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ESTIMATION OF DIFFERENT EXTRACTS OF *AMARANTHUS VIRIDIS* L FOR *INVITRO* ANTIOXIDANT ACTIVITY

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Abstract:

Back ground: *The plant Amaranthus viridis L (Amaranthaceous) is commonly known as "Never-fading flower" and "Slender amaranth" in Greek and English respectively. These plants have been traditionally used as antiulcer, antileprotic, antiviral and bronchodilator for treatment of asthma. As there has been an upsurge of interest in the therapeutic potential medicinal plants as antioxidant, hence, the aim of present investigation was to discern evidence if Ayurveda remedy can be worthy for providing exogenous antioxidant as diet.*

Objective: *Estimation of different extracts of Amaranthus viridis L for invitro antioxidant activity.*

Material and methods:*The invitro antioxidant activity of aqueous, ethanol and petroleum ether extracts of Amaranthus viridis L was appraised by diphenyl picryl hydrazyl scavenging, nitric oxide scavenging, hydrogen peroxide scavenging and reducing power using standard procedure. The IC_{50} was determined.*

Results: *Antioxidant activity of all extracts of Amaranthus viridis L was proved by declining in nitric oxide activity, hydrogen peroxide activity and diphenyl picryl hydrazyl free radical activity and also increasing in reducing power percentage. The aqueous extract of Amaranthus viridis L was found to be more effective than other extracts. Fifty percentage of inhibition concentration of aqueous extracts of Amaranthus viridis L was discerned to be 25.33µg/ml, 29.25 µg/ml, 35.17 µg/ml and 356.65 µg/ml for reducing power, diphenyl picryl hydrazyl free radical activity, nitric oxide activity and hydrogen peroxide activity respectively.*

Conclusion: *This investigation advocates that aqueous, ethanol and petroleum ether extracts of Amaranthus viridis L manifest potential antioxidant activity which can be considered as nutrient supplement.*

Keywords: *Amaranthus viridis L, invitro antioxidant activity, nutrient supplement.*

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1-INTRODUCTION:

These free radicals which are mostly produced during physiological and biological processes[1] that are recognized as agent involved in the ailments such as cancer, obstruction of arteries (atherosclerosis), central nervous system (CNS) disorder[2], Neurodegenerative disorder, immunosuppression, ischemic heart disease and storkes.[3] Oxidative stress considers an imbalance between reactive nitrogen species (Nitric oxide, nitrite, nitrogen dioxide and nitrate) or oxygen species (superoxide, hydrogen peroxide and hydroxyl radical) and a biological antioxidants in body. The human body has barrier system (endogenous antioxidant) to counter free radical and repair their damages for instance; catalase, superoxide dismutase, glutathione peroxide, vitamin C and vitamin E [3] while exogenous antioxidant which is derived from sources outside the living system such as diet. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are extensively used because they are effective and cheaper than natural ones. However, the toxicity of synthetic antioxidants and safety has raised important concerns.[4] Consequently, The investigation for natural antioxidants as therapeutic agents to conquer the role of free radicals in the pathogenesis of above diseases that is gaining global attention.[5] Medicinal herbs may contain a wide variety of free radical scavenging molecules, for examples phenolic compounds (e.g. phenolic acids, flavonoids, quinones, coumarins, lignans, stilbenes, tannins), nitrogen compounds (alkaloids, amines, betalains), vitamins, terpenoids (including carotenoids).[4] Nowadays, the extract from medicinal plants which are known to have some biologically active constituents are used in Ayurveda system for antioxidant activity purpose.[6]Therefore, present investigation was designed to estimation of different extracts of *Amaranthus viridis* L for *invitro* antioxidant activity.

2-MATERIALS AND METHODS:

2.1. Plant material and Preparation of extracts

The roots of *Amaranthus viridis* L were collected from Chennai, Tamil Nadu, India and authenticated by Amruta herbals company, Indore, Madhya Pradesh, India, a voucher specimen (AV-GRC-010) were preserved for future references. The roots materials (500g) were dried, powdered mechanically and the leaves powder was macerated in the solvents including water, ethanol 95% (v/v) and petroleum ether that undergoing mechanical shaking for 8 hours followed by filtration. The filtrate was evaporated at 60 oC in a vacuum dryer and the percentage yield of extracts is as follows: Aqueous: 23% w/w, Ethanol 10% w/w, petroleum ether: 3% w/w.

