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Studies on mineral profile and antioxidant potential of differentially processed lesser known green leafy vegetables (*Achyranthes aspera* L. and *Talinum triangulare* L.)

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Abstract
Indigenous leafy vegetables have been recognized as rich sources of natural antioxidants, hence having the power to perform preventive effects in opposition to serious disorders which are related to oxidative stress. They contain trace elements, which are act as dietary constituents and play important role in human system. Present study evaluated the proximate composition and mineral profile, contribution to nutritional significance, and antioxidant potential of two lesser known greens, *Achyranthes aspera* and *Talinum triangulare*. Different processing methods are adopted to assess their influence on nutritive value and antioxidant properties. Mineral contents were studied by employing ICP-MS (Inductively Coupled Plasma - Mass Spectrometer). Using different in vitro models, antioxidant potential of raw and processed samples were determined. Compared to raw, processed samples registered better values. Results revealed that cooking has influenced the mineral profile and antioxidant capacity. The presence of essential minerals such as Na, K, Zn and Mn were observed. Current study suggests that lesser known leafy vegetables efficiently contribute to the nutritional requirement and food security.

Keywords: antioxidants, minerals, leafy vegetables, *achyranthes aspera*, *talinum triangulare*

Introduction
Leafy vegetables are considered as primary food class and regular ingredient in the diet; because they can provide appreciable amount of nutrients in comparison to other fruit and seed plants. To sustain equilibrium among the increased population and agricultural output in most part of the developing nations, a lot of wild food plants which are possibly valuable for human being have been recognized. Starch based foods are the major staple food in underdeveloped regions, which supply energy and protein requirements [1]. Since the prehistoric periods, Green leafy vegetables (GLVs) are utilized as a food resource as they provide nourishment essential for the maintenance of life and help to humans stay healthy. In countryside where non-indigenous species are not accessible, traditional vegetables are valuable sources of nutrition [2].

The utilization of plant products is a primary necessity for human health, and leafy vegetables take a vital and nutritious role in this aspect, in particular for rural populations. The intake of leafy vegetables takes part in a key function in retaining a stable diet and aids to prevent the unending effects of malnutrition [3]. They are chief supplier of vitamins and trace elements required for the regular functioning of the body. Consumption of GLVs has reported to contribute in lowering the risk of cataract and they contain essential antioxidants in neutralizing free radicals.

Epidemiological studies point out that high intake of plant foods in the diet is correlated with reduced risk of constantly recurring health conditions, for example neurodegenerative and heart diseases [3]. To some extent, the positive outcomes of plant produces, particularly vegetables, are characterized to the biological activities of their phytochemical components, like phenolic compounds, vitamins, carotenoids, flavonoids, saponins and iridoids [4-7]. These biologically active compounds are accountable for a large number of biological properties, which include antioxidant, anti-inflammatory, and antimicrobial activities. Due to their antioxidant activity, [8] phenolic compounds have turn into a measurable indicator of the dietary value of food.

Indigenous leafy vegetables are given powerful defensive effects in opposition to major ailments related to oxidative damage [9] since they possibly supply phenolic compounds and other phytochemicals that will add antioxidant activity in diet [10]. The powerful chain-breaking moreover free radical scavenging ability is characterized by antioxidant activity of phenolic compounds; in that way they are providing protection against reactive oxygen species[7]. Reactive oxygen species (ROS) is a whole class of extremely reactive molecules obtained from the chemical processes of oxygen; furthermore, is frequently produced as derivatives of natural responses or tests on living organisms, a few of these ROS take part in supportive functions in cell structure. Tissue breakages as well as oxidative damage to nucleic acids and proteins are caused by overproduction of ROS [11]. RNS (reactive nitrogen species) and ROS are described free radicals and acting together to damage cells [12]. Free radicals are standing for a class of very reactive and intermediary chemical elements whose reactivity is gained through the occurrence of odd electron in their configuration, which are able to survive independently for very short time interval [13]. They carry out a lot of significant roles in our bodies includes managing the stream of blood through arteries, to fight with diseases and also to maintain our brain attentive and in focus. A few free radicals destroy cancer cells. But, in fact certain cancer drugs intend to raise the amount of free radicals in body [14].

Free radicals are on the other hand, not always beneficial. Their adverse effects were discovered in the last decade. Cell

membranes are made up of unsaturated lipid molecules which are mostly liable to influenced by free radicals [15]. Disturbance in the balance between ROS is able to occur as cells cannot wipe out the overload of free radicals produced. It can be stimulate an array of persistent along with progressive deterioration causing diseases, progression of aging and quite a lot of severe pathology (physical injuries, brain damage) [12].

Antioxidants are every substance that delay or reduces oxidative injury to an object molecule. Generally, the antioxidants can act by chain breaking reaction (eg. α-tocopherol), by reducing the concentration of ROS (eg. glutathione), or by removing the originated radicals (eg. superoxide dismutase) [16]. Eradication of artificial antioxidants in food applications has given extra impulsion to discover natural supply of antioxidants. In GLVs, presence of numerous powerful nutritional antioxidants (Vit A, C and E), microelements (Cr, Se, Mn, Fe etc.) and phytochemicals (flavonoids, phenolic compounds and tannins) have been found.

