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Chemical, Functional properties and Amino acid composition of Water leaf (*Talinum triangulare*)

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Abstract The proximate, minerals, functional properties and amino acid composition of water leaf *(Talinum triangulare*) were investigated. The leaf, stem and root contained: ash (26.00%, 10.01%, 38.22%), crude protein (28.22%, 24.12%, 25.11%), moisture content (10.10%, 18.00%, 11.10%), crude fat 7.45%, 6.45%, 7.12%, crude fibre (11.45%, 9.22%, 10.42%, 6.45% and carbohydrate (16.78%, 32.2% 8.03%) respectively. Potassium was the highest mineral in all the parts of the sample while sodium was the lowest. The soil also contained more minerals compared to leaf, stem and root. The sample contained: water absorption capacity (137.5%), oil absorption capacity (32.40%). foaming capacity (22.10%), foaming stability (4.30%), emulsion capacity 27.70%, emulsion stability (43.5%) and least gelation capacity (8.00%) respectively. Amino acid result showed that glutamic acid had the highest value of 57.87 mg/g crude protein while cystine had the least value of 8.13mg/g protein.

Keywords Water leaf, *Talinum triangulare*

Introduction

Vegetables are the cheapest and most available sources of protein, vitamins, minerals and essential amino acids [1]. They are added as supplements to provide useful and correct proportions of nutrients and some have medicinal properties useful for the invalids and convalescences. Consumable vegetables are those plants whose leaves or aerial parts have been integrated into a food chain for consumption over a large span of time. They are highly recommended because they have a relatively high nutritional value and their consumption gives daily food intake and adding flavour to the diet. Vegetables constitute essential components of the diet, by contributing nutrients and food properties that are important to human health. A healthy heart and circulation system could benefit from a balanced diet with adequate fruits and vegetables [2]. Epidemiological evidences support a significantly positive correlation between eating fruits and vegetables as well as cardiovascular health [3-4]. Increased awareness on the usefulness of vegetable inclusions in human food has enhanced their consumption as part of daily diet. Consumption of vegetables has been associated with a reduction in the incidences of chronic diseases of lifestyle such as cardiovascular diseases, coronary heart diseases and various types of cancer. *Talnum triangulare* belongs to the botanical family of portulacaceae which grows almost in every area and highly tolerant to drought. It is an erect perennial crop with swollen and obtuse angular, hairless and succulent stem that grows about 30-100cm tall; the branches are with two lateral and basal buds. The leaves are arranged spirally and nearly opposite. The leaf-blades are usually spoon-shaped with a size of measure about 3-15cm by 1-6 cm. It has a lot of economic importance, which include provision of food, income, employment and herbal medicine to the population [5]. It is mainly used as leafy vegetable for human consumption and its gastronomic use is most popular in local delicacies of Southern Nigeria most especially in Ekiti state. *Talinum triangulare* is rich in iron, calcium, vitamins A and C. Because of the presence of high level of oxalic acid, its consumption should be avoided or limited by those suffering from kidney disorders, gout, and rheumatoid arthritis. It is one of the most important leafy vegetables in Nigeria. The average nutritive value per 100g of edible portion of waterleaf is higher than that of lettuce, but lower than pumpkin (6). This work was designed to study the proximate, minerals of all the parts and amino acid of *Talinum trangulare* leaf, steam and root.

Materials and Methods

Sample collection and Preparation

The *Talinum triangulare* leaves were collected along Are-Afao, Iworoko, Ekiti, Ekiti State, Nigeria. The spot of collection is about 20 fts away from the road. The sample was separated into parts; leaf, stem and root respectively. They were later air-dried, made into flour using Kenwood blender and then stored in a freezer prior to analyses.

