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Biochemical Composition and Antioxidant Potential of Raw and Hydrothermal Treated of Two Underutilized Leafy Vegetables *Amaranthus dubius* and *Allmania nodiflora*

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Abstract
Synthetic and natural food antioxidants are used routinely in foods and medicine especially those containing oils and fats to protect the food against oxidation. Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical scavengers. Hence the plant nutrition plays a crucial role in the prevention of chronic diseases, as most of them can be related to diet. As the studies on the nutritional composition of wild food plants are limited and incomplete, and it is an area that demands. The present study is focused on the indigenous and underutilized plants *Amaranthus dubius* and *Allmania nodiflora.* The raw and processed samples were extracted using 70% acetone and 80% methanol. The samples were analyzed for the proximate composition, total phenolics and tannins content, flavonoid characterization, FRAP assay, Metal chelating activity, DPPH$^{\bullet}$, ABTS$^{\bullet+}$, Hydroxyl, Nitric oxide, Superoxide radical scavenging activities. Both the samples are highly rich in protein, dietary fiber, starch, micronutrients and bioactive compounds with low level of fat.

Keywords: Amaranthus dubius, Allmania nodiflora, Hydrothermal processing, Antioxidant potential

1. Introduction
Plants usually contain natural antioxidants that can scavenge free radicals and those natural antioxidants have the capacity to improve food quality and can also act as nutraceuticals to cease free radical chain reactions in biological system. There are at least 3000 edible plant species known to mankind, but just about 30 crops alone contribute to more than 90% of the world's calorie intake and only 120 crops are economically important at the national scale [18]. This shows that several hundreds of species remain discarded or unnoticed at the hands of various human societies. Among the edible plant diversity, many are nutritionally -important. Many tribal and rural families still conserve knowledge on a wide range of species for their food needs [24, 36, 7]. Oxygen is essential even then; its reaction with biological substrates during physiological process in the human body generates unstable compounds called free radicals. Oxidative stress in turn leads to the generation of various degenerative and chronic diseases like atherosclerosis, cancer, diabetes and so on [29, 41]. Therefore, it is necessary to delicate the balance between beneficial and harmful effects of free radicals. An antioxidant is a molecule that quench/prevents the generation of free radicals by acting as a reluctant and it is evolved to refer as the prevention of oxidative stress to the whole.

Worldwide there are about 795 million undernourished people who suffer chronic calorie deficiency due to issues related to food insecurity [18]. The leafy vegetables, fruits & whole grains are naturally occurring dietary antioxidants. The Food and Nutrition Board of the National Academy of Science (1998) defined regular consumption of such dietary antioxidant as a substance in foods may significantly decreases the adverse effects of reactive oxygen species and maintain the normal physiological function in humans. Leafy vegetable are widely consumed all over the world and have been eaten for centuries and are classified as GRAS (Generally Recognized As Safe). An abundance of research has shown that fresh Leafy vegetable contain important functional food components, such as β-carotene, ascorbic acid, riboflavin, and folic acid, as well as minerals [19]. Thermal processing is of great importance, considering the fact that only a small amount of leafy vegetables is consumed in the raw state, whilst most need to be processed for safety and quality. Processing may cause loss of antioxidant component and nutrient. Losses of antioxidant components and nutrients of some vegetables during processing have been previously reported [25]. Several studies have been showed that blanching is able to improve the palatability and bioavailability of natural occurring antioxidants in vegetables. Additionally, blanching would bring about a number of changes in physical characteristics and chemical composition of vegetables [48, 52, 37]. Showed that blanching had significant effect on the contents of ascorbic acid and total phenolics, and antioxidant activity of green leafy vegetables. Amaranthus spp. are a group of versatile food crops that consist of 60 species, of which three species are grown for edible grains and 17 species are grown for edible leaves. Amaranthus leaves are used as food in many countries from Africa, where they are consumed as infusions, salads, soups, sauces, or mixed with other vegetables or legumes [15]. It is very important to consider amaranth as potential antioxidant source due to tendencies for "new" natural ingredients demanded by consumers nowadays. The genus Amaranthus (family Amaranthaceae), including quinoa and amaranth species, is a valuable food source of nutrients with high quality proteins, vitamins, minerals and bioactive compounds such as phenolics. Amaranthus have also been commercially exploited for natural dyes, pharmaceuticals and skin lubricants [30]. *A. nodiflora* constitutes Alkaloids, glycosides, flavonoids, saponins, tannins, carbohydrate and essential oils, steroids,

carbohydrates, carotenoids, anthocyanins [7]. Thus in the present investigation the successive extraction of *A. dubius and A. nodiflora* was screened for in vitro antioxidant properties using standard operating procedures. The *A. dubius* (spleen spinach) *and A. nodiflora* distributed in tropical and subtropical regions belongs to the economically important family *Amaranthaceae*. The leaves of both the species are usually consumed in the cooked form as curry, From a nutritional perspective, *A. dubius* is valued as a leafy vegetable and includes vitamin A, B_6, C, riboflavin, folate, calcium, iron, magnesium, phosphorus, potassium, zinc, copper and manganese. It has 30% higher protein value than other pastas, such as rice, wheat, flour, oats, and rye. On therapeutic intersect it is employed to assist with healing of rashes of the skin and boils, migraine headaches, diarrhoea, leucorrhoea, soothing the stomach, as a diuretic, for bladder distress, excessive menstruation, hemorrhoids and dysentery. It can also assist with an improvement in antioxidants levels and rise in the red blood cell count especially for those who have been diagnosed with anemia.

However the available information regarding the nutrition and antioxidant activities of bioactive compounds in raw and thermal processing (Blanching) of *A. dubius and A. nodiflora* species are found to be meager. Therefore the present study has been carried out to analyze the proximate composition like moisture, ash crude fiber, crude protein, crude lipid and total carbohydrate content present in the leaf, stem and flower of *A. dubius* and *A. nodiflora* and study the total phenolic, tannin, and flavanoid content present in the leaf, stem and flower of *A. dubius* and *A. nodiflora*. The antioxidant activity of leaf, stem and flower of *A. dubius* and *A. nodiflora* extracts by $DPPH^{\cdot}$ radical scavenging assay, hydroxyl radical scavenging assay, metal chelating assay, $ABTS^{+}$ radical scavenging assay, FRAP and nitric acid assay also studied

2. Materials and Methods

2.1 Chemicals

Ferric chloride, 2, 2′–diphenyl-1picrylhydrazyl (DPPH), potassium persulfate, 2,2 azinobis (3-ethylbenzo-thiozoline-6-sulfonic acid) disodium salt (ABTS), 6-hydroxy -2,5,7,8-tetra-methylchroman 2- carboxylic acid (trolox), linoleic acid, ferrous chloride, ammonium thiocyanate, hydrogen peroxide, ferrous ammonium sulfate, ethylene diamine tetra acetic acid (EDTA) disodium salt and were obtained from Hi Media, Merck or Sigma. All other reagents used were of analytical grade.

2.2 Leaf samples and processing

The leafy vegetable *A. dubius* were purchased from Coimbatore, Tamil Nadu, India and *A. nodiflora* were collected from Nammakal, Tamil Nadu, India. From the plant source leaf, stem and flower have been separated. From all the parts of the plants, one part were dried at room temperature and considered as raw samples. The remaining part were blanched in water at 100º C in the ratio 1:10 (W/V). The remaining water after blanching was discarded and the processed samples were cut in to sample pieces and dried in room temperature. The raw and processed samples were ground in to fine powder and stored in separate screw capped bottles for further analysis.