Green leafy vegetables have been recognized as excellent sources of natural antioxidants for example tocopherols, vitamin C and polyphenols which are accountable for retaining healthiness and antagonistic towards coronary heart diseases and cancer. In developing countries where access to animal food is restricted, GLVs significantly contribute to fighting retinol deficiencies by being rich sources of the provitamin A and β-carotene, without any bioavailability issues [2]. The most important phytochemicals having been discovered in fruits as well as leafy vegetables which are engaged in making stable the free radicals consists of flavonoids, tannins and phenolic compounds [18-19].

Amaranthus plants have been accounted as one of the vegetables abundant in antioxidant elements. Polyphenols were established as the chief antioxidant elements in extracts of *A. Lividus* in a previous study [20-21]. *Achyranthes aspera L*. is dispersed as weed all through India, humid areas in Asia and other parts of the world. Existence of a group of naturally occurring compounds such as steroid alcohols, alkaloids, saponins, cardiac glycosides and ecdysterone from distinct parts of this plant is revealed by several phytochemical investigations [22]. *A. aspera* showed high amount of all necessary macronutrients including proteins, fats and carbohydrates and, micronutrients like vitamins and minerals [23]. *Talinum triangulare* (water leaf) is adaptable throughout the country. It spreads easily and becoming an agricultural weed. High moisture content was reported in *T. triangulare* in the previous study of proximate composition among four different leafy vegetables (*Corchorus olitorius, Ocimum gratissimum, Talinum triangulare* and *Telfaria occidentalis*) [24]. Results of earlier studies revealed that leaves of *T. triangulare* contain an appreciable amount of flavonoids, alkaloids, saponins and low level of toxicants like tannins, since it contains substantial amount of bioactive compounds [25]. Nutritionally, water leaf has been shown to possess the essential nutrients like β-carotene, minerals (such as calcium, potassium and magnesium), pectin, protein and vitamins [26]. While carotenoids are the biological antioxidants, high level of β-Carotenoids proved the vegetable is excellent for managing oxidative stress and cardiovascular diseases.

Increasing evidences put forward that majority of the bioactive and phytochemical components in plants impart physiological activities and can offer various health advantages for example, antioxidant, antibacterial and anti-inflammatory activity [27]. Epidemiological evidences indicate that benefits of vegetables on the danger of aging cannot entirely due to the antioxidants but also other phytochemicals present in them. Growing requirement for dietary antioxidant sources has generated the exploration of novel and cost-effective bioresources that are fulfilling several functions, and also have the free radical scavenging potential [28]. As it is very important and adds urgency to the search for new infection-fighting strategies, the knowledge of bioactive components in green leafy vegetables will help to discover the vegetables with good potential [29]. Lots of research inquiries have exposed the presence of compounds having biological effects in the food stuffs and influence of processing methods on them. Though both *A. aspera* and *T. triangulare* are rich in minerals, proteins and phytochemicals their fate after processing was not studied in deep yet. Hence, the current study is seek to evaluate the effect of indigenous processing techniques such as boiling and blanching, on proximate composition, mineral profile as well as antioxidant activity of *A. Aspera* and *T. triangulare*. Findings from this first round investigation might be offer advanced idea about antioxidant activity of the plants which may be useful in developing value-added foods.

Materials and Methods

Chemicals

Folin-ciocalteu reagent, Sodium carbonate, Poly(vinyl-polypyrrolidone), Sodium nitrite, Aluminium chloride, Sodium hydroxide, Ferric chloride, 2,2'-diphenyl-1-picrylhydrazyl (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), Ferrous chloride, Ammonium thiocyanate, Hydrogen peroxide, Ferrous Ammonium Sulfate, Ethylene Diamine Tetra Acetic acid (EDTA) disodium salt and were obtained from Hi Media, Merck and Sigma. All other reagents used were of analytical grade. All analysis was performed with UV- Visible Spectrophotometer (Cyberlab – UV 100, USA).

Samples and Processing Methods

The mature tender whole plant of *T. triangulare* and *A. aspera* were collected from Palakkad, Kerala, and Bharathiar University campus, Coimbatore, Tamil Nadu, India respectively and were identified morphologically. After collection the samples were washed with tap water to remove debris and damaged portions. The mature leaves of *A. aspera* and *T. triangulare* were separated from the stem completely and processing methods were adopted.

The leaves of *A. aspera* and *T. triangulare* were randomly divided into three equal parts. One part of leaves were chopped and dried at 40°C in incubator without any treatment. Second part was chopped and boiled at 100°C for 10 min in the ratio 1:10 (w/v), and third part of the sample was chopped and blanched in boiling water at 100ºC for 20 min in the ratio 1:10 (w/v). After decanting the water, boiled and blanched samples were cooled and dried separately. The dried materials were ground (particle size of about 0.25 mm) and stored for further analysis.

Proximate analysis

The Moisture content of raw and processed samples was determined using Moisture Analyzer MA35 (Sartorius AG, Germany) at 105°C. Micro-Kjeldahl method was used to determine the total nitrogen and a nitrogen-protein conversion

factor (N×6.25) is used for Crude protein determination. Crude lipid (Soxhlet extraction), Crude fiber and Ash contents (Gravimetric) were also determined based on the methods of AOAC, 1990. The proximate composition was expressed in g/100 g DM.