2.2. *Invitro* antioxidant activity:[7]

2.2.1. Nitric oxide scavenging activity

The Nitric oxide scavenging was determined according to Panda (2009). The extracts were prepared from 100, 200, 400, 600 and 800 µg/ml aqueous, ethanol and petroleum ether crude extracts; same concentrations were prepared for ascorbic acid as standard. Griess reagent was prepared by mixing equal amounts of 1% sulphanilamide in 2.5% phosphoric acid and 0.1% naphthylethylene diamine dihydrochloride in 2.5% phosphoric acid immediately before use. A volume of 0.5mL of 10mM sodiumnitroprusside in phosphate buffered saline was mixed with 1mL of the different concentrations of the extracts and incubated at 25∘C for 180mins. The extract was mixed with an equal volume of freshly prepared Griess reagent. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. A volume of 150 μL of the reaction mixture was transferred to a 96-well plate. The absorbance was measured at 546nm using a microplate reader. The percentage nitrite radical scavenging activity of the extracts and standard were calculated using the following formula:

$$\text{Nitric oxide Scavenged (\%)} = \frac{(A0 - A1)}{A0} \times 100$$

Where A0 = absorbance of control sample and A1 =absorbance in the presence of the samples of extracts or standard.

2.2.2. Reducing power assay

The reducing power was determined according to the method of Oyaizu (1986). About 2.5 ml of sample/std solution (100, 200, 400, 600 and 800 µg/ml) was mixed with phosphate buffer (2.5 ml, 0.2 M, PH 6.6) and Potassium ferricyanide (2.5 ml, 1%). The mixture was incubated at 50o C for 20 min. Aliquots of trichloroacetic acid (2.5 ml, 10%) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml, 0.1%). The absorbance was measured at 700 nm. A blank was prepared without adding standard or test compound. Increased absorbance of the reaction mixture indicates increase in reducing power which was calculated by using the following formula:

$$\text{Increase in reducing power (\%)} = \frac{\text{A Test} - \text{A blank}}{\text{A blank}} \times 100$$

2.2.3. Hydrogen peroxide scavenging assay

The Hydrogen peroxide scavenging was determined according to the method of Jayaprakah et al., (2004). A solution of hydrogen peroxide (20mM) was

prepared in phosphate buffered saline (PBS, pH 7.4). Various concentrations of 1ml of the extracts or standards in methanol were added to 2 ml of hydrogen peroxide solutions in PBS. The absorbance was measured at 230 nm, after 10 min against a blank, PBS without hydrogen peroxide.

$$\text{Hydrogen peroxide Scavenged (\%)} = \frac{\text{A Test} - \text{A blank}}{\text{A blank}} \times 100$$

2.2.4. DPPH (1,1 Diphenyl-2-picryls-hydrazyl) free radical scavenging activity

The DPPH scavenging was determined according to the method of Cotelle (1996). 3 ml of reaction mixture containing 0.2 ml of DPPH (100 µM in methanol) 2.8 ml of test solution, at various concentrations (100, 200, 400, 600 and 800µg/ml) of the extract fractions and ascorbic acid as standard was incubated at 37°C for 30 min absorbance of the resulting solution was measured at 517 nm. The percentage of inhibition was calculated using the following formula:

$$\text{DPPH Scavenged (\%)} = \frac{(A0 - A1)}{A0} \times 100$$

Where A0 = absorbance of control sample and A1 =absorbance in the presence of the samples of extracts or standard.

2.3. Statistical analysis

The data were exhibited as mean ± S.E.M. Statistical analysis was done by using means analysis of variance (ANOVA) followed by Dunnett' post hoc test, where the difference was considered statistically significant if $p < 0.05$.

