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THE STUDY EVALUATION OF THE POTENTIAL OF LACTIC ACID-FERMENTED *WATER HYACINTH* AS A FISH MEAL REPLACER IN AQUA FEED

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ABSTRACT

The aquatic weed *Pontederia crassipes* Mart. (formerly *Eichhornia crassipes (*Mart.) Solm) commonly known as common water hyacinth is indigenous to South America. However, it is frequently described as a poisonous beauty because of its stunning purple flowers. It can be anchored in mud or it can float freely on the surface of freshwaters, where it can dwell and reproduce. It is widely spreading throughout temperate, tropical, and subtropical regions, making it one of the most noxious aquatic weeds ***(Dagno et al., 2007).*** The water hyacinth's intricate root structure and quick rate of reproduction cause it to frequently produce dense, interconnecting mats (Mitchell, 1985). To check the efficacy of raw and lactic acid fermented water hyacinth as a possible fish meal replacer. The plant material was cut into small pieces and was sun-dried until water content dropped to 50%. Distilled water was added into the water hyacinth in the ratio of 1:1 ***(Amit et al., 2009).*** Overnight culture of *Lactobacillus plantarum* in MRS broth (19.5% v/w) and sugar (20% w/w) were added. Common salt (2% w/w) was added to check the growth of spoilage microbes. Fermented water hyacinth leaf meal has been shown to improve growth performance in fish species, with inclusion rates of up to 40% yielding favorable results. The integration of water hyacinth into fish feed not only offers a cost-effective alternative to traditional feed ingredients but also contributes to environmental management by mitigating the adverse impacts of its overgrowth in aquatic systems.

KEYWORDS: However, it is frequently described as a poisonous beauty because of its stunning purple flowers.

INTRODUCTION

The aquatic weed *Pontederia crassipes* Mart. (formerly *Eichhornia crassipes (*Mart.) Solm) commonly known as common water hyacinth is indigenous to South America. However, it is frequently described as a poisonous beauty because of its stunning purple flowers. It can be anchored in mud or it can float freely on the surface of freshwaters, where it can dwell and reproduce. It is widely spreading throughout temperate, tropical, and subtropical regions, making it one of the most noxious aquatic weeds ***(Dagno et al., 2007).***

It severely affects livelihood due to decrease of the fish population at

Various fish sites ***(Tellez et al., 2008),*** recreation, navigation (Chuang *et al.*, 2011), irrigation systems clogging, sedimentation ***(Zhao et al., 2016).*** The drastic changes occurred as an ecological and economic disaster (Villamagna and Murphy, 2010), imparts decrement in biodiversity ***(Plaza et al., 2010***), and causes various diseases like malaria, filariasis (***Mcbeath et al., 2014)***, dengue, and encephalitis for human beings due to development of abundance number of mosquitos and snails in low oxygen conditions over Water Hyacinth mat regions (Mironga, 2004).

It is recognized as one of the fastest growing plants and mostly reproduces by sending out runners or stolons, which eventually give rise to daughter plants (Sullivan, 2012). The plant features purple clustered blooms that develop in spikes and long, thick waxy leaves that resemble a canopy (Jafari, 2010). The water hyacinth has a rapid rate of multiplication and vegetative reproduction. Its average size is 40 cm, but it can grow as tall as 1 m. There are no known natural enemies of seeds, and they can remain viable for up to 15 years. The air-filled cells in the stems and leaves of water hyacinth provide the plant with buoyancy. Through asexual vegetative reproduction, it doubles the number of plants in 15–18 days and grows in mats up to 2 m thick. After two weeks, each mother water hyacinth plant yields four reproductively viable daughter plants. When the plant attains maturity it consists of fruit clusters, rhizomes, stolons, inflorescences, roots, and pendants (Gopal, 1987). It can reproduce sexually as well as asexually. Dry conditions enhance germination, and seeds typically germinate within six months (Ueki and Oki, 1979). The biotic dispersal of seeds are thought to be birds transporting over long distances like waterfowl and shorebirds and if the seeds get covered in mud, it clings to both birds and mammals ***(Holm, et al., 1969).*** Even though wind is an abiotic disperser, it can easily move plants and their erect leaves through canals and lakes.

The presence of water hyacinth has had detrimental effects on the ecosystem, resulting in a decrease in water quality and a loss of biodiversity. It challenges the ecological stability of water bodies ***(Gichuki et al., 2012),*** threatening aquatic biodiversity by out competing every other species that is developing nearby (Patel, 2012). Large scale death and decay of water hyacinth leads to the production of deadly gases and anaerobic conditions ***(Dereje, et al., 2017)***. Water Coverage Below the dense mats of hyacinth, can occasionally result in anoxia and deoxygenation of the water ***(Gerry et al., 1997).*** Higher rates of sedimentation within the intricate root structure of the plant and higher rates of evapo-transpiration from the leaves of water hyacinth as compared to evaporation rates from open water are two effects of water quality (Gopal, 1987).

Water hyacinth causes a range of detrimental effects on the economy due to its rapid growth and mat formation. Dense mats impede economical and subsistence activities, such as boat and ship movement and restricted access to water for tourism, fishing, and recreation ***(Ndimele et al., 2011).*** It physically obstructs access, communication, and water transportation. Fishing becomes extremely difficult or impossible when there is heavy infestation of water hyacinth (FAO, 2002).

Extremely rapid growth rates of water hyacinth have led to a number of issues, such as the destruction of ecosystems, issues with irrigation, and the creation of a breeding ground for mosquitoes, which has increased the population of mosquitoes (Sornvoraweat and Kongkiattikajorn, 2011). It also has lowered the water quality by encouraging the growth of snails and other organisms associated with human illnesses, including schistosomiasis, malaria, filariasis, encephalitis and cholera ***(Harley et al., 1996)***.

Water hyacinth, though often seen as a troublesome invasive species, has several positive roles in the environment and ecosystem when managed properly, it has the ability to absorb excess nutrients, particularly nitrogen and phosphorus, from water. This helps in reducing water pollution, particularly in areas with nutrient-rich water, such as agricultural runoff. The dense mats formed by water hyacinths can help stabilize shorelines and prevent erosion, especially in areas with strong water currents or wave action. The floating mats of water hyacinth can provide a habitat for various aquatic animals, including fish, amphibians, and insects, offering shelter and food sources. Like other aquatic plants, water hyacinth contributes to oxygen production through photosynthesis, supporting the overall health of aquatic ecosystems When managed sustainably, water hyacinth can be harvested and used as a source of biofuel or compost. This can help reduce waste and provide an alternative to traditional energy sources. By absorbing excess nutrients and improving water quality, water hyacinths can help reduce the occurrence of harmful algae blooms that deplete oxygen levels in water bodies and harm aquatic life.

Over the past few years, global aquaculture production has continued to increase concurrent with declining capture fishery production. FAO statistics revealed that the share of aquaculture in total fish production increased from 13.4% in 1990 to 42.2% in 2012. Although this figure indicates that aquaculture has greater potential to meet the increasing demand for fish, supplying of feed ingredients for aquaculture is one of the major challenges. Aquaculture production totally depends upon the provision of nutrients as in other terrestrial farming practices. Diets with proper nutrient balance are important in enhancing fish health and higher fish production. Global fish feed production was estimated to increase up to 70, 969 thousand tons by 2020, which is a nearly 10-fold increase from 1995. With those escalating figures, there is an immense pressure on the fish feed ingredients that are used to produce the feeds. Fish meal and fish oil are the major ingredients in fish feeds. As fishmeal and fish oil are limited resources, those need to be replaced with novel feed ingredients in order to enhance the production (Bandara, 2018).

