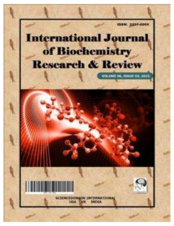

International Journal of Biochemistry Research & Review

24(3): 1-8, 2018; Article no.IJBCRR.44652
ISSN: 2231-086X, NLM ID: 101654445

Evaluation of the Antinutrients, Nutrients, Phytochemicals and Metals Content of Five Leafy Vegetables in Dengi Metropolis

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJBCRR/2018/44652
Editor(s):
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Complete Peer review History: http://www.sdiarticle3.com/review-history/44652

Original Research Article

Received 22 September 2018
Accepted 31 December 2018
Published 21 January 2019

ABSTRACT

Aim: To quantify the levels of amino acids, some metals, and phytochemicals/antinutritional factors in leafy vegetables: *Cucurbita pepo*, *Vitex doniana*, *Hibiscus cannabinus, Leptadenia hastata*, *Balanites aegyptiaca.*
Study Design: The research work is descriptive.
Place and Duration of Study: Department of Biochemistry, University of Jos between March 2016 and September 2016.
Methodology: Five samples each of the vegetables were collected at random from different locations in the area of study. Atomic Absorption Spectrophotometry was applied to analyse for metals. Levels of amino acids in samples were determined using the PTH amino acids analyser. Antinutrients were quantified using standard methods. One way ANOVA was used to analyse the data obtained at 95% level of significance.
Results: Mean levels of Pb and Cd were 0.030 ±0.01 ppm and 0.004 ± 0.001 ppm respectively (P>0.05) relative permissible limits. Mean Mg, Mn and Fe content were 1.084 ± 0.02 ppm, 0.069 ±

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0.01 ppm and 1.534±0.10 ppm respectively (*P*=.05). Amino acids profile indicated mean values (g/100 g proteins) of Glutamate 8.34, Aspartate 8.14, Leucine 8.34, Lysine 4.03, Isoleucine 3.30, Phenylalanine 4.17, Tryptophan 2.25, Valine 4.6, Methionine 1.12, Proline 2.84, Arginine 4.99, Tyrosine 2.75, Histidine 2.23, Cysteine 1.09, Alanine 3.71, Glycine 4.08, Threonine 2.88, Serine 2.99. Mean range of antinutrients were: tannins (g/100 g), 0.51±0.13 to 0.60±0.12, oxalates (mg/100 g), 0.14±0.14 to 0.60±0.20, phytates (mg/100 g), 1.70±0.01 to 4.10±0.01, saponins (g/100 g), 11.85±1.85 to 15.13±1.50, cyanogenic glycosides (mg/100 g), 4.82±1.30 to 7.59±1.20, total alkaloids and total flavonoids (g/100 g) were 16.22±1.61 to 19.37±1.23 and 9.87±1.32 to 14.71±2.30 respectively.

Conclusion: Although samples analysed contained significant amounts of antinutrients, they are very good sources of amino acids; especially Lysine, Methionine, Leucine, tryptophan which are essential; and mineral elements. Levels of lead and cadmium in the samples were lower than safe limits. These vegetables are good sources of nutrients. Their consumption will replenish nutrients to the cell thereby improving the well being of consumers.

Keywords: Amino acids; antinutrients; nutrients; metals; vegetables.

1. INTRODUCTION

Leafy vegetables have been shown to be valuable sources are generally good sources of nutrients, important protective foods, highly beneficial for the maintenance of health and prevention of diseases as they contain valuable food ingredients which can be utilized to build up and repair the body and are therefore indispensable constituent of human diet [1]. Some leafy vegetables have medicinal properties [2]. They are valuable in maintaining alkaline reserve in the body and are valued mainly for their high vitamin, dietary fibre and mineral content. The dark green leaves and deep yellow fruits provide a high amount of carotene, ascorbic acid and micro-minerals which play important roles in nutrient metabolism and slowing down of degenerative diseases [3]. These vegetables serve as valuable sources of nutrients especially in semi urban areas like Dengi where they exist in the open country. The feeding pattern of people in Dengi metropolis suggests a heavy reliance on many leafy vegetables commonly found in the town. The five leafy vegetables for this study are the most common ones found in town.