2.3 Solvent Extraction

After defatting by petroleum ether, the raw and processed leaf, stem and flower samples (15 g) were extracted by stirring with 100 ml of acetone (70:30; Acetone:H_2O) at 25º C for 48 h and filtering through whatmann No. 4 filter paper. The residues were re extracted with an additional 75 ml of acetone, as described above, for 3 h. The remaining residues left after the acetone extraction were again extracted with 100ml of methanol (80:20; Methanol: water) as described above. The solvent of the combined extract was evaporated under low temperature at 40º C in incubator (NSW make, New Delhi) respectively. The extract thus obtained was used directly for total phenolics and tannins estimation and also for the assessment of antioxidant activity through various *in vitro* assays. From the extract, a known volume was taken, dried in an oven at incubator temperature of 40º C (until sample getting a constant weight) and the recovery percent was calculated as equation;

$$\text{Recovery \%} = \frac{(\text{Extract + container (g)}) - (\text{Empty container (g)})}{\text{Sample weight (g)}} \text{x100}$$

2.4 Proximate analysis

The moisture content of raw and processed samples was determined using Moisture Analyzer MA35 (Sartorius AG, Germany) at 105 °C. Crude lipid (Soxhlet extraction), crude fiber and ash contents (gravimetric) were also determined based on the methods outlined in Association of Official Analytical Chemists (AOAC, 1990). Micro-Kjeldahl method was employed to determine the total nitrogen and a nitrogen protein conversion factor is used for crude protein (Nx6.25) determination. The crude carbohydrate (also called Nitrogen Free Extractives (NFE)) content was estimated by the difference. The proximate composition was expressed as g/100 g DM. The gross energy (KJ) was determined by multiplying the percentage of crude protein, crude lipid and NFE by 16.7, 37.7 and 16.7 respectively (Siddhuraju et al., 1996).

2.5 Estimation of total phenolics and tannins

The total phenolic content was determined according to Folin-Ciocalteau method (FCM). [46] The total phenolics and tannins were measured as gallic acid equivalents from gallic acid standard curve (3-15 µg range). For the assay, aliquots (100 µL) of extracts were taken in test tubes and the volume was made up to 1 mL with distilled water. Then 0.5 mL of Folin-Ciocalteau phenol reagent (1:1 with water) and 2.5 mL of sodium carbonate solution (20% w/v) were added sequentially in each tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725 nm against the reagent blank. For tannin estimation, the sample extracts were incubated with polyvinyl polypyrrolidone (PVPP) (100 mg) for 4 h at 4 °C. The phenolics and tannins were expressed as mg gallic acid equivalents (GAE)/g extract. From the above results, the tannin content of the sample was calculated as follows:

Tannin (%) =Total phenolics (%)-Non-tannin phenolics (%).

2.6 Total flavonoids

The total flavonoid content was measured by a spectrophotometric assay. [56] 1 ml aliquot of standard solution of Rutin at different concentrations (0–100 mg/l, external calibration with n = 6 concentrations) or sample was added to 10 ml volumetric flasks containing 4 ml water. At the onset of the experiment, 0.3 ml of 5 % $NaNO_2$ was added to the flask. After 5 min, 3 ml of 10 % $AlCl_3$ was added. At 6 min, 2 ml of 1M NaOH was added to the mixture. Immediately, the solution was diluted to a final volume of 10 ml with water and mixed thoroughly. The absorbance of the mixture was determined at 510 nm versus the prepared blanks. Total flavonoid content was expressed as mg rutin equivalents (RUT) per g extract.

2.7 Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacity of phenolic extracts of raw and processed *A. dubius* and *A. nodiflora* was estimated according to the procedure described by Benzie & Strain [8] as modified by Pulido *et al* [39] FRAP reagent (900 µl), prepared freshly and incubated at 37ºC, was mixed with 90 µl of distilled water and 30 µl of test sample, or methanol (for the reagent blank). The test samples and reagent blank were incubated at 37º C for 30 min in a water bath. The FRAP reagent contained 2.5 ml of 20 mmol/l TPTZ solution in 40 mmol/l HCl plus 2.5 ml of 20 mmol/l $FeCl_3.6H_2O$ and 25 ml of 0.3 mol/l acetate buffer, pH 3.6 [8]At the end of incubation the absorbance readings were taken immediately at 593 nm using a Spectrophotometer. Methanolic solutions of known Fe (II) concentration ranging from 100 to 2000 µmol/l ($FeSO_4.7H_2O$) were used for plotting the calibration curve. The parameter Equivalent Concentration (EC_1) was defined as the concentration of antioxidant has a ferric- TPTZ reducing ability equivalent to that of 1 mmol/l $FeSO_4.7H_2O$. EC_1 was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/l concentration of Fe (II) solution determined using the corresponding regression equation.

2.8 Metal chelating activity

The extracts (100 µl) were added to a solution of 2 mmol /l $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mmol/l ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm. The results were expressed as mg EDTA equivalent/g extract using the calibration curve of EDTA. Linearity range of the calibration curve was 0.5-2.5 µg in the reaction mixture [14].

2.9 Stable free radical scavenging activity using DPPH$^{\bullet}$ method

The radical scavenging activity of sample extracts was measured using DPPH radical by the method of Brand-Williams *et al* [10]. With some modifications. Extract of 0.1 mL prepared in methanol was mixed with 3.9 mL of DPPH$^{\bullet}$ (6×10^{-5} mol/l methanol) and incubated in dark for 30 min. Absorbance was read at 515 nm and the results were expressed mmol trolox equivalents/g extract.

2.10 Total antioxidant activity assay by radical cation (ABTS$^{\bullet+}$)

ABTS was dissolved in water to a 7 mM concentration, ABTS radical cation (ABTS) was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at temperature for 12-16 h before use. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated to 30º C to give an absorbance at 734 nm of 0.700± 0.02 in a 1cm cuvette. The stock solution of the sample extracts in ethanol were diluted such that, after introduction of a 10 µl aliquot of each dilution into the assay, they produced between 20-80 % inhibition of the blank absorbance. After the addition of 1 ml diluted ABTS solution to 10 µl of samples or Trolox standards (final concentration 0-15 µM) in ethanol OD (optical density) was taken at 30º C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of standard, and the percentage inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration [40] described by Siddhuraju and Becker [40]. The unit of total antioxidant activity (TAA) is defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extracts using the calibration curve of trolox. Linearity range of the calibration curve was 0.25-1.25 mm/l. The total antioxidant activity of ASC and BHA were also measured by ABTS method for comparison.

2.11 Nitric oxide scavenging activity assay

Nitric oxide generated from sodium nitroprusside (SNP) was measured by the Griess reaction. Nitric oxide interacts with oxygen to produce nitrite ions that can be observed by Griess reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide. Various concentrations of samples and sodium nitroprusside (SNP, 5 mM final concentration) in phosphate buffer saline, pH 7.4, in a final volume of 1 ml were incubated at 25° C for 150 min. A control experiment without samples but with equivalent amount of vehicles was conducted in an identical manner of the sample. After incubation, the reaction mixtures were mixed with Griess reagent (1% sulfanilamide and 0.1 % naphthylethylene diamine dihydrochloride in 5 % H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent coupling with naphthylethylene diamine was measured at 540 nm. The total antioxidant activity of ASC (500 µg) and QUE (500 µg) were also measured by nitric oxide scavenging method for comparison. The % Nitric oxide scavenging activity was calculated by the following equation;

(%) Nitric oxide scavenging activity = (Control OD- Sample OD)/Control OD x 100

2.12 Superoxide anion radical scavenging activity assay

The method used by Martinez for determination of the superoxide dismutase was followed with modification in the riboflavin-light-nitroblue tetra zolium (NBT) system [9]. Each 3 ml of reaction mixture contained 50 mM phosphate buffer (pH 7.8), 13 mM methionine, 2 µM riboflavin, 100 µM EDTA,

NBT (75 µM) and 1 ml of sample solution. The production of blue formazan was followed by monitoring the increase in absorbance at 560 nm after 10 min of illumination from a fluorescent lamp. The total antioxidant activity of BHA (150 µg) and TRO (150 µg) were also measured by superoxide anion radical scavenging method for comparison. The % superoxide radical scavenging activity was calculated by the following equation;

(%) superoxide anion radical scavenging activity = (Control OD-Sample OD)/Control OD x 100

2.13 Hydroxyl radical scavenging activity

The scavenging activities of the extracts of raw and processed leafy samples on hydroxyl radical were measured according to the method of Klein. Various concentrations (200 µg) of extracts were added to 1.0 ml of iron–EDTA solution (0.13 % ferrous ammonium sulphate and 0.26 % EDTA), 0.5 ml of EDTA solution (0.018 %) and 1.0 ml of DMSO (0.85 % v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 ml of ascorbic acid (0.22 %) and incubated at 80-90º C for 15 min in a water bath. After incubation the reaction was terminated by the addition of 1.0 ml of ice-cold TCA (17.5 % w/v). 3 ml of Nash reagent was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectrophotometrically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity was calculated by the following equation;

(%) HRSA = 1-(difference in absorbance of sample / difference in absorbance of blank) x 100

2.14 Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA), and the significance of the difference between means were determined by Duncan's multiple-range test ($P < 0.05$) using SPSS (Version 13.0, SPSS Inc., Wacker Drive, Chicago, USA). Values expressed are means of triplicate determinations ± standard deviation.

