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ANTIOXIDANT ACTIVITY OF ETHYL ACETATE EXTRACT OF GENUS *AMARANTHUS* LINN.

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Antioxidant activity, DPPH, Nitric-oxide, *Amaranthus*

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ABSTRACT: Plants possess good antioxidant capacity to get rid of free radicals that cause many lifestyle diseases especially cancer. Assessment of Antioxidant activity of *Amaranthus* Linn. leaves were evaluated by using *in- vitro* model. In the present study evaluation of ethyl acetate of extract of *Amaranthus* Linn. leaves with the help of two In-vitro antioxidant models were carried out for Nitric-oxide scavenging Activity and DPPH method. IC_{50} value was calculated and compared with standard Ascorbic acid. Ethyl acetate extract was found to be extremely effective in scavenging nitric-oxide (IC_{50} 584.4 µg/ml to 1072 µg/ml). In inhibition of DPPH Radical Scavenging activity (IC_{50} 260.6 µg/ml to 957 µg/ml). Ethyl acetate extract showed different levels of antioxidant activities in tested models.

INTRODUCTION: India, rich with wide variety of under- utilised plants can be explored in the modern medicinal system for curing ailments related to oxidative stress. Plants acting as a dietary supplement can convert the free radical components in the body to an healthy molecule that can function effectively in the human physiological activities [21]. Many medicinal plants act as these supplement and can function as a target molecule to convert the Reactive oxygen species (ROS) by scavenging the molecule and get rid of diseases associated with it.

All aerobic organisms can easily react with most biological molecules including proteins, lipids, lipoproteins and DNA by producing Reactive oxygen species (ROS).

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Reactive oxygen species (ROS), which consist of free radicals such as superoxide anion (O_2-) and hydroxyl (HO·) radicals and non-free radical species such as H_2O_2 and singled oxygen (O_2), are different forms of activated oxygen. Antioxidants regulate various oxidative reactions naturally occurring in tissues. Antioxidants can terminate or retard the oxidation process by scavenging free radicals, chelating free catalytic metals and also by acting as electron donors. Free radicals formation can result in many different diseases including nerve disorders, cancerous cell formation, inflammations of internal organs and other patho-physiological disorders.

Recently, there has been a considerable interest in finding the natural antioxidants from plant materials to replace synthetic ones. Plant phytochemicals not only counteract free radical induced oxidative stress but also overcome the side effects of synthetic antioxidants [25]. Amaranth grows rapidly and has a high tolerance to arid conditions and poor soils where traditional cereals cannot be grown.

Amaranth has been touted as a miracle grain, a super grain, and the grain of the future [18]. Grain Amaranth has got neutraceutical property that the flour obtained were used to prepare bakery products and can act as a good health supplement [3]. Amaranth, a legacy of the Atecs, Mayas, and Incas, continues to be an under-exploited plant with a promising economic value due to the variety of uses it can have and the benefits it can provide to producers, processors, and consumers [11].

The Amaranth plant is also attractive since it adapts itself to a large number of environments, grows with vigor, produces large amounts of biomass, and resists drought, heat, and pests [26, 23].

The pharmacological properties of Amaranth products are considered of vital importance [22]. Increasing the Antioxidant activity, Total Phenolic and flavonoid contents were observed in germination conditions during the seedling formation, as the plant mature the content varies [10]. For reducing tissue swelling the leaves are well thought-out to be constructive, and they have a cleansing effect too. The plant has also been used curatively for diarrhea, dysentery, excessive menstrual flow, ulcers and intestinal hemorrhaging.

The present study is an attempt to evaluate the antioxidant activity of *Amaranthus spinosus* Linn., *Amaranthus caudatus* Linn., *Amaranthus tricolor* Linn., *Amaranthus dubius* Mart., and *Amaranthus viridis* Linn.

MATERIALS AND METHODS:

Materials: The plant specimen proposed for the study was collected from places around Ernakulam city Kerala, India. The plants were authenticated and voucher specimen were placed in KFRI Peechi Thrissur, Kerala India.