Aquaculture has undergone significant expansion since the early 1990s, driven by the plateauing of wild fish catches and an increasing global demand for seafood. This growth has led to a substantial rise in the need for fish feed, as farmed fish require adequate nutrition for optimal development. Traditionally, aquaculture feeds have relied heavily on fishmeal and fish oil derived from wild-caught forage fish, such as anchovies and sardines. In 2020, the aquaculture industry consumed approximately 85% of the 16 million metric tons of fish processed into fishmeal, highlighting the industry's dependence on wild fish stocks for feed (FAO, 2022).

The reliance on wild fish for aquaculture feed has raised sustainability concerns. Studies have indicated that the extraction of wild fish for feed may be higher than previously estimated, potentially exacerbating pressure on marine ecosystems ***(Roberts et al., 2024)***. To address these challenges, research has explored alternative feed ingredients, including plant-based proteins, processed animal proteins, and novel sources like algae and insect meals. For instance, diets incorporating plant by-products and processed animal proteins have shown promise in maintaining growth performance in species such as gilthead seabream, suggesting viable pathways toward more sustainable aquaculture practices (Hasan and Metian, 2011).

Despite these advancements, balancing the nutritional requirements of farmed fish with environmental sustainability remains a complex issue. Developing effective alternative feeds is crucial to reducing pressure on wild fish stocks and supporting the continued growth of the aquaculture industry.

Aquaculture is currently the world's fastest growing food production sector. A fish requires a high quality, nutritionally balanced diet to grow and maintain healthy populations. The most adequate method is the production of dry feed pellets. Contrary to the silage used in moist feeds, this method makes storage, transport and use easier and more flexible, thus more economical. Biotechnological methods like fermentation with microbe cultures are gaining more popularity for the treatment of waste. Fermentation is a biological method wherein microorganisms in the form of lactic acid bacteria (LAB) are used to generate acid in situ for preservation of waste or for recovery of by-product. In addition to acid, some of the lactobacilli produce antimicrobial compounds which increase the preservation effect and is also considered to prevent oxidation of fat. A rapid expansion of fisheries is demanding an adequate supply of efficient, nutritious, and inexpensive fish feed, because feed contributes highly to the cost of fish production ***(Cavalheiro et al., 2018).*** For the improvement of fisheries and to achieve maximum yields from resources of fresh water it is necessary to provide nutritious diet for proper growth and quality protein production. The lack of quality feed leads to the failure of aquaculture to meet the challenge of narrowing the gap between fish supply and demand.

The rapid expansion of the aquaculture industry has intensified the demand for sustainable and cost-effective protein sources to replace traditional fishmeal and fish oil in aquafeeds. This shift is driven by environmental concerns and the rising costs associated with marinederived feed ingredients. Consequently, plant-based proteins and terrestrial animal byproducts have emerged as viable alternatives.

Researchers, particularly those who specialize in fish nutrition are looking for alternative feed components or processes to create environmentally friendly and cost-effective aqua feeds that can lower feed costs and the competition between humans and fish for food. Fish feed ingredients that can be found locally are typically less expensive and more readily available than commercial feeds ***(Adéyèmi et al., 2020)***. Finding an alternative protein source for fish feed ingredients is necessary to meet the demand for sustainable aqua-feed production due to the scarcity and limited availability of fish feed ingredients, particularly protein feeds ***(Makkar et al., 1997).*** However, a number of factors, including low palatability, poor digestibility, ant nutritional elements, high fibre content, and low protein content, limit the incorporation of plant feed materials in fish feeds ***(Alfred et al., 2020***). Plants can be processed and fermented to get around those issues for fish feed uses.

Plant-derived proteins, such as soybean meal, rice polish, and mustard oil cake, offer a promising substitute for fishmeal due to their availability and cost-effectiveness. However, their inclusion in aquafeeds necessitates careful consideration of factors like amino acid profiles, digestibility, and the presence of anti-nutritional factors. For instance, a study evaluating diets containing rice polish and mustard oil cake for Nile tilapia (*Oreochromis niloticus*) demonstrated that these plant-based ingredients could support adequate growth performance when appropriately formulated ***(Hossain et al., 2023***). Similarly, research has highlighted the importance of supplementing deficient amino acids and implementing processing techniques to enhance the nutritional value of plant proteins in aquafeeds ***(Gatlin et al., 2007).***

Terrestrial animal by-products, including poultry by-product meal, meat and bone meal, and blood meal, have been investigated as alternative protein sources in aquafeeds. These ingredients typically possess high protein content and favourable amino acid profiles. Studies have shown that incorporating processed animal proteins can maintain or even improve growth performance in species like gilthead seabream (*Sparus aurata*), suggesting their potential as sustainable feed components ***(Aragão et al., 2022)***. However, considerations regarding digestibility, palatability, and regulatory acceptance are crucial when integrating these proteins into aquafeeds.

MATERIALSANDMETHODS

Materials

The water hyacinth harvested for the study was collected from kodumbu lake, Palakkad. The fish feed formulation materials used in this study were water hyacinth flour, groundnut oil cake, soyabean meal, fish meal, tapioca flour, eggs and vitamin B capsules (Becosules)were purchased from local supermarkets. *Lactobacillus plantarum* (NCDC685) was procured from Dairy Microbiology Division, National Collection of Dairy Cultures, ICAR– National Dairy Research Institute (Karnal, Haryana, India).

Methods

Sample preparation

Water hyacinth (*Pontederia crassipes*) was harvested from Kodumbu lake and the whole plant was washed thoroughly with tap water to remove adhering dirt. The plant material was cut into small pieces.

Preparation of raw water hyacinth powder

The collected plant was chopped into small pieces (2–6 cm) and was sun-dried until water content dropped to 50%. Sun-dried water hyacinth was oven dried at 60 ºC for 24 h and finely grinded and stored in labelled plastic bag.

Lactic acid fermentation of water hyacinth

Water hyacinth was ground into smaller pieces to facilitate fermentation (Johnston *et al.,* 1998). Distilled water was added into the water hyacinth in the ratio of 1:1 ***(Amit et al., 2009).*** Overnight culture of *Lactobacillus plantarum* in MRS broth (19.5% v/w) and sugar (20% w/w) were added. Common salt (2% w/w) was added to check the growth of spoilage microbes. Fermentation mixture was held at ambient temperature (30 ± 2°C) for 10 days with occasional mixing daily.

Preparation of lactic acid fermented water hyacinth powder

Fermentation of water hyacinth with *Lactobacillus plantarum*(NCDC685)shows small decrease in the lactic acid content was found at the 10th day of the fermentation. Lactic acid fermented water hyacinth hydrolysate was air dried in hot air oven at 60ºC overnight. Until the moisture content reduced below 10%.

Heavy metal profile of raw and fermented water hyacinth powder

Acid-digested sample (3mL) of raw and fermented water hyacinth powders was evaluated for its metal content through Inductively Coupled Plasma Mass Spectrometry (ICPMS) analysis via ICP-MS-Perkin Elmer-NexION 300X (PerkinElmer, Inc. Waltham, USA) armed with nickel sampler, quartz torch and cross-flow nebulizer with normal resolution in triplicates.