3. Result and Discussion

3.1 Proximate composition

Leafy vegetable are usually cooked before consuming. It brings about a number of changes in physical characteristics and chemical composition. By considering the importance of processing, it is essential to determine the effect of processing methods on nutritional value of these two underutilized leafy vegetable plant parts. The proximate composition of raw and processed samples of leaf, stem and flower of *A. dubius* and *A. nodiflora* were analysed and listed in Table.1 The moisture content of *Amaranthus* sample was ranging from 4.57-8.74%. The highest moisture was significantly absorbed in the ADSR (8.74%). Protein is an essential macronutrient for growth and maintenance of body tissues. The highest protein content was significantly observed in blanched sample of *A. nodiflora* stem (30.13%). The protein content of raw leaf of *A. nodiflora* was similar to that of *A.caudatus* (20.59%), but they are relatively low when compared to that of *A. dubius***.** [1,2]All the processed blanched samples exhibited highest protein content than the respective raw samples except the flower of *A. nodiflora* (8.23%). The increase in protein content is probably due to reductions corresponding to solubilization of carbohydrates, oligosaccharides, minerals and total dietary fiber contents during each treatment [13]. plant food that provides more than 12% of its calorific value from protein is considered good source of protein. Therefore, all the *Amaranthus* samples meet this requirement. The values of ash content of the samples were ranging from 40.63-8.31%. This requires investigation to ascertain the species of mineral elements as they are essential for tissue functioning and a necessity in daily requirement for human nutrition. The ash content of *A. dubius* (25.38%) and *A. Nodiflora* (17.43%) leaf were found to be higher than the previously report of *A. hybridus* leaf (13.8%). All the processed samples exhibited the decreased ash content than the raw samples. The substantial reduction of ash content in the processed samples might be due to the leaching of both micro and macro elements into the blanched solutions. The *Amaranthus* leaf sample is poor source of lipid. The lipid content of *A. nodiflora* leaf raw and blanched was compared to that of *A. hybridus* (4.65% DW). [21] The lipid content of all processed blanched sample were found to be reduced. A diet providing 1-2% of its caloric of energy as fat is said to be sufficient to human beings as excess fat consumption is implicated in certain cardiovascular disorders such as atherosclerosis, cancer and aging [6]. The carbohydrate content was significantly found to be high in flower of *A. nodiflora* raw and blanched sample. The carbohydrate content of *A. dubius* (54.25%) and *A. nodiflora* (49.38%) were similar to that of *A. hybridus* leaf (52.18%) and found to be higher than reported for *Senna obtusfolia A. Incurvatus and M.balsamina* leaves, respectively [22]. The recommended dietary allowance (RDA) values for children, adults, pregnant and lactating mothers are 130g, 130g, 175g and 210 g, respectively. It implies that respective daily requirement can be met when 100 g dried leaves are consumed. The processed blanching increases the total carbohydrate content in the *A. dubius* leaf (58.37 %) and stem (48.98 %) than the raw. The crude fiber content of the *A. nodiflora* sample was ranging from 0.62-5.59. The crude fibre content of *A. dubius* (1.31%) and *A.* nodiflora (5.47%) leaf was higher when compared to *I. batatas* (7.20%), *T. triangulare* (6.20%) *P.guineeses* (6.40%), *Corchorus olitorius* (7.0%) *Vernonia amagydalina* (6.5%) [1, 6] Adequate intake of dietary fibre can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer The RDA of fibre for children, adults, pregnant and lactating mothers are 19 – 25, 21-38, 28 and 29 g, respectively. This shows that the plants are capable of contributing 34 - 45, 23 - 41, 31 and 30% of their respective daily requirement when 100 g dried leaves are consumed and as such could be valuable sources of dietary fibre in human nutrition. The energy values of *Amaranthus* samples were ranging from 7.70 -13.68 MJ/Kg. The processed blanched sample of *A.dubius* leaf (13.28 MJ/Kg) and stem (11.73 MJ/Kg) showed increased energy values than the respective raw sample, were as other sample exhibited the decreased energy values than the raw samples. The effect of this food processing on nutrient content will depend on the sensitivity of the nutrient to the various conditions prevailing during the process, such as heat, oxygen, pH and light. The nutrient retention may vary with a combination of conditions, such as the characteristics of the food being processed and the concentration of the nutrient in the food. Similar results have also been reported in *G. max*, *P. lunatus* and *M. pruriens* when leaf samples were subjected to the autoclaving process [3].

3.2 Extract Yield Percentage, Total Phenolic and Tannin content

The extract yields, total phenolics and tannins content of raw and processed sample of acetone and methanol extracts of *A. dubius* and *A. nodiflora* samples are presented in Table-2. The extract yield percent of the raw and processed samples ranged from 2.10-36.40%. The highest yield percentage was significantly showed in ANSBA (36.40%). On the other hand, arrange all the samples acetone extracts showed highest percentage yield on comparison with methanol extracts. This indicated that acetone was found to be more suitable solvent for extracting the phytochemicals than the methanol. methanol was a better extraction solvent for amaranth [33]. The total phenolic and tannin content was determined according to the colorimetric Folin–Ciocalteu method with tannic acid as a standard compound. A wide range of total phenolic and tannin content were found in leaf, stem and flower of *A. dubius* and *A. nodiflora* are shown in Table 2. The phenolic and tannin content of acetone and methanol extracts of leaf, stem and flower of *A. dubius* and *A. nodiflora* were ranging from 65.47-280.47 mg GAE/g extract and 42.14-143.33 mg GAE/g extract. General, arrange all the samples the acetone extracts showed the highest phenolic and tannin content than the methanol extracts. Results showed that significantly the highest phenolics were presented in the ANFBA (280.47 mg GAE/g extract) following, the highest tannin content were presented in the ANSBA (143.33 mg GAE/g extract). Interestingly, except *A. nodiflora* stem extracts, all the samples showed highest phenolic and tannin content in the processed blanching samples extracts than the respective raw sample. From the above results, blanching process showed different effects on different plant parts of amaranth species, with some showed increased phenolic content while others decreased. Similarly, Oboh, reported an increase in phenolic content up to 200% after blanching in green leafy vegetables [37]. This increment was suggested due to breakdown of tannins during heating process, and increased their extractability. Increase in phenolic content of microwave treated bean compared to raw form was reported by Roy *et al*. observed an increase of 18% in phenolic content in steamed broccoli compared to raw [42]. Generally, phenolics found in fruits and vegetables are bonded to dietary fiber, proteins or to sugars in plants to form complex structures. Thermal processing disrupts the cell membranes, cell walls and hydrolyzes these bonds making it more available during extraction [11, 42]. Formation of phenolic compounds at high temperature due to availability of precursors formed by non-enzymatic interconversion between phenolic molecules [53]. In contrary, few studies have shown that phenolic compounds are sensitive to heat whereby blanching of vegetables for few minutes could cause a significant loss of phenolic content which can leach into boiling water [4]. Amin *et al* reported a loss of 71% in total phenolic content in blanched *Amaranthus* compared to raw one and they also found a similar loss of phenolic content in olive oil after microwave heating [4]. In our study the reduction of phenolic content in the processed *A. nodiflora* stem sample of both extracts than the raw sample were similar to that of Amin. Blanching for up to 15 min may affect the antioxidant activity and phenolic content in raw amaranth sample [4].

