Full Length Research Paper

Evaluation of the nutritional quality of four unexplored wild food plants from Arunachal Himalayas for the formulation of cost effective fish feeds

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Wild food plants used by the tribes of Arunachal Himalaya (Northeast India) found to be highly nutritious to maintain general balance diet. A study was conducted to evaluate the nutritional potential of four commonly found, unexplored wild food plants namely, Angiopteris evecta, Amaranthus viridis, Celosia argentea and Monochoria vaginalis used by the tribes of Arunachal Himalaya to ascertain their feasibility for the formulation of cost effective commercial fish feeds. Study revealed that M. vaginalis and A. evecta stood to be the most promising species in terms of nutritional content out of the four wild food plants investigated for low cost and balanced fish diet.

Key words: Wild food plants, cost effective fish feeds, nutritional potential, antinutrient, Arunachal Himalaya.

INTRODUCTION

Indigenous food plants play an important role in both human and animal feed particularly in the remote areas of North East India, like Arunachal Himalayas. Local plants supply a large proportion of the protein, vitamins and minerals to the human and animals. In addition, some plants are also used as medicinal agent. Wild edible plants are rich in several nutrients (e.g., vitamins), but information about their nutritional availability is scarce. In fact significant effort has been directed toward evaluating the nutritive value of different non-conventional feed resources, including terrestrial and aquatic macrophytes to formulate nutritionally balanced and cost effective diets for fish and poultry (Ray and Das, 1995).

Feed is considered as the most critical input for augmenting fish production. A fish accept a wide variety of agricultural by-products, food plants in the form of pelleted or dough feed. Present fish production of Northeastern region of India is about 7% of the total inland fish production in the country, and is just sufficient to provide about 6 kg per capita of the fish to its present population against the standard nutritional requirement of 12 kg per capita (FAO, 2004). To partially offset the demand, substantial quantity of fish is imported daily into the region from other States of the country but transportation cost from Indian major city up to remote locality of Arunachal Himalayas is slightly higher than expected (Hui et al., 2005).

Therefore, there is a great demand for scientifically developed fish feed for this region. It is generally advisable to make the use of local resources as fish feed as far as possible so that the farmers and fishery entrepreneurs do not have to import the feed materials from other States. Present huge transportation cost of the region can be drastically cut off by ensuring available cost effective feeds in their own locality as the region is

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gifted with huge natural resource potential especially wild edible plant species along its altitudinal gradient. Perusal of ethnobotanical literature of the region has confirmed that there are abundant of wild food plants which grow throughout the year and these plants are consumed by the local people of Arunachal Himalayas both wild and cultivated (Murtem, 2000). Some of these plants are considered highly nutritious which are widely used by 28 local tribal communities as food supplements (Hui et al., 2008). However, few nutritional evaluation works has been done on wild food plants of other region of world but such laboratory report is not available till date on four selected wild food plant found in Arunachal Himalayas (Kalita et al., 2007; Borah et al., 2009). Therefore, present study was undertaken to investigate the nutritional and anti-nutritional components of four wild food plants of Arunachal Himalayas, namely, *Angiopteris evecta*, *Amaranthus viridis*, *Celosia argentea* and *Monochoria vaginalis* to ascertain the feasibility for formulation of cost effective fish feeds.

**MATERIALS AND METHODS**

**Samples preparation**

Sample of fresh, tender and green leaves, stem, roots, fruits and flowers of *A. evecta*, *A. viridis*, *C. argentea* and *M. vaginalis* were collected from several wetland and marshy areas of middle and foothill belt of Arunachal Himalaya and taxonomically identified. The samples were washed under running water and blotted shade dry. The moisture content of the samples was determined at 60°C (AOAC, 1990). The dried material obtained was ground to a fine powder and stored at -5°C in air-tight containers prior to further analysis.

