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Proximate composition analysis of plant-based, Non-conventional feed ingredients having potential as ingredient in fish feed

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Abstract
The aquaculture sector is well known to feed the human population since time immemorial. Fish feed plays an important role in the growth and development of the aquaculture industry. The use of non-conventional plant protein sources in the feed industry has been in practice for various advantages like sustainability, availability, cost-effectiveness, etc. Numerous plant leaves of Moringa, Sesbania, *Leucaena leucocephala*, alfalfa, *Medicago sativa*, *Ipomoea sp*., cassava, cucumber, squash, *Carica papaya*, white cowpea, green mung-bean, jackfruit, Mexican fire plant, cocoyam, black jack, banana, etc. and aquatic plant leaves of azolla, water hyacinth, duckweeds, water lettuce, Indian star grass, duck lettuce, water fern, etc. have been used in the fish feed industry. They are used in the form of fresh and raw, dried, powdered, cooked, fermented, concentrates, etc. It can be used directly in the feed (in case of herbivorous fishes), supplementary or partial replacement to fish meal in the aqua feed formulation. The paper is an attempt to study and analyse the nutrient composition (crude protein, ether extract and crude fibre) of different plant sources that can be a potential replacer of conventional feed ingredient such as de-oiled rice bran and fishmeal. For this purpose the leaves or samples were collected from different places and analysis of proximate composition was done using procedures of AOAC (2005).

Keywords: Non-conventional, feed ingredient, proximate analysis, ether extract, crude fibre

Introduction
Natural food in any water body can support only a small population of fish whereas the intensive culture of fish species with quality fish feed, prepared from indigenous as well as less expensive sources of plant and animal food materials allow high stocking density and thereby increases the fish production in many folds [1]. There is a high demand and low supply of fish meal in the market due to intensive trash fish-catching practices and increasing competition with the animal feed industry because of which it has become essential to find out the alternative non-conventional feed ingredients which are locally available that can be used as alternate protein sources to replace the existing commercial bran. Protein sources are extremely necessary while keeping the cost of feed reduced to affordable limits.
Alternate fish feed ingredients from plant origin include local grasses, leaves, oils and aquatic plants. These non-conventional plant ingredients are good source of protein and energy and can possibly be used to replace existing conventional sources in feed for major carps [2]. The plant origin feed stuffs including bran, polishes, grains, leaves, stoves, cobs, roots and grasses are placed in energy rich feed stuffs with high carbohydrate and fiber content, while plant origin cakes are low cost and easily available protein sources as compare to animal protein source such as fish meal. Plant proteins are potential ingredient as that can be used as an alternative to fishmeal in fish feed preparation. Plant sources are also being used as a partial or complete replacer of de-oiled rice bran. Many plant sources consist of a considerably rich amount of proteins and contain essential amino acid (EAA) profile, but they lack one or more EAA. This problem can be dodged by making use of the mixture of plant protein sources rather than incorporating a single plant protein sources to obtain the adequate EAA profile [3].
Among the world's 190 or so countries only 17 of them hold about 70 percent of total biodiversity, earning them the title "megadiverse." India is one of these megadiverse countries with 2.4% of the land area, which accounts for 7-8% of the species of the world, including about 91,000 species of animals and 45,500 species of plants documented in its bio-geographic regions. The nation is known as a region of genetic diversity and falls in the global hotspot of bio-diversity. India has three of 34 "global biodiversity hotspots" that are unique, biologically rich areas that are facing much conservation threats. Providing access to biodiversity information is of much importance to promote conservation, management and sustainable use of resources and has great potential to increase the existing and future value of the country's

Biodiversity for a sustainable society. There is a substantial value of biodiversity for sustaining and nourishing human communities like the ecosystem of two great mountain chains, the Himalayas and the Western Ghats that directly or indirectly support millions of people in India.

The majority of the edible plants are neglected which grow naturally in the wild and do not have to be tended before producing edible parts. Such edible wild plants can significantly increase sustainability by reducing the risk of over-dependence on a limited number of crops.