Proximate Analysis

The moisture and ash contents were determined using the air oven and dry ash method [7]. The sample was analyzed for crude fat and crude protein according to the methods described by [8]. Nitrogen was determined by micro-Kjedahl method described by (AOAC, 2005) and the percentage nitrogen was converted to crude protein by multiplying by 6.25. The crude fibre was determined by adding 2g (W_1) of the sample into 500ml conical flask; 200ml of boiling 1.25% of H_2SO_4 was added and boiled for 30minutes. The mixture was filtered through muslin cloth and rinsed with hot distilled water. The sample was scrapped back into the flask and 200ml of boiling 1.25% NaOH was added and allowed to boil again for another 30 minutes; filtered and then rinsed with 10% HCl twice with industrial methylated spirit and allowed to drain and dry. The residue was scrapped into a crucible, dried in the oven at 105 °C, allowed to cool in a desicator and weighed (W_2); then placed in muffle furnace at 300 °C for 30 minutes and finally allowed to cool at room temperature and weighed again (W_3).

$$\%\text{ crude fibre} = \frac{W_2 - W_3}{W_1} \times 100$$

The carbohydrate content was calculated by method of difference.

$$\%CHO = \{100 - (\%\text{ moisture} + \%\text{ Ash} + \%\text{ crude fibre} + \%\text{Crude fat} + \%\text{ Crude protein})\}$$

Mineral Analysis

The minerals were analyzed by dry ashing the sample at 550 ºC to constant weight and dissolving the ash in 100 ml standard flask using distilled deionized water with 3ml of 3M HCl. Sodium and potassium were determined by using a flame photometer (Model 405, Corning, U.K). All other minerals were determined by Atomic Absorption Spectrophotometer (Perkin & Elmer model 403, USA).

Determination of Functional properties

The water and oil absorption capacities of the sample were determined using the method [9]. 10cm^3 of water was added to 1.0g sample in a centrifuge tube. The suspension was mixed vigorously using votex mixer. This was then centrifuged at 3500 rpm for 25 minutes and the volume of the supernatant left after centrifuging was noted. Water bound was calculated from the difference in the initial volume of the solvent used and the final volume after centrifuging. The same procedure was used for oil absorption capacity but oil replacing water in above process.

The slight modified procedure of Sathe and Salunkhe [10] was used to determine least gelation concentration. Sample slurries of 2, 4, 6, 8, 10, 12, 14 and 16 were prepared in 5ml of distilled water. The test tubes containing these slurries were heated for one hour in boiling water followed by rapid cooling under running tap water. The test tubes were then cooled for 2hours at 4 ºC. The least gelation concentration was determined as concentration which did not fall or slip when the test tubes were inverted.

The method of Coffman and Garcia [11] was employed to determine foaming capacity and stability. 1g of the sample was whipped with 50ml distilled water for 5 minutes in a Kenwood blender and later poured into a 100 ml graduated flask to study the foaming stability (volume increase %). The foaming capacity was calculated according to the following equation.

$$\text{Volume increase \%} = \frac{\text{volume after whipping - volume before whipping}}{\text{Volume before whipping.}} \times 100$$

The emulsion capacity and stability was determined by the method of Yasumatsu et al [12]. The emulsion (2g) sample, 20ml distilled water, 20ml executive oil was prepared in a calibrated centrifuge tube. The emulsion was centrifuged (Gallen kamp) at 3,500xg for 5min. the ratio of the height of the emulsion layer to the total height of the mixture was calculated as the emulsion capacity expressed in percentage. The emulsion stability was estimated by heating the emulsion contained in a calibrated centrifuge tube at 80 °C for 30min in a water bath, cooling for 15min under running tap water and centrifuging (Gallen Kamp) at 3,500xg for 15min. the emulsion stability, expressed as a percentage, was calculated as the ratio of the height of the emulsified layer to the total height of the mixture.

The dependence of protein solubility with pH was determined by mixing, for 5 mins, 2g of flour with 50cm^3 of prepared salt solution in a magnetic stirrer at room temperature (25 ^{o}C). The pH of resulting solution was adjusted to the desired value using either 0.1M HCI or 0.1M NaOH: samples were centrifuged for 30 mins and the protein content of the supernatant determined by micro-Kjeldehl method [8].

Determination of Amino acid

The amino acid profile was determined using the method described by Spackman et al. [13]. The sample was dried to constant weight and defatted using Soxhlet extractor. After the defatting process, the defatted sample (2g) was weighed into a glass ampoule; 7ml of 6MHCl was added and oxygen was expelled by passing nitrogen into the glass ampoule sealed with Bunsen burner flame and placed in an oven present at 105±5 ºC for 22 hours. The ampoule was allowed to cool before broken at the tip and the content was filtered to remove the organic matters. The filtrate was then evaporated to dryness at 40 ºC under vacuum in a rotavapor. The residue was dissolved in 5ml of acetate buffer (pH 2.0) and stored in specimen bottles which were kept in the freezer. The hydrolysate (7.5µL) was dispensed into the cartridge of the Technicon Sequential Multi-Analyser (TSM) using a syringe. The TSM analyser is designed to separate and analyse neutral, acidic and basic amino acids of hydrolysate. The amount of amino acid was obtained from the chromatogram peaks. The whole analysis lasted for 76 minutes and the gas flow rate was 0.50mL per minute at 60 ºC with reproducibility consistent within ±3%.

