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Evaluation of nutritional value and *in vitro* antioxidant potential of differentially processed underutilized leafy vegetables

[*1] Aathira M, [2] Sudarsa KS, [3] Siddhuraju P

[1] Research scholar, Bioresource Technology Lab, Department of Environmental Sciences, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

[2] Master degree student, Bioresource Technology Lab, Department of Environmental Sciences, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

[3] Professor, Bioresource Technology Lab, Department of Environmental Sciences, School of Life Sciences, Bharathiar University, Coimbatore, Tamil Nadu, India

Abstract
The influence of domestic cooking regimes like boiling and blanching on nutritional value and antioxidant potential of two underexploited leafy vegetables of tribal utilization: *Vigna unguiculata* and *Cucurbita pepo* was studied. Boiling at 100°C for 15 min. was found to be better with enhanced fibre content, crude ash and high retention of total phenolics, tannins and thereby antioxidant capacity when compared to blanching for 20 min. in boiling water. Among the analysed minerals presence of essential minerals including Na, k, Zn, Mn were found to be prominent. The results illustrated that these unconventional greens offer considerable potential as functional food ingredients owing to the presence of dietary fibre, minerals and trace minerals, phenolic compounds etc, which produce different biological activities and also to their high antioxidant capacity.

Keywords: *v. unguiculata*, *C. pepo*, biochemical composition, cooking, bioactive compounds, antioxidant potential

1. Introduction
For consumption of plant-based foods some degree of cooking or processing is done which also increases their edibility and palatability. It has taken over centuries to develop domestic methods of food processing to increase consistency of final food product through attractive flavour and taste. But none of the epicure realized that cooking process was making their foods more digestible, microbiologically safer and more or less nutritive depending on the selected cooking technology [41]. Overwhelming epidemiological evidences indicates that diets rich in fruits and vegetable are associated with a lower risk of several degenerative diseases. It is well known that food processing can have many effects, not all of which result in a loss of quality and health properties. Selecting the correct method of food preparation is as important as selecting the right food, whereas only a little amount of food is eaten as raw, while most of them (Eg: vegetables) should be processed for economic, safety and quality reasons. On the basis of these considerations, the study of the influence of food processing on naturally occurring antioxidants is a key factor in the search of the technological conditions necessary to preserve or improve their original activity and bioavailability [11].

Plant foods including green leafy vegetables are major part of well-balanced diet which acts as a hoard of minerals, antioxidant and vitamins. Some of the commonly consumed leafy vegetables are amaranth, spinach, fenugreek, coriander, etc. Apart from these there are various types of underutilized leafy vegetables, which are available seasonally, and practically only meagre information is available in relation to their nutrient content and anti-nutritional factors. Assorted research findings have unleashed the health benefits of green leafy vegetables viz. anti-carcinogenic, anti-bacterial and anti-diabetic effects. These health benefits are attributed, at least in part, to their antioxidative compounds (polyphenolic constituents, carotenoids like lutein) [18]. Compared to conventional cultivated species, wild vegetables are hardy, require less care, and are a rich source of micronutrients including provitamin-A, *β*-carotene, ascorbic acid and dietary fibre [46, 32]. Recognizing the need for identification of such green leafy vegetables, which are believed to be nutritious, may help in achieving nutritional (micronutrient) security. Unfortunately, wild vegetables are currently underutilized, and have been neglected by researchers and policy makers. Their promotion and integration into human diets could assist in their protracted use and consequent conservation [15].

Pumpkin, *Cucurbita pepo* L., (English name; summer pumpkin) is an herbaceous, monoecious, annual plant of the Cucurbitaceae family. The importance of *Cucurbita pepo* as a vegetable crop has been long recognized worldwide due to its high nutritional and economic value. Pumpkin (*Cucurbita pepo*) is an annual green leafy vegetable which grows wildly and is also widely distributed, cheap and cultivated for its leaves, fruits and seeds, but in certain foreign countries it is largely underutilized. It is an excellent source of natural pigments for instance chlorophyll a (Chl-A), chlorophyll b (Chl-B), that play an important role in its visual appearance and the nutritional value within the human diet [8]. Young leaf of pumpkin is used as an indigenous leafy vegetable and in this form; processing or pre-treatment is not required or done before cooking unlike many other leafy vegetables. About 43.8% of *C.pepo* leaf constitutes protein which is comparable to soybean. This is one of the most palatable leafy vegetable with wide range of genetic variability,

both in vegetative and reproductive characteristics. The shoot tips, leaves, flowers, fruits and seeds of *C. pepo* are cooked to prepare leafy vegetable dishes [31, 34].