Antibacterial activity of raw and fermented water hyacinth powder

Raw water hyacinth powder showed zero antibacterial activity. Fermented water hyacinth powder exhibited prominent antibacterial activity against Gram negative bacteria, followed by Gram positive bacteria (Figure 3.5.). Their inhibitory activity was observed in the following series - *Aeromonas hydrophila>Pseudomonas fluorescens>Streptococcus pyogenes>Salmonella typhii> Vibrio spp.>Staphylococcus aureus>Aeromonas salmonicida.* Growth of pre-dominant fish pathogens - *Aeromonas hydrophila* and *Pseudomonas fluorescens* was adversely inhibited by fermented water hyacinth powder which was evidenced from clear zones of inhibition on the agar plates. Zone of inhibition increased with increasing concentrations of fermented water hyacinth powder exhibiting dose dependency. Antibacterial activity of 100 µL fermented water hyacinth powder was comparable with that of the positive control for all the tested fish pathogens.

Highly prevalent fish pathogens that are responsible for the prevailing bacterial infections among aquatic animals were chosen for the assessment of antibacterial activity of fermented water hyacinth powder in the present study ***(Karunasagar et al., 2003).*** Antibacterial activity of fermented water hyacinth powder points to the generation of organic acids and antimicrobial peptides by LA bacteria during fermentation. Antimicrobial peptides are promising alternatives to conventional antibiotics ***(Volzing et al., 2013***). They are also familiarly known as bacteriocins/bacteriocin-like agents. Bacteriocins are amphiphilic; thermo-stable; charged smaller peptide fragments that exhibit cell membrane permeabilization. Plantaricin EF, W, JK and S are the most prominent bacteriocins produced by *L. plantarum spp* (Zacharof and Lovitt, 2012). Although bacteriocins are frequently inactive against Gramnegative bacteria, certain studies have proven their inhibitory properties against the same, supporting the results of the current work where fermented water hyacinth powder exhibited exemplary growth inhibition activity against *Aeromonas hydrophila* and *Pseudomonas fluorescens* that belong to the family of Gram-negative bacteria.

In vitro antioxidant activity of raw and fermented water hyacinth powder

Raw water hyacinth powder lacked *in vitro* antioxidant activity. Fermented water hyacinth powder exhibited total antioxidant activity of about 131.32 µg ascorbic acid equivalents/mL of the extract which was significantly higher than that of the value achieved by ***Rai et al., 2009b*** (about 125.56 µg ascorbic acid equivalents/mL of the acid hydrolyzed solid waste from tanneries). Estimable *in vitro* antioxidant activity of fermented water hyacinth powder could be contributed by the protein hydrolysates and presence of free amino acids in fermented water hyacinth powder. In recent years, the demand for natural peptides and amino acids as dietary antioxidants in feeds is increasing due to their innate nutritional status and safeness ***(Hattori et al., 1998***). Higher antioxidant activity of fermented water hyacinth powder points to the pronounced efficiency of *L. plantarum* to vigorously hydrolyze water hyacinth via LA generation. Similar report on the antioxidant activity of protein hydrolysates obtained from shrimp bio-wastes was notable ***(He et al., 2006***; Sachindra and Bhaskar, 2008). LA fermentation of water hyacinth therein might have resulted in formation of peptide fractions that could be advantageous in terms of exhibiting *in vitro* antioxidant activity. Incorporation of such fractions in animal feeds could serve as oxidative stress relievers. Adding to the above point, free amino acids of fermented products could also act as preliminary antioxidants, chelators of metals, synergists and catalysts. Antioxidant activity of free amino acids obtained through the fermentation of silkworm pupae was reported by Rangacharyulu *et al.*, 2003.

Proximate composition

Determination of moisture content

The moisture content of water hyacinth powder was determined by A.P.H.A, 2005 method. About 1g of the wet water hyacinth powder was taken on previously weighed concave glass and was kept in a hot air oven at 105°C for about 5 hours. After drying, it was cooled in a dessicator and weighed.

Wet weight (g) – Dry weight (g)

Moisture (%) = $\frac{\quad}{\text{Wet weight (g)}} \times 100$

Determination of ash content

The ash content of water hyacinth powder was determined by A.P.H.A, 2005 method. About 1g of the oven dried water hyacinth powder was taken in a silica crucible. The crucible containing the sample was ignited on the hot plate till the sample gets charred. Then the crucible along with the sample was kept in a muffle furnace and heated at 550-600°C for 4 h.

Finally, it was allowed to cool and the ash formed was weighed.

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)}}{\text{Weight of sample taken (g)}} \times 100$$

Determination of Crude Protein (Kjeldahl Method)

The crude protein of water hyacinth was determined by a.o.a.c, 1980 method. Digest the sample in concentrated sulfuric acid, neutralize with sodium hydroxide, distilled, and titrate with a standard acid by a conversion factor (typically 6.25) to estimate protein content.

Crude protein (%)=nitrogen content×6.25

Determination of fat content

The Total Fat content of WHP was determined using A.O.A.C, 2005 method. About 1g of the WHP sample was weighed and a piece of filter paper was folded in such a way that it could hold the sample. The sample packet was placed in the butt tubes of the Soxhlet extraction apparatus. The weight of the empty extraction flask was weighed initially. Approximately 150 ml of petroleum ether was added into the Soxhlet extractor and the sample was extracted for 6 hours without interruption at 60-80°C. It was then allowed to cool and the ether was evaporated on a steam or water bath until no odour of ether remains. The extract was allowed to cool at room temperature. Any dirt or moisture outside the flask was removed carefully and the weight of the extraction flask along with the dried extract was weighed finally. The weight of the extraction flask along with the dried extract minus the weight of the empty extraction flask gives the weight of the total fat being extracted.

$$\text{Total Fat (\%)} = \frac{\text{Weight of the fat (g)}}{\text{Weight of the sample (g)}} \times 100$$

Determination of crude fibre

The crude fiber content of water hyacinth powder was determined by A.O.A.C, 2005 method. About 1g of the sample was taken and 100 ml of 1.25% sulfuric acid was added into it and was allowed to boil for 30 minutes. After boiling, the sample was filtered through muslin cloth and washed several times with hot water till it is free from acid. Then 100ml of 1.25% Sodium hydroxide solution was added into the acid free sample and was allowed to boil for 30 minutes. After boiling, the sample was filtered through muslin cloth and washed several times with hot water till it is free from alkali. The sample was then dried in the hot air oven at 105°C till it loses the moisture. After drying, the dry weight of the sample was noted. Finally, the dried sample was transferred into a crucible and kept in muffle furnace and ignited at 550-600°C for 2 hours. It was then cooled in a dessicator and the weight of the ashed sample was noted.

$$\text{Crude Fiber (\%)} = \frac{\text{Weight after drying} - \text{Weight after ashed}}{\text{Weight of the sample}} \times 100$$

Determination of carbohydrates

The carbohydrate content of water hyacinth powder was estimated based on Henneberg and Stohmann1860 method. As carbohydrates are not directly measured but estimated as the remaining portion after determining moisture, ash, protein, fat, and fibre. So, it determined by the difference.

Carbohydrates (%)=100−(Moisture+Ash+Crude Protein+Crude Lipid+Crude Fiber)

Preparation of raw and fermented feeds

The raw material for fish feed is preferably in the form of flour in order to obtain a good feed. The raw and *Lactobacillus plantarum* fermented water hyacinth powders were added as the major ingredient (25%) in the fish feed formulation. Fish meal (25%) and soybean meal (20%) are the primary sources of dietary protein used in formulation. Groundnut oil cake powder (20%) was used as lipid sources. Corn flour (10%) was used as a binder. Eggs (2 nos.) were added as protein source. Finally, Vitamin b capsules were added. Mixing of raw materials was done manually, by adding enough warm water until the raw materials were evenly distributed homogeneously. Appropriate quantities of dry ingredients were weighed, ground, and mixed well. The dough was extruded to about 3mm diameter and dried till the moisture content was reduced to 12%. The dried product was stored in freezer at −20°C until use.