Extraction: The plant material was washed, shade dried coarsely powdered. Then the drug was dried and was extracted with Ethyl acetate and extract was filtered. Then the filtrate was concentrated over water bath and dried in a vaccum desicator.

Phytochemical Investigation of Extract: Different chemical constituents present in Ethyl acetate extract were subjected to the tests by Kokate and Trease & Evans [13, 7].

***In-vitro* Antioxidant Method:**

DPPH Radical Scavenging Activity: [4, 11] In different vials, 1ml of extract solution and standard were taken. To these solutions, 5 ml of methanolic solution of DPPH was added, shaken well and the mixture was incubated at 37 ^{0}C for 20 min. The absorbance was measured against methanol as a blank at 517 nm. The absorbance of DPPH taken as a control was measured. Percentage anti-radical activity can be calculated by using following formula:

% Anti-radical activity = Control Absorbance - Sample Absorbance/ Control Absorbance× 100

IC_{50} value was calculated using formula:

$$IC_{50}= a + b (50)$$

$$B=SX.Y/ SX2$$

$$A= \bar{y}-b\bar{x}$$

Nitric oxide Scavenging Activity: Nitric oxide (NO) was generated from sodium nitroprusside (SNP) and was measured by the Griess reagent. SNP in aqueous solution at physiological pH spontaneously generates NO which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavengers of NO compete with oxygen leading to reduced production of NO [5]. SNP (10 mM) in phosphate buffer saline (PBS) was mixed with different concentration of extracts (100-1000µg/ml) and incubated at 25 °C for 180 minutes.

The samples from the above were reacted with Griess reagent (1% sulphanilamide, 0.1% naphthyl-ethylene-diamine dichloride and 3% phosphoric acid). The absorbance of the chromophores formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthyl-ethylene-diamine-dichloride was read at 546 nm. Inhibition of nitrite formation by the plant extracts and the standard antioxidant ascorbic acid were calculated relative to the control.

Nitric Oxide scavenged (%) = A control − A test A control X 100

Where, A control = Absorbance of control reaction and A test = Absorbance in the presence of the samples of extracts/Ascorbic acid.

IC_{50} Value of the extracts: The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in) µg/ml of extracts that inhibits the formation of DPPH radicals by 50%. IC_{50} was calculated for all the extracts by plotting the percentage of DPPH radicals/NO scavenged versus the concentration of extract. Lower the IC_{50} value, higher the radical scavenging effect.

Statistical Analysis: All the experiments were evaluated statistically with SPSS version 20.0, the results were represented in mean±SEM (standard error of mean). One way analysis of varience (ANOVA) followed by DMRT (Duncan's Multiple Range test) to find out any significant difference in the antioxidant potential among genus *Amaranthus* Linn.

RESULTS AND DISCUSSION: In the phyto-chemical and phamacological studies, *Amaranthus* Linn. posses many secondary metabolites which has got numerous function both for plants and animals. In humans, these metabolites have beneficial effects including antioxidant, anti-inflammatory effects, modulation of detoxification enzymes, stimulation of the immune system, modulation of steroid metabolism and antibacterial and antiviral effects [12]. *A. spinosus* showed the presence of different phytochemicals that has got antimicrobial and antioxidant activity and work support that the plant posses antioxidant activity [2]. For the determination of antioxidant potential various *in vitro* assay systems was used like DPPH radical scavenging assay and Nitric oxide scavenging assay since, evaluation of antioxidant properties of plants cannot be carried out accurately by single universal method.

So, therefore it is important to carry out more than one type of antioxidant capacity measurement to cover the various mechanism of antioxidant action [8]. Thus the present work undertake in evaluating the antioxidant activity of *Amaranthus* Linn. species using two *in vitro* antioxidant models.

In phytochemical screening of ethyl acetate extract showed positive test for Flavonoids, Tannins, Saponins, Phenolic compounds. Phenol, flavonoid metabolites are correlated with the antioxidant activity and these metabolite have direct role in freeradical scavenging activity [25]. These secondary metabolite has got immense role in acting against variety of biological activity concerned with human metabolism.

