Evaluation of some edible leaves as potential feed ingredients in aquatic animal nutrition and health

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Manuscript history
Received 6 July 2017 | Revised 19 February 2018 | Accepted 5 March 2018 | Published online 8 March 2018

Citation
Olusola SE and Olaifa FE (2018) Evaluation of some edible leaves as potential feed ingredients in aquatic animal nutrition and health. Journal of Fisheries 6(1): 569–578. DOI: 10.17017/jfish.v6i1.2018.266

Abstract
In this study, the potential benefits of ten edible leaves (Manihot esculents, cassava leaf; Colocasia esculenta, cocoyam leaf; Talinum triangulare, water leaf; Telfairia occidentalis, fluted pumpkin leaf; Carica papaya, pawpaw leaf; Amaranthus chlorostachys, green leaf; Moringa oleifare, drumstick leaf; Vernonia amygdalina, bitter leaf; Ipomoea batatas, sweet potato leaf and Basella alba, Malabar spinach ‘Amunututu’ to aquatic animal nutrition and health were studied along with proximate, mineral and phytochemical compositions. Results show that these edible leaves were a good source of protein and the highest crude protein was obtained in C. papaya (32.6%) while the lowest in C. esculenta (14.7%). The highest and lowest total ash was found in T. triangulare (34.6%) and C. papaya (11%) respectively. The result showed that the edible leaves are rich in wide variety of secondary metabolites of phytochemical constituents such as tannins, alkaloids, flavonoids, saponins, glycosides oxalates and phytate which can act against different diseases. Results suggest that inclusion of edible leaves may be nutritionally beneficial and this could promote growth, immune system and enhance disease resistance properties and subsequently very potential to reduce the cost of highly priced supplementary feeds.

Keywords: Edible leaves; proximate composition; minerals; animal nutrition; phytochemical; fish feed

1 | INTRODUCTION

The increasing cost of feed stuffs in animal production have been identified as a serious impediment in meeting the demand for animal protein particularly in developing countries (Adejinmi et al. 2000; Hossain et al. 2009; Mohsin et al. 2012a, 2012b). However, the ever-increasing cost of animal feeds makes it necessary to explore the use of alternative feed ingredients that are cheaper, locally available and of low human preference (Agbede et al. 2002; Tuleun et al. 2009; Kamal et al. 2010; Galib et al. 2013; Asadujjaman and Hossain 2016) such alternatives include the uses of edible leaves (vegetables). Vegetables are rich sources of vital ingredients in healthy and balanced human and animal diets without quantitative restriction (Aletor and Adeogun 1995; Okoli et al. 1998; Osuagwe 2008).

They are important low cost foods containing low levels of fat and high levels of vitamins, minerals, fibre and...
2.2 | Plant leaves preparation

The plants were washed with distilled water and allowed to air dry at room or ambient temperature for two weeks. The leaves (200 g) were blended into fine powder and stored in air tight container until required.

2.3 | Analytical methods

Each sample of the edible leaves was replicated thrice and analysed for their proximate composition according to the methods of Association of Official Analytical Chemists (AOAC 2005).

2.4 | Determination of the minerals

Each of the edible leaves (10 g) was ashed (dry ashing) at 550 °C for 6 hours in an electric muffle then diluted in 5 ml of 10% HCl. This was filtered and made up to mark in 50 ml volumetric flask, the filtrate was used for the analysis and this was carried out at the Department of Aquaculture and Fisheries Management, University of Ibadan, Nigeria from January to March, 2014 in Atomic absorption/emission spectrophotometer (Buck Scientific, model 200-A) for calcium and iron while phosphorus was determined by the use of the UV visible spectrometer following Nwanna and Olusola (2014).

2.5 | Determination of phytochemical in edible leaves

Phytochemical tests for bioactive constituents were carried out on portions of the residual material using standard phytochemical procedures:

Colour tests for alkaloids: 500 mg of plant material was extracted with 500 ml of methanol for 20 minutes, on a water bath. The extract was then filtered off and allowed to cool. This extract was dispensed in 2 ml of portions into four different test tubes. Either the Dragendorff’s or Hager’s or Mayer’s or Wagner’s alkaloidal reagent was added to each tube and the presence or absence of colours of any precipitates was noted in each test tube.