Results and Discussion

Table 1: Proximate composition of water leaf (T*alinum triangulare*)

Proximate analysis	Composition %		
	Leaf	**Stem**	**Root**
Ash	26	10.01	38.22
Moisture	10.1	18	11.1
Crude Fat	7.45	6.45	7.12
Crude fibre	11.45	9.22	10.42
Crude protein	28.22	24.12	25.11
Carbohydrate	16.78	32.2	8.03

The proximate analysis of the leaf, stem, and root samples are shown in Table 1. Moisture content of a particular vegetable usually depends on age, agronomic particles, freshness, method of drying and duration before use [14]. The moisture contents of oil seeds are generally low and that is why they would probably not be susceptible to microbial attack [15]. The moisture content of leaf, stem and root of the samples were shown to be 10.10%, 18.00% and 11.10% respectively. The moisture values were higher than those reported by Ogungbenle [15] for gourd seeds (3.46%) and for soy beans (5.38%) reported by Temple et al [16]. This shows that the susceptility of *Talinum triangulare* to microbial attack would be higher than those of gourd seeds and soy beans. The ash content was fairly high. The intake of this vegetable would be expected to contribute a large proportion of the mineral requirement in the body. The ash content for the leaf, stem and root were 26.00%, 10.01% and 38.22%. These values are high compared to some legumes such as the cream coat variety of Bambara groundnut (4.30%), kersting's groundnut (3.2%), brown (3.7%) and white coat variety of cowpea (3.6%) reported by Olaofe et al. [19], (4.12%) for soy beans

(16) and (2.9%) for groundnut [18]. It is also comparably higher than those vegetables like *Basella alba L* (2.0%) and *Amaranthus cruentus* (2.5-2.9%) [14]. It worth noting that, the leaf has the highest proportions of nutrients when compared to the stem and the root. The root had the highest value of ash (38.22%) than the leaf and stem. *Talinum triangulare* had crude protein content of leaf (28.22%), stem (24.12%) and root (25.11%) respectively. The protein is more concentrated in the leaf than other parts of the plant. This value of crude protein in the leaf was higher than those of Iima bean flour (26.2%) [18], *Amaranthus cruentus* (4.0 – 6.0%), *Adansonia digitata* (3.8%) [14] but lower than those reported for soy beans (43%) by Apata and Ologhodo [20] and calabash seed (35.9%) [21]. Therefore, *Talinum triangulare* is a good nutrient supplement especially in foods that are low in protein but high in carbohydrate. It is also a cheap source of protein than animal products e.g. meat, fish, egg, poultry e.t.c.

The fat contents of the leaf, stem and root of *Talinum triangulare* were 7.45%, 6.45%, 7.12% respectively. It had a low value of fat when compared to some oil seeds like calabash seed (43%) [21], soy beans (18%) and groundnut (43%) [20] but higher than that of *Cactus pear cladodes* flours (2.30%) by Cervantes et al. [22]. This indicates that the plant is not a good source of oil for domestic and industrial purposes. The crude fibre values for the leaf, stem and root were 11.45%, 9.22% and 10.42% respectively. These values were higher than those of dry okra fruit (8.85%) reported by Ogungbenle et al. [23], quinoa seeds (9.50%) by Ogungbenle [24], *Amarantus hybridus L* (8.61%) were 16.78%, 32.2% and 8.03% respectively. The carbohydrate content in the leaf was higher than those of gourd seeds (9.38%) reported by Ogungbenle [15], pumpkin (6.93%) [17] but lower than 46.5% reported for *Pleurotus tuber regium* [25].