**Proximate analysis**

Moisture, ash, ether extract (EE), crude fibre (CF) and nitrogen-free extract (NFE) were determined by the methods of the Association of Official Analytical Chemistry (AOAC, 1990). The crude protein content was determined by Kjeldahl procedure, AOAC method 920; factor N x6.25 was used to convert nitrogen into crude protein. The crude lipid was analyzed gravimetrically using Soxlet apparatus (petroleum ether extraction, boiling point 60 to 80°C). Ash content was determined by incineration the dried sample in a muffle furnace (petroleum ether extraction, boiling point 60 to 80°C). Ash content was used as a standard. For the estimation of crude fibre system (Fibro Plus, Model Kalvat FES2) with repeated treatment of dilute H2SO4, followed by dilute NaOH and washing by water. The carbohydrate (NFE) content of feed was determined as the weight difference using moisture, crude protein, lipid and ash content data (Carrel et al., 1956).

Gross energy was determined by using an adiabatic bomb calorimeter (IKAC-7000) using benzoic acid as a standard. Determination of α-tocopherol and carotenoids contents of plant materials first includes extraction of total lipid material from dried plant powder (Folch et al., 1957), followed by extraction and estimation of α-tocopherol and carotenoids levels by procedure of Baker and Frank (1968). For the extraction of ascorbic acid (Vitamin-C), 5.0 g plant materials were ground using a pestle and mortar in 50 ml of 4% (w/v) oxalic acid solution and filtered through a Whatmann filter paper (No. 100). The ascorbic acid content was then determined volumetrically using 2,6-dichlorophenol indophenols dye (Sadasivam and Manickam, 1992).

**Mineral analysis**

The mineral contents of the plants namely, Na, K, Fe and Ca, were estimated by flame photometry, whereas Mg contents were measured by atomic absorption spectrophotometer (Carl Zeiss) using standard reference chemicals. The total phosphorus content was determined as described by Umoren et al. (2005).

**Anti nutritional components**

To determine trypsin inhibitors in the samples benzoil-DL-arginine-paranitroaniline (BAPNA) was used as substrate. The activity of trypsin inhibitors in the samples was determined by using benzoil-DL-arginine-paranitroaniline (BAPNA) as a substrate. The trypsin inhibitory activity was expressed as the amount of trypsin inhibitor (TI), in grammes, present per 100 g of sample (Kakade et al., 1974). Total phenols (tannin) from the plants were isolated as described by Makkar (1994) and then estimated by the Folin-Denis reagent (Makkar and Goodchild, 1996). The tannin content of the samples was calculated as tannic acid equivalents from a standard graph. The phytic acid content of the samples was determined spectrophotometrically using a Hitachi U 2000 UV-vis spectrophotometer (Vaintraub and Lapteva, 1988). Phytic acid was used as a standard. For the estimation of calcium oxalate, the procedure of Jones (1988) was followed.

**Statistical analysis**

Data are presented as mean ± SD. One-way analysis of variance (ANOVA) were carried out to compare the different values.

**RESULTS AND DISCUSSION**

**Proximate composition**

The proximate composition of the four wild food plants namely, *A. evecta*, *A. viridis*, *C. argentea* and *M. vaginalis* are presented in Table 1. Moisture and lipid contents were nearly identical in all the samples. Among these four plants *M. vaginalis* possess the highest amount of crude protein (30.6%), followed by *A. evecta* (20.5%); *A. viridis* and *C. argentea* had comparatively less crude protein (5.2 to 5.8%). The ash content in these plants ranged between 10.1 to 30.7%, the highest amount being displayed by *A. evecta* and the lowest by *A. viridis*. Total carbohydrate (including starch) content of *A. viridis* (79.9%) and *C. argentea* (79.0%) are almost equal and significantly higher than the other two, whereas *A. evecta* possessed the highest amount of crude fibre (20.6%, w/w) followed by *M. vaginalis* (14.2%) and *A. viridis* (3.8%).

Carbohydrates are important sources of energy in fish diet, the dietary carbohydrate requirement may vary depending upon the fish species; e.g. herbivorous fish can metabolize carbohydrates better than carnivorous species (Cowey and Sargent, 1979; Furuichi and Yone, 1981). It is quite reasonable to assume that because of the high carbohydrate and low protein contents, *M. vaginalis* and *A. evecta* may be used as supplementary feed in commercial fish diet, in particular, for the
Table 1. Proximate composition of four wild food plants from Arunachal Himalaya (Northeast India) on dry matter basis (%). Each value represents mean ± SD of three determinations.