Leafy vegetables might contain significant levels of trace elements, heavy metals, amino acids as well as antinutrients. Leafy vegetables can contribute substantially to food security in the rural areas where people's diet is based on mostly carbohydrates and legumes which are high in calories but deficient in essential micronutrients and proteins [4].

Antinutritional factors reduce the nutritive values of many plants due to their natural inherence in the plants. They are capable of eliciting deleterious effect in man and animals [5].Oxalate tends to render calcium unavailable by binding to the calcium ion to form complexes [1,6]. Apart from their fruits, plant seeds are also rich sources of nutrients [7]. Phytic acid acts as a strong chelator forming protein and mineral-phytic acid complexes thereby decreasing protein and mineral bioavailability [8]. Phytate is associated with nutritional diseases such as rickets and osteomalacia in children and adult, respectively. Tannins are water soluble phenolic compounds with a molecular weight greater than 500 and with the ability to precipitate proteins from aqueous solution. They occur in all vascular plants. Tannins bind to proteins making them bio-unavailable [9]. This work seeks to quantify both the nutrients and antinutrients in these plants samples.

Heavy metals such as arsenic, cadmium, lead are toxic to cells [10], thus it is important to determine their levels in especially in plant-based foods and diets. In general, information on edibility and therapeutic properties of wild plants is scanty but data on their nutritional composition and mineral content is negligible [11]. Manganese is an essential trace element, which plays an important role as a cofactor for many enzyme systems such as hexokinase and superoxide dismutase. At high level however, it can cause damage to the brain [12]. Magnesium is another nutrient required in the plasma and extra cellular fluid, where it helps in maintaining osmotic equilibrium. It is required in many enzyme–catalysed reactions, especially those in which nucleotides participate where the reactive species is the magnesium salt (eg$MgATP^{2-}$). Lack of Mg is associated with abnormal irritability of muscle and convulsions whereas excess level is implicated in depression [13].

Iron is necessary for the formation of haemoglobin and also plays an important role in oxygen and electron transfer in human body [14], also in the functioning of the central nervous system as well as oxidation of carbohydrates, proteins and fats [15].Cadmium is a heavy metal that causes both acute and chronic poisoning; adverse effect on kidney, liver, vascular and immune system [16]. Chronic exposure to chromium may result in liver, kidney and lung damage [17].

Lead causes both acute and chronic poisoning with the kidney and liver as primary targets. It inhibits the catalytic action of δ-amino levulinic acid dehydratase (Porphobilinogen Synthase) in the haem biosynthetic pathway therefore exerting a toxic effect on the vascular and immune system [18].

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Chemicals and reagents

All reagents and chemicals were of analytical grade from British Drug Houses.

2.1.2 Equipment used

OHUAS (Ohaus Harvard Trip Balance) digital balance, applied biosystems PTH (phenylthiohydantoin) amino acid analyzer and Soxhlet assembly set up.

Raw samples of the leafy vegetables were collected from different farms in and around Dengi metropolis. They were destalked, washed with distilled water and air dried. Samples were pulverised in ceramic mortar and pestle. This was followed by sieving the samples to obtain fine particles 10 g each for the analysis using the Enodecott machine.

2.2 Amino Acids Analysis

The amino acid profile was determined using the method described by [19]. Where each of the dried samples were defatted(by refluxing 100 g of the air-dried powdered samples with 250 ml petroleum ether for 4 hours, the resulting residue was then dried and subjected to aqueous extraction using Soxhlet assembly. Extracts were thereafter evaporated and loaded into the “applied biosystems PTH amino acid analyzer” which separated and analysed free acidic, neutral and basic amino acids of the hydrolysate.

2.3 Determination of Mineral Elements Content

The minerals content of the different samples was evaluated for Mn, Mg, Fe, Cd, Cr and Pb by dry ashing of dried powdered sample (5 g) in a muffle furnace set at 775°C. The ash obtained was dissolved in 5 mL of 20% HCl and analysed using the atomic absorption spectrophotometer at their respective wavelengths of maximum absorption (λ_{max}) thus: 385 nm, 285.5 nm, 405 nm, 357.8 nm, 582 nm, 389.6 nm for Mn, Mg, Fe, Cd, Cr and Pb in that order.