Mineral Analysis

A. aspera and *T. triangulare* leaf samples were digested using triacid. Triacid was prepared with Nitric acid, Sulphuric acid and Perchloric acid in the ratio of 9:2:1. 10 mL of triacid was added to 200 mg of sample and digested at 80°C in fume hood. After digestion the samples were made up to 100 mL with distilled water (HPLC Grade). 3 – 4 mL of samples were used to analyze all the 17 minerals through the ICP-MS (Nex Ion 300 X, Perkin Elmer, USA).

Solvent Extraction

The raw and processed samples (15g) were extracted separately with 70% acetone and 80% methanol (1:7 w/v) by occasional stirring for 48 h at room temperature. The extracts were filtered, and pooled. The filtrates were re-extracted with 70% acetone and 80% methanol (1:5 w/v) for 24 h at room temperature and filtering through WhatmannNo.4 filter paper. Both the extracts were mixed. The solvent of pooled extracts was evaporated under low temperature at 40°C in incubator (NSW, New Delhi). The extract gained was used for further analysis and the percentage of recovery was calculated by following equation:

$$\text{Recovery \%} = \frac{[(\text{Extract + container weight (g)}) - \text{Empty container (g)}]}{\text{Sample weight (g)}} \times 100$$

Estimation of Total phenolics and Tannins

The total phenolics and tannins were quantified as tannic acid equivalents (TAE) [30] from tannic acid standard curve (3–15 µg range). For the test, 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 consecutively to each tube. Later on swirling the reaction mixture, 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 treated with 100 mg of polyvinyl polypyrrolidone (PVPP) and incubated at 4° C for 4 h. Then the samples were centrifuged at 3000 rpm and the supernatant was collected. This supernatant comprised only with simple phenolics other than tannins (tannins would have been precipitated along with PVPP). Then using this supernatant, tannins were estimated by same method as phenolics. The analysis was carried out in triplicate and the phenolics and tannins were expressed as mg tannic acid equivalents (TAE)/g extract. The tannin content of the samples was determined as follows:

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

Estimation of Total flavonoids

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 of water. At the beginning of the experiment, 0.3 mL of 5% sodium nitrite was added to the flask. After 5 min, 3 mL of 10% aluminium chloride solution was added. At 6 min, 2 mL of 1 mol/L sodium hydroxide was added to the mixture. Instantly, the solution was diluted to a final volume of 10 mL with water and mixed systematically. The absorbance was measured at 510 nm against the prepared blanks with UV-Visible Spectrophotometer. Total flavonoid content was expressed as mg rutin (RUT)/g extract [31].

Ferric reducing antioxidant power (FRAP) assay

The antioxidant ability of phenolic extracts of raw and processed samples was estimated [32]. 900 µL of freshly prepared FRAP reagent incubated at 37°C, was mixed with 90 µL of distilled water and 30 µL of test sample, or methanol (for the reagent blank). The samples and reagent blank were incubated at 37° C for 30 minutes 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. At the end of incubation, absorbance readings were taken immediately at 593 nm using a UV-Visible 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 which 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 test equal to theoretical absorbance value of a 1 mmol/L concentration of Fe (II) solution, which was determined using the related regression equation.

Metal chelating activity

100 µL of the extracts were added to 0.05 mL of $FeCl_2$ (2 mmol/L) solution. The reaction was initiated by the addition of 0.2 mL of 5 mmol/L ferrozine, and the mixture was shaken strongly and kept for standing at room temperature for 10 minutes. Absorbance of the solution was then measured at 562 nm using a Spectrophotometer. 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 [33].

Stable free radical scavenging activity using DPPH˙ method

The radical scavenging activity of raw and processed sample extracts, ASC (Ascorbic acid) and BHA (Butylated Hydroxyl Anisole) were measured with regard to hydrogen donating or radical scavenging ability, using the DPPH method [34] with minor alterations. 0.1 mL of extract was prepared in methanol and mixed with 3.9 mL of DPPH (6×10^{-5} mol/L methanol) solution. The solution was incubated at room temperature for 30 minutes and at the end of incubation period, decrease in absorbance was determined at 515 nm with a spectrophotometer. The trolox standard was prepared in the range of 0-2.5 mmol/L. The concentration of DPPH was calculated from trolox standard graph and expressed as mmol trolox equivalents/g extract.