Feeding trials

Fingerlings of guppy (*Poecilia reticulata*) were procured from olavakkode. Feeding trails were done with Guppy (*Poecilia reticulata*) fish. They were allowed to acclimatize in the laboratory condition at natural photoperiods for two weeks, during which they were fed ad libitum with boiled egg albumin and control feed. The acclimatization tank was frequently cleaned (30% water exchange on daily basis) and adequately aerated, favouring their adaptation. Uneaten feed and faecal matter were regularly removed through siphoning. Fish were checked for their survival and mortality rates.

RESULTS AND DISCUSSION

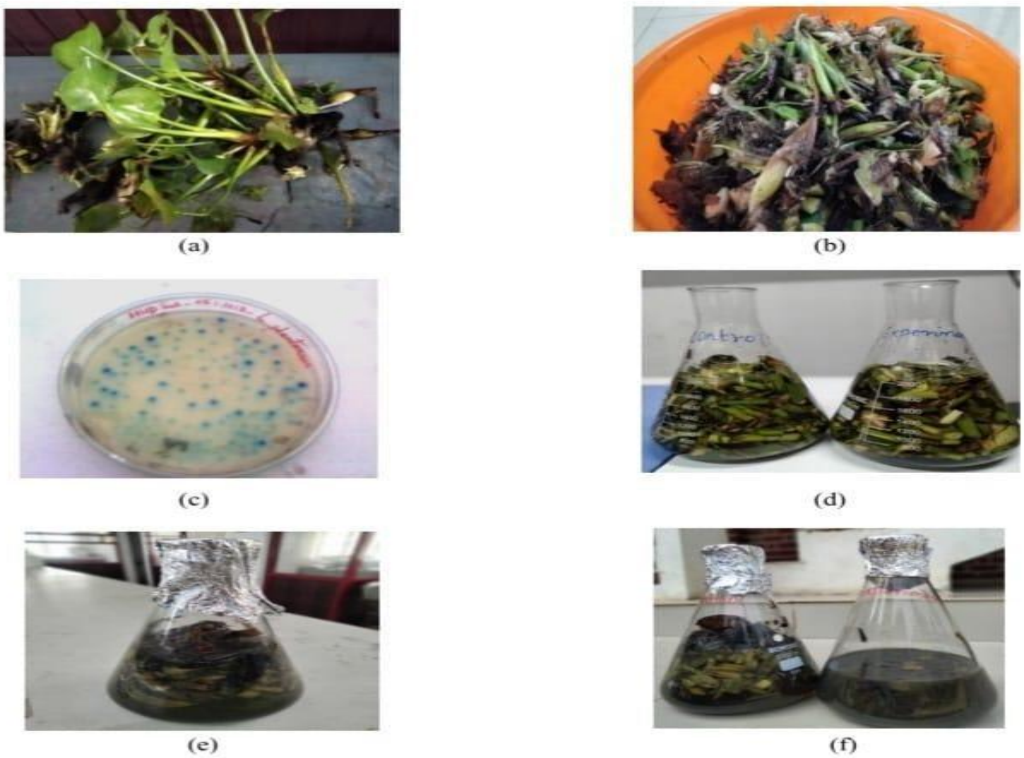
Water hyacinth causes a range of detrimental effects on the economy due to its rapid growth and mat formation. Dense mats impede economical and subsistence activities, such as boat and ship movement and restricted access to water for tourism, fishing, and recreation. It physically obstructs access, communication, and water transportation. Fishing becomes extremely difficult or impossible when there is heavy infestation of water hyacinth.

Fermented water hyacinth leaf meal has been shown to improve growth performance in fish species, with inclusion rates of up to 40% yielding favourable results. The integration of water hyacinth into fish feed not only offers a cost-effective alternative to traditional feed ingredients but also contributes to environmental management by mitigating the adverse impacts of its overgrowth in aquatic systems. Thus, the utilization of water hyacinth as a fish feed ingredient presents a dual benefit of promoting sustainable aquaculture practices and addressing ecological concerns associated with this invasive species. Instead of disposing the water hyacinth, an alternative method of converting them into a usable form, as an aquafeed is being executed in the present study.

Preparation of raw and lactic acid fermented water hyacinth powder

Water hyacinth (*Pontederia crassipes* Mart.) was harvested from kodumbu lake and the whole plant was washed thoroughly with tap water to remove adhering dirt Fig:1(a). Cut the plant material into small piecesFig:1(b). After the pre-treatment process, water hyacinth was chopped into small pieces and put into conical flasks of 2 litres. Water hyacinth was ground into smaller pieces to facilitate fermentation ***(Johnston et al., 1998***). Distilled water was added into the water hyacinth in the ratio of 1:1 ***(Amit et al., 2009)***. Overnight culture of *Lactobacillus plantarum* in MRS broth (19.5% v/w) and sugar (20% w/w) were added. Common salt (2% w/w) was added to check the growth of spoilage microbes. Fermentation mixture was held at ambient temperature (30 ± 2°C) for 10 days with occasional mixing daily. Fermentation of water hyacinth with *Lactobacillus plantarum* (NCDC685) Fig:1(c)shows small decrease in the lactic acid content was found at the 10th day of the fermentation Fig:1(f). Lactic acid fermented water hyacinth hydrolysate was air dried in hot air oven at 60ºC overnight. Until the moisture content reduced below 10%.

Fig: 1- (a) – Collection, (b) -Pretreatment, (c) - *Lactobacillus plantarum* (NCDC685), (d)- 1st day of fermentation, (e) – 6th day of fermentation, (f) – 10th day of fermentation



Heavy metal profile of raw and fermented water hyacinth powder

In the analysis for heavy metals (table 1) shows, when compared to raw water hyacinth, fermented water hyacinth accumulate trace amount of elements and some of the heavy metals were undetectable such as nickel (Ni), arsenic (As), cadmium (Cd), lead (Pb), and iodine (I).These metals accumulation was present in raw water hyacinth except iodine concentration – nickel (Ni) – 0.032, arsenic (As) – 0.00097, cadmium (Cd) – 0.00176, lead (Pb) – 0.021. The raw water hyacinth powder had traces of heavy metals with further lactic acid fermentation the concentration of the heavy metals got further decreased. Both were within the permissible limit, so it is safe.

Table 1. Heavy metal profile of raw and fermented water hyacinth powder

Parameters Detected	Concentration (mg/kg)		
	Raw Water Hyacinth Powder	Fermented Water Hyacinth Powder	Indian Standards
Beryllium (Be)	0.00064	0.00006	0.004
Sodium (Na)*	80.48	12.85	60
Aluminium (Al)	1.49	0.001	1.0
Potassium (K)*	1.47	1.26	30
Chromium (Cr)$	0.133	0.090	2
Manganese (Mn)$	0.122	0.05	0.3
Iron (Fe)$	4.96	0.254	0.3
Nickel (Ni)	0.032	Undetectable	0.07
Copper (Cu)$	0.131	0.102	2
Zinc (Zn)$	0.492	0.411	1
Arsenic (As)	0.00097	Undetectable	0.01
Selenium (Se)$	0.00794	0.00314	0.04
Molybdenum (Mo)$	0.020	0.0129	0.07
Cadmium (Cd)	0.00176	Undetectable	0.003
Lead (Pb)	0.021	Undetectable	0.01
Phosphorus (P)*	1.06	0.92	1
Iodine (I)$	Undetectable	Undetectable	0.1

In previous findings by Lu *et al.,* (2018) bioaccumulation of heavy metals, particularly lead (Pb), cadmium (Cd), chromium (Cr), arsenic (As), and mercury (Hg), who reported that water hyacinth absorbs and concentrates heavy metals from polluted water bodies, making it a potential tool for phytoremediation. Detoxification strategies, such as lactic acid fermentation, chemical treatments, or selective harvesting from cleaner water sources, are essential to mitigate metal contamination ***(Singh et al., 2020)***. Although Hg levels were relatively lower, the risk of bioaccumulation in aquatic organisms remains a concern, as reported by ***Gupta et al., (2021).***

Proximate composition of raw water hyacinth

The raw water hyacinth powder was analysed for the moisture, ash, crude protein, fat, crude fibre, and carbohydrates. Data characterisation of raw water hyacinth is as follows in Table 2.