<table>
<thead>
<tr>
<th>Component</th>
<th>A. evecta</th>
<th>A. viridis</th>
<th>C. argentea</th>
<th>M. vaginalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>84.4 ± 0.7</td>
<td>86.0 ± 1.1</td>
<td>80.1 ± 1.5</td>
<td>81.0 ± 1.2</td>
</tr>
<tr>
<td>Crude protein (Nx 6.25)</td>
<td>20.5 ± 1.2</td>
<td>5.2 ± 0.2</td>
<td>5.8 ± 0.5</td>
<td>30.6 ± 0.3</td>
</tr>
<tr>
<td>Crude lipid (ether extract)</td>
<td>0.64 ± 0.2</td>
<td>1.02 ± 0.1</td>
<td>1.09 ± 0.8</td>
<td>1.03 ± 0.4</td>
</tr>
<tr>
<td>Ash</td>
<td>30.7 ± 1.9</td>
<td>10.1 ± 1.5</td>
<td>10.5 ± 1.3</td>
<td>17.1 ± 1.5</td>
</tr>
<tr>
<td>Total carbohydrate (NFE + Crude fibre)</td>
<td>48.16 ± 1.2</td>
<td>79.9 ± 2.1</td>
<td>79.0 ± 1.6</td>
<td>51.27 ± 1.6</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>20.6 ± 0.63</td>
<td>3.8 ± 0.3</td>
<td>2.7 ± 0.1</td>
<td>14.2 ± 0.2</td>
</tr>
</tbody>
</table>

Table 2. Energy values, vitamin contents and mineral ion concentrations in the leaves of four wild food plants from Arunachal Pradesh (Northeast India). Values are mean ± SD of triplicate determinations.

<table>
<thead>
<tr>
<th>Properties</th>
<th>A. evecta</th>
<th>A. viridis</th>
<th>C. argentea</th>
<th>M. vaginalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy values</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gross energy (kcal/100 g)</td>
<td>397.94 ± 2.2</td>
<td>379.75 ± 2.1</td>
<td>372.84 ± 1.7</td>
<td>428.78 ± 1.3</td>
</tr>
<tr>
<td>P/E (mg protein/kcal)</td>
<td>51.52 ± 0.2</td>
<td>13.69 ± 0.2</td>
<td>15.56 ± 0.3</td>
<td>71.37 ± 0.1</td>
</tr>
<tr>
<td>Vitamin content</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin E (mg/100 g)</td>
<td>27.5 ± 0.5</td>
<td>28.1 ± 0.5</td>
<td>29.5 ± 0.2</td>
<td>29.0 ± 0.3</td>
</tr>
<tr>
<td>Vitamin C (mg/100 g)</td>
<td>1.0 ± 0.1</td>
<td>2.0 ± 0.2</td>
<td>1.2 ± 0.1</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>Carotene (mg/100 g)</td>
<td>0.03 ± 0.001</td>
<td>0.1 ± 0.02</td>
<td>0.03 ± 0.002</td>
<td>0.04 ± 0.01</td>
</tr>
<tr>
<td>Mineral ion concentration</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ca (g/100 g)</td>
<td>0.02 ± 0.01</td>
<td>0.012 ± 0.001</td>
<td>0.013 ± 0.201</td>
<td>0.6 ± 0.01</td>
</tr>
<tr>
<td>K (ppm)</td>
<td>23.7 ± 1.2</td>
<td>78.2 ± 2.5</td>
<td>83.3 ± 2.3</td>
<td>61.0 ± 1.8</td>
</tr>
<tr>
<td>Mg (g/100 g)</td>
<td>0.2 ± 0.02</td>
<td>0.03 ± 0.001</td>
<td>0.01 ± 0.004</td>
<td>0.1 ± 0.01</td>
</tr>
<tr>
<td>Na (ppm)</td>
<td>10.0 ± 0.8</td>
<td>12.1 ± 0.3</td>
<td>3.2 ± 0.4</td>
<td>11.6 ± 0.6</td>
</tr>
<tr>
<td>Fe (g/100 g)</td>
<td>0.1 ± 0.02</td>
<td>0.05 ± 0.01</td>
<td>0.02 ± 0.001</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>P (ppm)</td>
<td>1.4 ± 1.0</td>
<td>5.1 ± 1.2</td>
<td>3.9 ± 1.1</td>
<td>4.3 ± 0.1</td>
</tr>
</tbody>
</table>

energy formulation of herbivorous fish feed.