Mineral composition

It was generally observed that the soil contained the highest proportions of minerals than other parts such as leaf, stem and root. Modern diets which are rich in animal proteins and phosphorus may promote the loss of calcium in the urine [26]. This has led to the concept of the Ca/P ratio. If the Ca/P (Low Calcium, high phosphorus intake) is, more than the normal amount of calcium may be lost in the urine decreasing the calcium level in bones [27]. Food is considered good if the ratio is above one and poor if the ratio is less than 0.5. [28]. This means that the studied samples were good in Ca/P ratio. The Ca/P ratio of leaf, stem and root were 0.92, 1.43 and 1.41 respectively which means that consumption of this meal will not lead to loss of calcium in the urine. Na/K, in the body is of great concern for prevention of high blood pressure. A Na/K ratio of 0.6 is recommended [28].

Table 2: The mineral composition of the leaf, stem and root of water leaf (*Talinum triangulare)* (g/100g)

Mineral	Composition			
	Leaf	**Stem**	**Root**	**Soil**
Na	3.72	4.33	2.50	85.54
K	2.60	5.20	3.50	93.75
Mg	2.24	1.21	0.62	37.58
Ca	0.83	0.43	0.51	41.33
P	0.90	0.30	0.36	37.58
Zn	0.08	0.11	0.09	12.54
Fe	0.40	0.34	0.38	1.34
Cr	0.03	0.06	0.04	0.85
Cu	0.02	0.02	0.01	5.22
Mn	0.01	0.01	0.02	1.36
Na/K ratio	1.43	0.83	0.71	0.91
Ca/P ratio	0.92	1.43	1.41	1.0

The samples had Na/K values slightly higher than 0.6 therefore consumption of the sample must be moderate to avoid high blood pressure disease. Generally, minerals from plant sources are less bio-available than those from animal sources [29]. In this study, Na, K, Mg and Ca contents (g/100g) were particularly higher and the leaf is richer in Na with a value of (3.72g/100g), K (2.60g/100g), Mg (2.24g/100g), Ca (0.83g/100g) and other elements like Fe, P, Zn, Cr, Cu, Mn were very low. Na is required for balance of extracellular body fluid. Calcium is responsible for the formation of bone [24]. Deficiency of Ca and P lead to a disease common among women called Osteomalacia (Bone thinning) and Osteoporosis (Adult ricket) [30]. Mg ions regulate over 300 biochemical reaction in the body through their role as enzyme co-factors. They also play a vital role in the reactions that generate and use of ATP, the fundamental unit of energy within the body's cells. This value (2.24 g/100g) was higher than that of fermented *Parkia biglobosa* (2.30 g/100g) [31]. The high Na/K ratio for the sample is an indication that it may not reduce the incidence of hypertension as it would not reduce high blood pressure, which is the major cause of cardiovascular disease [32] while the moderately high calcium in the sample would help to circumvent bone diseases such as rickets, osteoporosis and osteomalacia [31].

Table 3: Functional properties composition of water leaf (*Talinum triangulare*)

Functional Properties	Composition (%)
Water Absorption capacity	137.5
Emulsion stability	43.5
Emulsion capacity	27.7
Foaming capacity	22.1
Foaming stability	4.30
Oil absorption capacity	32.4
Least gelation concentration (w/v)	8.0

Water absorption capacity (WAC) of *Talinum triangulare* was 137.5 % (Table 3). This value was comparatively lower than those reported for benniseed (182.00%) and quinoa seeds (147%) but higher than that of pearl millet (115%) [33]. It is also found to be higher than that of melon seeds (12%) [17], but comparable with that of soy flour [34]. The high WAC of *Talinum triangulare* makes it useful as soup thickener. The oil absorption capacity (OAC) of *Talinum triangulare* (32.4 %) is lower than the values reported for most legumes such as melon seeds (122.00%) [17], pigeon pea flour (89.70 %) [35], sun flower flour (270%) [34] and *Cucumeropsis vulgaris* (302%) [36]. The capacity of a protein to absorb fat had been attributed to the physical entrapment of oil by numerous non-polar side groups of the protein [37]. The result of OAC showed that it would not be a good flavour retainer. The least gelation concentration (LGC) was 8.0%w/v. This value was lower than the values reported for pigeon pea (12%w/v) [35] and full-fat fluted pumpkin (30%w/v) [38], but comparable with that reported for the brown species of lima beans (8.0 % w/v) [19]. The ability of protein to form gel provide a structural matrix for holding water, flavour, sugars and food ingredients and this is useful in food application and new product development, thereby providing and added dimension to protein functionality [39]. The emulsion capacity and stability were 27.7% and 43.5% respectively (Table 3). The emulsion capacity was lower than the values reported for benniseed (63.00%), pearl millet (89.00%) and quinoa (104.0%) (33) but higher than the values reported for soy bean flour (18%) and wheat flour (7-11%) [34]. The emulsion stability after 5h was 43.5%. This value was lower than 86.7% reported for *Dalium guineese* pulp (40) but higher than that 29.0% for *Celosia spicata* (41). The value of foaming capacity was 22.1%. This value was lower than those reported for soy bean flour (66%) [34] and pigeon pea (68%) [35]. The foaming stability of 30 min (4.30%) was lower than that of soy flour (14.8%) and pigeon pea flour (20.0%) [35], but compared favourably with those values reported for full – fat fluted pumpkin seeds (5.0%) [38], quinoa seeds (2.0%) [33] sesame flour defatted (10.10%), raw (8.00%) [41].