2.4 Determination of Antinutrients

Tannins were quantified according to Bainbridge et al. [20], total oxalate quantified applying Day and Underwood method [21]. Phytate content was determined by the Reddy and Love method [22]. Saponin was quantified applying Makkar et al. method [23]. Total flavonoids content of the samples was determined using the Folin-Ciocalteu method. For cyanogenic glycoside, the method of Aritomi et al was applied [24].

2.5 Statistical Analysis

The statistical method employed for all the analysis was one way ANOVA and all results are means of three determinations (±SD). P =.05 was considered significant.

3. RESULTS AND DISCUSSION

This research sought to assess the amino acids, antinutrients, phytochemicals and metals composition of five commonly consumed leafy vegetables in Dengi metropolis. Eighteen amino acids were analysed in different proportions in the vegetables. The amino acid contents are generally high in all samples with the highest, based on dry weight, observed in *Letadenia hastata* (93.20 g/100 g protein) others range between 71.41 g to 74.62 g/100 g proteins. Level of Glutamic Acid was the highest amongst other amino acids in all samples (with an average 11.19 g/100 g proteins) followed by Aspartic Acid with an average of 7.92 g/100 g proteins whereas Cysteine and Methionine were low with average of 1.37 g/100 g proteins. All the samples analysed contained high levels of Glutamate with *Letadenia hastata* have the highest level at 12.11 g/g proteins. Levels of Cysteine and Methionine in *Balanites aegyptiaca* were 0.78 g/100 g and 0.78 g/100 g proteins respectively. The samples contain essential amino acids such as Methionine, Lysine, Leucine, Isoleucine, Tryptophan, Phenylalanine, Valine and Histidine albeit the levels were lower than the non essential amino acids. Leucine stimulates muscle strength and growth, regulate blood sugar level by moderating insulin into the body during and after exercise and can even help prevent depression by the way it acts on neurotransmitters in the brain [25]. Lysine is responsible for proper growth and in the production of carnitine (a nutrient responsible for converting fatty acids into fuel to lower cholesterol). It also helps the body absorb calcium for further bone strength and also aids in collagen production. Methionine helps form cartilage in the body through the use of sulphur. Histidine is involved in transport neurotransmitters to the brain and also helps overall muscle health within each muscle cells. Valine is needed for optimal muscle growth and repair [26].

Table 2 shows the mineral content of the vegetables. The permissible limit of iron in edible plants is 20 ppm [27]. Iron is necessary for the formation of haemoglobin and also plays an important role in oxygen and electron transfer in human body [27] and normal functioning of the central nervous system and in the oxidation of carbohydrates, proteins and fats [28], the highest iron content of 2.223 ppm was found in the leaves of *Leptadenia hestata* while the leaves of *Balanites aegyptiaca* contain the least iron content of 0.772 ppm, the leaves of *Vitex doniana, Hibiscus cannabinus* and *Cucurbita pepo* have significantly higher iron content of 1.081, 0.916 and 0.831 ppm, respectively. An average culinary preparation contains about 300g of the fresh leaves of vegetables and this would result in an intake of 30 g dry weight leaves per serving portion. Therefore 1.081, 0.916 and 0.831 ppm in *Vitex doniana*, *Hibiscus cannabinus and Cucurbita pepo respectively*, will contribute up to, in mg, 3.2, 2.7 and 2.3/serving portion of Fe respectively, to the recommended dietary allowance of Fe (10-15 mg/day) [15].

Manganese level, in ppm, was found to be 0.133 in *Leptadenia hastata,* 0.065 in *Hibiscus cannabinus,* 0.041 in *Balanites aegyptiaca*, 0.061 in *Cucurbita pepo* and 0.051 in *Vitex doniana* with respectively.

In Table 2, level of Mg, in ppm, was 1.091 in *Cucurbita pepo*, which is the highest, compared to others whose content ranged from 0.915 to 1.080. Mg is required in the plasma and extra cellular fluid, where it helps in maintaining osmotic equilibrium [1]. It is required in many enzyme – catalysed reactions, especially those in which nucleotide participate where the reactive species is the magnesium salt, $MgATP^{2-}$. Deficiency of Mg is associated with abnormal irritability of muscle, and convulsions. Excess Mg predisposes to depression [1].