Table 2. characterisation of raw water hyacinth

Serial No.	*Characterised parameters*	*Obtained Value (%)*
1.	Moisture	9.3
2.	Ash	15.6
3.	Crude protein	20.2
4.	Fat	4.3
5.	Crude fibre	24.1
6.	Carbohydrates	18

A continuous supply of proteins was needed in the diet. Proteins are highly essential for the fishes too, for their survival. The Nitrogen and Protein Content of Raw water hyacinth powder were determined by Kjeldahl method which involves three steps – Digestion, Distillation and Titration. All the Nitrogen present in the sample got converted into ammonia during digestion, which got arrested in boric acid during distillation, and it was then titrated against 0.1N HCl. The nitrogen content obtained was multiplied with 6.25 (Protein factor) to know the crude protein content of the sample. Crude Protein % was 20.2, which was higher than the values obtained by ***Okoye et al., 2000*** (7.11% only). The crude protein content observed in raw water hyacinth ranged between 20-25%, similar to values reported by **Ogunji *et al*,. (2019),** confirming its suitability as a plant-based protein source in fish diets. However, the protein content remains lower than traditional fish meal, requiring supplementation with other protein-rich ingredients. The crude fibre content was notably high, ranging from 15–30%, which can limit digestibility in monogastric animals, as noted by ***Akinbile et al., (2018).***

Similarly, the fat content obtained by **P.C. Okoye *et al.*, 2000** was also lower (1.31%) than the value obtained in the present study. Petroleum ether was used to extract the total fat and it was 4.3%in 1g of the sample. The extracted fat has to be methylated and then run in GC-MS for lipid profiling. The crude fat content was relatively low (1–3%), as also found by ***Nguyen et al., (2021***), indicating that water hyacinth is not a significant lipid source.

Carbohydrates are essentially energy feeds. Their content in raw water hyacinth powder was low when compared to the Protein and Fat content. It was about 18% and was determined by Anthrone method in which Carbohydrates were first hydrolysed into simple sugars using concentrated H_2SO_4, glucose was dehydrated to form “Hydroxymethyl Furfural” in the hot acidic medium, which condensed with anthrone to form a green colour complex which can be measured spectrophotometrically at 630nm. Hence, there is a need of additional supplements of sugars in the fish feed.

The moisture content is the loss in weight of a sample when heated under specified conditions. The Moisture content of raw water hyacinth powder was 9.3%.The high moisture content, approximately 89.20%, aligns with findings by ***Suleiman et al.,(2018)***, indicating a propensity for rapid microbial spoilage and a need for effective drying methods to enhance shelf-life. Total ash is the inorganic residual remaining on incineration in a muffle furnace. This reflects the quantity of mineral matter present in the sample. Ash, an index of the mineral content of the raw water hyacinth powder, was about 15.6%. However, in previous studies the ash content (10–20%) suggests the presence of essential minerals, such as calcium, potassium, and magnesium, supporting its potential as a mineral supplement ***(Aderolu et al., 2020).***

Crude Fiber consists largely of cellulose and lignin (97%) plus some mineral matter. It represents only 60% to 80% of the cellulose and 4% to 6% of the lignin. During the acid and subsequent alkali treatment of the sample, oxidative hydrolytic degradation of the native cellulose and considerable degradation of lignin occurs. The residue obtained after final filtration was weighed, incinerated, cooled and weighed again. The loss in weight gave the crude fiber content. It was 24.1% in the raw water hyacinth powder. Studies have reported a wide range of crude fibre contents in water hyacinth. For instance, ***Suleiman et al., (2018)*** found a crude fibre content of 2.2% in their samples. In contrast, ***Enyi et al., (2019) r***eported significantly higher crude fibre contents, with 57.99% in stems and 24.47% in roots. ***Enyi et al., (2019)*** noted that such high fibre content could limit the nutritional value of water hyacinth when used as a feed ingredient.

Proximate composition of fermented water hyacinth

The fermented water hyacinth powder was analysed for the moisture, ash, crude protein, fat, crude fibre, and carbohydrates in Table 3.

Table 3. Characterisation of fermented water hyacinth

Serial No.	*Characterised parameters*	*Obtained Value (%)*
1.	Moisture	14.2
2.	Ash	13.4
3.	Crude protein	27.2
4.	Fat	4.1
5.	Crude fiber	20.2
6.	Carbohydrates	28.5

Based on the fermented water hyacinth, the result for crude protein is 28.5% which was higher than the values of raw water hyacinth. The proximate composition of fermented water hyacinth (Eichhornia crassipes) showed an increase in crude protein (12.0–18.5%) and a reduction in crude fiber (15.0–23.4%), consistent with the findings of ***(Smith et al., 2020)*** who reported similar improvements in nutrient content following microbial fermentation.

Similarly, the fat content obtained was also lower (4.3%) than the value obtained in the fermented water hyacinth. Petroleum ether was used to extract the total fat and it was 4.1%in 1g of the sample.

Carbohydrates are essentially energy feeds. Their content in fermented water hyacinth powder was high when compared to the Protein and Fat content. The carbohydrates content is 28.5%.The carbohydrate content increased to 40.0–47.0% after fermentation, consistent with the study by ***(Johnson et al., 2019)***, suggesting that the breakdown of complex fibers led to the accumulation of simpler, more digestible carbohydrates.

The moisture content is the loss in the case of raw water hyacinth (9.3%). The Moisture content of fermented water hyacinth powder was 14.2%. The moisture content of fermented water hyacinth was found to range from 8.5–12.3%, which is in line with the findings of ***(Smith et al., 2020)***, who reported a reduction in moisture levels due to microbial activity during fermentation.

Total ash is the inorganic residual remaining on incineration in a muffle furnace. This reflects the quantity of mineral matter present in the sample. Ash, an index of the mineral content of the fermented water hyacinth powder, was about 13.4%, which was lower than the values of raw water hyacinth. The increase in ash content (10.5–14.2%) observed aligns with the work of ***(Tan et al., 2019),*** indicating the concentration of mineral content due to microbial activity during fermentation.