3.3 Total flavonoid content

Flavonoid is a typical phenolic that possesses antioxidant activities. As components of vegetables and fruits they are regularly presented in human foods. However, there were only a few reports on the identification and quantification of flavonoids in food legumes, including common beans and peas. [47] Table 2 shows the total flavanoid content of raw and processed samples of leaf, stem and flower of *A. dubius* and *A. nodiflora*. The flavonoid content of samples was ranging from 24.84-878.89 mg RUT/g extract. The highest flavonoid contents were significantly observed in the ANFBA (878.89 mg RUT/g extract). The methanol extracts of *A. dubius* stem blanched (45.60 mg RUT/g extract) and *A. nodiflora* leaf blanched (158.33 mg RUT/g extract) samples showed highest flavonoid content than the acetone extracts, were in all other samples acetone extracts showed highest flavonoid content than the methanol extracts. Except *A. nodiflora* leaf, all the samples showed highest flavonoid content in the processed blanching sample than the raw extracts. By estimating the total flavonoid content, we observed that autoclaving and dry heating significantly increased the extractability of these compounds in millets. The result supports a previous observation [20] where in steam cooking of broccoli increased total content of flavonoid as well as glucosinolates comparing to fresh broccoli. The increasing release of flavonoid is a promising sign, that antioxidant activity related to their level would be positively balanced. We believe that the increase in flavonoid content is primarily due to the increased release of phytochemicals from the matrix to make it more accessible in the extraction. Thermal processing disrupts the cell membranes and cell walls and releases phytochemicals from the insoluble portion of the millets, which increases the pool of flavonoids. The flavonoids have been well studied for their potential health benefits that include antiproliferate, anticarcinogenic, and antioxidant activity [28].

3.4 Antioxidant activity

3.4.1 Ferric reducing/antioxidant power (FRAP) assay

The ability of the plants extracts to reduce ferric ions was determined using the FRAP assay developed by Benzie and Strain [8]. An antioxidant capable of donating a single electron to the ferric-TPTZ (Fe (III)-TPTZ) complex would cause the reduction of this complex into the blue ferrous-TPTZ (Fe (II)-TPTZ) complex which absorbs strongly at 593 nm. Therefore, the antioxidant potential of acetone and methanol extracts of raw and processed leaf, stem and flower of *A. dubius* and *A. nodiflora* were estimated for their ability to reduce TPTZ-Fe (III) complex to TPTZ-Fe (II) (Table 3). All the extracts revealed good ferric reducing ability ranging from 7420.28-42217.39 mmol Fe (II)/g extract). The highest ferric reducing ability was observed in the ANFRA (42217.39 mmol Fe (II)/g extract). The methanol extracts of *A. dubius* leaf (24231.88 mmol Fe(II)/g extract), and stem (11478.26 mmol Fe(II)/g extract) blanched sample and *A. nodiflora* leaf (26797.10 mmol Fe(II)/g extract) and flower (22913.04 mmol Fe(II)/g extract) blanched sample showed highest reducing ability than the acetone extracts. On the other hand, all the other samples showed highest ferric reducing activity in acetone extracts than the methanol. The processed sample showed decreased ferric reducing activity in acetone extracts of *A. dubius* stem (7710.14 mmol Fe (II)/g extract), *A. nodiflora* leaf (18855.07 mmol Fe (II)/g extract) and flower (11362.31 mmol Fe (II)/g extract) and in methanol extracts of *A. nodiflora* leaf (26797.10 mmol Fe (II)/g extract) and stem (15304.34 mmol Fe (II)/g

extract) samples. Interestingly, all the other extracts showed highest reducing activity in the processed samples. However, when compared with the standards, ascorbic acid (730676.32 mmol Fe (II)/g extract) and trolox (641061.80 mmol Fe (II)/g extract) the entire sample extracts showed significantly lowest ferric reducing ability. FRAP assay was used by several authors for the assessment of antioxidant activity in various food product samples had most of the secondary metabolites are redox-active compounds that would be picked up by FRAP assay [23, 38].

3.4.2 Metal chelating activity

Presence of transition metal ions in a biological system could catalyze the Haber-Weiss and Fenton-type reactions, resulting in the generation of hydroxyl radicals (OH•). However, these transition metal ions could form chelates with the antioxidants, which results in the suppression of OH• generation and inhibition of peroxidation processes of biological molecules.[12] The extent of chelation of ferrous ions by the extracts of raw and blanched leaf stem and flower samples of *A. dubius* and *A. nodiflora* are listed in Table 3. The extents of chelation of ferrous ions by the *Amaranths* species were ranging from 52.96-7.46 mg EDTA/g extract. The highest chelating ability was significantly showed in the ANSRM (52.96 mg EDTA/g extract). On the other hand, all the methanol extracts of *Amaranths* species showed highest chelating ability than the acetone extracts. The processed (blanched) sample showed decreased chelating ability in acetone extracts of *A. dubius* leaf (34.08 mg EDTA/g extract) and stem (9.68 mg EDTA/g extract), *A. nodiflora* leaf (14.29 mg EDTA/g extract) and in methanol extracts of *A. dubius* leaf (11.00 mg EDTA/g extract) and *A. nodiflora* stem (19.15 mg EDTA/g extract). Interestingly, all the other processed showed highest chelating ability than the raw sample extracts. On comparison with the standard BHA (10.49 mg EDTA/g extract) all sample extracts exhibited the highest chelating except ADSBA (9.68 mg EDTA/g extract) and ANSRA (7.46 mg EDTA/g extract). The spectroscopic studies showed that green leafy vegetables are capable of chelating metal ions. Both raw and processed sample extracts had shown good metal chelating activities, and hence can serve as potential reducing agent. Flavonoids are known for their reducing properties and chelating action for metal ions. [26]The chelating agents may inhibit lipid oxidation by stabilization of transition metals. Reports are there with compounds bearing ortho-dihydroxyl groups having roles in chelating transition metal ions. Our study apparently exhibited that all the amaranth extracts promoted the formation of metal complex. [32] It has been reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, thereby stabilizing the oxidized form of the metal ion. Earlier results conducted upon the aerial part extract of *Ornithogalum sintenisii* showed higher Fe2+ - chelating properties [17].

3.4.3 DPPH• scavenging activity

DPPH• scavenging method has been widely used to evaluate the antioxidant activity of extracts due to simple, rapid, sensitive and reproducible procedure. DPPH radical is a stable organic free radical with adsorption maxima at 517nm. It loses this adsorption when accepting an electron or a free radical species, which results in a visually noticeable discoloration from purple to yellow. [57] The free radical scavenging activity of acetone and methanol extracts of raw and processed leaf, stem and flower of *A. dubius* and *A. nodiflora* were listed in Table 3. All the sample extracts exhibited the good scavenging activity ranging from 23014.83-245607.9 mmol trolox/g extract. The highest radical scavenging activity was observed in ANFBA (245607.9 mmol trolox/g extract). The processed sample exhibited the decreased radical scavenging activity in the acetone and methanol extract of *A. dubius* leaf (46436.88 mmol trolox/g extract; 47571.26 mmol trolox/g extract) and acetone extract of *A. nodiflora* stem (46611.4 mmol trolox/g extract). Our results of decreased radical scavenging activity of processed sample are in agreement with Xu and Chang, who has reported a decrease in DPPH activity of boiled green pea, yellow pea and chick pea due to leaching of soluble antioxidant components into boiling water. [54] On the other hand, the acetone and methanol extracts of *A. dubius* stem and methanol extracts of *A. nodiflora* stem and flower exhibited the equal radical scavenging activity on both raw and processed sample. The previous studies have also shown that some of the antioxidant components in vegetables remain unchanged after cooking [16]. Interestingly, the acetone extract of *A. nodiflora* leaf (58599.96 mmol trolox/g extract) and flower (245607.9 mmol trolox/g extract) and methanol extract of *A. nodiflora* leaf (59414.39 mmol trolox/g extract) exhibited highest radical scavenging activity on processed samples. Some authors as similar to our study reported that an increase in antioxidant capacity on processing samples. [4, 48, 52, 54] Fluctuation of antioxidant capacity in vegetables could be attributed to the characteristics of antioxidant components. Previous research on vegetables indicated that processing caused increased antioxidant potential due to improvement of antioxidant properties of naturally occurring compounds or formation of novel compounds such as Maillard reaction products, which have antioxidant activity. In addition, thermal treatments also could break the glucosides of flavonoids to form aglycones which possess higher antioxidant properties. On the other hand, leaching of polar phenolic compounds into water during boiling and degradation or formation of new compounds (not limited to phenolic compounds) can contribute to lower antioxidant activity [48, 54]. However, on comparison with the standards ascorbic acid (493310.06 mmol trolox/g extract) and BHA (386368.04 mmol trolox/g extract) all the sample extracts exhibited the lowest radical scavenging activity.