Inhibition of DPPH radical: Free radical molecule DPPH accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radicals was determined by the decrease in its absorbance at 517 nm, which is induced by antioxidants. The potential decrease in the concentration of DPPH radical due to the scavenging ability of *Amaranthus* Linn. and Ascorbic acid (reference standard) showed significant free radical scavenging activity *A.viridis* 95.1%, *A. spinosus* 98.2%, *A. dubius* 91.4%, *A.caudatus* 64.7% and *A. tricolor* 53.8% of inhibition, respectively, at 100 µg/ml. The IC_{50} (the inhibitory concentration at which there is 50% reduction of free radical) of *Amaranthus* Linn. was in the range 260.6 to 957 µg/ml in **Table 1**. The significant decrease in the concentration of the DPPH radical is due to the scavenging ability of *Amaranthus* Linn. Another report showed that the peptides derived from Amaranth species got antifungal and antioxidant property [6]. **Table 3** showed the mean and standard error of five selected plants showing significant difference among antioxidant capacity with p value is less than 0.05.

Nitric oxide scavenging activity: The scavenging of nitric oxide by *Amaranthus* Linn. and Ascorbic acid was concentration dependent. There was a moderate inhibition of nitric oxide formation, with the maximum inhibition being of *A. viridis* 79.1% and *A. caudatus* 78% at 100 µg/ml and Ascorbic acid showing 83.5% respectively. The IC_{50} of *Amaranthus* Linn. was found to be in the range of 584.4 µg/ml to 871.1 µg/ml respectively as shown in **Table 2**.

Similar work done to evaluate antioxidant activity of hydroalcoholic extract of *A. spinosus* L. showed IC_{50} value of 525µg/ml [17, 16]. *Amaranthus viridis* Linn., *A. lividus* Linn. and *A. paniculatus* Linn. also showed antioxidant activity [27, 19, 15]. *A. viridis* showed good antioxidant potential and showed the presence of phenol in phytochemical profiling of methanol extract [1, 20].

Table 4 showed the significant difference among antioxidant capacity since p value is less than 0.05.

Nitric oxide was generated from sodium nitroprusside and measured by the Greiss reduction. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrate ions that can be estimated by use of Greiss reagent. Scavengers of nitric oxide compete with the oxygen, leading to reduced production of nitric oxide [9]. Significant scavenging activity was observed for *A. dubius* and *A. caudatus*, though all the other species of *Amaranthus* Linn. also exhibited the activity [24]. Major compounds like squalene and tocopherols content in the leaf extract of *Amaranthus* Linn. plants exhibited antioxidant property from few reported works [14]. Thus work has to be carried out in the detection of these compounds responsible for similar property. Thus the *Amaranthus* Linn. has potent antioxidant and free radical scavenging effects in different *in vitro* antioxidant models.

TABLE 1: COMPARISON OF DPPH ASSAY ON GENUS *AMARANTHUS* LINN.

DPPH Assay	
Name of Plants	**IC_{50} µg/ml**
AV	349.4
AS	295.2
AD	260.6
AC	675.7
AT	957
Ascorbic	393.3

AV- *Amaranthus viridis* Linn. AS- *Amaranthus spinosus* Linn. AD - *Amaranthus dubius*, Mart.
AC- *Amaranthus caudatus*, Linn. AT- *Amaranthus tricolor* Linn.

TABLE 2: COMPARISON OF NITRIC OXIDE SCAVENGING ASSAY ON GENUS *AMARANTHUS* LINN.

Nitric Oxide Scavenging Activity	
Name of Plants	**IC_{50}µg/ml**
AV	447.4
AS	871.1
AD	629
AC	584.4
AT	1072
Ascorbic	230.2

AV- *Amaranthus viridis* Linn. AS - *Amaranthus spinosus* Linn. AD - *Amaranthus dubius*, Mart.
AC- *Amaranthus caudatus*, Linn. AT- *Amaranthus tricolor* Linn.