Energy values, vitamin contents and mineral ion concentrations

Table 2 exhibited remarkably similar amount of gross energy value (372.84 kcal/100 g to 428.78 kcal/100 g) in all the four plants. Again P/E value is highest in M. vaginalis (71.37 mg protein/kcal) followed by A. evecta (51.52 mg protein/kcal) and lowest in A. viridis (13.69 mg protein/kcal).

Protein is the major as well as the most expensive component in fish feed (Kalita, 2006). The P/E ratio in fish diet should range from 80 mg protein/kcal to 100 mg protein/kcal to attain the maximum growth (Akand et al., 1991; Arockiaraj et al., 1999). Therefore, on the basis of high gross energy, and P/E values, it may be inferred that M. vaginalis and also A. evecta are suitable for incorporation in fish diet.

Vitamin content of the four food plants are shown in Table 2. Vitamin E or α-tocopherol and vitamin C or ascorbic acid was similar in the four plants. Carotenoid content of A. viridis was significantly higher than the other three food plants.

Vitamins are unavoidable constituents of fish diet. Basically, vitamin E is a lipid soluble antioxidant present in the biological membranes and it renders stability to the membranes (De Silva and Anderson, 1995). It is considered as a primary defense against lipid peroxidation, protecting polyunsaturated fatty acids in cell membranes from free radical attack through its free radical quenching activity in biomembranes (Jacobson, 1987). It has been reported that approximate dietary vitamin E requirements for Cirrhinus mrigala and Laboe rohita ranged between 99 mg/kg and 132.0 mg/kg of dry diet, respectively for the normal growth and development of fish (Sau et al., 2004). According to Table 2, vitamin E in the four plants, are exhibited to be within 275 to 290 mg/kg, which is again above the normal range of the
basic requirements of the carps. Fishes also require dietary supply of vitamin C for their survival and normal growth (Sahoo, 2006). Their dietary vitamin C requirement is around 10 mg/100 g diet (Mitra and Mukhopadhyay, 2003).

Carotenoid requirement for Indian major carps can be fulfilled by providing 4 to 6 mg carotenoid / kg of diet (ADCP, 1983). However, the carotenoid concentrations in these food plants are much lower as compared to Ipomoea (Kalita et al., 2007). It is worth to be mentioned here that this vitamin is mainly required for ornamental fish for pigmentation purpose (Meyers, 1994), and carps may not require a high content of this vitamin in their diet. Therefore, results of the present study indicate that sufficient amounts of vitamin E and C are present in these plants under study to meet the requirements of these vitamins for the proper growth and development of the fish species.

Mineral composition of the four food plants are illustrated in Table 2. Significant variation was noticed in mineral contents of these plants, which may be due to the differences in their genus and species level. As shown in Table 2, magnesium and iron were the most abundant metals present in M. vaginalis and A. evecta. Again, potassium and phosphorus levels were more in M. vaginalis, C. argentea and A. viridis. Sodium content was high in A. viridis, M. vaginalis and A. evecta. Calcium content is highest in M. vaginalis (0.6 mg %). Interestingly, M. vaginalis showed high amount of minerals, followed by A. evecta, A. viridis and C. argentea. Minerals, or inorganic elements, are needed by animals to maintain many of their metabolic processes and to provide material for major structural elements (e.g. skeleton). Mineral elements play an important role in regulating many vital physiological processes in the body, such as regulation of enzyme activity (cofactor or metalloenzyme), skeletal structures (e.g. calcium and phosphorus), neuromuscular irritability and for the clotting of blood (calcium).

In fish and crustaceans, a dietary requirement has been established for only 11 minerals (Kaushik, 2001), which are calcium (Ca), phosphorus (P), potassium (K), magnesium (Mg), iron (Fe), zinc (Zn), manganese (Mn), cobalt (Co), copper (Cu), iodine (I) and selenium (Se). Mg has a relationship with the protein concentrations in the blood serum of fish because 25% of the total serum Mg is bound to albumin and 8% to globulin (Kroll and Elin, 1985). Non-availability of adequate quantities of minerals in diet affects fish growth and may cause irrecoverable deficiency diseases (DeSilva and Anderson, 1995). Although, diet is the main source of minerals for fish, some minerals can be absorbed from the environment (Lalli and Bishop, 1977). But even then, despite the large amount of Ca in the water, the presence of a minimum amount of dietary Ca (≈ 2.0 ppm) causes an increase in the final weight of the fish, indicating the absolute requirement of Ca in the fish diet (Chavez-Sanchez et al., 2000).