3.4.4 $ABTS^{\bullet+}$ scavenging activity

Proton radical scavenging is an important attribute of antioxidants. ABTS, a protonated radical, has characteristic absorbance maxima at 734 nm, which decreases with the scavenging of the proton radicals. [34]The amaranth sample exhibited the good scavenging proton radical ranging from 1500.0-20395.83 mmol trolox/g extract (Table 3). The highest radical scavenging activities were observed in acetone extract of *A. nodiflora* leaf raw sample (20395.83 mmol trolox/g extract). The methanol extracts of *A. dubius* leaf (10229.17 mmol trolox/g extract), *A. dubius* stem (raw) (9666.67 mmol trolox/g extract) and *A. nodiflora* leaf (blanched) (11479.17 mmol trolox/g extract) exhibited the highest radical scavenging activity than the acetone extracts, whereas all the other samples exhibited highest radical scavenging activity in the acetone extract. The decreased radical scavenging activity on processed samples were exhibited in acetone extract of *A. dubius* leaf (5541.67 mmol trolox/g extract), *A. nodiflora* leaf (4500.00

mmol trolox/g extract) and in methanol extract of *A. nodiflora* stem (1500.0 mmol trolox/g extract). The great loss of antioxidant activity in the processed sample is due to the fact that, they are sensitive to the aqua thermal process which reduces the polyphenols in the samples. Interestingly, all the other samples exhibited the highest radical scavenging activity on the processed samples. However, when compared with the standards, ascorbic acid (597916.67 mmol trolox/g extract) and BHA (654356.06 mmol trolox/g extract) all the sample extracts exhibited the lowest radical scavenging activity. The increased radical scavenging activity in the processed samples of our study were similar to Siddhuraju and Manian, reports that increased activity in processed samples of horse gram might be due to the bound insoluble fractions of other compounds. [44]On the other hand, the formation of tannin-protein complexes, both in soluble and insoluble complexes, as the result of conventional food/seed processing have also been shown to be potential free radical scavenger and radical sinks [45].

3.4.5 Hydroxyl radical scavenging activity

The hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells. This species is considered to be one of the quick initiators of the lipid peroxidation process, abstracting hydrogen atoms from unsaturated fatty acids [27]. Fig 1 shows that the hydroxyl radical scavenging activity of leaf, stem and flower of *A. dubius* and *A. nodiflora* extracts. All the extracts exhibited radical scavenging activity ranging from 22.97%-84.18%. The highest radical scavenging activity was significantly observed in the acetone extracts of *A. nodiflora* flower (84.18%) and leaf (83.92%) blanched samples. The acetone extracts of all *Amaranthus* sample exhibited the highest radical scavenging activity than methanol extracts. The radical scavenging activity of processed blanched sample of acetone extracts of *A. dubius* stem, *A. nodiflora* leaf, stem and flower on comparison with the raw were found to be insignificant. On the other hand, reduced radical scavenging activities were significantly showed in acetone extract of *A. dubius* leaf on comparison with raw sample. Interestingly, the other entire blanched sample exhibited highest radical scavenging than the raw extract. However, when compared with the standard catechin (82.46%), ANLBM (83.92%) and ANFBM (84.18%) significantly exhibited the equal radical scavenging activity, whereas all the other samples significantly showed lowest radical scavenging activity. The ability of extracts of *Amaranthus* samples to quench hydroxyl radicals seems to directly relate to prevention of propagation of the process of lipid peroxidation and the extract seems to be a good scavenger of active oxygen species, thus reducing rate of chain reaction. Yen and Hsieh attributed that the hydroxyl radical scavenging potential is combined effects of reducing power, donation of hydrogen atoms and scavenging of active oxygen [55].

3.4.6 Nitric oxide radical scavenging activity

In addition to reactive oxygen species, nitric oxide is also implicated in inflammation, cancer and other pathological conditions [35]. Nitric oxide (NO) is defence molecule with cytotoxic, microbiocidal and microbiostatic activities. Sodiumnitroprusside (SNP) will release nitric oxide when dissolved in PBS solution and reacts with oxygen to form nitrite. SNP solution under aerobic conditions, in the presence of various extracts with Griess reagent, can be used to evaluate the scavenging effect on nitric oxide of the extract. In the present study the extract competes with oxygen to react with (NO) and thus inhibits generation of anion. The percentage inhibition of acetone and methanol extracts of raw and processed samples of leaf, stem and flower of *A. dubius* and *A. nodiflora* are presented in the Fig 2. All the sample extracts exhibited radical scavenging activity ranging from 22.55%-34.13%. The highest radical scavenging activity was significantly observed in ANLBM (34.13%) and ANSRA (33.69%). The methanol extracts of *A. dubius* leaf blanched (23.92%), *A. dubius* stem raw (27.16%) and blanched (28.27%), *A. nodiflora* leaf (34.13%) and *A. nodiflora* flower (33.22%) blanched samples exhibited the highest radical scavenging activity than the acetone extracts. On the other hand, all the other samples exhibited the highest radical scavenging activity in acetone extracts than the methanol. The decreased radical scavenging activity samples were observed in acetone extract of *A. dubius* leaf (22.55%) and *A. nodiflora* stem (27.06%) than the raw sample. The processed blanched samples of methanol extract of *A. dubius* leaf (23.92%) and acetone extract of *A. dubius* stem (26.12%) significantly exhibited equal radical scavenging activity when compared with the raw sample. Interestingly, are the other processed samples exhibited highest radical scavenging activity respective than the raw sample. However, when compared with the standards quercetin (57.93%) and ascorbic acid (62.13%), all the sample extracts significantly exhibited lowest radical scavenging activity. The nitric oxide radical ($NO^{\bullet}$) which is produced *in vivo* by a variety of cell types, is an important bio-regulatory molecule with a number of physiological functions. However, under oxidative stress this reactive nitrogen species (RNS) reacts with other reactive species to produce more toxic RNS and reactive oxygen species (ROS) [49, 51, 50].

3.4.7 Superoxide radical scavenging activity

Superoxide anions ($O_2^{\bullet-}$) are the most common free radicals whose concentration increase under conditions of oxidative stress and are generated either by auto-oxidation processes or by enzymes and produce other cell damaging free radicals and oxidizing agents [31]. The percentage inhibition of superoxide radical of acetone and methanol extracts of raw and processed samples of leaf, stem and flower of *A. dubius* and *A. nodiflora* were presented in the Fig 3. All the *Amaranthus* sample extracts exhibited radical scavenging activity ranging from 39.52%- 77.61%. The highest radical scavenging activity was significantly observed in ADSBM (77.61%). The methanol extracts of *A. dubius* leaf raw (58.49%), blanched (70.78%), *A. dubius* stem blanched (77.61%), *A. nodiflora* flower raw (67.15%) and blanched (68.25%) sample exhibited the highest radical scavenging activity than the acetone extracts of the respective samples. On the other hand, the other *Amaranthus* sample showed highest radical scavenging activity in the acetone extract than the methanol. The decreased radical scavenging activities of processed sample were significantly observed in the acetone (41.47%) and methanol (39.52%) extracts of *A. nodiflora* stem than the raw sample. The insignificant radical scavenging activity of processed sample on comparison with raw were exhibited in the methanol extract of *A. nodiflora* leaf (59.46%), acetone (56.81%) and methanol (57.70%) extracts of *A. nodiflora* flower. Interestingly, all the

other amaranthus on comparison with the raw sample significantly exhibited the highest superoxide radical scavenging activity. However, when compared with standards BHA (68.33%) and trolox (48.31%) as showed in Fig 3, some samples exhibited highest radical scavenging activity, while other samples showed lowest radical scavenging activity. The reduced radical scavenging activities of processed samples of our study were similar to Siddhuraju reported in cowpea [43]. The lowest activity of hydrothermally processed samples may be attributed to the partial loss of active phytocompounds in the blanching process as well as decanting of liquid.