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

ABTS was dissolved in water to a 7 mmol/L concentration. ABTS stock solution reacts with 2.45 mmol/L Potassium persulfate (final concentration) to produce ABTS radical cation ($ABTS^{\cdot+}$) and let the mixture to stand in the dark at room temperature for 12-16 hours prior to use. Before starting the

assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated to 30°C to give an absorbance of 0.700 ± 0.020 at 734 nm in a 1 cm cuvette. The stock solution of sample extracts in ethanol was diluted in such a way that, after the introduction of 10 µL aliquot of each dilution into the assay, they produced within 20-80 %inhibition of blank absorbance. After the addition of 1 mL of diluted ABTS solution to 10 µL of samples or Trolox standards (final concentration 0-15 µmol/L) in ethanol, OD (optical density) was taken at 30°C just 30 minutes after the initial mixing. Suitable solvent blanks were also run in each assay. Triplicate determinations were prepared at each dilution of standard and percentage inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration [31]. The unit of total antioxidant activity is defined as the concentration of Trolox having equivalent antioxidant activity and expressed as µmol/g sample extracts using the calibration curve of trolox. Linearity range of the calibration curve was 0.25-1.25 mmol/L. The total antioxidant activity of ASC and BHA were also measured by this method for comparison.

Statistical analysis

The data were brought to one-way Analysis of Variance (ANOVA), and the significance of the difference between means was 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 determination ± Standard deviation.

Result and Discussion

Proximate composition

Proximate composition of the leaves of *A. aspera* and *T. triangulare* were summarized in Table 1a. The parameters determined were Moisture, Ash, Crude protein, Crude lipids, and Crude fiber. The moisture content observed for dried flour of raw and processed samples are ranged from 6.61-9.24%. There was no difference in moisture content among raw and processed samples. Leafy vegetables with relatively higher moisture content are prone to deterioration; therefore proper care should be taken for their preservation. On the other hand, high moisture content has positive influences, which can helps to attain better activity for water dissolved enzymes and co-enzymes that are required in metabolic activities [35-36]. The protein content of these leafy vegetables was ranged from 19.23-25.76 g/100g DW. *A. aspera* has been found to be more protein content than *T. triangulare*. The highest protein content was registered in *A. aspera* raw leaves (25.76 g/100g). Protein content achieved in this study was little higher than earlier reports[1]. Plant foods with above 12 % energy content have been considered as good supply of proteins [35]. Among the samples studied, lipid content was found to be higher in *T. triangulare* boiled leaves (12.33 g/100g DM) and the range was 3.35-12.33 g/100g. Ash content showed a wide range from 2.16 - 5.05 g/100g with *A. aspera* boiled and *T. triangulare* raw samples. Occurrence of high ash content indicates the sample is a storehouse of minerals [38]. The leafy vegetables employed in this study were exhibited great fiber content in contrast to previous works (Chinese cabbage- 2.2 g/100g) [39].

Table 1a: Proximate composition of *A. aspera* and *T. triangulare* leaves

Samples	Moisture content (%)	Lipid (g/100g)	Ash (g/100g)	Fiber (g/100g)	Protein (g/100g)
AR	8.59^{b}± 1.25	4.89^{e}± 0.44	3.73^{c}± 0.04	7.57^{e}± 1.69	25.76^{a}± 3.71
ABO	6.61^{e}± 0.83	5.38^{d}± 3.25	2.16^{f}± 0.02	37.77^{a}± 14.45	21.25^{c}± 1.46
ABL	9.25^{a}± 0.35	3.35^{f}± 0.27	2.32^{e}± 0.02	31.23^{b}± 13.86	19.23^{cd} ± 2.32
TR	8.1^{c}± 0.16	6.72^{c}± 0.59	5.05^{a}± 0.09	7.0^{e} ± 0.53	19.24^{cd}± 0.52
TBO	8.21^{d}± 0.59	12.33^{a}± 2.54	3.59^{d} ± 0.02	12.73^{d} ± 2.39	22.26^{b}± 3.30
TBL	8.44^{b}± 0.29	9.38^{b}± 0.86	4.26^{b} ± 0.36	25.37^{c}± 23.95	21.41^{c} ± 2.49

AR- *A. aspera* Raw, ABO- *A. aspera* Boiled, ABL- *A. aspera* Blanched, TR- *T. triangulare* Raw, TBO- *T. triangulare* Boiled, TBL- *T. triangulare* Blanched

Values are means of triplicate determination ± standard deviation. Mean values followed by different letters in a column are significantly different ($P<0.05$).

Mineral profile

Mineral profile of *A. aspera* and *T. triangulare* leaves are presented in Table 1b. From the analysis presence of essential minerals such as Na, Al, K, Zn, Mn were confirmed. Sodium and potassium are the main cations located within and outside the cell correspondingly, and engaged in the regulation of plasma volume, acid-base balance and, nerve and muscle contraction [40]. The result showed that all raw and processed samples having high amount of Na except *T. triangulare* blanched sample. Potassium was detected only in *A. aspera* boiled and blanched samples. The *T. triangulare* boiled sample having high amount of Fe (26.00 mg/100g). Iron content of the considered leafy vegetables is more than the suggested dietary allowance meant for males (1.37mg/day) and females (2.94 mg/day) [41]. Fe is a key component in the human body, which performs several biochemical functions such as, infection control, cell mediated immunity and oxygen binding in haemoglobin. It can act as the main catalytic centre in various enzymes as the cytochrome oxidase [42-43]. Cu is a necessary micronutrient, can act as biocatalyst, and have need for body pigmentation additionally with iron, to keep up a healthy central nervous system and prevent anemia. When considering Cu content in plants, most of them contain fewer amounts which are insufficient for usual development and is make sure through synthetic and inorganic fertilizers. The analyzed leaf samples have high Cu content than previously studied Nigerian leafy vegetables [44]. Zn is essential for nerve function, male fertility and reproduction especially in the development of testes and ovary [45]. The amount of Mn and Zn was high in *T. triangulare* (14.18-16.71 mg/100g and 12.35-13.9 mg/100g) than *A. aspera* (6 - 8.4 mg/100g and 3.95-4.67 mg/100g) respectively. Therefore, utilization of these leafy vegetables can lessen the possibility of Zn deficiency which can result in retarded growth and delayed sexual maturity. Highest aluminium content was detected in *A. aspera* raw sample (28.75 mg/100g). Both raw and processed samples of *T. triangulare* have shown very less amount of Mg (0.09-0.094 mg/100g). All the samples showed very low amount of Cd (0.014 – 0.019 mg/100g) and trace amounts of Se, Be, Cr and Pb.

