A. viridis* 85.41%, 83.28%, 78.82%, 68.89% and 34.24% respectively. There was a marked change in scavenging activity of plant and standard by change in concentration. Maximum scavenging activity of the plant and Ascorbic acid was observed at highest concentration (250µg/ml).

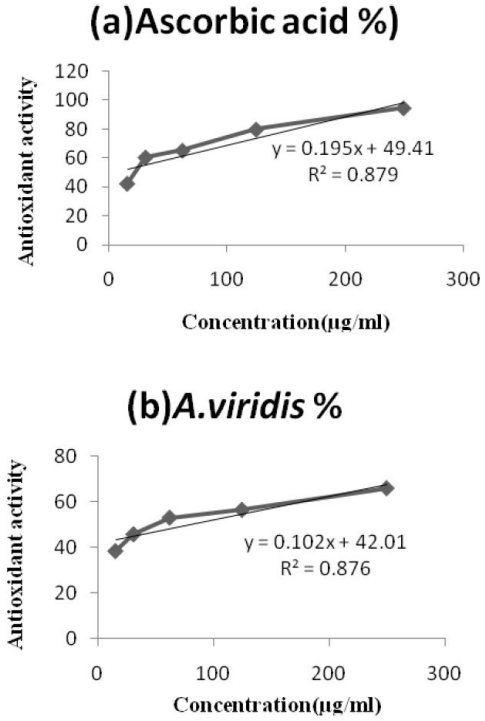

Fig4. Correlation curve of antioxidant activity% of (a) Ascorbic acid (b) *Amaranthus viridis* by Phosphomolybdenum assay

4. Hydrogen Peroxide Scavenging Activity

IC_{50} value of *Amaranthus viridis* was 52.77and Ascorbic acid had 47.89 (Fig 5).

Table 6: Percentage Scavenging Activity of *Amaranthus viridis* and Ascorbic acid by Hydrogen peroxide Assay at Different Concentrations.

Conc. (µg/ml)	(%)Scavenging Activity	
	Ascorbic acid	*A. viridis*
250	72.9	65.80
125	62.98	60.09
62.15	52.99	53.12
31.25	47.80	47.01
15.62	41.69	43.99

Results for percentage scavenging activity of the plant ethanolic extract and ascorbic acid are displayed in Table 6. It was observed that scavenging activity is directly related with concentration. Following concentrations of plants extract and ascorbic acid were

used: 250µg/ml, 125µg/ml, 62.15µg/ml, 31.25µg/ml and 15.62 µg/ml. Scavenging activity of *A.viridis* was 65.80%, 60.09%, 53.12%, 47.76 and 43.99 followed by ascorbic acid having 71.9%, 62.98%, 52.9%, 47.80% and 41.69% respectively.

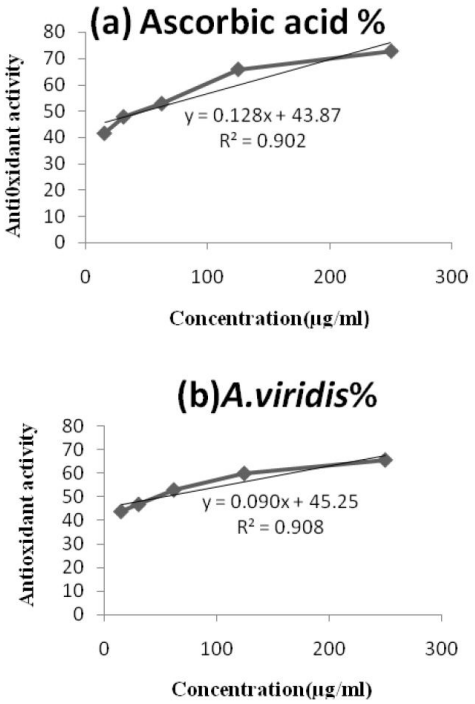

Fig5. Correlation curve of antioxidant activity% of (a) Ascorbic acid (b) *Amaranthus viridis* by Hydrogen peroxide scavenging assay