Table 1: Proximate composition of raw and processed leaf stem and flower of *A. dubius* and *A. nodiflora.*

Samples	Moisture content (%)	Crude ash (g/100 g) DM	Crude fiber (g/100 g) DM	Crude lipid (g/100 g) DM	Crude protein (g/100 g) DM	Carbohydrates (g/100 g) DM	Calorific value (MJ/Kg) DM
ADLR	6.30^{e}±0.14	25.38^{b}±1.22	6.70 ^{j}±0.18	1.31^{b}±0.72	12.36±0.12	54.25	11.63
ADLB	6.14^{f}±0.08	16.38^{c}±1.60	7.99^{h}±0.06	3.14^{ab}±0.13	14.12±0.45	58.37	13.28
ADSR	8.74^{a}±0.18	40.63^{a}±10.44	13.62^{f}±0.10	1.16^{b}±0.57	15.71±0.13	28.34	7.70
ADSB	7.45^{c}±0.11	24.63^{b}±7.04	14.81^{e}±0.04	1.36^{b}±0.70	18.22±0.10	48.98	11.73
ANLR	4.57^{i}±0.06	17.43^{c}±1.06	7.53^{i}±0.02	5.47^{a}±1.11	20.19±0.11	49.38	13.68
ANLB	6.18^{f}±0.16	17.00^{c}±2.73	10.25^{g}±0.10	5.59^{a}±1.61	26.45±0.15	40.71	13.32
ANSR	5.47^{h}±0.15	8.31^{d}±1.82	23.95^{b}±0.09	0.62^{b}±0.53	29.03±0.14	38.09	11.44
ANSB	6.57^{d}±0.10	9.97^{cd}±0.62	27.05^{a}±0.07	3.64^{ab}±4.82	30.13±0.13	29.3	11.29
ANFR	7.61^{b}±0.13	7.44^{d}±1.46	20.00^{d}±0.14	2.55^{ab}±1.63	9.32±0.09	60.69	16.12
ANFB	5.76^{g}±0.21	7.06^{d}±0.23	23.26^{c}±0.04	1.42^{b}±1.18	8.23±0.11	60.03	11.93

ADLR- *A. dubius* leaf raw, ADLB- *A. dubius* blanched, ADSR- *A. dubius* stem raw, ADSB- *A. dubius* stem blanched, ANLR- *A.nodiflora* leaf raw, ANLB- *A.nodiflora* leaf blanched, ANSR- *A.nodiflora* stem raw, ANSB- *A.nodiflora* stem blanched, ANFR- *A.nodiflora* flower raw, ANFB- *A. nodiflora* flower blanched. Values are means of triplicate determination ± standard deviation. Mean values followed by different letters in a column are significantly different (P<0.05)

Table 2: Extract yield, Total Phenolics, Tannins and Flavanoid Content of raw and processed leaf, stem and flower *A. dubius* and *A. nodiflora* extracts

Samples	Extract yield (%)	Total phenolics[a] (mg of GAE/g extract)	Total tannins[a] (mg of GAE/g extract)	Total flavanoids[b] (mg of RUE/g extract)
ADLRA	13.30	91.90^{i}±11.09	83.33^{gh}±9.29	123.33^{g} ± 5.51
ADLBA	9.70	131.42^{de}±6.54	107.61^{de}±5.77	384.22^{b} ±3.29
ADSRA	20.20	106.19^{h}±8.72	96.66^{efg}±7.33	24.84^{lm}±1.27
ADSBA	18.00	116.66^{fgh}±8.72	102.85^{def}±7.95	34.00^{klm}± 1.48
ANLRA	9.30	109.09^{gh}±16.05	102.38^{def}±13.57	281.33^{c}±31.67
ANLBA	12.30	113.33^{fgh}±4.59	105.71^{def}±4.28	79.33 ^{i}± 4.08
ANSRA	15.40	211.90^{b}±9.29	88.09^{g}±11.54	127.67 ^{g}± 4.00
ANSBA	36.40	146.19^{c}±7.19	143.33^{a}±8.72	213.44^{d}±21.43
ANFRA	7.30	149.52^{c}±10.03	135.71^{ab}±15.90	280.00^{c}±17.68
ANFBA	5.80	280.47^{a}±8.37	122.85^{bc}±9.36	878.89^{a}±10.18
ADLRM	11.70	71.90^{jk}±2.70	43.09^{l}±4.36	46.27^{jk}±3.14
ADLBM	3.30	110.47^{fgh}±4.75	63.33^{ij}±6.15	188.56^{e}±3.15
ADSRM	3.70	65.47^{k}±5.36	59.28^{jk}±3.27	17.69^{m}±0.38
ADSBM	3.80	131.90^{de}±3.66	42.14^{l}±3.77	45.60^{jk}±6.24
ANLRM	2.10	109.76^{fgh}±4.75	73.80^{hi}±8.93	77.60^{i}±7.25
ANLBM	2.30	120.47^{efg}±7.15	113.57^{cd}±2.57	158.33^{f}±7.55
ANSRM	3.10	91.42^{i}±4.28	43.57^{l}±4.68	27.20^{klm}±1.75
ANSBM	3.20	79.04^{ij}±4.06	47.85^{kl}±3.97	42.67^{d}±1.06
ANFRM	2.20	123.57^{ij}±2.85	52.38^{jkl}±5.72	55.37^{j}±5.08
ANFBM	2.60	148.33^{cd}±3.93	91.42^{fg}±3.97	101.11^{h}± 8.33

Each value is expressed as mean ± standard deviation (n = 3).ADLRA- *A. dubius* leaf raw acetone extract; ADLBA-*A.dubius* leaf blanched acetone extract; ADSRA- *A.dubius* stem raw acetone extract; ADSBA- *A.dubius* stem blanched acetone extract; ANLRA-*A. nodiflora* leaf raw acetone extract; ANLBA- *A.nodiflora* leaf blanched acetone extract; ANSRA-*A.nodiflora* stem raw acetone extract; ANSBA- *A.nodiflora* stem blanched acetone extract; ANFRA-*A.nodiflora* flower raw acetone extract; ANFB- *A.nodiflora* flower blanched acetone extract; ADLRM-*A.dubius* leaf raw methanol extract; ADLBM- *A.dubius* leaf blanchedmethanol extract; ADSRM-*A.dubius* stem raw methanol extract; ADSBM-*A.dubius* stem blanched methanol extract; ANLRM-*A.nodiflora* leaf raw methanol extract; ANLBM- *A.nodiflora* leaf blanched methanol extract; ANSRM-*A.nodiflora* stem raw methanol extract; ANFBM- *A.nodiflora* stem blanched methanol extract; ANFRM-*A.nodiflora* flower raw methanol extract; ANFBM-*A.nodiflora* flower blanched methanol extract.

[a]Total phenolic and tannin content are expressed as Galic acid equivalent (GAE); [b]Flavanoids are expressed as rutin equivalents (RUE). Mean values followed by different letters in a column are significantly different (P<0.05).