For aquaculture, most of the small-scale farmers depend on traditional feed ingredients such as rice bran, wheat flour, maize bran, mustard oil cake, etc. Nowadays there is an emerging problem as these resources are being consumed by animal husbandry and for human consumption which makes them scarce due to which the aqua industry has to compete with them leading to costlier feed. Besides this, the problem is the deficit of knowledge and awareness of benefits of using alternatives like plant-based ingredients such as leaf meal. Studies have been carried on multiple leaves and some have been found having the potential to be used as a replacer of traditional fish feed ingredients. Some of these potential replacers include leaves of *Ipomea batatas*, *Leucaena leucocephala*, *Cajanus cajan*, *Pawpaw*, *Sesbania*, *Cassava*, *Helianthus multiflorus*, banana, cabbage and many more. Also the awareness to the importance of adherence to a proper feeding schedule is crucial.

In fresh water aquaculture fish farmers consider these cakes as suitable feed ingredient than fish meal. Most of the plant protein is obtained from plant seeds, bran, cakes, grains and flour which are placed in energy rich ingredients as these have less than 20% protein content except oil cakes that has more than 20% protein content. These plant origin protein and energy sources are now being widely used in carp fish feed industries due to their easy availability and optimum cost. It was proved and reported that the overall production of aquaculture increased with artificial balanced diet [4]. Reports are there of rearing of grass carp, *Ctenopharyngodon idella* with cattle fodder [5].

In India, we know aquaculture is immensely dependent on carp culture. Since it a landlocked state, freshwater culture can only be practiced. Major species being cultured are both IMCs and EMCs (Rohu, Catla, Mrigal, Common carp, Grass carp and silver carp); catfishes (*Clarias batrachus*, *Heteropneustes fossilis*, *Wallago attu*), etc. In fish culture protein is most important nutrient. Fish meal is best known as a protein source but it maximizes the input cost of feed and makes it difficult for the majority of small-scale fish farmers in rural areas to afford. In a quest to enhance aquaculture production which consequently improves food security, and reduce the level of poverty in developing countries, a search for affordable and locally available feedstuffs is being advocated [6].

Many locally available plants and plant-based non-conventional ingredients have been studied for inclusion in poultry and livestock feeds [7] among which only a few have been evaluated for their potential use as fish feeds. When leaves of some plants were examined for nutritional composition by performing the proximate analysis by the method of AOAC (2005), it was discovered that some contained high crude protein and low crude fiber. From this it was understood that some of these leaves has a potential to be used as feed ingredients in aquaculture. This concludes that the transformation of plant stuffs into affordable and quality feed can make a contribution in improving fish production. In this paper we are going to discuss some of the locally available plant leaves and their nutritional values (crude protein, crude lipid, and crude fiber) that have been analysed and found to have potential to replace conventional protein sources.

Materials and Methods

a. Collection of plant materials

The fresh plants were collected from a Krishi Vigyan Kendra farm in Bihar state, India and few were collected from other places. Identification and authentication of the plants was done by the scientist working there.

b. Processing of plant materials

Multiple non-conventional feed ingredients from plant source were collected, air dried at 28 ℃ for 15 days. They were grounded into fine powder form using an electric blender and stored in a cool dry container until use. Biochemical analyses such as crude protein, ether extract, crude fibre, etc. were carried out in the Fish Physiology and Biochemistry lab of Central Institute of Fisheries Education, Mumbai using internationally established procedures of AOAC (2005).

c. Moisture analysis

Ten grams of the fine grinded sample was taken in a pre-weighed Petri dish and placed in an oven at 105 ℃ for overnight, followed by placing in desiccators for half an hour. Moisture free sample was weighed to calculate the percentage of moisture as follows:

Weight of moisture = Weight of sample before moisture extraction - Weight of sample after moisture extraction.

$$\text{Moisture (\%)} = \frac{\text{Weight of moisture}}{\text{Sample Weight}} \times 100$$

2.4 Protein analysis: It was done by following Kjeldhal's method [8].

2.5 Fat analysis: Fat was extracted from samples by Soxhlet method and total fat was calculated through formula:

$$\text{Percentage of Fat} = \frac{\text{Weight of fat}}{\text{Sample Weight}} \times 100\text{merit}$$

2.6 Fibre: Fibre estimation was done in fibro-tech (Tulin equipment, India) apparatus.

Table 1: List of plant-based, non-conventional feed ingredients having potential to be used as fish feed ingredient and their nutrient composition