Cowpea (*Vigna unguiculata*) is a leguminous plant which belongs to the family *Fabaceae* and is perceived to have originated from Africa. It is largely grown in warm and hot regions of Africa, Asia and the Americas [44]. The crop is valuable for its high nutritional quality (high in proteins and minerals), drought and heat tolerance, and relative short maturity [33]. The seeds are a major source of plant proteins and vitamins for man, feed for animals, and also a source of income. The young leaves and immature pods are eaten as vegetables. Cowpea has much variability within the species. Being one of the important tropical dual-purpose legumes all parts of cowpea plants are used for food or fodder. It is used as leafy vegetable, grain, as fresh cut-and-carry foliage and for hay and silage. The plant is used at all stages of growth as a vegetable crop, tender shoot tips and leaves are consumed when they reach the seeding stage while immature pods and seeds are consumed during the fruiting stage. Cowpea leaves, like many green leafy vegetables, are an excellent source of micronutrients like folate and minerals in the human diet. Mostly vegetables are harvested between 4 and 9 weeks of maturity. In case of cowpea leaves 8 weeks old vegetables will provide highest total folate content and highest amount of both di- and monoglutamates (>70%). In cowpea leaves folates principally existed as poly-γ- glutamate forms consisting of 4 to 7 glutamic acids. Older leaves are good source of folate with a longer poly-γ-glutamic acid chain. Thus harvesting time plays an important role in optimizing the intake of micronutrients. Harvesting at the 6th week after planting is the optimal maturity to obtain high levels of both total folates and minerals. They provide a low-cost and abundant supply of minerals such as calcium (Ca),magnesium (Mg), sodium (Na), potassium (K), phosphorus (P), iron (Fe), zinc (Zn), manganese (Mn), copper (Cu) and selenium (Se). The leaves are mostly consumed as cooked/blanched vegetables. Certain tribal communities of Nigeria uses cowpea tender leaves as important ingredient in variety soups like spinach. They are also eaten as a relish with maize staple (pap or sadza). In rural South Africa they play an important role as famish foods and may be cooked (fresh or dry) with tomato or peanut sauce and served alone or with meat. Leaves are boiled/ blanched, drained, sun-dried and then stored for later use [22, 47, 10, 48]. Apart from nutritional and nutrceutical uses cowpeas are also used as green manure, employed in a rotary scheme with other annual crops or in fruit plantations to increase or sustain soil fertility [35].

Many research investigations have revealed the presence of bioactive compounds in the food materials and influence of processing methods on them. Though both *C. pepo* and *V. unguiculata* is rich in minerals, proteins and phytochemicals their providence after processing was not studied in deep yet. Therefore, the present study is aimed to compare effect of culinary treatment and domestic cooking methods like boiling and blanching, on proximate composition, mineral profile and antioxidant activity of *C. pepo* and *V. unguiculata* to identify optimal process which reduce degradation of biologically active metabolites. Furthermore, findings from this preliminary investigation may provide better idea about adequate domestic processing that might help to increase intake of active metabolites thereby enhancing their functionality and reduce the risk of chronic diseases.

2. Materials and Methods

2.1 Chemicals

Butylated hydroxyanisole (BHA), 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), Folin-Ciocalteau phenol reagent, ferrous chloride, ferrous ammonium sulfate, ethylene diamine tetra acetic acid (EDTA) disodium salt and ferric chloride were obtained from Hi Media, Merck and Sigma. All chemicals were of analytical grade. All analysis was performed with UV-visible spectrophotometer (Cyber lab-UV 100, USA).

2.2 Samples and processing methods

The mature tender leaves of *V. unguingulata* and *C. pepo* were harvested prior to their flowering period at Palakkad, Kerala State, India. Upon arrival at the laboratory, samples were washed with water to remove debris and damaged portions. The leaves were separated from stem completely and divided into three equal batches. One part of the sample was cut into small pieces and air dried. The second part was boiled in water at 100 °C for 15 min in the ratio of 1:10 (w/v). The third part of the respective sample was blanched in boiling water (at 100 °C) for 20 min in the ratio of 1:10 (w/v). After boiling and blanching, the remaining water was discarded and the respective processed samples were dried at 45 °C. After drying, all the raw and processed samples were ground to fine powder (particle size of about 0.25nm) and stored in separate screw capped bottles at room temperature for further analysis.

2.3 Biochemical Composition

The moisture content of raw and processed samples was determined using Moisture Analyzer MA35 (Sartorius AG, Germany) at 105°C. Micro- Kjeldahl method was employed to determine the total nitrogen and a nitrogen-protein conversion factor 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 outlined in Association of Officinal Analytical Chemists (AOAC, 1990). The Proximate composition was expressed as g/100g DM (dry matter).