Frothing test for saponins: Water extract was obtained by boiling on the water bath. The extract was transferred into a test tube and shaken vigorously then was left to stand for 10 minutes and the result noted. A thick persistent froth indicates saponins.

Ferric chloride solution test for tannins: Water extract was treated with 15% ferric chloride test solution. The resultant colour was noted. A blue colour indicates condensed tannins, a green colour indicated hydrolysable tannins.

Test for flavonoids: Water extract of the sample was reduced to dryness on the boiling water bath. The residue was treated with diluted NaOH, followed by addition of...
dilute HCl, solubility and colour was noted. A yellow solution with NaOH, which turns colourless with diluted HCl confirm flavonoids.

Borntrager’s test for anthraquinone derivatives: Chloroform extract of the material was obtained by boiling on the water bath. To 2 ml of this extract, 1 ml of dilute (10%) ammonia was added and the mixture was shaken. Any colour change was recorded. A pink-red colour in the ammoniacal (lower) layer shows anthracene derivatives.

Fehling’s test for reducing sugars (in glycosides): The residue was re-dissolved in water on the water bath. To 2 ml of the solution, in the test tube was added, 1 ml each of Fehling’s solutions A and B. The mixture was shaken and heated in a water bath for 10 minutes. The colour obtained was recorded. A brick-red precipitate indicates reducing sugar.

2.6 | Statistical analysis

Proximate composition of the edible leaves and minerals resulting from the experiment were subjected to one-way analysis of variance (ANOVA) using SPSS (Statistical Package for Social Sciences, version 15.0). Duncan new multiple range test was used to separate means of significant treatment (p = 0.05).

3 | RESULTS

3.1 | Proximate composition

The proximate composition of studied edible leaves showed the highest dry matter in *M. oleifera* and the lowest in *C. esculenta*, the highest and lowest crude protein was recorded in *C. papaya* and *M. esculents* respectively. The highest and lowest ash contents were recorded in *T. triagulare* and *C. papaya* respectively. All these varied significantly (p < 0.05) among leaf types. The highest and lowest ether extract was obtained in *T. occidentalis* and *T. triagulare* respectively. Crude fibre was the highest in *M. oleifera* and the lowest in *A. chlorostachys*, which also varied significantly (p < 0.05) among leaves. The nitrogen free extract (NFE) was highest in *I. batatas* and lowest in *M. oleifera* (Table 1).

| Leases | Dry matter | Crude protein | Ether extract | Crude fibre | Ash | NFE |
|--------|------------|---------------|---------------|-------------|-----|-----|
| Manihot esculents (Cassava leaf) | 25.60±0.00 | 14.69±0.01 | 8.90±0.02 | 15.63±0.03 | 16.07±0.02 | 45.22±0.03 |
| Colocasia esculenta (Cocoyam leaf) | 8.23±0.02 | 24.95±0.04 | 10.66±0.01 | 12.08±0.05 | 12.42±0.02 | 39.89±0.01 |
| Talinum triangulare (Water leaf) | 9.68±0.04 | 21.09±0.02 | 1.47±0.06 | 10.34±0.01 | 34.56±0.01 | 32.54±0.02 |
| Telfairia occidentalis (Fluted pumpkin leaf) | 13.63±0.03 | 21.17±0.05 | 12.94±0.07 | 12.79±0.08 | 13.86±0.02 | 39.24±0.04 |
| Carica papaya (Pawpaw leaf) | 24.60±0.00 | 32.60±0.08 | 0.80±0.01 | 7.30±0.04 | 11.00±0.03 | 48.30±0.05 |
| Amaranthus chlorostachys (Green leaf) | 11.40±0.06 | 26.30±0.02 | 5.30±0.00 | 8.80±0.03 | 19.30±0.05 | 40.30±0.09 |
| Moringa oleifera (Drumstick leaves) | 88.62±0.05 | 26.62±0.02 | 5.34±0.06 | 18.97±0.01 | 12.01±0.10 | 25.68±0.03 |
| Vernonia amygdalina (bitter leaf) | 21.20±0.03 | 19.70±0.01 | 4.50±0.01 | 18.95±0.05 | 12.85±0.08 | 44.00±1.05 |
| Ipomoea batatas (Sweet potato leaf) | 12.45±0.05 | 24.65±0.01 | 3.58±0.03 | 9.10±0.02 | 11.47±0.05 | 51.20±0.01 |
| Basella alba (Malabar spinach) | 9.80±0.02 | 22.10±0.00 | 3.80±0.01 | 10.35±0.05 | 20.50±0.02 | 43.25±0.08 |