Table 1b: Mineral profile of *A. aspera* and *T. triangulare* leaves

Mineral	AR (mg/100g)	ABO (mg/100g)	ABL (mg/100g)	TR (mg/100g)	TBO (mg/100g)	TBL (mg/100g)
Be	0.149 ^{f}± 0.02	0.204^{a}±0.03	0.199^{b}±0.01	0.16^{e}±0.004	0.16^{d}±0.04	0.180^{c}±0.05
Na	43.92^{a} ± 1.83	21.13^{c}±6.35	22.55^{c}±4.02	41.86^{ab}±6.35	41.3^{b}±4.42	13.60^{d}±23.5
Mg	0.401^{a}± 0.02	0.25^{c}±0.01	0.3183^{b}±0.036	0.09^{d}±0.01	0.091^{d}±0.003	0.094^{d}±0.01
Al	28.75^{a} ± 5.59	19.67^{e}±3.98	19.42^{e}±3.08	23.6^{c}±0.99	25.23^{b}±1.22	22.18^{d}±6.64
K	ND	0.096±0.006	0.12±0.01	ND	ND	ND
Ca	ND	ND	ND	ND	ND	ND
Cr	0.715^{f}±0.11	0.735^{e}±0.03	0.89^{c}±0.10	0.80^{d}±0.06	0.96^{b}±0.11	1.03^{a}±0.19
Mn	6.0^{d}±0.30	6.6d±0.73	8.4^{c}±1.56	14.18^{b}±2.03	16.72^{a}±0.3617	16.03^{a}±1.57
Fe	21.18^{c}±1.97	16.65^{e}±3.04	19.07^{d}±3.50	24.02^{b}±2.09	26.00^{a}±0.92	24.03^{b}±2.62
Ni	0.38^{c}±0.007	0.35^{d}±0.009	0.35^{d}±0.0094	0.77^{a}±0.09	0.74^{b}±0.04	0.75^{b}±0.053
Cu	1.8^{d}±0.12	1.5^{e}±0.94	1.12^{f}±0.14	2.12^{c}±0.31	2.245^{b}±0.18	2.62^{a}±0.49
Zn	4.09^{d}±0.32	4.67^{c}±0.55	3.95^{e}±0.49	12.35^{ab}±0.51	13.9^{a}±0.71	13.5^{a}±2.01
As	ND	ND	ND	ND	ND	ND
Se	0.02^{a}±0.004	0.01^{b}±0.005	ND	0.02^{a}±0.003	0.02^{a}±0.005	0.02^{a}±0.008
Mo	0.07^{b}±0.11	0.03^{d}±0.009	0.02^{e}±0.01	0.15^{a}±0.06	0.05^{c}±0.004	0.07^{b}±0.04
Cd	0.02^{a}±0.003	0.02^{a}±0.004	0.01^{a}±0.01	0.02^{a}±0.001	0.02^{a}±0.004	0.02^{a}±0.001
Pb	0.22^{b}±0.03	0.20^{c}±0.09	0.15^{e}±0.03	0.21^{b}±0.02	0.17^{d}±0.02	0.39^{a}±0.14

AR- *A. aspera* Raw, ABO- *A. aspera* Boiled, ABL- *A. aspera* Blanched, TR- *T. triangulare* Raw, TBO- *T. triangulare* Boiled, TBL- *T. triangulare* Blanched

Extract yield, Total Phenolics and Tannins

Recovery is a main step for acquiring extracts with adequate yields and strong antioxidant activity. The percentage of recovery, total phenolics and tannin content of both raw and processed samples of *A. aspera* and *T. triangulare* are shown in Table 2. In this study, the influence of two different solvents on the efficient extraction of phenolics from leaf part of the plant was investigated. Each sample was extracted using solvent systems of different polarities, which are acetone and methanol with definite amount of distilled water (7:3 and 8:2 respectively). It was seen that 70 % aqueous concentration of acetone was found to be most efficient for extraction of polyphenols in comparison to 80% aqueous methanol. The highest recovery (16%) was registered in boiled leaves of *T. triangulare* acetone extract and the lowest values (3.6 %) were registered for blanched leaves of *A. aspera*. From these results, it is observed clearly that, addition of some amount of water enhances the extraction efficiency.