Table 3: DPPH radical, FRAP, ABTS cation radical and metal chelating activity of raw and processed leaf, stem and flower of *A. dubius* and *A. nodiflora* extracts

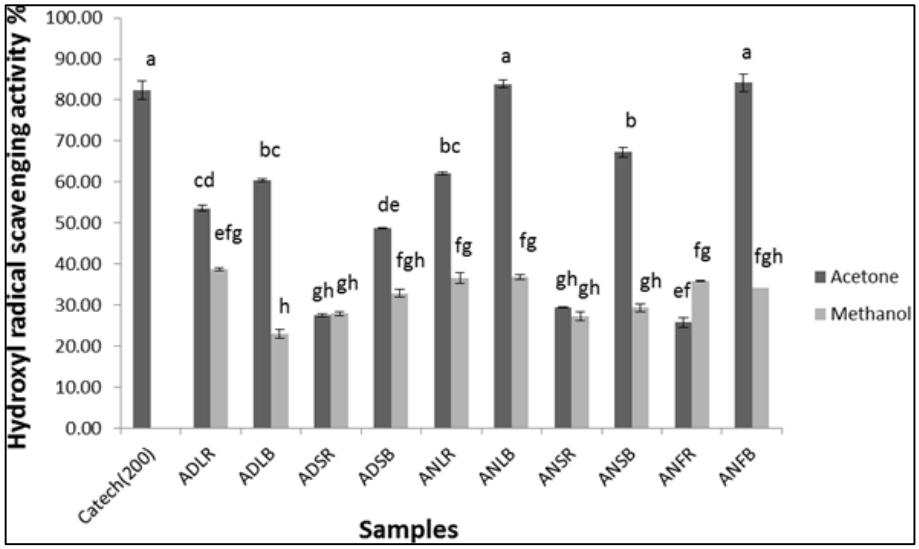
Samples	FRAP[b] (mom lFe (II)/g extract)	Metal chelating activity (mg EDTA/g extract)	TEAC[a] (mmol/g extract)	
			DPPH assay	ABTS assay
ADLRA	17536.23^{c}±308.46	39.42^{g}±0.45	54721.74^{e}±88.86	8708.33^{c}±425.43
ADLBA	18188.40^{c}±181.01	34.08^{h}±0.38	46436.88^{f}±181.64	5541.67^{c}±260.21
ADSRA	8188.40^{c}±326.32	20.57^{l}±0.80	54023.66^{e}±88.86	5229.17^{c}±260.21
ADSBA	7710.14^{c}±239.45	9.68^{r}±0.27	55361.64^{e}±177.72	14770.83^{c}±629.15
ANLRA	34043.47^{c}±230.06	30.82^{i}±0.13	23014.83^{g}±57.71	20395.83^{c}±381.88
ANLBA	18855.07^{c}±239.45	14.29^{n}±0.38	58599.96^{e}±146.36	4500.00^{c}±496.08
ANSRA	22710.14^{c}±437.67	7.46^{s}±0.24	58735.7^{e}±146.39	6520.83^{c}±236.62
ANSBA	26202.89^{c}±196.05	26.87^{j}±0.28	46611.4^{f}±133.29	7729.17^{c}±95.47
ANFRA	42217.39^{c}±501.41	11.05^{o}±0.79	183827.8^{d}±726.58	11458.33^{c}±200.91
ANFBA	11362.31^{c}±289.49	19.45^{m}±0.24	245607.9^{c}±786.95	13083.33^{c}±366.22
ADLRM	14376.81^{c}±109.41	41.68^{e}±0.17	53713.4^{e}±88.86	1416.67^{c}±236.62
ADLBM	24231.88^{c}±262.07	11.00^{p}±0.20	47571.26^{f}±153.22	10229.17^{c}±260.21
ADSRM	7420.28^{c}±205.47	21.02^{l}±0.27	54799.3^{e}±209.74	9666.67^{c}±200.91
ADSBM	11478.26^{c}±115.03	23.9^{k}±0.80	54198.18^{e}±204.29	13270.83^{c}±401.82
ANLRM	29869.56^{c}±86.95	44.54^{d}±0.70	57068.06^{e}±209.74	9833.33^{c}±95.47
ANLBM	26797.10^{c}±478.91	47.48^{c}±0.30	59414.39^{e}±286.91	11479.17^{c}±157.29
ANSRM	15695.65^{c}±230.06	52.96^{a}±0.30	56544.5^{e}±461.73	2208.33^{c}±95.47
ANSBM	15304.34^{c}±156.76	49.15^{b}±0.44	55574.95^{e}±187.00	1500.0^{c}±437.50
ANFRM	20072.46^{c}±305.38	27.48^{j}±0.20	57300.76^{e}±116.34	4958.33^{c}±130.10
ANFBM	22913.04^{c}±285.10	40.25^{f}±0.54	58231.53^{e}±153.91	9000.00^{c}±62.50
ASC	730676.32^{a}±91181.57	-	493310.06^{a}±11253.9	597916.67^{b}±104877.7
TRO	641061.80^{b}±55986.56	-	-	-
BHA	-	10.49^{q}±0.06	386368.04^{b}±9622.07	654356.06^{a}±61742.13

Each value is expressed as mean ± standard deviation (n = 3). ADLRA- *A. dubius* leaf raw acetone extract; ADLBA-*A.dubius* leaf blanched acetone extract; ADSRA- *A.dubius* stem raw acetone extract; ADSBA- *A.dubius* stem blanched acetone extract; ANLRA-*A. nodiflora* leaf raw acetone extract; ANLBA- *A.nodiflora* leaf blanched acetone extract; ANSRA-*A.nodiflora* stem raw acetone extract; ANSBA- *A.nodiflora* stem blanched acetone extract; ANFRA-*A.nodiflora* flower raw acetone extract; ANFB- *A.nodiflora* flower blanched acetone extract; ADLRM-*A.dubius* leaf raw methanol extract; ADLBM- *A.dubius* leaf blanchedmethanol extract; ADSRM-*A.dubius* stem raw methanol extract; ADSBM- *A.dubius* stem blanched methanol extract; ANLRM-*A.nodiflora* leaf raw methanol extract; ANLBM- *A.nodiflora* leaf blanched methanol extract; ANSRM-*A.nodiflora* stem raw methanol extract; ANFBM- *A.nodiflora* stem blanched methanol extract; ANFRM-*A.nodiflora* flower raw methanol extract; ANFBM-*A.nodiflora* flower blanched methanol extract. ASC, Ascorbic acid; TRO, Trolox; BHA,Butylated hydroxyl anisole. Mean values followed by different letters in a column are significantly different (P<0.05).

[a] TEAC (Trolox equivalent antioxidant capacity) were assayed by the ABTS and DPPH methods. Data expressed as millimoles of trolox equivalents per g extract

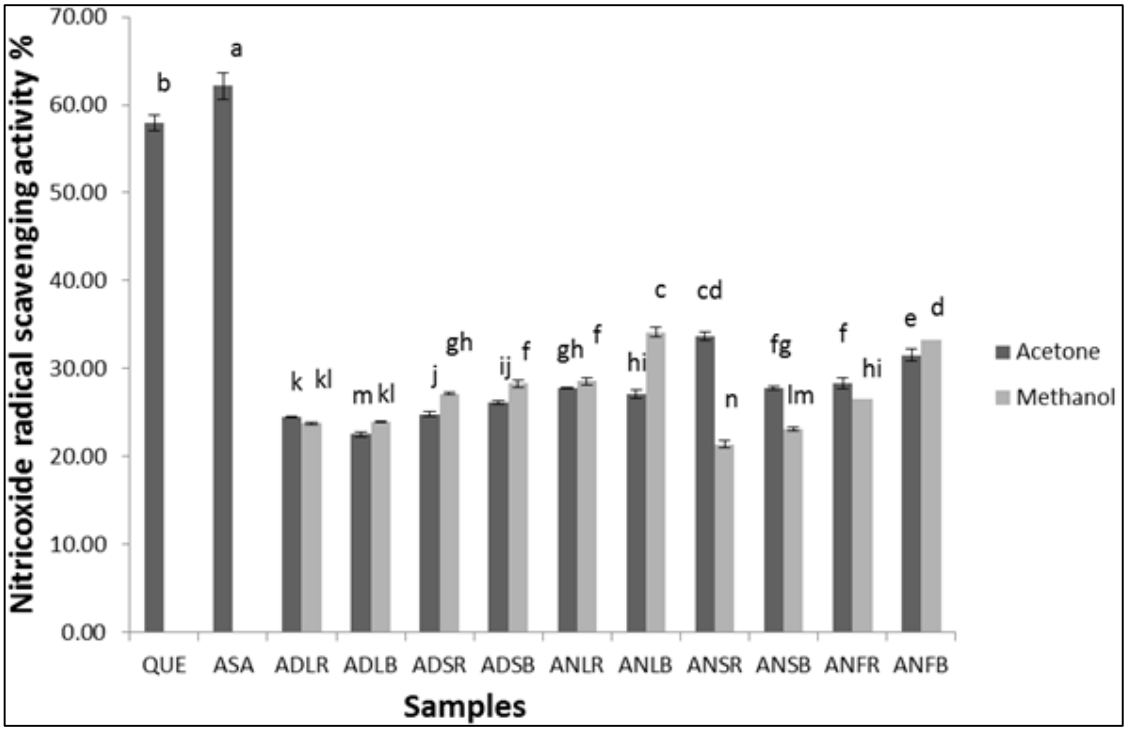
[b] Concentration of substance having ferric-TPTZ reducing ability expressed as mmol Fe (II) equivalents.