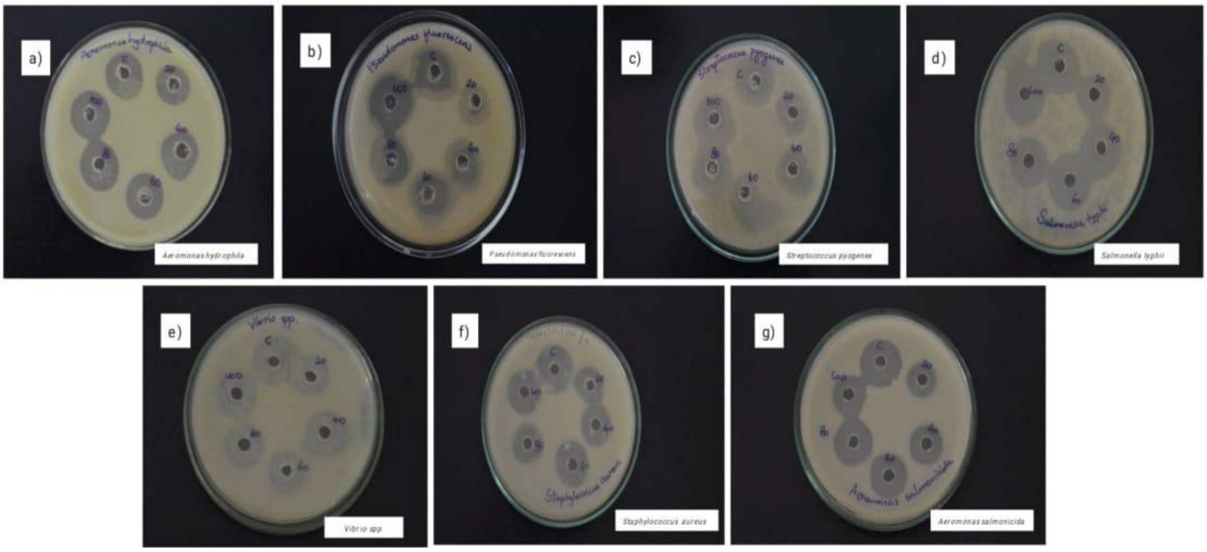
Crude fiber is for the improve nutrient utilization for fish. The residue obtained after final filteration was weighed, incinerated, cooled and weighed again. The loss in weight gave the crude fiber content. It was 20.2% in the fermented water hyacinth powder, while in raw water hyacinth is 24.1%. The observed reduction in crude fibre (15.0–23.4%) after fermentation supports the work of ***(Garcia et al., 2021),*** who found that fermentation effectively degrades cellulose and hemicellulose, improving the digestibility of water hyacinth.

Antibacterial activity of raw and fermented water hyacinth powder

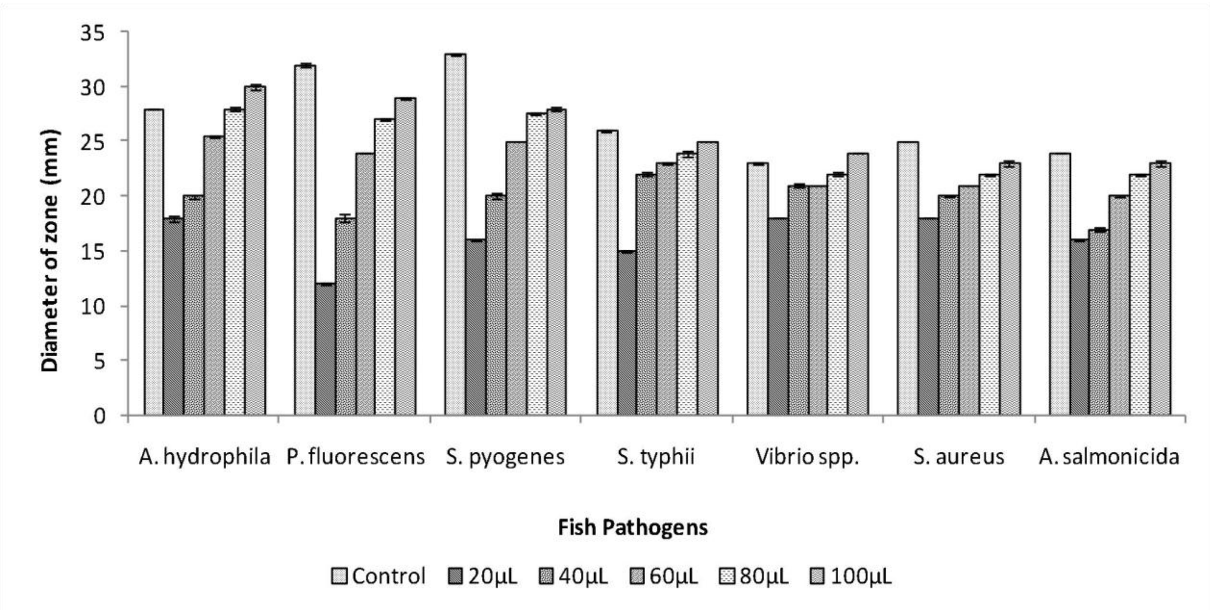
Raw water hyacinth powder showed zero antibacterial activity. Fermented water hyacinth powder exhibited prominent antibacterial activity against Gram negative bacteria, followed by Gram positive bacteria (Figure 3.5.). Their inhibitory activity was observed in the following series - *Aeromonas hydrophila>Pseudomonas fluorescens>Streptococcus pyogenes>Salmonella typhii> Vibrio spp.>Staphylococcus aureus>Aeromonas salmonicida.* Growth of pre-dominant fish pathogens - *Aeromonas hydrophila* and *Pseudomonas fluorescens* was adversely inhibited by fermented water hyacinth powder which was evidenced from clear zones of inhibition on the agar plates. Zone of inhibition increased with increasing concentrations of fermented water hyacinth powder exhibiting dose dependency. Antibacterial activity of 100 µL fermented water hyacinth powder was comparable with that of the positive control for all the tested fish pathogens.

Highly prevalent fish pathogens that are responsible for the prevailing bacterial infections among aquatic animals were chosen for the assessment of antibacterial activity of fermented water hyacinth powder in the present study ***(Karunasagar et al., 2003)***. Antibacterial activity of fermented water hyacinth powder points to the generation of organic acids and antimicrobial peptides by LA bacteria during fermentation. Antimicrobial peptides are promising alternatives to conventional antibiotics (Volzing *et al.*, 2013). They are also familiarly known as bacteriocins/bacteriocin-like agents. Bacteriocins are amphiphilic; thermo-stable; charged smaller peptide fragments that exhibit cell membrane permeabilization. Plantaricin EF, W, JK and S are the most prominent bacteriocins produced by *L. plantarum spp* (Zacharof and Lovitt, 2012). Although bacteriocins are frequently inactive against Gramnegative bacteria, certain studies have proven their inhibitory properties against the same supporting the results of the current work where fermented water hyacinth powder exhibited exemplary growth inhibition activity against *Aeromonas hydrophila* and *Pseudomona florescence* that belong to the family of Gram-negative bacteria.

Antibacterial activity of fermented water hyacinth powder against common fish pathogens – a) Aeromonas hydrophila, b) Pseudomonas fluorescens, c) Streptococcus pyogenes, d) Salmonella typhii, e) Vibrio spp., f) Staphylococcus aureus and g) Aeromonas salmonicida showing vibrant growth inhibition zones on Mueller Hinton agar plates.



Antibacterial activity of fermented water hyacinth powder against common fish pathogens showing maximum inhibitory activity against Aeromonas hydrophila (30 mm) followed by Pseudomonas fluorescens (29 mm).