Scientific name/ Common name	Identification with Pictures	Crude Protein (%)	Ether Extract (%)	Crude Fibre (%)	Description
Ipomoea batatas CN: Sweet potato, Sakarkand (Hindi)		22.98	3.18	10.17	Family- Convolvulaceae sweet-tasting roots, native to tropical America
Ficus palmata CN: Punjan fig, Anjiri (Hindi), Heibam (Manipuri)		16.36	2.79	16.45	Family- Moraceae, one of the tallest wild fig commonly found in Mid-Himalayan region
Leucaena leucocephala CN: Su-babul Note: Additional source of carotene, Vit A, riboflavin, Vit K & Xanthophyll		23.32	4.12	19.18	Family: Fabaceae, native to southern Mexico, excellent source of high-protein cattle fodder, fodder contains mimosine, a toxic amino acid
Litsea cubeba CN: Mountain pepper, Mejankeri (Assamese)		22.37	4.92	10.38	Family- Lauraceae, native to China and SE Asia, produces oil used for skincare
Canavalia gladiata CN: sword bean or scimitar bean, Bari seam (Hindi)		15.48	2.14	25.34	Family- Fabaceae, domesticated plant species often eaten as a vegetable in Africa and Asia
Cassava CN: Tarukandah (sanskrit), sakarkhand (Hindi)		17.83	4.88	9.36	Family- Euphorbiaceae native to South America, edible starchy tuberous root, source of carbohydrates
Elaeocarpus floribundus CN: Indian olive, Chorphon (Manipuri)		16.97	4.58	19.68	Family- Elaeaocarpaceae, known for attractive, pearl-like fruit which are colorful
Cissus adnata CN: Kokngouyen, Charpate (Nepali)		18.43	4.92	20.21	Family- Vitaceae, woody vine species found in Asia and Australia
Ricinus communis CN: Castor bean or castor oil plant, Arandi (Hindi)		25.36	2.34	9.35	Family-Euphorbiaceae, Source of castor oil, seeds contain between 40- 60% oil that is rich in triglycerides, mainly ricinolein
Zanthoxylum acanthopodium CN: Lemon pepper, Tejphal Timur (Hindi)		17.65	5.18	15.22	Family: Rutaceae, native to warm temperate and subtropical areas worldwide

Hibiscus sabdariffa CN: Roselle, Lal Ambari (Hindi)		18.02	2.96	11.69	Family- Malvaceae, native to West Africa, used as vegetable for food preparation
Cynodon dactylon CN: Barmuda grass, Dhoob, scutch grass		12.67	1.08	21.32	Family: Poaceae, Native to eastern hemisphere, exhibit anti-stress and improved fertility in animals
Bombax ceiba CN: Simul (Bengali), semal (Hindi)		13.14	3.95	20.47	Family: Malvaceae, widely planted in parks and on roadsides because of its beautiful red flowers
Sesbania aculeata ***Sesbania bispinosa*** (seed) CN: Dhaincha		34.30	4.8	11.24	Family: Fabaceae, native to Asia and Africa, grows as a common noxious weed, used as green manure, rice straw, wood and fodder
Artocarpus heterophyllus CN: Kathal, Jackfruit		13.49	2.67	20.32	Family: Moraceae, native to South India, popular food item throughout the tropical regions of the world
Chenopodium album CN: Lambs quarters *Bathua*		17.29	2.31	10.19	Family: Amaranthaceae, weedy annual plant, extensively cultivated and consumed in Northern India as a food crop
Moringa oleifera CN: Sajana Drumstick tree Munga (Bihar)		20.94	4.86	12.16	Family: Moringaceae, drought-resistant tree, native to the southern foothills of the Himalayas in north-western India
Eichhornia crassipes CN: water hyacinth		17.15	2.02	16.44	Family: Pontederiaceae, aquatic plant native to Amazon basin, Free-floating, perennial, problematic invasive species.