Phenolic compounds are present in plants, which are applied as major constituents of both human and animal diets [46]. Fruit and vegetables deliver the greatest drug store against growing prolonged diseases, reflecting that they hold a huge collection of antioxidant components. Polyphenols make a major contribution to free radical scavenging capacities. Antioxidant activity is directly related to total phenolic content, which could be observed as a main sign of antioxidant properties of plant extracts [47-48]. Phenolic compounds can perform as antioxidants through mechanisms including both free radical scavenging and metal chelation.

The effects of two different solvent systems in extracting polyphenols and antioxidants from the selected leafy vegetables were quantitatively measured and compared. Table 2 shows the total phenolic content (TPC) and tannin contents of two solvent extractions from the samples. The outputs were expressed as mg TAE/g DW basis, so as to exclude the effect of moisture content to quantification. The TPC of leafy vegetable samples of current study ranged from 140.3-315.3 mg of TAE/g extract for acetone and 125.5-324.0 mg of TAE/g extract for methanol (Table 2). It was higher in *T. triangulare* boiled leaves (324.0 mg of TAE/g extract) and lower in *A. aspera* blanched leaves (125.5 mg of TAE/g extract). The variation in TPC was characterized to various aspects including genotype, agronomic practices, post-harveststorage and, climatic and geographical locations.

Tannins are major groups of antioxidant polyphenols, have attracted lot of interest for the reason that, their multifunctional properties advantageous to human well-being. They have been well thought-out as cardio protective, anti-inflammatory and anti-mutagenicagents [49]. The tannin content of samples was ranged from 133.1-296.3 mg TAE/g for acetone extract and 118.7-308.5 mg TAE/g for methanol extract. The highest tannin content was found in *T. triangulare* boiled leaves and lowest value was recorded in *A. aspera* blanched leaves. Tannins were compounded with organic compounds such as proteins, starches and digestive enzymes thus reduce the dietary importance of foods [50]. They inhibit protein absorption and reduce iron availability [51]. So, minimum level of tannins in the diet is recommended. These leafy vegetables possess moderate range of tannins so mostly preferred for human diet.

Total flavonoids

Flavonoids are plant organic compounds that are not directly involved in metabolic activities and abundant constituents of fruits and vegetables, which including flavonols, flavones, flavnones and anthocyanins [52]. The total flavonoid, described as Rutin equivalent antioxidant capacity. Table 2 showed the total flavonoid content of raw and processed *A. aspera* and *T. triangulare* leaves. All the samples have lower amount of flavonoid when compared to total phenolics. Boiled leaves of *T. triangulare* in 70 % acetone extract possessed the maximum total flavonoid content (52.94 mg of RUE/g extract) and raw leaves of *T. triangulare* in 80 % methanol have the lowest (17.33 mg of RUE/g extract). As evaluations were made among two solvent extractions, 70% acetone regarded as the best effective solvent for extraction of flavonoids from raw and processed leafy vegetables. This solvent system was every so frequently used in many studies as an extraction solvent for flavonoids [53]. Certain studies have revealed that flavonoids and associated polyphenols significantly supplement to the total antioxidant activity of fruits and vegetables [54]. Presence of flavonoids such as myricetin, quercetin, kaempferol etc. has been described in

LVs [55]. *In vitro* and studies related to health and disease conditions were advised that the intake of diets with huge quantities of flavonoids will defend against human diseases for the reason that their free-radical scavenging activity also has defense mechanism contrary to oxidative stress [56].

Table 2: Extract yield, Total Phenolics, Tannins and Flavonoid content of Aqueous Acetone and Methanol extracts of *A. aspera* and *T. triangulare* leaves

Samples	Extract Yield (%)	Phenolics[a] (mg of TAE/g extract)	Tannins[a] (mg of TAE/g extract)	Flavonoids[b] (mg of RUE/g extract)
ARA	8.2	169.63[f]± 4.88	159.34[f]± 4.95	26.72[e] ± 1.26
ABoA	8.8	220.30[b] ± 15.63	209.11[b]± 16.45	43.05[b]± 0.38
ABlA	3.6	140.30[h] ± 12.35	133.06[h]± 11.65	24.55[g]± 3.87
ARM	3.8	145.48[h]± 5.30	136.94[h]± 5.34	22.55[h] ± 0.83
ABoM	4.4	173.93[e]± 13.10	164.88[e]± 12.99	38.77[c]± 1.51
ABlM	5.0	125.48[i]± 6.05	118.69[i]± 5.82	20.16[i]± 1.30
TRA	14.2	198.07[c] ± 19.41	183.27[c]± 17.48	32.16[d] ± 2.13
TBoA	16	315.26[a]± 15.78	296.26[b]± 17.85	52.94[a]± 5.30
TBlA	9.8	185.33 [d]± 1.78	173.37[d]± 2.53	35.11[c]± 3.42
TRM	8.8	194.22[c]± 16.26	181.71[c]± 15.88	17.33[j] ± 0.5
TBoM	8.2	324.00[a]± 19.11	308.54[a]± 18.18	31.5[e]± 1.87
TBlM	8.2	157.04[g]± 12.60	147.05[g]± 12.99	26.61[f]± 1.02

ARA- *A. aspera* Raw Acetone, ABOA- *A. aspera* Boiled Acetone, ABLA- *A. aspera* Blanched Acetone, TRA- *T. triangulare* Raw Acetone, TBOA- *T. triangulare* Boiled Acetone, TBLA- *T. triangulare* Blanched Acetone, ARM- *A. aspera* Raw Methanol, ABOM- *A. aspera* Boiled Methanol, ABLM- *A. aspera* Blanched Methanol, TRM- *T. triangulare* Raw Methanol, TBOM- *T. triangulare* Boiled Methanol, TBLM- *T. triangulare* Blanched Methanol.