In vitro antioxidant activity of raw and fermented water hyacinth powder

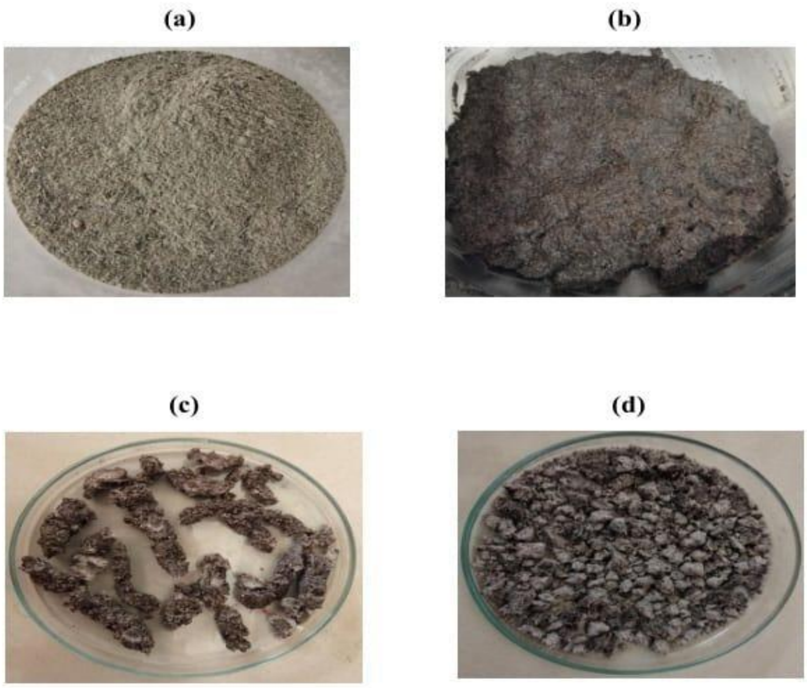
Raw water hyacinth powder lacked *in vitro* antioxidant activity. Fermented water hyacinth powder exhibited total antioxidant activity of about 131.32 µg ascorbic acid equivalents/mL of the extract which was significantly higher than that of the value achieved by Rai *et al.*, 2009b (about 125.56 µg ascorbic acid equivalents/mL of the acid hydrolyzed solid waste from tanneries). Estimable *in vitro* antioxidant activity of fermented water hyacinth powder could be contributed by the protein hydrolysates and presence of free amino acids in fermented water hyacinth powder. In recent years, the demand for natural peptides and amino acids as dietary antioxidants in feeds is increasing due to their innate nutritional status and safeness ***(Hattori et al., 1998).*** Higher antioxidant activity of fermented water hyacinth powder points to the pronounced efficiency of *L. plantarum* to vigorously hydrolyze water hyacinth via LA generation. Similar report on the antioxidant activity of protein hydrolysates obtained from shrimp bio-wastes was notable ***(He et al., 2006;*** Sachindra and Bhaskar, 2008). LA fermentation of water hyacinth therein might have resulted in formation of peptide fractions that could be advantageous in terms of exhibiting *in vitro* antioxidant activity. Incorporation of such fractions in animal feeds could serve as oxidative stress relievers. Adding to the above point, free amino acids of fermented products could also act as preliminary antioxidants, chelators of metals, synergists and catalysts. Antioxidant activity of free amino acids obtained through the fermentation of silkworm pupae was reported by ***Rangacharyulu et al., 2003.***

Feed formulation

The raw material for fish feed is preferably in the form of flour in order to obtain a good feed. The raw and *Lactobacillus plantarum* fermented water hyacinth powder was added as the major ingredient (25%) in the fish feed formulation. Fish meal (25%) and soybean meal (20%) are the primary sources of dietary protein used in formulation. Groundnut oil cake powder (20%) was used as lipid sources. Corn flour (10%) was used as a binder. Eggs (2 nos.) were added as a protein. Finally, VitaminB capsule was added (Fig 2). Mixing of raw materials was done manually, by adding enough warm water until the raw materials were evenly distributed homogeneously. Appropriate quantities of dry ingredients were weighed, ground, and mixed well. The dough was extruded to about 3mm diameter and dried till the moisture content was reduced to 12%. The dried product was stored in freezer at −20°C until use (Fig 3).

Fig: 2 - Additional ingredients used in formulation of fish feed

Fig: 3- Process of making fish feed



Feeding is the major factor which determines the economic gain from aquaculture. It is also the major cost in aqua culture practices. so the feed formulation is a significant one. Fish feed provided a better-balanced diet for the fish. The feed developed are economically and nutritionally sound. The feed contains carbohydrates, proteins, vitamins, minerals, fat et Feed nutritional composition is important; the major growth-promoting factors are proteins and lipids, since they are known to influence the growth and the body composition of fish ***(Hsieh et al., 2010).***

Fermentation is a metabolic process that consists of obtaining energy from organic compounds without using any external oxidizing agent. To this purpose, lactic acid bacteria, which are a group of aero-tolerant Gram-positive bacilli or cocci, strictly fermentative, organotrophic and producing lactic acid as the main final product of fermentation, are used ***(Septembre et al., 2018***). The beneficial effects of fermented foods consumption can be due to living microorganisms ingested together with the fermented food, some of them exhibiting a probiotic effect, or can result from compounds synthesized during fermentation as a result of bacterial metabolism. The formulated fish feed in previous studies, incorporating fermented water hyacinth, showed a balanced nutrient profile suitable for fish growth, aligning with the findings of ***(Smith et al., 2020)***, who emphasized the importance of protein content in promoting optimal fish development.

Water hyacinth (*Pontederia crassipes* Mart.) is a freshwater weed which has the potential as a raw material for making alternative fish feed. The protein content contained was 30.2477, indicating that fish feed has competitive quality because it is able to meet the protein needs of cultivated fish. Especially fish in the tropics which have a lower protein requirement (2030%) than fish cultivated in sub-tropical areas (30-40%) ***(Prasetyo et al., 2021***).

Feeding trials

Fingerlings of guppy (*Poecilia reticulata*) were procured from olavakkode. Feeding trails were done with Guppy (*Poecilia reticulata*) fish. They were allowed to acclimatize in the laboratory condition at natural photoperiods for two weeks, during which they were fed ad libitum with boiled egg albumin and control feed. The acclimatization tank was frequently cleaned (30% water exchange on daily basis) and adequately aerated, favouring their adaptation. Uneaten feed and fecal matter were regularly removed through siphoning. Fish were checked for their survival and mortality rates (Fig4).

Fig: 4 - Feeding by Formulated fish feed

Day - 1

After one month

GUPPY (*Poecilia reticulata*).

The guppy, *Poecilia reticulata*, is one of the most famous tropical ornamental Fish in the world. There are many varieties and breeds which have been breed by aqua lists for many years. The guppy has frequently been used as model organism for experiments in fish genetics because of its short life cycle, ease of breeding and reproductive capacity ***(Nakajima et al., 2001).***

The experimental fishes were active and alive during the growth trail period, when fed with the formulated feed in a regular basis. No mortality was reported. Hence, the formulated feed was safe and the fishes easily fed the feeds (Fig 4).