Cadmium concentration, in ppm, of the samples ranged from 0.003 in *Hibiscus cannabinus* to 0.004 in *Leptadenia hestata*. These values are below the permissible limit of 0.212 in edible plant [29]. In medicinal plants however, the permissible limit by WHO is 0.310. The low level of Cd in all the samples means they are safe for consumption.

As for Pb content of the samples, *Leptadenia hestata* had 0.002 ppm whereas *Balanites aegyptiaca* contained 0.055 ppm. These levels in the samples are below the permissible level, 0.43 ppm [29].

Results of the phytochemical analysis are presented in Table 3. Antinutritional factors have been shown to limit the use of many plants due to their ubiquitous occurrence as natural compounds capable of eliciting deleterious effect in man and animals [30]. The major antinutritional factors commonly found in green leafy vegetables are phytic acid, oxalic acid and tannins [31]. High levels of phytates and oxalates have been shown to inhibit the absorption and utilization of minerals such as calcium by animals including man [32]. Tannins decrease protein quality by reducing the digestibility and palatability; they interfere with absorption of iron and a possible carcinogenic effect [33].

The oxalate content in these vegetables ranged between 0.14 mg/100 g in *Vitex doniana* to 0.60 mg/100 g in *Cucurbita pepo*. These values are below the established toxic level which is between 15-30 g [34]. The phytate level was between 1.7 mg/100 g in *Cucurbita pepo* to 4.1 mg/100 g in *Leptadenia hastate*. Results obtained are below the toxic level [27]. According to Agbaire [35] a phytate diet of 1-6% over a long period of time decreases the bioavailability of minerals in mono gastric animals.

Cyanogens are glycosides of a sugar, sugars and cyanide containing aglycone. Cyanogens can be hydrolyzed by enzymes to release a volatile HCN gas [36]. Excess cyanide ion inhibits the cytochrome oxidase which stops ATP formation and so tissues suffer energy deprivation and death follows rapidly. High level of HCN has been implicated for cerebral damage and lethargy in man and animal [19]. In table 3, the levels were 7.59 mg/100 g in *Cucurbita pepo* and 4.82 mg/100 g *in Balanites aegyptiaca* which are below lethal level as indicated on the Table 3. As for saponins, level ranged between 11.85 mg/100 g in *Leptadenia hastata* to 15.13 mg/100 g in *Vitex doniana*. Saponins are glycosides containing polycyclic aglycone moiety of either C_{27} steroid or C_{30} triterpenoids attached to a carbohydrate. High saponin level has been associated with gastroenteritis manifested by diarrhoea and dysentery [37].

Tannins are water soluble phenolic compounds with a molecular weight greater than 500 and with the ability to precipitate proteins from aqueous solution. They occur in all vascular plants. Tannin binds to proteins making them bio unavailable [38]. From the results, the level obtained was between 0.51 g/100 g in *Balanites aegyptiaca* and 0.60 g/100 g in *Vitex donina*. There was no significant difference in the Tannin content of the vegetables ($p>0.05$) relative reference standard level.

Alkaloids have been implicated in the inhibition activities of many bacterial species [39]. The levels of alkaloids in the various leafy vegetables analysed ranges from 16.22 g/100 g observed in *Letadenia hastata* and 19.37 g/100 g in *Vitex doniana.* Plant phenolics such as flavonoids have been shown to have antioxidant properties and also contribute to their medicinal significance [40]. In this work, the levels of flavonoids ranged from 9.87 g/100 g in *Balanites aegyptiaca* to 14.71 g/200 g in *Vitex doniana*. Consumption of these vegetables would further enhance the capacity of the cell to mop up the highly reactive oxygen radicals generated due to oxidative metabolic reactions that occur in cells.