NFE, Nitrogen Free Extract; mean values in each row with similar superscripts are not significantly different (p > 0.05)

3.2 | Mineral composition

The mineral composition of studied leaves is presented in Table 2. The highest calcium was recorded in *M. oleifera* and lowest in *T. occidentalis* (Table 2). The highest and lowest phosphorus were recorded in *M. oleifera* and *B. alba* respectively and varied significantly (p < 0.05) among leaf types. The highest iron and calcium/phosphorus ratio was obtained in *V. amygdalina* and *M. oleifera* respectively which also varied significantly (p < 0.05; Table 2).

3.3 | Phytochemical properties

Preliminary phytochemical screening of studied edible leaves for secondary metabolites showed the presence of saponins, tannins, alkaloids, oxalates, glycosides, phytate and flavonoids. The values of these metabolites were in higher quality (+++), moderate quality (+++) and small quality (+). However, some phytochemical were not detected in some of these leaves (Table 3).

4 | DISCUSSION

Minerals are required for normal growth, activities of muscles and skeletal development (such as calcium), cellular activity and oxygen transport (iron), and the regulation of acid-base balance (phosphorus). Iron is useful in prevention of anemia and other related diseases (Oluyemi et al. 2006). Deficiency of these nutrients and minerals are known to affect the performance and health of animals (Merck 2005).
Edible leaves as potential feed ingredients

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TABLE 2 Minerals content and calcium/phosphorus ratio of studied leaves (mg 100 g⁻¹ food; N = 3)

| Leaves                                  | Calcium       | Phosphorus   | Iron          | Ca:P ratio |
|-----------------------------------------|---------------|--------------|---------------|------------|
| Manihot esculents (Cassava leaf)        | 39.00±0.03d   | 22.00±0.01e  | 2.02±0.00c    | 1.80±0.02b |
| Colocasia esculenta (Cocoyam leaf)      | 409.00±2.00   | 51.40±0.01i  | 1.21±0.01b    | 7.96±0.02j |
| Talinum triangulare (Water leaf)        | 37.00±0.01c   | 20.00±0.00i  | 7.15±0.00d    | 1.85±0.01b |
| Telfairia occidentalis (Fluted pumpkin leaf) | 28.00±0.05b   | 21.00±0.02d  | 6.90±0.01k    | 1.33±0.00a |
| Carica papaya (Pawpaw leaf)             | 58.60±0.01e   | 26.30±0.04f  | 0.31±0.02c    | 2.23±0.02c |
| Amaranthus chlorostachys (Green leaf)   | 190.00±0.02h  | 39.00±0.01b  | 4.60±0.02j    | 4.87±0.03f |
| Moringa oleifare (Drumstick leaves)     | 440.00±0.02j  | 70.00±0.05i  | 7.00±0.04h    | 6.29±0.07k |
| Vernonia amygdalina (bitter leaf)       | 97.00±0.03f   | 18.00±0.01b  | 7.50±0.03j    | 5.39±0.01g |
| Ipomoea batatas (Sweet potato leaf)     | 98.10±0.04i   | 27.60±0.00g  | 3.03±0.01d    | 3.56±0.02d |
| Basella alba (Malabar spinach)          | 15.00±0.01a   | 4.00±0.01a   | 4.50±0.00a    | 3.76±0.01a |

Mean values in each row with similar superscripts are not significantly different (p > 0.05)