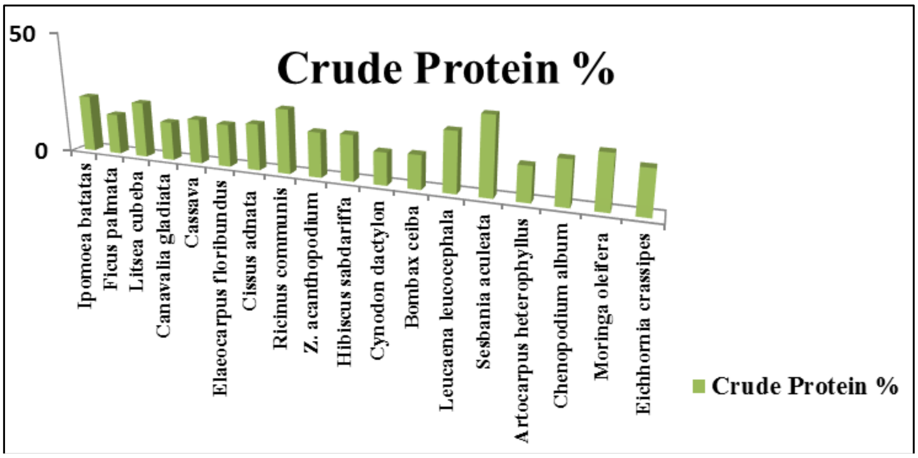

Fig 1: Crude Protein concentration in different samples

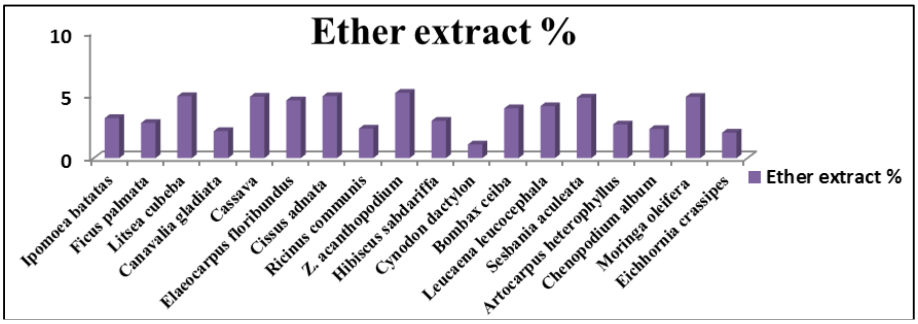

Fig 2: Ether extract concentration in different samples

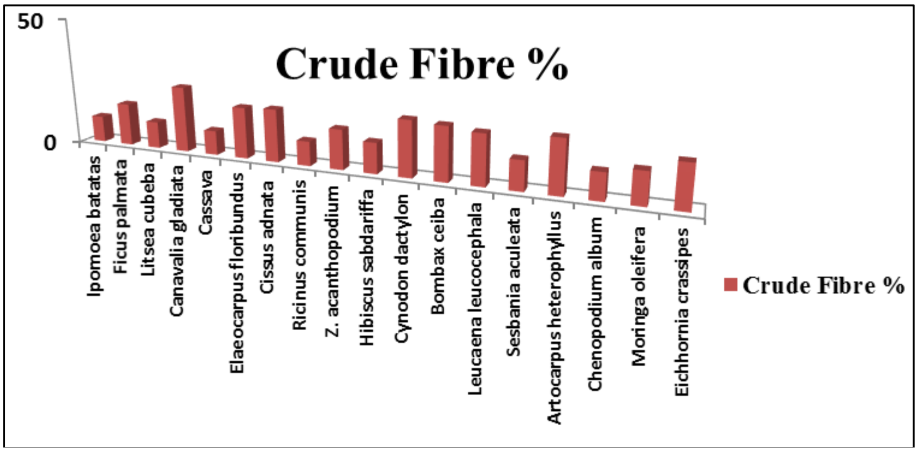

Fig 3: Crude fibre concentration in different samples

Result

a. Protein

Out of the 18 analyzed samples (Table. 1), 4 are in the range of 20-26% crude protein while the remaining 13 samples are within 10-20% CP and the highest CP was reported in sesbania leaves (30.30%). Their CP ranged from 12.67% (C. dactylon) to 30.3% (S. aculeata). This was followed by R. communis (25.36%), L. leucocephala (23.32%) and I. batatas leaves (22.98%) and the lowest CP was found in C. dactylon (12.67) (Fig: 1)

b. Ether Extract /Fat

Z. acanthopodium was recorded the highest ether extract (EE) value of 5.18% followed by L. cubeba and C. adnata (4.92% in both). Ether extract of other leaves were below 4.88%. Lowest EE was reported in C. dactylon (1.08%) (Fig: 2).

c. Crude Fibre

The crude fibre (CF) content was high in some of the ingredient such as 25.34% in C. gladiata, 21.32% in C. dactylon and 20.47% in B. ceiba. R. communis leaves and cassava leaves had CF content of 9.36 and 9.35%, respectively showing the lowest content. The ideal fibre required for feed ingredient is 2% to 8% (Fig: 3).