3-RESULTS AND DISCUSSION:

Antioxidants compounds can fight with free radicals and protect our body against various diseases. Various techniques have been delineated to determine the *in vitro* antioxidant activity for rapid screening of compounds, if substances that have low *in vitro* antioxidant activity, will definitely exhibit low *in vivo* antioxidant activity.It has been reported that phenolic compounds can manifest antioxidant activity due to redox properties so they can scavenge free radicals either by donating H+ atom with electron or delocalized unpaired electron by reason of having extended conjugated aromatic system; in addition, they have metal chelators potential.[8] Table 1 displays the percentage increase of nitric oxide scavenging activity of different extracts of *Amaranthus viridis* L. As the nitric oxide scavenging activity is recorded in terms of percentage inhibition, it is observed that all the extract have demonstrated dose dependent increase in the nitric oxide scavenging activity. The 800µg of ascorbic acid (as standard) has 79.66% nitric oxide scavenging property. The 800 µg/ml of both aqueous and ethanol extracts of *Amaranthus viridis* L has shown maximum nitric oxide scavenging 67.21% and 59.68% respectively. Aqueous extract of *Amaranthus viridis* L exhibited good antioxidant activity with IC50 value of 35.17µg/ml compared to IC50 value of ascorbic acid as standard 15.47 µg/ml.

Table 1: Percentage of nitric oxide scavenging activity of different extracts of *Amaranthus viridis* L

Conc µg/ml	% Scavenging			
	ASC	AV(aq)	AV(E)	AV(PE)
100	47.43±0.13	46.93±0.3*	39.51±0.1***	28.15±0.06***
200	61.25±0.24	50.54±0.2***	43.33±0.1***	31.86±0.51***
400	66.67±0.27	55.23±0.3***	48.63±0.2***	36.45±0.22***
600	72.03±0.34	61.23±0.4***	54.53±0.4***	41.83±0.37***
800	79.66±0.66	67.21±0.2***	59.68±0.3***	52.80±0.48***
IC50	15.47	35.17	66.65	101.51

n = 3, values are mean ± S.E.M, one way ANOVA followed by Dunnet's multiple test. P values: *< 0.05, ** < 0.01 and *** < 0.001 as compared to ascorbic acid treated group as standard. ASC: Ascorbic acid, AV: *Amaranthus viridis* L, Aq: Aqueous, E: Ethanol extract, extract, PE: petroleum ether extract.

Table 2 indicates the percentage increase of reducing power of different extracts of *Amaranthus viridis* L. All extracts significantly (P<0.001) increase the reducing power as compared to the ascorbic acid as standard. The 800µg of ascorbic acid (as standard) has 97.54% reducing property. The percentage increase in reducing power was found to be 62.37% and 75.76% in 800µg of ethanol and aqueous extracts of *Amaranthus viridis* L respectively. Aqueous extract of *Amaranthus viridis* L manifested considerable reducing power activity with IC50 value of 25.33µg/ml compared to IC50 value of ascorbic acid as standard, 10.81 µg/ml.

Table 2: Percentage increase of reducing power of different extracts of *Amaranthus viridis* L

Conc µg/ml	% Increase of reducing power			
	ASC	AV(aq)	AV(E)	AV(PE)
100	69.13±0.51	44.40±0.0***	37.35±0.3***	26.93±0.1***
200	82.34±0.51	55.64±0.7***	48.42±1.1***	35.53±0.8***
400	91.93±0.19	63.40±0.2***	55.55±0.1***	41.46±0.2***
600	95.43±0.12	72.58±0.2***	59.67±0.3***	42.45±0.4***
800	97.54±0.20	75.76±0.7***	62.37±0.0***	45.81±0.4***
IC50	10.81	25.33	51.80	118.5

n = 3, values are mean ± S.E.M, one way ANOVA followed by Dunnet's multiple test. P values: *< 0.05, ** < 0.01 and *** < 0.001 as compared to ascorbic acid treated group as standard. ASC: Ascorbic acid, AV: *Amaranthus viridis* L, Aq: Aqueous, E: Ethanol extract, extract, PE: petroleum ether extract.