IV. DISCUSSION

Discovery of new medicinal drugs become possible by the screening of plants for their active compounds which have significant treatment and protection roles against various diseases [13]. Phytochemical screening of *Amaranthus viridis* showed positive results for alkaloids, saponins, flavonoid, tannins and total phenolics, while it showed negative results for terpenoids and resins. These results for *A. viridis* are almost same as in some previous studies [14] and [15]. Flavonoids are very important group of phenolics compound with antioxidant activity which help to minimize the mortality rates in people those are eating a lot of plant-based foods. Zutphen Elderly Study showed that there is inverse association between flavonoid intake and heart diseases and cancer [16]. Phenolics compounds and flavonoids are abundantly found in plants and have biological effect such as antiinflamatory, antioxidant, anticarcinogenic etc [17]. Saponins are natural glycosides which have a variety of pharmacological affects such antifungal, antiparasitic, anti-inflammatory, expectorant, vasoprotective, hypocholesterolemic, immunomodulatory, hypoglycemic, molluscicidal and many others [18]. Alkaloids are helpful to make powerful pain killer for human medication [19]. Tannins are used for the cancer prevention due to its anti-cancer activity [20]. It is group of polymeric phenolics so used to kill microorganism [21]. So, due to the presence of these phytochemicals in *Amaranthus viridis* this plant can be recommended to use in medicines such as pain killers, antibiotics, anticeptic, anti-inflamatory agent and diaphoretic.

All over the world medicinal properties of plants have been evaluated due to the presence of antioxidants. It is reported that antioxidants are compounds that reduce the oxidative damage caused by free radicals present in human body .This oxidative damage is responsible for causing many serious diseases in human such as diabetes mellitus, cancer, arthritis, atherosclerosis and neurodegenerative diseases [22]. Natural source of antioxidants are plants, fruits, leaves, stem and bark of plants contain flavonoids, flavanole and phenolics compounds which act as an antioxidants and have preventive role against cancer and heart diseases [23]. *Amaranthus viridis* is widely used species as wild vegetables and also used as herbal remedies in rural areas due to its therapeutic values. Antioxidant analysis of this plant was done to investigate its antiradical activity by using four assays, each assay gave different results because every assay has its own chemistry. Overall results showed that this plant has high free radical scavenging activity, it might be due to the presence of large quantity of total phenolics compounds that were also determined in this study. There is positive relationship between phenolics compounds and antioxidant activity of plant species, because of having good redox properties and hydrogen donating ability [24].

DPPH-assay is a stable free radical, sensitive and rapid method which is used to analyze the anti oxidant activity of plant extracts. It was clear from results of this study that scavenging effect on DPPH radical is directly proportional to concentration of sample (250µg/ml to 15.62µg/ml). IC_{50} value of *Amaranthus viridis* is 27.97µg/ml which is same to the previous work [25] and [26] and if the IC_{50} value was low it mean scavenging activity is high. So *Amaranthus viridis* has high antioxidant activity in this assay.
Another assay used for determine antioxidant activity of plant extract is ABTs cation radical assay. Antioxidant activity of single compounds and complex mixtures was investigated by using ABTS radical cation which is a common organic radical. Hydrogen atoms or electron donating capacity of antioxidant species to inactivate this radical cation was assessed by decolonization of ABTS+ [27]. So it means that if antioxidant species high in concentration, it can donate more electron to ABTS and reduced and decolorized it rapidly. *Amaranthus*

viridis showed significant scavenging effect in this assay.

Phosphomolybdenum assay was also used for spectrophotometeric quantification of total antioxidant capacity. Molybdenum Mo (VI) is reduced into green colored compound phosphate Mo (V) due to the presence of antioxidant. As shown in results antioxidant activity of samples was increased by the increase in concentration of samples. In Phosphomolybdenum assay plants extract showed highest antioxidant activity which is comparable to that of ascorbic acid.

Generally, hydrogen peroxide is not much reactive, but inside a cell, it may produce hydroxyl radical [28]. So, the elimination of H_2O_2 is very important to protect antioxidant in cell. In H_2O_2 assay, H_2O_2 absorbance was decreased as it oxidized by antioxidant species (plant extract and ascorbic acid), Ascorbic acid, extracts of *A. viridis* have ability to oxidize H_2O_2 by donating electrons, this ability of oxidizing was increased with increase in concentration.

In above four assays Correlation between concentration of sample and free radical(DPPH, ABTS, MO, H_2O_2) was also calculated in terms of IC50 values (which is a concentration of sample need for 50% scavenging of the free radicals), low IC50 value means scavenging ability of samples in that assay was high.

CONCLUSION

The results of present study showed that extract of *A. viridis* leaves contain high amount of flavonoides and phenolic contents and exhibited high antioxidant activity. Previous documentations showed that high scavenging activity is related to the presence of hydroxyl group in phenolic compounds structure. As free radicals are highly involved in the pathogenesis of a lot of diseases so free radical scavengers can be a preventive measure for those diseases. Thus observed radical scavenging activity of *A. viridis* leaf extract can be exploited for disease prevention as well as nutraceutical application.

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