2.4 Mineral profiling

Mineral profiling of raw and processed *V. unguiculata* and *C. pepo* leaf samples were performed, using PerkinElmer inductively coupled Ar plasma quadrupole mass spectrometer (Q-ICP-MS) model NexIon 300X, according to the method previously described by Mastro *et al* [27] with slight modifications. Instrumental details including operating conditions are summarized in Table 1. Ar gas of purity 99.9% was used for ICP-MS determination. Nitric acid, Sulphuric acid and Perchloric acid (in the ratio 9:2:1) were used for sample treatment. All reagents were of analytical grade. HPLC water was used for preparations of sample dilution and standards. Commercially available 100 mg L^{-1} standard solutions (Perkin Elmer pure plus) of the elements analyzed were used. Diluted working solutions were prepared daily by serial dilutions of the stock solution. All glassware and plastic bottles used were cleaned by rinsing several times with double distilled water. All samples and standards were stored in polyethylene bottles (50 mL).

Table 1: Instrumental characteristics and settings for ICP-MS

Instrument	PerkinElmer NexIon 300X
ICR RF Power	500- 1600 W
N	MEINHARD® Concentric Nebulizer
Interface	Sampler and skimmer cones in Ni; hyper skimmer in Al
Argon flows	Nebulizer, 0- 2 L/min : Plasma, 16; auxiliary, 1.2 (all in L min−1)
Solution delivery	0.85 mL min−1
Detector voltages	−3000 V (analog); 500-2500 V (pulse)
Scanning conditions	Sweeps per reading, 20; readings per Replicate, 1; number of replicates, 3
Scanning mode	Standard
Vacuum	Analytical zone, <2.2 10^{-06} Torr

2.5 Solvent extraction

The raw and processed samples (each 15 g) were extracted with 70% Acetone and 80% methanol (1:7 w/v) separately for 48h at room temperature. The extracts were centrifuged, filtered and pooled. The residues were re-extracted with an additional 75 ml of aqueous acetone and methanol, as described above, for 24 h. The solvent of the combined extract was evaporated under low temperature at 40 °C in incubator (NSW, 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 incubator at a temperature of 40 °C (until sample getting a constant weight) and the recovery percent was calculated by following equation;

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

2.6 Estimation of total phenolics and tannin contents

The total phenolics and tannins were measured as gallic acid equivalents [25] 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 (%).

2.7 Total flavonoids

The total flavonoid content was measured by a spectrophotometric assay [21 outlined by Siddhuraju and Becker [42]. A total of 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 1 mol/L 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 against the prepared blanks. Total flavonoid content was expressed as mg rutin (RUT)/g extract.

2.8 Ferric reducing/antioxidant power (FRAP) assay

The antioxidant capacity of phenolic extracts of raw and processed *V. unguiculata* and *C. pepo* were estimated according to the procedure by Pulido *et al*. [36]. FRAP reagent (900 µL), prepared freshly and 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 test samples and reagent blank were incubated at 37 °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. 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 2 000 µmol/L (FeSO4.7H2O) were used for plotting the calibration curve. The parameter equivalent concentration (EC1) was defined as the concentration of antioxidant has a ferric-TPTZ reducing ability equivalent to that of 1 mmol/L FeSO4.7H2O. EC1 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.

2.9 Metal chelating activity

The extracts (100 mL) 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 mg [12].

2.10 Stable free radical scavenging activity using DPPH· method

The radical scavenging activity of raw and processed sample extracts was measured using DPPH radical by the method of Brand-Williams *et al*. [9] with slight modification. Extract of 0.1 mL prepared in methanol was mixed with 3.9 mL of DPPH· (6×10-5 mol/L methanol). The solution was incubated at room temperature for 30 min and the decrease in absorbance at 515 nm was determined at the end of incubation period 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.

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

ABTS was dissolved in water to a 7 mmol/L concentration, ABTS radical cation (ABTS•+) was produced by reacting ABTS stock solution with 2.45 mmol/L 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 30 °C to give an absorbance at 734 nm of 0.700±0.020 in a 1 cm 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 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 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 Re *et al.*, [39] described by Siddhuraju and Becker [43]. The unit of total antioxidant activity 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 mmol/L. The total antioxidant activity of ASC and BHA were also measured by ABTS method for comparison.