Table 3 reveals the percentage increase of hydrogen peroxide scavenging of different extracts of *Amaranthus viridis* L. It is observed that all the extract have demonstrated dose dependent increase in the hydrogen peroxide scavenging activity. The 800µg of ascorbic acid has shown maximum hydrogen peroxide scavenging (44.19%). Aqueous extract of *Amaranthus viridis* L as well as ethanol extract of *Amaranthus viridis* L showed activities at par with ascorbic acid as standard and these differences were found to be P<0.001. IC50 value of aqueous extract of *Amaranthus viridis* L was found to be 356.65 µg/ml compared to IC50 value of ascorbic acid as standard, 203.15 µg/ml.

Table 3: Percentage of hydrogen peroxide scavenging activity of different extracts of *Amaranthus viridis* L

Conc µg/ml	% Scavenging			
	ASC	AV(aq)	AV(E)	AV(PE)
100	37.39±0.13	27.66±0.3***	17.96±0.6***	8.37±0.08***
200	39.100±0.02	30.58±0.0***	21.02±0.0***	11.35±0.01***
400	41.02±0.05	31.16±0.0***	23.48±0.2***	12.15±0.0***
600	42.04±0.33	32.23±0.2***	24.87±0.3***	12.87±0.0***
800	44.19±0.66	33.26±0.3***	26.77±0.4***	13.62±0.3***
IC50	203.15	356.65	400.95	628.63

n = 3, values are mean ± S.E.M, one way ANOVA followed by Dunnet's multiple test. P values: *< 0.05, ** < 0.01 and *** < 0.001 as compared to ascorbic acid treated group as standard. ASC: Ascorbic acid, AV: *Amaranthus viridis* L, Aq: Aqueous, E: Ethanol extract, extract, PE: petroleum ether extract.

Table 4 demonstrates the percentage increase of DPPH scavenging activity of different extracts of *Amaranthus viridis* L. As the DPPH radical scavenging activity is recorded in terms of % Inhibition, it is observed that all the extract have demonstrated dose dependent increase in the DPPH scavenging activity. The 800µg of ascorbic acid (as standard) has 101.09% DPPH scavenging property. The 800µg of both aqueous and ethanol extracts of *Amaranthus viridis* L has displayed maximum DPPH scavenging 99.69% and 99.61% respectively. IC50 value of aqueous extract of *Amaranthus viridis* L was perceived to be 29.25 µg/ml compared to IC50 value of ascorbic acid as standard 21.83µg/ml.

Table 4: Percentage of inhibition of DPPH free radical activity of different extracts of *Amaranthus viridis* L

Conc µg/ml	% Inhibition			
	ASC	AV(aq)	AV(E)	AV(PE)
100	92.29±0.13	90.76±0.4*	89.51±0.28***	.23±0.12***
200	96.81±0.42	95.76±0.17	94.54±0.1**	79.53±0.15***
400	97.85±0.29	96.44±0.28	96.36±0.96***	85.73±0.5***
600	99.97±0.02	97.57±0.32***	97.51±0.12***	89.05±0.49***
800	101.09±0.08	99.69±0.21	99.61±0.23	91.98±0.37***
IC50	21.83	29.25	36.06	51.90

n = 3, values are mean ± S.E.M, one way ANOVA followed by Dunnet's multiple test. P values: *< 0.05, ** < 0.01 and *** < 0.001 as compared to ascorbic acid treated group as standard. ASC: Ascorbic acid, AV: *Amaranthus viridis* L, Aq: Aqueous, E: Ethanol extract, extract, PE: petroleum ether extract.

The pervious phytochemical analysis of *Amaranthus viridis* L revealed the presence phenolic compounds such as flavonoids and tannin, saponins.[9] The quantity of phenolic compounds extracted from *Amaranthus viridis* L was found to be solvent dependent; hence the aqueous extract of *Amaranthus viridis* L exhibited superior antioxidant activity as compare with other extracts.

4-CONCLUSION:

The results of *invitro* evaluations suggest that aqueous extract of *Amaranthus viridis* L may be useful in defense against illness such as cancer, obstruction of arteries (atherosclerosis), central nervous system (CNS) disorder, neurodegenerative disorder, etc., due to antioxidant properties. However, further investigations on the *in vivo* antioxidant activity and establish the mechanism of action of antioxidant activity of *Amaranthus viridis* L can be warranted.

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