[c] Concentration of substance having chelating ability expressed as mg EDTA equivalents per g extract



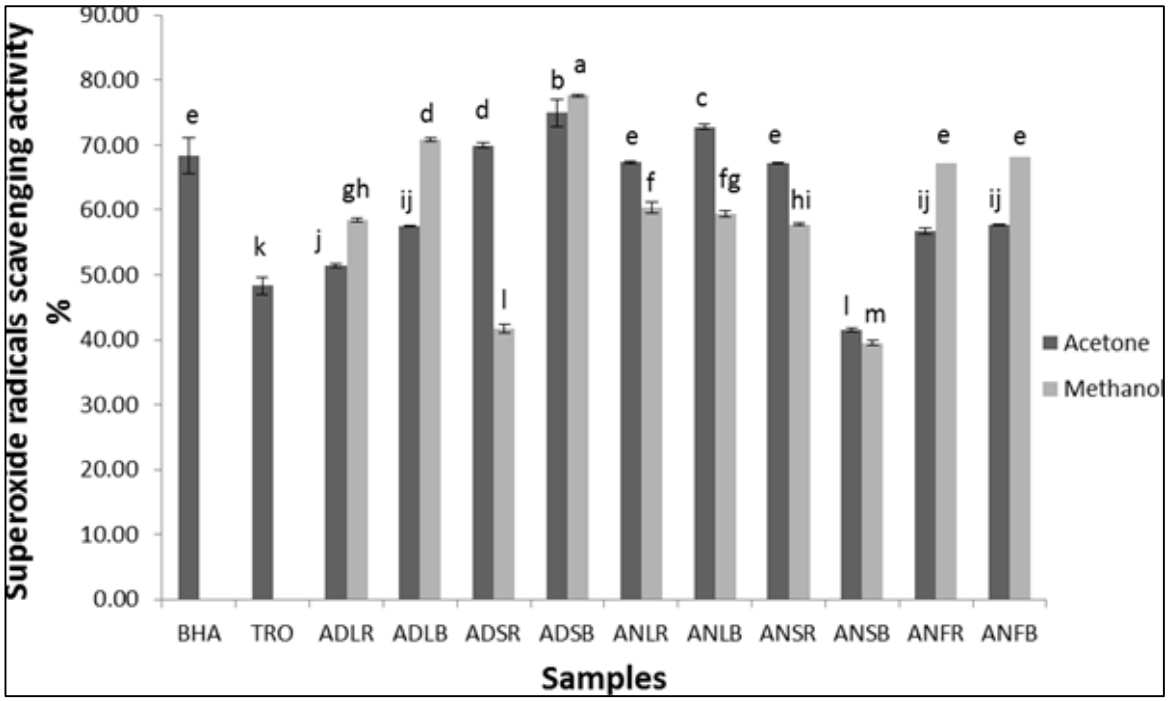
Hydroxyl radical scavenging activity of acetone and methanol extracts of *A. dubius* and *A. nodiflora* leaf stem and flower of at the concentration of 200µg/ml in the reaction mixture; Catechin, ADLR- *A. dubius* leaf raw, ADLB- *A. dubius* blanched, ADSR- *A. dubius* stem raw, ADSB- *A. dubius* stem blanched, ANLR- *A.nodiflora* leaf raw, ANLB- *A.nodiflora* leaf blanched, ANSR- *A.nodiflora* stem raw, ANSB- *A.nodiflora* stem blanched, ANFR- *A.nodiflora* flower raw, ANFB- *A.nodiflora* flower blanched. Values are means of triplicate determination ± standard deviation. Bars having different letters are significantly different (P<0.05)

Fig 1: Hydroxyl radical anion scavenging activity of raw and processed leaf, stem and flower of *A. dubius* and *A. nodiflora* extracts.



Nitricoxide radical scavenging activity of acetone extracts of *A.dubius* and *A.nodiflora* leaf stem and flower at the concentration of 500µg/ml in the reaction mixture; QUE;querce, ASC;Ascorbic acid, ADLR- *A. dubius* leaf raw, ADLB- *A. dubius* blanched, ADSR- *A. dubius* stem raw, ADSB- *A. dubius* stem blanched, ANLR- *A.nodiflora* leaf raw, ANLB- *A.nodiflora* leaf blanched, ANSR- *A.nodiflora* stem raw, ANSB- *A.nodiflora* stem blanched, ANFR- *A.nodiflora* flower raw, ANFB- *A.nodiflora* flower blanched.. Values are means of triplicate determination ± standard deviation. Bars having different letters are significantly different (P<0.05).

Fig 2: Nitricoxide radical scavenging activity of raw and processed leaf, stem and flower of *A. dubius* and *A. nodiflora* extracts.



Superoxide radical scavenging activity of acetone extracts of *A.dubius* and *A.nodiflora* seeds at the concentration of 150µg/ml in the reaction mixture; BHA-Butylated hydroxyl toluene; TRO; Trolox; ADLR- *A. dubius* leaf raw, ADLB- *A. dubius* blanched, ADSR- *A. dubius* stem raw, ADSB- *A. dubius* stem blanched, ANLR- *A.nodiflora* leaf raw, ANLB- *A.nodiflora* leaf blanched, ANSR- *A.nodiflora* stem raw, ANSB- *A.nodiflora* stem blanched, ANFR- *A.nodiflora* flower raw, ANFB- *A.nodiflora* flower blanched. t. Values are means of triplicate determination ± standard deviation. Bars having different letters are significantly different (P<0.05)

Fig 3: Superoxide anion radical scavenging activity of raw and processed leaf, stem and flower of *A. dubius* and *A. nodiflora* extracts.

4. Conclusion

Indigenous food plants are usually considered inferior and associated with a low standard of living. In order to remove this low self-esteem with regard to our local food plants, there is need to present them in a state that is acceptable in current food markets. In addition, Indigenous food plants are adapted to withstand adverse local conditions. This aspect should be taken advantage of in their cultivation for commercial purposes. This is also the reason why wild food plants are a major food source during times of famine. Studies on the nutritional composition of wild food plants are limited and incomplete, is an area that demands. Such knowledge will enhance the appreciation of wild food plants by local communities and can promote their appreciation as alternative sources of nutrition against the threatening of commercial available vegetable. Based on the advantageous of the indigenous plants, the underutilized *Amaranthus* species *A. dubius* and A. *nodiflora* were analyzed for proximate, phytochemical composition and antioxidant activity on the both raw and processed sample. Both the samples are highly rich in protein, dietary fiber, starch, micronutrients and bioactive compounds with low level of fat. The food industry should help consumers as regards the optimal cooking methods for maintenance of the antioxidant and nutrient properties of vegetables. The fact that the antioxidant activity of some *Amaranthus* vegetables plant parts increased with cooking suggests that the prooxidant activity was due to peroxidases which were inactivated at high temperatures. Because the antioxidant capacity of plant tissues is strongly influenced by a number of factors, it is very difficult to extrapolate the effects of the consumption of processed plants foods on human health. However, this study may contribute to performing more accurate estimations of antioxidants in the diet. Also ‘‘ready-to-eat” vegetables show antioxidant activity after they have been submitted to heat treatment to increase their shelf-life. Blanching of vegetable does not cause the loss of nutrients and antioxidant properties. In the samples, blanching might actually increase the availability of the natural occurring antioxidant components besides improving the palatability of the vegetable. Therefore, moderate blanching time and proper handling of vegetable are important in order to preserve the antioxidant and nutritional properties. The proper conservation and cultivation of these underutilized leafy vegetable will serve as a low cost effective food and can solve the present problems in overcoming scarcity of conventional crops in human consumption.

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