2.12 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 Biochemical composition

Biochemical composition of *V. unguiculata* and *C. pepo* leaves are summarised in Table 2. The moisture content of *V. unguiculata* processed leaves ranged from 11.19% to 15.015%. In case of *C. pepo* it is from 12.81% to 15.97%. The obtained results are higher when compared to Nigerian leafy vegetables (*Amaranthus hybridus*, *Hibiscus sabdariffa* L, *Telfaria occidentialis*, *Vernonia amygdalina* L, *Basella alba* L, *Gongronema latifolium* and *Ocimium gratissimum* L) studied by Asaolu *et al*. [5]. Moisture content was highest for *C. pepo* blanched sample followed by *C. pepo* boiled and *V. unguiculata* blanched sample (15.015%). Relatively higher moisture content reveals that the leafy vegetable are prone to deterioration, so appropriate care should be taken for their preservation. But a positive impact of high moisture content is that it helps to achieve greater activity for water soluble enzymes and co-enzymes that are involved in metabolic activities [20]. Blanched leaves of *V. unguiculata* had 7.5 g/100g of ash content when compare with boiled leaf sample of *C. pepo* (5.5 g/100g) which is the least of the leafy vegetable investigated. Presence of high ash content means the sample is a good repository of minerals [19]. Fibre content also showed a broad variation of between 8.61g/100g to 15.44 g/100g. Higher value of fibre content was observed for *V. unguiculata* boiled leaves, but, obtained value is lower compared to previous investigation in *V. unguiculata*. The level of lipid content analyzed in the sample ranged from 7.96 g/100g to 13.33 g/100g. Plant foods which provide more than 12 % of their calorific value from proteins have been shown to be good source of proteins [3]. Protein analysis of present samples is such that 21.37 g/ 100g being the highest shown by *V. unguiculata* raw sample and lowest value for *C. pepo* boiled sample (5.12 g/100g). As concern proteins content, cooking processing used in this study caused reduction after 15 min boiling and 20 min blanching. This reduced protein contents of cooked leafy vegetables could be attributed to the severity of thermal process during cooking to protein degradation.

Table 2: Biochemical composition of Vigna unguiculata and Cucurbita pepo

Sample	Moisture (%)	Crude Ash (g/100g)	Crude Lipid (g/100g)	Crude Fibre (g/100g)	Crude Protein (g/100g)
VR	11.19^{c} ±0.79	5.17^{d}±0.76	10.26^{c}±0.74	10.09^{d}±1.73	21.37^{a}±5.45
VBO	12.33^{b}±0.88	6.17^{c}±0.578	13.33^{a}±0.82	15.44^{a}±0.39	14.64^{b}±0.76
VBL	15.015^{a}±1.77	7.5^{a}±2.89	12.91^{b}±0.64	12.53^{c}±1.44	13.81^{c}±0.73
CR	12.81^{b}±1.83	5.17^{d}±0.76	7.96^{e}±1.90	8.61^{e}±1.11	6.74^{d}±3.31
CBO	15.015^{a}±1.77	5.5^{e}±0.5	8.79^{d}±0.43	13.68^{b}±0.57	5.12^{e}±2.72
CBL	15.97^{a}±1.86	6.83^{b}±0.58	10.69^{c}±0.83	12.49^{c}±1.21	6.35^{d}±4.99

Each value is expressed as mean ± standard deviation (n=3). VR - *V. unguiculata* raw, CR - *C.pepo* raw, VBO - *V. unguiculata* boiled, CBO- *C.pepo* boiled, VBL- *V.unguiculata* blanched, CBL - *C.pepo* blanched. Mean values different letters in a column are significantly different ($p<0.005$)

3.2 Mineral profiling

Mineral profile of processed leaves of both *V. unguiculata* and *C. pepo* are given in Table 3. From the analysis presence of essential minerals such as Na, K, Zn, Mn were confirmed. Sodium and potassium are important intracellular and extracellular cations respectively, which are involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction [2]. Sodium functions as the "osmotic skeleton" of the extracellular fluid. The minimum requirement for a healthy person is 500 mg of sodium for adults and for infants and children, 58mg/day [38]. Sodium has shown the highest amount (126.5 mg/100g) with *V. unguiculata* raw leaves which is not sufficient for adult but adequate for children. So this leafy vegetable should be taken in excess to meet the requirement. Mineral present in least amount is Cd (0.005 mg/100g) in *C. pepo* boiled leaves. Mn, Fe, Al, Cu and Zn have shown higher values next to sodium. Mn is the cofactor for the enzymes Superoxide dismutase (SOD), arginase, and glycosyl transferase. Moreover the Mn ions take part in activation of enzymes like phosphoenol pyruvate carboxy kinase and glutamine synthetase. Mn requirement for an adult is between 2-5 mg/d. In case of deficiency it results in growth failure, skeletal abnormalities, impaired reproductive function, abnormal insulin metabolism and glucose tolerance [38]. Analyzed leafy