[a-] Total phenolic and tannin content are expressed as Tannic acid equivalent (TAE),

[b-] Flavonoids are expressed as Rutin (RUE) equivalents.

DPPH radical scavenging activity

DPPH radical scavenging activity was examined in each sample with reference to inhibition ability of a pre-formed free radical by antioxidants. DPPH assay is one of the most extensively used methods for monitoring the antioxidant activity of plant extracts. The assay is based on the extents of the antioxidants ability to scavenge the stable radical of DPPH. DPPH is a stable nitrogen-centered free radical, which turn outs violet color in methanol solution. DPPH radicals react with appropriate reducing agents and for the duration of which, the electrons turn into paired off and the solution loses colour stoichiometrically dependent on the number of electrons taken up [57]. In the experiment, solution was gradually reduced to a yellow colored product (diphenylpicryl hydrazine) with the addition of extracts in a way of increasing concentration. The absorbance was measured at 517 nm. The capacity of both 70% acetone and 80% methanol extracts to quench the DPPH radical was moderately strong in all samples (1809.00 - 6356.45 TE mmol/g extract). The strongest activity was detected in blanched leaves of *T. triangulare* in 70% acetone extract and blanched leaves of *A. aspera* in 80% methanol extract.

ABTS$^{\cdot+}$radical cation decolorization assay

DPPH is often used in the evaluation of free radical scavenging ability; though, it has the drawback of color interference and sample solubility. Hence, free radical scavenging ability of the vegetable extracts was additionally studied using a temperately stable nitrogen-centered radical species, ABTS$^{\cdot+}$. The ABTS$^{\cdot+}$ radical based model of free radical scavenging capacity has the benefit of being extra flexible, at the same time both non-polar and polar samples can be evaluated, and spectral hindrance is minimized and the absorption maximum used is 760 nm [58]. The ABTS assay has been commonly used to assess antioxidant activities of constituents in foods and beverages because of its relevance in aqueous and lipid phases. Results showed that *A. aspera* were highly effective in reducing the stable radical ABTS than *T. triangulare*. Boiled leaves of A. *aspera* in 70% acetone demonstrated a stronger ABTS radical scavenging activity than other samples (145425.34 TE mmol/g extract).The lowest value was registered in blanched leaves of A. *aspera* in 80% methanol extract (17708 TE mmol/g extract). In the advanced version of this test, a stable ABTS radical cation, which has blue-green chromophore absorption, was produced before the adding up of antioxidants as a result of the oxidation of ABTS with Potassium persulphate [59]. The antioxidant capacity of natural products include carotenoids, phenolic compounds, and some plasma antioxidants, is found out by the decolorization of ABTS, by assessing the decline of radical cation as the percentage inhibition of absorbance at 734 nm. The absorbance of the reaction mixture of ABTS and an antioxidant is compared to that of Trolox standard, and results are indicated in terms of Trolox equivalent antioxidant capacity (TEAC). Among all samples, *A. aspera* blanched leaves showed the poor scavenging activity to the ABTS radical.

Ferric reducing/ antioxidant power

Reducing power is a novel antioxidation protection method which is influenced by electron and hydrogen atom transfer [60]. The ferric reducing/antioxidant power is an easy and shortest test of assessing antioxidant capacity [61]. This assay determines the electron-donating capability of antioxidants using potassium ferricyanide reduction method. Antioxidant compounds reduce the ferric ion/ferricyanide complex to ferrous form [62] and they act as reducing agents by giving a hydrogen atom to this ferric complex, as a result breaking the radical chainreaction [63]. The FRAP value of the two leafy vegetables was varied from 1884.84- 12762.79 mmol Fe (II)/g extract DM. The maximum reducing power being detected in 70% acetone extract of raw leaves of *T. triangulare* and *A. aspera*. The enhanced level of total reductant ability in processed samples monitored in the current study is in good agreement with the earlier reports [64] wherein it is observed that thermal processing is completely conserved the biologically active compounds (polyphenols, flavonoids, flavanols and tannins) and total antioxidant capacity of leafy vegetables. The enhanced reducing activity in processed samples was justified by reason of the formation of redox-active

secondary metabolites (Maillard reaction and Amadori rearrangement products) and the breaking of composite phenolic compounds at high temperature became soft or causing distraction of plant cell walls [65]. Their elevated activities are also aligned with TPC. Phenolic compounds that had been recognized from *Amaranthus* species are Hydroxyl benzoic acid, Gallic acid, Vanilic acid, Chlorogenic acid and salicylic acid [66]. In addition, flavonoid derivatives such as quercetin and kaempferol appear to be their main elements that may be accountable for the antioxidant activity [67].