TABLE 3 Phytochemical properties of studied leaves

| Leaves                                  | Tannin | Saponins | Cyanides | Oxalates | Phyate | Glycosides | Alkaloids | Flavonoids |
|-----------------------------------------|--------|----------|----------|----------|--------|------------|-----------|------------|
| Manihot esculents (Cassava leaf)        | + + +  | + + +    | + + +    | + + +    | +      | ND         |           |            |
| Colocasia esculenta (Cocoyam leaf)      | + + +  | + + +    | + + +    | + + +    | +      | ND         |           |            |
| Talinum triangulare (Water leaf)        | + +    | ND       | +        | +        | +      | +          |           |            |
| Telfairia occidentalis (Fluted pumpkin leaf) | + +    | + + +    | + + +    | + +      | ND     | +          |           |            |
| Carica papaya (Pawpaw leaf)             | + + +  | + + +    | + + +    | + + +    | +      | ND         |           |            |
| Amaranthus chlorostachys (Green leaf)   | + + +  | + + +    | + + +    | + + +    | +      | +          |           |            |
| Moringa oleifare (Drumstick leaves)     | +      | +        | ND       | +        | +      | +          |           |            |
| Vernonia amygdalina (bitter leaf)       | + +    | +        | ND       | +        | +      | +          |           |            |
| Ipomoea batatas (Sweet potato leaf)     | +      | +        | +        | +        | +      | ND         |           |            |
| Basella alba (Malabar spinach)          | + +    | ND       | +        | +        | +      | +          |           |            |

+, available in small quality; + +, available in moderate quality; + + +, available in high quality; ND, Not Detected

The presence of important nutrients like crude protein, crude fibre, low crude fat and fatty acid and high ash contents in all the studied leaves indicate that these leaves could be used as a nutritionally valuable and healthy ingredient to improve animal health and growth performance. However, there were variations in values of proximate composition of these plants when compared to other findings (e.g. Idris 2011; Ogbe and Affiku 2011; Oluwalana et al. 2011). The differences in the composition may be due to the differences in the locality of its growth and the stage at maturity prior to harvesting.

Cassava leaf (M. esculents) is a significant source of potential alternative protein resource for both human and animals (Fasuyi 2005a). The results of this study showed high protein content and mineral composition which comply with Hidajat and Wargiono (2002) who reported that high protein content and nutritive value in cassava leaves may contribute to alleviate nutritional deficiency in poor countries. Also, cassava leaves could be a solution to micronutrient undernourishment due its high vitamin and mineral contents (Hidajat and Wargiono 2002).

Cocoyam leaves (C. esculenta) is an herbaceous plant which belongs to the family Araceae and its leaves are eaten as vegetable by human, having ß-carotene, iron, protein, vita-
mains and folic acid which protects against anemia (Sukamoto 2003). The results of proximate composition contrast to the report of Ogunlakin et al. (2012) whose values were lower than the one obtained in this study. Cocoyam leaves has been said to have the potential of being used in ruminant nutrition (Babayemi and Bankole 2009). It has been fed to snails (Okon et al. 2012), fish (Aderolu et al. 2009), pigs (Aguwunobi et al. 2002) and poultry (Adejumo and Ologhobo 2012). Ndabikunze et al. (2011) reported high minerals value such as potassium, phosphorus, magnesium, and calcium in cocoyam leaves and this is in agreement with the present study. Phytochemical analysis revealed presence of some secondary metabolites that agrees with Olajide et al. (2011) that the use of cocoyam leaves in animal nutrition is, however, limited by the presence of anti-nutritional factors.

Water leaf (T. triangulare) is a common edible leafy vegetable, belonging to the family Portulacaceae. Different ethnic tribes in Nigeria referred to it as follows: Edos- Adodororo, Efik- Mmongikong, Igbo- Ntioke or Ofibekeke, Tiv- Ashwe, Yoruba- Gbure and Yagbaland in Kogi State- Adegbere. The present results indicated that water leaf is a good source of protein and minerals. This agreed with the findings of Disu (2010) who reported that water leaf is eaten cooked as a pet-herb and in soups, as a condiment in sauces or raw in salad, besides, very rich in mineral salts and amino acids as well as having anti-scrotic properties i.e. prevention against scurvy. Water leaf contains carotenoids such as lutein and zeaxanthin which act as a stimulant and in a way influencing the immune cells of the eyes (Shakuntala and Shadaksharawamy 1985). Basuvi (2005b) reported that consumption of vegetables such as water leaf helps toward off heart diseases, control blood pressure and cholesterol level, prevent some types of cancer, avoid a painful intestinal ailment called diverticulosis, and guard against cataract and muscular degeneration two common causes of vision loss.