The result of the variation of solubility of *Talinum triangulare* leaf flour with pH is depicted in Fig 1. The minimum protein solubility was at pH 3.0, which may correspond to the isoelectric point of the protein [24]. The pH observed for minimum protein solubility is lower than the value reported for pearl millet and quinoa flours (pH 6.0) and benni seed (pH 5.0), but compared favourably with the value reported for white melon (pH 3.0) [15] but lower than that of fluted pumpkin seeds (pH 4.0) [38]. Minimum protein solubility of *Talinun triangulare* in the acid region of pH indicates its potential usefulness in the formulation of carbonated beverages and low acid foods [24].

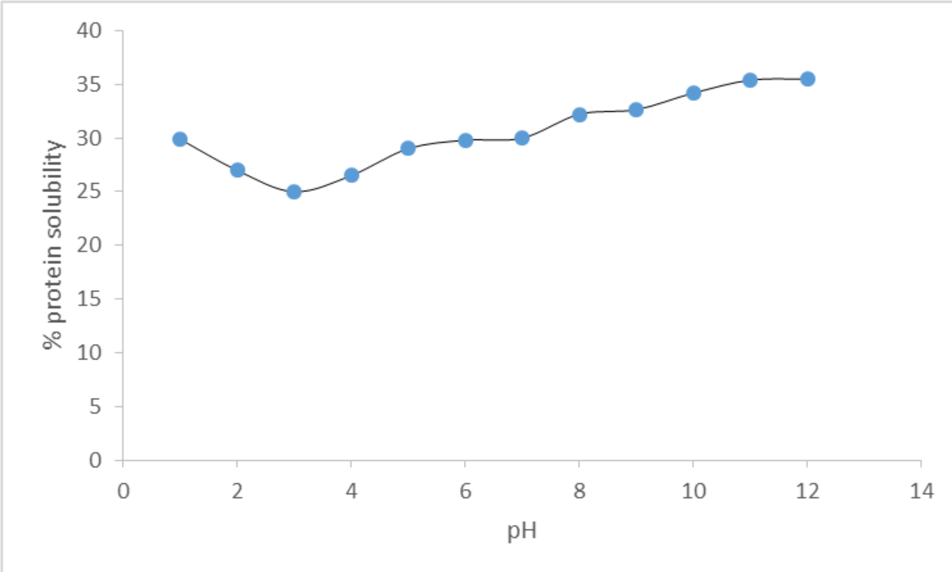

Figure 1: Variation of protein solubility (PS) of Talinum triangulare flour with pH

Amino acid	Composition (mg/g crude protein)
Alanine	38.25
Aspartic acid	43.81
Arginine	37.25
Glycine	34.93
Glutamic acid	57.87
Histidine	12.56
Isoleucine	34.43
Lysine	16.56
Methionine	13.75
Cystine	8.13
Leucine	5.6.37
Serine	25.18
Threonine	25.62
Phenylalanine	38.81
Valine	88.75
Tyrosine	28.25
Tryptophan	11.37

Table 5: Essential, non-essential, acid, basic, neutral total sulphur and total aromatic amino acids (mg/g crude protein) with isoelectric point of water leaf *(Talinum triangulare)*

Amino acid	Value (mg/g)
Total amino acid (TAA)	521.88
% TAA	100.00
Total non-essential amino acid (TNEAA)	273.66
% TNEAA	52.43
Total essential amino acid (TEAA)	248.22