TABLE 3: DPPH SCAVENGING ACTIVITY OF FIVE *AMARANTHUS* SPECIES

Concentration (µg/ml)	*Amaranthus viridis* Linn.	*Amaranthus spinosus* Linn.	*Amaranthus dubius* Mart.	*Amaranthus caudatus* Linn.	*Amaranthus tricolor* Linn.	Ascorbic Acid
100	25.408 ^{e} ±0.413	32.812 ^{e} ±0.20	36.278 ^{e} ±0.179	22.626 ^{e} ±0.150	15.666 ^{e} ±0.152	28 ^{e} ±0.707
200	40.632 ^{d} ±0.418	47.996 ^{d} ±0.336	43.604 ^{d} ±0.168	27.088 ^{d} ±0.327	21.812 ^{d} ±0.31	36.8 ^{d} ±1.01
500	64.308 ^{c} ±0.434	60.688 ^{c} ±0.173	74.208 ^{c} ±0.412	42.462 ^{c} ±0.217	29.224 ^{c} ±0.345	57.4 ^{c} ±0.4
700	84.256 ^{b} ±0.337	83.068 ^{b} ±0.306	81.436 ^{b} ±0.126	53 ^{b} ±0.291	38.168 ^{b} ±0.134	70.6 ^{b} ±0.5
1000	94.854 ^{a} ±0.331	98.036 ^{a} ±0.209	91.284 ^{a} ±0.157	62.666 ^{a} ±1.991	53.642 ^{a} ±0.130	87.2 ^{a} ±0.37

Each value is expressed as mean±Std. Error done in triplicates. Data analysed by SPSS version 20.0 by Duncan's Multiple Range test(α=0.05).Mean values followed by different Superscript in the columns are significantly different

TABLE 4: NO SCAVENGING ACTIVITY OF FIVE *AMARANTHUS* SPECIES

Concentration (µg/ml)	*Amaranthus viridis* Linn.	*Amaranthus spinosus* Linn.	*Amaranthus dubius* Mart.	*Amaranthus caudatus* Linn.	*Amaranthus tricolor* Linn.	Ascorbic Acid
100	25.408 ^{e} ±0.413	32.812 ^{e} ±0.204	36.278 ^{e} ±0.179	22.626 ^{e} ±0.150	15.666 ^{e} ±0.152	28 ^{e} ±0.707
200	40.632 ^{d} ±0.418	47.996 ^{d} ±0.336	43.604 ^{d} ±0.168	27.088 ^{d} ±0.321	21.812 ^{d} ±0.311	36.8 ^{d} ±1.01
500	64.308 ^{c} ±0.434	60.688 ^{c} ±0.137	74.208 ^{c} ±0.412	42.462 ^{c} ±0.217	29.224 ^{c} ±0.345	57.4 ^{c} ±0.40
700	84.256 ^{b} ±0.337	83.068 ^{b} ±0.306	81.436 ^{b} ±0.126	53 ^{b} ±0.291	38.168 ^{b} ±0.134	70.6 ^{b} ±0.509
1000	94.854 ^{a} ±0.331	98.036 ^{a} ±0.209	91.284 ^{a} ±0.157	62.666 ^{a} ±1.991	53.642 ^{a} ±0.130	87.2 ^{a} ±0.374

Each value is expressed as mean±Std. Error done in triplicates. Data analysed by SPSS version 20.0 by Duncan's Multiple Range test(α=0.05). Mean values followed by different Superscript in the columns are significantly different

By comparing the R^2 value between DPPH assay and Nitric oxide scavenging activity, the result showed that R^2 value of Nitric oxide scavenging activity was 0.952 and while the R^2 of DPPH assay were 0.870.Thus from the present study conducted the R^2 value Nitric oxide scavenging activity were more positively correlated than the DPPH assay in the selected *Amaranthus* species.

CONCLUSION: In brief summarizing of the above results, it is well clear that all the five *Amaranthus* species posses antioxidant capacity and can act as antioxidant plant substitutes from the present work. This under-utilised plant can throw light to ethno-pharmacological importance further confirming the pharmacological basis in the use of the plants in traditional medicine for the treatment of diseases associated with oxidative stress. Further studies are still needed on the isolation and identification of bioactive components responsible and to clarify the *in vivo* potential of this plants in the management of human diseases resulting from oxidative stress.

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CONFLICT OF INTEREST: There is no conflict regarding the result availed in the data provided.

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