Fluted pumpkin leaves (T. occidentalis) are a tropical vine grown in West Africa as a leafy vegetable and for its edible seeds. Common names for the plant include fluted gourd and fluted Pumpkin. It is known as ‘Ugu’ (Igbo language) in eastern parts of Nigeria. These leaves are sometimes called ‘pumpkin leaves’ in English. The plant is dioecious, perennial, and drought-tolerant. It is usually grown trellised. The young shoots and leaves are the main ingredient of Nigerian ‘edikang ikong soup’. The plant is cultivated for its edible seeds and young shoots and leaves. The seeds are cooked and eaten like beans and the shoots and leaves are eaten like vegetables. The results of the proximate composition of the present study are in agreement with Akoroda (1990) but contrast to Basuvi and Nonyerem (2007) who reported higher values than the values found in the present study. Fluted pumpkin leaves are rich source of protein, oil, vitamin and minerals which enhances, nourish, protect, and heal the body. The presence of calcium, phosphorus and iron from the present study support the report of Ajibade et al. (2006) that the leaves are low in crude fibre but rich source of folic acid, calcium, zinc, potassium, cobalt, iron, vitamin A, C, and K. Consumption of the leaves assist to combat certain disease due to the presence of antioxidant and antimicrobial properties, its minerals (iron) vitamins (A and C) and high protein contents (Kayode and Kayode 2011).

Pawpaw (C. Papaya) leaves is a tropical herbacious plant. It bears fruits which may be yellowish green, yellow or orange in colour when ripe. Pawpaw is a powerhouse of nutrients and is available throughout the year. It is a rich source of threes powerful antioxidant vitamin C, vitamin A and vitamin E; the minerals, magnesium and potassium; the B vitamin pantothenic acid and folate and fibre (Aravind et al. 2013). The result of the study shows high protein source, crude fibre, ash and low fat which was in accord with the report of FAO (2001) that it contains appreciable amount of macro and micro nutrients required by animals for growth and development such as protein, carbohydrate, minerals, vitamins and fat content in little to no amount.

Amaranthus chlorostachys consists of hardy, weedy, herbaceous, fast-growing, cereal-like plants (Opute 1979) with a vegetable yield of 4.5 tons dry matter ha⁻¹ after 4 weeks (Grubber and van Sloten 1981). This is one of those rare plants whose leaves are eaten as a vegetable while the seeds are used as cereals (Oke 1983). Amaranth leaves are combing with condiments to prepare soup in Nigeria (Oke 1983).

Drumstick leaf (M. oleifera) is a non-conventional plant with substantial nutritional value (Sanchez-Machado et al. 2010). It serves as a good source of protein, fat and an excellent source of calcium, iron or copper and zinc. Vitamin A is the most prominent vitamin essential for immune protection against all infections. It is a bio-enhancer of drug, and nutrient due to its antibiotic activity. The result of the study complies with Ferreira et al. (2008). It has nine essential amino acids that comprise the sulphur – containing amino acid methionine and costive (Makkar and Becker 1997) higher than levels recommended by the FAO (Ferreira et al. 2008). The leaves treat different ailments such as abnormal blood pressure, respiratory disorders, inflammation of mucous membranes, hepatitis, impotency, infertility and joint pains (Fahey 2005).

Bitter leaf (V. amygdalina) is a shrub or small tree of 2–5 m belonging to the family Asteraceae. It has petiolate leaves of about 6 mm diameter and elliptic shape. The leaves are green with a characteristic odour and a bitter taste (Singha 1996). In many parts of Nigeria, the plant has been domesticated (Igile et al. 1994). It is known as ‘Ewuro’ in Yoruba, ‘Etidot’ in Ibibio, ‘Onugbu’ in Igbo and ‘Chusa-diki’ in Hausa tribes in Nigeria (EgedeWig 2010). This plant grows under a range of ecological zones in Africa and produces large mass of forage and is drought tolerant; it is found in homes in villages as fence post and pot-herb (Bonsi et al. 1995). The result of the study shows that bitter leaf is a good source of protein, fibre and minerals, this support the findings of Ejo et al. (2005) that it is an excellent source of vitamin C, total
carotenoid and minerals. These are used as vegetable in meals to stimulate the digestive system, and as a treatment for fever. A wide array of phytochemical has been shown to be present in *V. amygdalina*. The presence of oxalates, phytate and tannins have been reported (Ejoh et al. 2007; Eleyinmi et al. 2008), as well as flavonoids (Tona et al. 2004) were in accord with the present study. Bitter leaf extracts have been shown to exhibit profound ethno-medical and pharmacological properties such as anti-diabetic, anti-malarial, anti-helminthic and antibiotic properties (Farombi 2003).