Plants appear to be able to accumulate many nutrient minerals from water and soil. Studies done by Guittikar et al. (1966) informed that the mineral requirements of the body are supplied by leguminous plants, especially Mg and Cu. Again, report of Riche and Brown (1999) revealed that the incorporation of plant protein feed stuffs into fish meal diets for rainbow trout increases P availability in the fish. Thus, it may be assumed that presence of adequate amounts of minerals in the selected food plants may increase the growth of fish under study to a great extent, if incorporated in their diet.

### Anti-nutritional factors

Anti-nutritional contents of the four plants are illustrated in Table 3. Highest trypsin inhibition was detected in C. argentea (1.41%), followed by M. vaginalis (1.31%) and lowest in A. evecta (1.01%). Calcium oxalate concentration was highest in A. viridis (0.9%) followed by C. argentea (0.8%). Tannin concentration was highest in A. evecta (0.02%) and lowest in M. vaginalis (0.002%). Again, phytate was highest in A. viridis and C. argentea (0.08%), followed by A. evecta (0.07%) and lowest in M. vaginalis (0.04%). Interestingly, among these four food plants, M. vaginalis possessed the lowest amount of anti-nutrients followed by A. evecta.

The anti-nutritional factors (ANFS) may be defined as those substances generated in natural food stuffs by the normal metabolism of species and by different mechanisms.

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**Table 3. Concentration of some anti-nutritional factors in the leaves of four wild food plants of Arunachal Himalaya (component in g%).**

<table>
<thead>
<tr>
<th>Component</th>
<th>A. evecta</th>
<th>A. viridis</th>
<th>C. argentea</th>
<th>M. vaginalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin inhibitor</td>
<td>1.01 ± 0.2</td>
<td>1.12 ± 0.1</td>
<td>1.41 ± 0.5</td>
<td>1.31 ± 0.9</td>
</tr>
<tr>
<td>Calcium oxalate</td>
<td>0.4 ± 0.1</td>
<td>0.9 ± 0.01</td>
<td>0.8 ± 0.1</td>
<td>0.3 ± 0.02</td>
</tr>
<tr>
<td>Tannin</td>
<td>0.02 ± 0.01</td>
<td>0.006 ± 0.001</td>
<td>0.004 ± 0.001</td>
<td>0.002 ± 0.002</td>
</tr>
<tr>
<td>Phytate</td>
<td>0.07 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.02</td>
<td>0.04 ± 0.02</td>
</tr>
</tbody>
</table>

Values are mean ± SD of triplicate determinations.
(e.g. inactivation of some nutrients, diminution of the digestive process, or metabolic utilization of feed) which affect the growth rate (Roeder, 1995) to the animals. It is known to cause a growth depressing effect in tilapia and rohu fish (Jackson et al., 1982). Phytates bind minerals like calcium, iron, magnesium and zinc and make them form insoluble complexes (Nelson et al., 1968) that are not readily broken down, thus making them unavailable in the digestive tract. They also form strong complexes with proteins that can lead to reduced digestibility of the latter biomolecule (Richardson et al., 1985).

Although, this study shows that antinutritional factors are present in these food plants being studied, their concentrations are within the tolerable limit of fish, reinforcing the future use of these plants for the formulation of cost effective and artificial fish diet (Kalita et al., 2008).

Conclusions

The combined data related to protein, vitamins and mineral composition of the four tested wild food plants in the present study reinforces the utilization of these plants as promising sources of nutrition in both human and fish diet. Moreover, *M. vaginalis* and *A. evecta* could be used as important sources of proteins, vitamins and minerals favourable for including in the fish diet. In spite of the anti-nutritional factors in these food plants, their levels were within tolerable limits and consumption of these plants would not result in any adverse effect on the growth of both human being and fish, documenting their further uses for the formulation of balanced fish diet.

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