vegetables provide Mn more than the recommended daily requirement. Fe is an important trace element in the human body, it plays numerous biochemical roles including, control of infection, cell mediated immunity, oxygen binding in haemoglobin and acting as an important catalytic centre in many enzymes as the cytochrome oxidase [16]. The iron content of the studied leafy vegetables is more than the recommended dietary allowance for males (1.37mg/day) and females (2.94 mg/day) [14]. Cu is another essential micronutrient, functions as biocatalysts, required for body pigmentation in addition to iron, maintain a healthy central nervous system and prevent anaemia. When considering Cu content in plants, most of them contain fewer amounts which are inadequate for normal growth and is ensured through artificial and inorganic fertilizers. In the present study *V. unguiculata* processed and raw sample possess Cu content in the range 1.72 mg/ 100g to 2.273 mg/100g which is in agreement with the recommended daily allowance (RDA) of 2 mg/day [38]. Also, *C. pepo* processed sample registered higher value (9.23 mg/100g) for copper suggesting that consumption of both the leafy vegetables is able to meet the daily requirement. In addition analysed plant leaf samples have Cu content more than Nigerian leafy vegetables [28]. Zn content of both, processed and raw leafy samples ranged from 4.142 mg/100g to 7.9 mg/100g which is slightly lower than the RDA (9-11 mg/d) [38]. Zn is important for nerve function, male fertility and reproduction especially in development of testes and ovary [4]. Therefore, surplus consumption of these leafy vegetables can reduce the risk of Zn deficiency which can result in retarded growth and delayed sexual maturity. Se, Be, Cr, Cd and Pb are found in trace amounts in the analysed samples.

Table 3: Mineral profile (mg/100g) of *V.unguiculata* and *C.pepo* Leaves

Minerals	VR	CR	VBO	CBO	VBL	CBL
Be	0.006^{e}±0.001	0.208^{c}±0.195	0.009^{d}±0.001	0.413^{b}±0.0167	0.009^{d}±0.001	0.663^{a}±0.071
Na	126.5^{a}±158.916	25.55^{d}±2.988	33.217^{c}±6.120	25.433^{d}±0.321	41.412^{b}±5.139	32.25^{d}±6.586
Mg	0.172^{e}±0.012	0.357^{c}±0.223	4.954^{a}±8.354	0.478^{b}±0.002	0.158^{f}±0.008	0.196^{d}±0.241
Al	13.933^{c}±3.155	17.3^{a}±1.15	13.067^{c}±1.358	17.483^{a}±2.846	12.45^{d}±4.838	16.533^{b}±3.066
K	0.079^{c}±0.007	0.222^{b}±0.203	0.40^{a}±0.025	0.053^{e}±0.003	0.462^{a}±0.041	0.066^{d}±0.015
Ca	ND*	ND*	ND*	ND*	ND*	ND*
Cr	0.480^{f}±0.082	1.297^{a}±0.487	0.837^{e}±0.247	1.25^{b}±0.1	0.675^{d}±0.122	1.08^{c}±0.393
Mn	16.75^{c}±2.861	10.383^{d}±7.765	17.333^{b}±3.396	7.3^{e}±0.15	21.017^{a}±2.843	5.102^{f}±3.678
Fe	13.89^{c}±1.937	19.583^{a}±0.557	15.45^{b}±2.066	15.267^{b}±0.175	13.883^{c}±3.754	11.603^{d}±9.040
Ni	0.37^{e}±0.057	0.385^{d}±0.024	0.382^{d}±0.089	0.682^{c}±0.042	6.247^{a}±6.086	0.718^{b}±0.040
Cu	1.72^{e}±0.442	7.133^{b}±10.542	2.308^{d}±0.742	9.233^{a}±0.325	2.273^{d}±0.218	4.308^{c}±4.279
Zn	5.22^{c}±0.487	6.183^{b}±1.402	7.017^{a}±1.314	5.75^{c}±0.180	7.9^{a}±0.264	4.142^{d}±2.701
As	ND	ND	ND	ND	ND	ND
Se	0.009^{e}±0.0002	0.037^{b}±0.051	0.029^{c}±0.024	0.015^{d}±0.001	0.009^{e}±0.002	0.312^{a}±0.259
Mo	ND	ND	ND	ND	ND	ND
Cd	0.227^{a}±0.190	0.007^{e}±0.003	0.009^{d}±0.004	0.005^{f}±0.001	0.012^{c}±0.001	0.159^{b}±0.105
Pb	0.255^{c}±0.244	2.859^{a}±3.719	0.201^{e}±0.016	0.275^{b}±0.015	0.213^{d}±0.038	0.159^{f}±0.105

Each value is expressed as mean ± standard deviation (n=3). VR - *V. unguiculata* raw, CR - *C.pepo* raw, VBO - *V. unguiculata* boiled, CBO- *C.pepo* boiled, VBL- *V.unguiculata* blanched, CBL- *C.pepo* blanched. Mean values different letters in a column are significantly different ($p<0.005$)
ND* - Not detectable; ND – Not detected.