Discussion

Among the feed ingredients analyzed S. aculeata contained a good proportion of CP (30.3%), CF (11.24%) and ether extract (4.8%), which suggests its utilization in the aquafeed formulation. Sesbania leaves are a rich source of proteins, vitamins, minerals, trace elements and antioxidants. The medicinal products made from it include simple preparations to complex ones such as oil solution, soft extract, membranous preparations, vitamin E, other antioxidants, etc. This is followed by R. communis with CP (25.36%), CF (9.35%) and EE (2.34%); L. leucocephala with CP (23.32%), CF (19.18%) and EE (4.12%); I. batatas with CP (22.98%), CF (10.17%) and EE (3.18%). All the above ingredients are claimed to be rich source of vitamins, trace elements and amino acids. These ingredients are reputed to have considerable medicinal value.

Protein is essential nutrient required in fish feeds to supply amino acids which are necessary for growth and maintenance of fish [9]. Crude protein analysis showed that all proposed ingredients has a higher protein content exceeding above 10%, with S. aculeate (30.30%) having the highest value. These indicate the potential of proposed ingredient to replace or substitute fish meal and de-oiled rice bran which is commercially used as protein component in fish feeds. Replacement of fish meal with another plant-protein source is important for sustainable aquaculture [10]. The reason for the higher protein-energy ratio is because fish require less energy for maintenance and the synthesis of uric acid [11]. Lower energy requirements for heat increment and maintenance mean that a higher protein: energy ratio is required in aquaculture diets [12]. Reports indicated the replacement of 15% of protein from soybean meal was feasible for stinging catfish [13].

Lipid plays significant roles as sources for metabolic energy especially for development including propagation and movement [14]. The study showed that all proposed ingredients contain a certain amount of lipid with Z. acanthopodium having the highest lipid content which is 5.18%. Lipid rich feedstuffs are prone to rancidity resulting off flavor and nutritional loss. Crude fibre content was analyzed because it is the important source of energy and dietary fibre. Catfish, which is omnivorous, are able to digest decent amount of crude fibre in their diets [15]. Studies conducted on the use of terrestrial plants for feeding Oreochromis shiranus and Tilapia rendalli showed that consumption ranged from 9-90% of plant dry matter per day [16]. The best fish growth occurred in a 50:50 ratio registering 0.98 and 0.91% specific growth rate per day [17]. A similar study conducted by using cabbage, pumpkin leaves and maize bran recorded specific growth rates of 0.16 to 0.51 for cabbage and maize bran inputs [18]. This shows that non-conventional, plant-based feed sources have a potential to be used in aquafeed formulation, thus providing nutritional security to the aquaculture sector.

Conclusion

In conclusion, aquaculture is currently one of the fastest-growing food sectors in the world. Due to the increasing demand for fish, aquaculture has forged into heavy intensification. In such a highly intensive system, nutritionally balanced, good quality feed should be provided to fish which will otherwise impair growth and productivity. Therefore to ensure that fishes are fed with a nutritionally balanced diet, formulated species-specific quality feed demand has increased over the last few years. The cost of fish meal, which has been the chief protein source in aquafeed, continues to be heightened whereas its availability has declined. Thus, the quest for another alternative protein source to partially or completely replace fishmeal and de-oiled rice bran becomes imperative in the sector. However, complete replacement of fishmeal using animal protein source are limited and expensive, thus the exploration of unconventional plant sources became essential as an alternative protein source. It is believed that use of plant protein sources could ease the availability of feedstuff and reduce the feed cost [19]. With greater research and experimentation, the limitations of using plant-sourced feedstuffs such as the presence of anti-nutritional factors, varying amino acid contents, etc. have been reduced by implying various processing techniques such as soaking, fermentation, heating, drying, disintegration to finer particles or concentrates, etc. Hence, many of the plant leaves are used in fish feed formulation as protein source most of which is a partial replacement of DORB and fish meal. Their use in the feed industry is sustainable, environment-friendly and cost-effective. The use of these feed ingredients can be advocated to the local farmers and they should be supported in gaining the first hand knowledge of these feedstuffs, thus providing nutritional security to aquaculture. Therefore, the study can provide essential information for possible plant-based, non-conventional fish feed ingredients.

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