% TEAA with Histidine	47.56
Total essential amino acid (TEAA) without Histidine	235.66
% TEAA without Histidine	45.16
Total neutral amino acid (TNAA)	342.46
% TNAA	65.62
Total acidic amino acid (TAAA)	101.68
% TAAA	19.48
Total basic amino acid (TBAA)	66.37
% TBAA	12.71
Total sulphur amino acid (TSAA)	21.87
% TSAA	4.22
Total aromatic amino acid (TArAA)	67.06
% TArAA	12.84
P-PER	2.01
Calculated iso-electric point	2.70

Amino Acid Composition

Table 4 shows the amino acid composition of *Talinum triangulare*) in mg/g crude protein. Glutamic acid had the highest value of 57.87mg/g and Leucine took the second position with value of 56.37 mg/g crude protein) while cystine had the least value of 8.13mg/g crude protein. The glutamic acid of the sample followed the similar trend reported for Nigerian dried okra seeds [43], *Kola acuminate*, *Garania kola* and *Anacardium occidentale* [44] where glutamic acid was the most abundant and also in some oil seeds [17]. It was observed that the value of glutamic acid in *Talinum triangulare* was higher than that of *Cactus pear cladodes* flours (6.35 mg/100g crude protein) [22]. The values presently reported for valine (88.75 crude protein), tyrosine (28.25 crude protein) and phenylalanine (38.81 crude protein) were found to be higher than those of *Cactus pear cladodes flours* (3.73 crude protein), tyrosine (15.02 crude protein) and (3.02 crude protein) [22]. The total amino acid (TAA) was 521.88mg/g crude protein (Table 5). This value was lower than those reported for dehulled African yam bean (917.48mg/g crude protein) [45], walnut flour (757.1mg/g crude protein) [46] and *Monodora myristica* (648.9mg/g crude protein) [47] but higher than those reported for *B. Sapida* (91.33mg/g crude protein) [30] and raw pearl millet (439mg/g crude protein) [48]. The total non – essential amino acids (TNEAA) was 273.66mg/g crude protein. This value was lower than reported for African yam bean [45] and Nigerian dried okra seeds [43]. The TNEAA was 52.43%. This indicates that TNEAA formed the bulk of the amino acids present in the sample. The values of TEAA was 248.22 mg/g crude protein (with histidine) while 235.66 mg/g crude protein was for TEAA without histidine. TEAA (with Histidine) was found to be lower when compared with some melon seeds (534.4mg/gcp) [17], but was found to be higher than 226.86mg/g crude protein for *B. sapida* [30].

TEAA (with histidine) was also lower than those of gourd seeds (536mg/g crude protein) [17], soy bean (444mg/g crude protein) [49], cowpea (426 mg/g crude protein) [50] *Cajanus cajan* (426 mg/g crude protein) [51]. The % cystine in TSAA was 4.22%. This is an indication that *Talinum trangulare* is good for consumption and the protein may prevent the precipitation of zinc in the intestine and amino acids such as cystine and peptides which facilitate zinc uptake by the mucosal cells [52]. It may be useful in this form. The total neutral amino acid (TNAA) was 342.46mg/g crude protein and the % TNAA was 65.62%. TAAA was 101.68mg/g crude protein and the % TAAA was 19.48% while TBAA was 66.37mg/g crude protein and the %TBAA was 12.71%, which was the least concentrated amino acid in the sample. The value of the iso- electric point (IP) calculated for the amino acid was 2.70 (Table 5). The predicted protein efficiency ratio (P-PER) was calculated to be 2.01. The experimentally determined P-PER usually ranged from 0.0 for a very poor protein to a maximum possible of just over 4.0 [53]). P-PER of the sample was higher than that of *Pleutorus tuber regium* (1.31) [25] and Nigeria dried okra seeds (2.31) [43]. The shows that *Talimum triangulare* would likely be better utilized in the body than the Nigeria driedokra seeds. Since the P-PER value of the sample was low, it indicates that there would be high level of physiological utilization of the protein. The lower is the level of P-PER in the diet, the better the quality of the protein. To obtain the maximum metabolic efficiency, it is imperative to maintain a nutritive balance of various amino acids.

Conclusion

It can be concluded that water leaf (*Talinum triangulare)* is a good source of nutrients, essential minerals for normal growth and body development. The results obtained also indicate its potential food for both man and animals.

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