Metal chelating activity

The assay was employed to find out chelating activity of Fe^{2+}. It was based on the chelation of metal ion (Fe^{2+}) with ferrozine to give a red colored complex. Ferrozine can form complexes by means of quantity with Fe^{2+}. In the presence of other chelating agents, the development of complex is disturbed; consequently the red color of the complex is drops off. As a result, measurement of the rate of color reduction permits evaluation of chelating activity of the coexisting chelator [68]. A promising connection among total flavonoids and Fe-chelating activity was suggested in an earlier study [69]. The results expose that all the extracts chelate Fe (II) moderately. 70% acetone extract of boiled leaves of *T. triangulare* showed highest activity among all samples. The lowest activity was registered for 70% acetone extract of blanched leaves of *T. triangulare*. Chelating agents creates σ-bonds with a metal, and they reduce the redox potential hence stabilizing the oxidized form of metal ion; for the reason that, they are efficient as secondary antioxidants [66]. The development in the Fe (II) chelating ability is related to that of DPPH radical scavenging, $ABTS^{+\cdot}$ radical scavenging capacity and reducing power. The total antioxidant capacity is a combination of diverse antioxidant mechanisms, comprising free radical scavenging ability, reducing power and Fe (II) chelating ability [58].

Table 3: DPPH radical, FRAP, ABTS cation radical and Metal chelating activity of aqueous Acetone and methanol extracts of *A. aspera* and *T. triangulare* leaves

Samples	FRAP[b] (mmol Fe(II)/g extract)	Metal Chelating[c] (mg EDTA/g extract)	ABTS[a] (TEAC mmol/g extract)	DPPH[a] (TEAC mmol/g extract)
ARA	10892.7^{b}± 128.74	0.87^{g}± 0.11	133095.01^{b}± 3602.80	2031.02^{e}± 87.99
ABoA	1884.84^{i}± 73.25	0.72^{h}± 0.41	145425.34^{a}± 13937.43	1832.73^{h}± 16.56
ABlA	9893.41^{c}± 142.99	1.68^{b}± 0.03	108848.82 ^{c}± 6705.49	6052.31^{b}± 149.86
ARM	2741.37^{g}± 108.09	1.23^{d}± 0.15	104704.17^{c}± 18256.45	2067.52^{e}± 64.80
ABoM	1899.12^{i} ± 108.09	0.99^{f}± 0.01	137654.12^{b}± 3602.80	1809.00^{h}± 30.61
ABlM	6215.08^{e}± 101.27	1.10^{e}± 0.02	17708.00^{h} ± 3001.15	6064.48^{b}± 93.64
TRA	12762.79^{a}± 86.04	0.51^{i}± 0.03	24525.95^{g}± 1915.19	1941.00^{g}± 57.97
TBoA	3721.62^{f}± 150.40	2.11^{a}± 0.12	25313.43^{g} ± 1960.40	2294.40^{d}± 112.23
TBlA	7033.54^{d}± 140.59	0.31^{j}± 0.14	30535.69^{d} ± 1307.53	6356.45^{a}± 95.60
TRM	2056.15^{h}± 186.31	0.41^{j}± 0.12	25168.37^{g}± 1476.88	2276.76^{d}± 67.35
TBoM	3916.72^{f}± 87.22	2.09^{a}± 0.15	34597.45^{e}± 1238.72	2468.98^{c}± 93.55
TBlM	7471.3^{d}± 86.04	1.42^{c}± 0.04	28069.63^{f} ± 1023.44	2007.91^{f}± 40.24

Each value is expressed as mean ± standard deviation (n=3). ARA- *A. aspera* Raw Acetone, ABOA- *A. aspera* Boiled Acetone, ABLA- *A. aspera* Blanched Acetone, TRA- *T. triangulare* Raw Acetone, TBOA- *T. triangulare* Boiled Acetone, TBLA- *T. triangulare* Blanched Acetone, ARM- *A. aspera* Raw Methanol, ABOM- *A. aspera* Boiled Methanol, ABLM- *A. aspera* Blanched Methanol, TRM- *T. triangulare* Raw Methanol, TBOM- *T. triangulare* Boiled Methanol, TBLM- *T. triangulare* Blanched Methanol.

[a-] TEAC (Trolox equivalent antioxidant capacity), (mmol equivalent Trolox performed by using $ABTS^{\bullet+}$ DPPH radical cation.

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

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

Current study is a novel approach to reveal the antioxidant potential and nutritional benefits of two lesser known greens, *Achyranthes aspera* and *Talinum triangulare*. The study focused on influence of processing methods on their proximate composition, mineral profile, phytochemical contents as well as effect on their antioxidant potential. The food elements with high level of phenolic content revealed comparatively greater antioxidant activity. The results indicate that cooking is suitable treatment for leafy vegetables, and including them is good for a balanced diet. For that reason, promotion and probably profit-orientation of these leafy vegetables ought to been encouraged and delivered for wider consumption. Advance research is supposed to consider next to antioxidant activity along with various physiological effects of cooked leafy vegetables.

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