Table 1. Amino acid composition of five leafy vegetables in Dengi metropolis

Amino acids g/100 g proteins	*Cucurbita pepo*	*Vitex doniana*	*Hibiscus cannabinus*	*Letadenia hastata*	*Balanites aegyptiaca*	MEAN	S.D	C.V %
Leucine	8.14	6.48	7.29	8.34	6.48	7.35	0.9	12.03
Lysine	4.03	3.63	3.97	5.54	4.37	4.31	0.7	17.11
Isoleucine	3.3	3.01	2.94	4.78	3.4	3.49	0.8	21.47
Phenylalanine	4.17	4.43	4.34	5.58	4.7	4.64	0.6	12
Tryptophan	2.25	1.26	2.1	2.86	1.12	1.92	0.7	37.78
Valine	4.5	3.59	4	5.61	4.09	4.36	0.8	17.69
Methionine	1.12	1.23	1.71	2	0.8	1.37	0.5	34.95
Proline	2.84	3.14	2.84	4.57	2.23	3.12	0.9	27.96
Arginine	4.99	6.11	5.33	7.83	4.99	5.85	1.2	20.47
Tyrosine	2.75	3.09	3.09	4.13	3.09	3.23	0.5	16.23
Histidine	2.23	2.17	2.39	2.11	2.65	2.31	0.2	9.39
Cysteine	1.09	1.21	1.57	2.18	0.78	1.37	0.5	39.22
Alanine	3.71	4.4	3.94	5.4	4.28	4.35	0.7	14.95
Glutamic acid	9.99	11.28	11.58	12.11	10.97	11.19	0.8	7.06
Glycine	4.08	4.11	3.75	4.46	3.89	4.06	0.3	6.61
Threonine	2.88	2.99	3.27	3.38	2.88	3.08	0.2	7.51
Serine	2.99	3.29	3.13	3.83	3.25	3.3	0.3	9.69
Aspartic Acid	8.31	8	7.38	8.49	7.44	7.92	0.5	6.33
TOTAL	**73.37**	**73.42**	**74.62**	**93.2**	**71.41**	**77.2**	**9**	**11.68**

Table 2. Levels of metals in five leafy vegetables in Dengi metropolis

Samples	Pb	Cd	Mg	Mn	Fe
			Concentration (ppm)		
Cucurbita pepo	0.0229±0.004	0.003±0.001	1.0871±0.002	0.0557±0.001	0.7997±0.003
Vitex doniana	0.0302±0.002	0.0036±0.001	1.1371±0.010	0.0499±0.001	1.0799±0.100
Hibiscus cannabinus	0.0396±0.021	0.029±0.001	1.1255±0.004	0.0653±0.004	0.9159±0.012
Letadenia hastata	0.0021±0.001	0.037±0.010	0.9951±0.010	0.1331±0.000	2.2231±0.900
Balanitesaegyptiaca	0.0552±0.012	0.041±0.001	1.0753±0.002	0.0405±0.001	0.7723±0.123
permissible levels	0.10	0.05	------	------	------

Values represent means of triplicate determination± SD

Table 3. Antinutrients and phytochemicals content of five leafy vegetables in Dengi metropolis

Samples	Tannins (g/100 g)	Oxalate (mg/100 g)	Phytate (mg/100 g)	Saponins (g/100 g)	Cyanogenic Glycosides (mg/100 g)	Alkaloids (g/100 g)	Flavonoids (g/100 g)
Cucurbita pepo	0.53± .012	0.60±0.20	1.70±0.01	12.49±1.20	7.59±1.20	17.45±2.51	13.72±1.21
Vitex doniana	0.60±0.12	0.14±0.01	2.10±0.21	15.13±1.50	5.33±1.11	19.37±1.23	14.71±2.30
Hibiscus cannabinus	0.59±0.13	0.32±0.12	3.14±0.12	13.35±2.35	4.93±2.13	17.10±2.50	10.38±1.30
Letadenia hastate	0.55±0.21	0.22±0.03	4.10±0.01	11.85±1.85	6.21±1.20	16.22±1.61	13.22±1.23
Balanites aegyptiaca	0.51±0.13	0.31±0.01	3.08±0.02	13.48±3.12	4.82±1.30	18.13±2.31	9.87±1.32

The values represent the mean of three determinations± SD

4. CONCLUSION AND CONTRIBUTION TO KNOWLEDGE

From these results, leaves of the vegetables constitute rich sources of amino acids and mineral elements. The vegetables are therefore rich alternatives (and/or supplements) which can replenish the cellular requirements of the nutrients. Also, samples contain antinutritional factors such as oxalates and phytates but at low levels. The content of flavonoids and alkaloids in appreciable amounts in the samples is critical given the therapeutic/medicinal use of phytochemicals. Hence, leafy vegetables could contribute to the alleviation of protein malnutrition and micronutrient deficiencies if consumed.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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