3.3 Extract yield and Total phenolic content

The recovery percentage of raw, boiled and blanched samples of *V. unguiculata* and *C. pepo* were ranged from 5.2% to 11.38% (acetone extract), 7.6% to 13.6% (methanol extract) and 3.6% to 8.2% (acetone extract), 3.2% to 9.6% (methanol extract) respectively (Table 4). Phenolics are powerful antioxidants which act in a structure-dependent manner. They can scavenge reactive oxygen species (ROS), and chelate transition metals which play vital roles in the initiation of deleterious free radical reactions. Total phenolic content could be regarded as an important indication of antioxidant properties of plant extracts [45]. The screening of acetone and methanol extracts of two lesser known leafy vegetables revealed that there was a wide variation in the amount of total phenolics ranging from 78.07 mg TAE/g extract to 354.52 mg TAE/g extract (Table 4). Acetone extracts has given good results with phenolic assay and this may be due to high solubility of phenolic compounds in more polar solvents. The combined use of water and organic solvent may facilitate the extraction of chemicals that are soluble in water and/or organic solvent [13]. In the present study processed sample has registered higher values than raw samples, highest value of phenolics was found to be with acetone extract of *V. unguiculata* boiled sample (354.52 mg TAE/g extract). In vegetables phenolics are found as complex structures bounded to dietary fiber, proteins or sugars which are hydrolysed by hydrothermal processing and thus become more available. Present investigation reveals the resistant nature of phenolics to thermal treatment and the result obtained was comparable with results of *B. diffusa* after 10 min boiling [29]. Lowest value was with processed methanol extract of *C. pepo* leaves (78.07 mg TAE/g extract). Plant tannins have attracted a lot of attention in recent years because of their multifunctional properties beneficial to human health. They have been considered to be cardioprotective, anti-inflammatory, anti-carcinogenic and anti-mutagenic [23]. Higher values of tannin content were registered for processed *V. unguiculata* acetone extract (340.10 mg TAE/g extract) and lower value of tannin content were shown by processed *C. pepo* methanol extract(73.26 mg TAE/g extract) (Table 4).

Table 4: Extract yield, Total phenolics,Tannins and Flavanoid content of *V.unguiculata* and *C.pepo* leaves

Samples	Extract yield (%)	Total phenolics**	Total tannins**	Total flavanoids*
VRA	11.38	291.11^{b}±10.95	274.68^{b}±8.23	22.22^{c}±0.85
VRM	13.6	259.85^{c}±5.80	247.47^{c}±3.85	17.67^{d}±0.5
CRA	8.2	165.33^{f}±2.22	155.17^{f}±3.66	128.22^{a}±9.16
CRM	9.6	112.00^{g}±1.54	105.12^{g}±0.75	12.33^{g}±0.33
VBOA	5.2	354.52^{a}±3.73	340.10^{a}±4.91	96.61^{b}±64.21
VBOM	7.6	265.19^{c}±24.11	250.59^{c}±24.36	18.83^{d}±0.58
CBOA	4.4	189.78^{e}±10.69	178.16^{e}±8.82	17.5^{d}±2.90
CBOM	3.2	181.63^{e}±7.56	170.59^{e}±7.93	13.78^{f}±0.53
VBLA	8.8	296.74^{b}±25.66	280.32^{b}±23.60	24.28^{c}±2.01
VBLM	8.8	214.52^{d}±10.67	202.72^{d}±11.02	15^{e}±0.29
CBLA	3.6	158.07^{h}±19.79	151.90^{f}±19.63	15.39^{e}±0.75
CBLM	3.4	78.07^{i}±1.85	73.26^{h}±2.17	11.05^{h}±0.42

Each value is expressed as mean ± standard deviation (n=3).VRA- *V.unguiculata* raw acetone, VRM- *V. unguiculata* raw methanol, CRA- *C.pepo* raw acetone, CRM-*C.pepo* raw methanol, VBOA- *V.unguiculata* boiled acetone, VBOM- *V.unguiculata* boiled methanol, CBOA-*C.pepo* boiled acetone, CBOM- *C.pepo* boiled methanol, VBLA- *V.unguiculata* blanched acetone, VBLM- *V.unguiculata* blanched methanol, CBLA-C.pepo blanched acetone, CBLM-*C.pepo* blanched methanol.