Sweet potato (*I. batatas*) leaves are usually used to feed monogastric and ruminant animals under subsistence farming systems. Sweet potatoes contain a large amount of protein with a high amino acid score. The result of the study shows that sweet potato is a good source of protein, fibre and minerals and this support the report of Antia et al. (2006) who reported that leaves of the sweet potato are highly digestible, fairly rich in protein, a dietary source of vitamins, minerals, antioxidants, dietary fibre and essential fatty acid and free from toxin. Bioactive compounds contained in sweet potato leaves could contribute to health promotion and chronic disease prevention.

The result of this study revealed that some secondary metabolites are present in sweet potato leaves which agrees with the findings of Soetan and Oyewole (2009) who reported that these plants generally contain chemical compounds (such as saponins, tannins, oxalates, phytate, trypsin inhibitors, flavonoids and cyanogenic glycosides) known as secondary metabolites, and which are biologically active. Secondary metabolites may be applied in nutrition and as pharmacologically-active agents (Soetan and Oyewole 2009). They have antibacterial and anti-parasitic properties. Plants are also known to have high amounts of essential nutrients, vitamins, minerals, fatty acids and fibre (Gafar and Itodo 2011).

Flavonoids (quercetin) have inhibitory activity against disease causing organisms in animals. Preliminary research indicates that flavonoids may modify allergens, viruses and carcinogens and so may be biological response modifiers. In vitro studies show that flavonoids also have anti allergic, anti inflammatory, anti microbial, anti cancer and anti diarreal activities (Cushnie and Lamb 2011). Tannins are plant polyphenols, which have ability to form complexes with metal ions and with macro-molecules such as proteins and polysaccharides (Dei et al. 2007). Dietary tannins are said to reduce feed efficiency and weight gain in animal (Dei et al. 2007). Environmental factors and the method of preparation of samples may influence the concentration of tannins present. Tannin presence influences protein utilization and build defence mechanism against microorganism (Cushnie and Lamb 2011).

Saponins are glycosides, which include steroid saponins and triterpenoid saponins. High levels of saponins in feed affect feed intake and growth rate in animal (Dei et al. 2007). Saponins (in excess), causes hypcholesterolaemia because it binds cholesterol making it unavailable for absorption (Soetan and Oyewole 2009). Saponins also have haemolytic activity against red blood cell (Ogbe and Affiku 2011). Saponin-protein complex formation can reduce protein digestibility (Ogbe and Affiku 2011). Saponins reduced cholesterol by preventing its reabsorption after it has been excreted in the bile. Proper food processing would reduce anti-nutrients (Akinleye et al. 2011).

Phytate is an organically bound form of phosphorus in plants. Phytate in foods are known to bind with essential minerals (such as calcium, iron, magnesium and zinc) in the digestive tract, resulting in mineral deficiencies (Bello et al. 2008). They bind minerals to form insoluble salts, thereby decreasing their bioavailability or absorption (Muhammad et al. 2011).

Oxalate binds with calcium to form calcium-oxalate crystals which are deposited as urinary calcium (stones) that are associated with blockage of renal tubules (Blood and Radostit 1989). Hydrogen cyanide is toxic when ingested by monogastric animals in large quantity. Soaking of plant materials or boiling in water is said to reduce toxic effects and improves utilization in terms of feed intake and protein digestibility (Dei et al. 2007).

5 | CONCLUSION

There is a need to explore and develop food and feed from alternate sources (e.g. non-conventional plant leaves); especially in view of rapidly growing population and the ever growing demands to improve agricultural system. Under these circumstances edible plant leaves may play a significant role.

Diet formulation with adequate ingredient is therefore the foundation on which animal nutrition is built. The nutritive values of studied locally available plant leaves tend to justify the continuous investigation and utilization of their potentials in animal feed. Possibly, the leaves from these plants could be useful as feed supplement and as medicine in animal nutrition to improve health and growth performance. Exploitation of these cheaper nutrient sources will lead to increased aquatic animal production. Further studies are recommended on inclusion of these leaves into the diets of aquatic animals at different inclusion levels to determine the optimum level for maximum production.

ACKNOWLEDGEMENTS

We wish to appreciate the efforts of Mr AO Isaac for his technical assistance during the study. Also, we would like to thank the Editorial Team and anonymous reviewers of Journal of Fisheries for thorough review of this manuscript.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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