** -mg of Tannic acid equivalent (TAE)/g extract; *-mg of Rutin equivalents (RUE)/g extract.

3.4 Total flavonoid content

Flavonoids are a class of plant secondary metabolites that are abundant components of fruits and vegetables, including flavonols, flavan-3-ols, flavones, flavnones and anthocyanins [26]. *In vitro* and epidemiological studies suggest that the consumption of flavonoid-rich foods protects against human diseases because of their free-radical scavenging activity and protection against oxidative stress [30]. Total flavonoid in the present study was estimated using colorimetric method which involves formation of stable acid complexes between the $AlCl_3$ reagent and the C-4 keto group; and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminium chloride forms labile acid complexes with the ortho-dihydroxyl groups in the A- or B-ring of flavonoids [6]. From the investigated sample it was observed up to 15 min of boiling (96.61 mg RUE/g extract) and 20 min of blanching (24.28 mg RUE/g extract) at 100°C, *V. unguiculata* acetone extract showed an increase in amount of flavonoids. This result is in agreement with the findings of Adefegha and Oboh [1] indicating a possible release of some flavonoids during the cooking of the green leafy vegetables. But, on contrary the methanol extract of boiled (18.83 mg RUE/g extract) and blanched (15 mg RUE/g extract) sample showed slight decrease. In the case of *C. pepo*, boiling and blanching for both acetone (17.5 mg RUE/g extract and 15.39 mg RUE/g extract) and methanol extract (13.78 mg RUE/g extract and 11.05 mg RUE/g extract) has shown significant decrease in flavonoid content. This might be due to the fact that, flavonoids being unstable compounds have been destroyed due to thermal treatment [37].

3.5 *In vitro* antioxidant potential

Antioxidant potential of the greens were analyzed by DPPH, ABTS, FRAP and Metal chelating activity (Table 5). DPPH, a stable free radical at ambient temperature is used to evaluate the antioxidant activity of natural compounds, accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Decrease in absorbance at 515 nm determines the antioxidant induced reduction capability of DPPH radicals. In the present study acetone extracts has registered better scavenging activity for DPPH compared to methanol extracts. *V.unguiculata* (Boiled) has registered higher activity (7854.83 mmol TEAC/g extract) among the investigated samples. Also for both the samples processing has increased the scavenging activity of the sample. Preliminary radical scavenging activity of plant extracts are tested using ABTS radicals. They accept an electron or free radical species, which results to discoloration of blue-green chromophore ABTS+. This is an excellent tool for determining the antioxidant activity of hydrogen-donating anti-oxidants (scavengers of aqueous phase radicals) and of chain-breaking antioxidants (scavengers of lipid peroxyl radicals) [30]. Processed sample of *V. unguiculata* (boiled) has shown highest value for ABTS assay (175888.50 TEAC/g extract). In both antiradical assays processed sample of *V. unguiculata* (boiled) has registered higher activity. In general, plant polyphenols are considered to be related to radical scavenging activity. Interestingly the boiled *V. unguiculata* has shown higher content for total phenolics and fairly high amount of flavonoids. The obtained results are in cogency with the underutilized leafy vegetables investigated by Nagarani *et al*. [29].

Metal catalysed oxidation is considered to be one of the reasons for Fenton type reaction which ultimately result in lipid peroxidation. Reduced iron complexes (Fe^{2+}) react with lipid peroxides (ROOH) to give alkoxy radicals and peroxy radicals, which may further stimulate chain reaction. Thus Fe^{2+} chelation is an important antioxidative mechanism which retards metal-catalyzed oxidation [30, 7, 40].The assay used to determine chelating capacity of the samples is based on the principle of red coloured complex fomation due to chelation of Fe^{2+} with ferrozine and decolouration of this complex in the presence of chelating agent measured at 562 nm. The metal chelating activity of *V. unguiculata* and *C. pepo* were 0.03 - 1.27 mg EDTA/g extract and 0.11 - 1.37 mg EDTA/g extract, respectively. Compared to synthetic antioxidants such as BHA (10.59 mg EDTA/g extract) the obtained values for raw and processed leaf extracts of both samples were found to be low. Among the samples, methanol extract of boiled *C. pepo* showed higher chelating activity with 1.37 mg EDTA/g extract and acetone extract of raw *V. unguiculata* registered lower chelating activity (0.03 mg EDTA/g extract). This might be due to enrichment of phenolic compounds after processing. A possible positive relation between phenolic compounds especially

flavonoids was suggested by Loizzo *et al*. [24] correlates with obtained result for methanol extract of processed *C. pepo* leaves. Reducing power is a novel antioxidant defence mechanism; the two mechanisms that are available to affect this property are electron transfer and hydrogen atom transfer [32].The reducing powers of extracts were assessed based on their ability to reduce Fe (III) to Fe (II). The determination of reductant capacity of the sample were performed by FRAP assay ranged from 29.95 mmol Fe(II)/g extract to 31.92 mmol Fe(II)/g extract when both acetone and methanol extracts were considered. Processed samples of *V. unguiculata* and *C. pepo* have shown better reducing capacity compared to raw sample. Boiled leaves of *C. pepo* have shown highest reducing capacity and can be due to preservation bioactive compounds like phenolics, tannin and flavonoids. Similar result was obtained in the investigations done on underutilized leafy vegetables *B. diffusa* and *P. oleracea* [29].

Table 5: Effect of boiling and blanching on the antioxidant activities of *V. unguiculata* and *C. pepo*

Samples	FRAP mmol Fe(II)/g extract	Metal chelating activity (mg EDTA/g extract)	DPPH assay (TEAC mmol/g extract)	ABTS assay (TEAC mmol/g extract)
VRA	30.16^{d}±0.51	0.03^{j}±0.07	2565.09^{e}±37.68	171847.48^{c}±2588.33
VAM	31.20^{b}±0.51	1.27^{b}±0.30	2236.01^{f}±70.78	3423.71^{l}±1041.69
CRA	29.95^{e}±0.15	0.11^{i}±0.04	2088.20^{h}±32.66	164283.49^{e}±6123.01
CRM	30.32^{d}±0.78	1.19^{c}±0.04	1968.98^{j}±17.60	32214.28^{j}±772.34
VBOA	31.38^{b}±0.44	0.17^{h}±0.19	7854.83^{a}±142.95	175888.50^{a}±179.47
VBOM	31.90^{a}±0.39	0.63^{b}±0.02	7345.90^{b}±112.21	34825.41^{f}±342.40
CBOA	31.07^{c}±0.04	1.01^{d}±0.18	2096.11^{g}±78.50	170086.01^{d}±1401.69
CBOM	31.92^{a}±0.49	1.37^{a}±0.23	2019.46^{i}±20.51	33436.95^{h}±1105.73
VBLA	30.97^{d}±0.59	0.22^{g}±0.13	2391.73^{d}±92.87	174230.65^{b}±1424.49
VBLM	30.65^{d}±0.73	0.93^{e}±0.12	2178.83^{f}±56.22	32981.04^{i}±1557.97
CBLA	30.26^{d}±0.09	0.41^{f}±0.30	6808.60^{c}±82.58	33996.48^{g}±543.17
CBLM	31.20^{d}±0.51	0.94^{e}±0.28	1915.45^{k}±10.38	31157.39^{k}±54.11
BHA	350278.70±735.70	10.49±0.06	814172.70±187.00	654356.10±617.10
RUT	172898.60±272.70	-	748175.20±598.00	432942.70±233.10
TAN	565217.40±427.70	-	848540.10±547.00	751041.70±632.30

Each value is expressed as mean ± standard deviation (n=3).VRA- *V.unguiculata* raw acetone, VRM- *V. unguiculata* raw methanol, CRA- *C.pepo* raw acetone, CRM-*C.pepo* raw methanol, VBOA- *V.unguiculata* boiled acetone, VBOM- *V.unguiculata* boiled methanol, CBOA-*C.pepo* boiled acetone, CBOM- *C.pepo* boiled methanol, VBLA- *V.unguiculata* blanched acetone, VBLM- *V.unguiculata* blanched methanol, CBLA-*C.pepo* blanched acetone, CBLM-*C.pepo* blanched methanol.

Trolox equivalent antioxidant capacity (TEAC), (mmol equivalent Trolox performed by using $ABTS^{\cdot+}$ and $DPPH^{\cdot}$

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

Concentration of substance having metal chelating ability expressed as mg EDTA equivalents per g extract.

4. Conclusion

Vitamins and minerals rich foods find an unavoidable place in human nutrition. In that case green leafy vegetables are considered as important food crops. Compared to cultivated greens wild ones are resilient, adaptive and tolerant to adverse climatic condition but lack of awareness and popularization of technologies for utilization they remained underutilized. Current study is a novel approach to reveal the antioxidant potential and nutritional benefits of two lesser utilized greens *V. unguiculata* and *C.pepo*. In all processed samples high amount of bioactive compounds are determined and has given better antioxidant activity compared to raw sample. In *V. unguiculata* the antioxidant capacity was in positive correlation with presence of high amount of phenols, tannins and flavonoids. Also higher reducing and chelating capacity of *C.pepo* in the present investigation is directly proportional to high phenolic content present. Mineral analysis revealed the enhancement of micronutrients when subjection to hydrothermal processing. Further investigation to elucidate nutritional and functional perspectives through *in vivo* studies is needed. In conclusion, traditional processing of these lesser known leafy vegetables has increased their antioxidant potential and nutritional quality and can be advocated for wider consumption in daily life for their possible health benefits and additional investigation for development of value added products from these leafy vegetables is an urgent concern.

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