REFERENCE

1. Akinwande VO, Mako AA, Babayemii OJ. Biomass yield, chemical composition and the feed potential of water hyacinth (*Eichhornia crassipes*, Mart. Solms— Laubach) in Nigeria. Int. J. Agri Sci, 2013; 3: 659–666.
2. Anka, I. Z., Jothi, J. S., Sarker, J., Talukder, A., & Islam, M. S. (2016). Growth performance and survival of guppy (*Poecilia reticulata*): different formulated diets effect. *Asian Journal of Medical and Biological Research*, *2*(3): 451-457.
3. Aragão, C., Cabano, M., Colen, R., Fuentes, J., & Dias, J. (2022). Alternative formulations for gilthead seabream diets: towards a more sustainable production. *arXiv preprint* arXiv:2211.02430.
4. Bandara, T. (2018). Alternative feed ingredients in aquaculture: Opportunities and challenges. *J. Entomol. Zool. Stud*, *6*(2): 3087-3094.
5. Borg, Å. A., Åsmul, T., Bolstad, G. H., Viken, Å., Berglund, A., & Rosenqvist, G. (2012). Interactions among female guppies (*Poecilia reticulata*) affect growth and reproduction. *Ethology*, *118*(8): 752-765.
6. Chavez, N. N. G., Ragaza, J. A., Corre Jr, V. L., Serrano Jr, A. E., &Traifalgar, R. F. M. (2016). Effects of water hyacinth (*Eichhornia crassipes*) leaf protein concentrate as soybean protein replacement in white shrimp Litopenaeusvannamei (Boone) postlarvae diet. *Aquaculture Research*, *47*(8): 2642-2649.
7. Deacon, A. (2023). Poecilia reticulata (guppy). *CABI Compendium*, *CABI Compendium*, *68208*.
8. Dorothy, M. S., Raman, S., Nautiyal, V., Singh, K., Yogananda, T., & Kamei, M. (2018). Use of potential plant leaves as ingredient in fish feed-a review. *International Journal of Current Microbiology and Applied Sciences*, *7*(7): 112-125.
9. Edwards, P., Kamal, M., & Wee, K. L. (1985). Incorporation of composted and dried water hyacinth in pelleted feed for the tilapia *Oreochromis niloticus* (Peters). *Aquaculture Research*, *16*(3): 233-248.
10. El-Sayed, A. F. M. (2003). Effects of fermentation methods on the nutritive value of water hyacinth for Nile tilapia *Oreochromis niloticus (L.)* fingerlings. *Aquaculture*, *218*(1- 4) Emshaw, Y., Getahun, A., & Geremew, A. (2023). Effect of different levels of fermented water hyacinth leaf meal on feed utilization and performance of juvenile nile tilapia. *Online Journal of Animal and Feed Research*, *13*(1): 55-62.
11. Falayi, B.A. (2003). Techniques in Fish Feed Manufacture. In Proceeding of the Joint Fishery Society of Nigeria / National Institute for Freshwater Fisheries Research/Special programme for food security National workshop on Fish feed development and feeding practices in aquaculture (Eyo, A.A. Ed). Held at National Institute for Freshwater Fisheries Research, New Bussa, 15th – 19th September, 2003; 43 – 55.
12. Fang, Y.X.; Guo, X.Z.; Wang, J.K. and Liv, Z.Y. (1986). Effects of different animal manure on fish farming. In: The Asian Fisheries Forum (J.I. Madean Ed.) Asian Fish Soc. Manila, Philippines, 117 – 120.
13. FAO. (2022). *The State of World Fisheries and Aquaculture 2022*. Food and Agriculture Organization of the United Nations.
14. Gatlin, D. M., Barrows, F. T., Brown, P., Dabrowski, K., Gaylord, T. G., Hardy, R. W.& Wurtele, E. (2007). Expanding the utilization of sustainable plant products in aquafeeds: a review. *Aquaculture Research*, 38(6): 551-579.
15. Gaurav, G. K., Mehmood, T., Cheng, L., Klemeš, J. J., & Shrivastava, D. K. (2020). Water hyacinth as a biomass: A review. *Journal of Cleaner Production*, *277*: 122214.
16. Geoffery, G. R., Athithan, S., Pereira, J. J., Amirtharaj, K. S., Jeyashakila, R., & Ruby, P. (2024). Nutritional Evaluation and Cost Analysis of Water Hyacinth (*Eichhornia crassipes*) as a Replacement of Fish Meal in the Diets of GIF Tilapia. *Indian Journal of Animal Research*, *1*: 7.
17. Hailu, D., Negassa, A., & Kebede, B. (2020). Evaluation of water hyacinth (*Eichhornia crassipes*) as a phytogenic diet for Nile tilapia (*Oreochromis niloticus*). *Int. J. Fish. Aquat. Stud*, *8*: 210-218.
18. Hasan, M. R., & Metian, M. (2011). *Demand and supply of feed ingredients for farmed fish and crustaceans: Trends and prospects*. Food and Agriculture Organization of the United Nations.
19. Hogendoorn, H. (1981). Controlled propagation of the African Catfish (Clarias lazera, C&V): Effect of feeding regime in fingerlings culture. Aquaculture, 24: 123 – 131.
20. Hossain, M. A., Islam, M. A., & Nahar, A. (2023). Effects of substituting plant-based protein sources for fish meal in the diet of Nile Tilapia (*Oreochromis niloticus*). *Archives of Agriculture and Environmental Science*, 8(3): 360-366.
21. Igbinosun, O.R. and Amako, D. (1988). Investigation into probable use of water hyacinth (*Eichhornia crassipes*) in Tilapia feed formulation. Nigeria Institute for Freshwater Fisheries Research Technical Paper, 39: 3-9.

22. Jafer Sadique, K., Pandey, A., Khairnar, S. O., & BT, N. K. (2018). Effect of molassesfermented water hyacinth feed on growth and body composition of common carp, Cyprinus carpio. *J. Entomol. Zool. Stud*, *6*: 1161-1165.
23. Kithsiri, H. P., Sharma, P., Zaidi, S. S., Pal, A. K., & Venkateshwarlu, G. (2010). Growth and reproductive performance of female guppy, Poecilia reticulata (Peters) fed diets with different nutrient levels. *Indian Journal of fisheries*, *57*(1): 65-71.
24. Konyeme, J. E., Sogbesan, A. O., & Ugwumba, A. A. A. (2006). Nutritive value and utilization of water hyacinth (*Eichhornia crassipes*) meal as plant protein supplement in the diet of Clarias gariepinus (Burchell, 1822)(Pisces: Clariidae) fingerlings. *African Scientist*, *7*(3): 127-133.
25. Kusemiju, K. (1988). Strategies for effective management of water hyacinth in the creeks and lagoons of southwestern Nigeria. In: Oke, K.L.; A.M.A. Imevbore and T.A. Farri, Op.cit., 39-45.
26. Kusemiju, K. and O.S. Akingboju (1988). Comparative growth of sarotherodonmelanotheron (Ruppell) on formulated fish feed and water Hyacinth diets. Op.cit., 196-203.
27. Lalitha P, Sripathi SK, Jayanthi P. Secondary Metabolites of Eichhornia crassipes (Water hyacinth): A Review (1949 to 2011). Natural Product Communications. 2021; 7(9).
28. Lata N, Debuy A. Quantification and identification of alkaloids of *Eichhornia crassipes*: the world's worst aquatic plant. Journal of Pharmacy Research, 2010; 3(6): 1229-1231.
29. Lee, S. W., Farid, M. R., Wendy, W., & Zulhisyam, A. K. (2016). Water hyacinth, *Eichhornia crassipes* (Mart.), leaf as an alternative protein source for Siamese Gourami, Trichogaster pectoralis. *Int. J. of Aquatic Science*, *7*(2): 58-62.
30. Mahmood, S., Khan, N., Iqbal, K. J., Ashraf, M., & Khalique, A. (2018). Evaluation of water hyacinth (*Eichhornia crassipes*) supplemented diets on the growth, digestibility and histology of grass carp (Ctenopharyngodonidella) fingerlings. *Journal of Applied Animal Research*, *46*(1): 24-28.
31. Malik, M. A., Sardar, P., Munilkumar, S., Varghese, T., Kumar, S., Gupta, G., ... & Bhat, N. M. (2024). Comparative evaluation of raw and fermented *Eichhornia crassipes* leaf meal in the diet of Cyprinus carpio fingerlings: Effects on growth performance, body composition and haemato-biochemical responses. *Animal Feed Science and Technology*, *310*: 115947.
32. Mazid, M.A.; Zaher, M.; Begum, N.N.; Aliu, M.Z.; Nahar, F. (1997). Formulation of costeffective feeds from locally available ingredients for carp polyculture system for increase production. Aquaculture, 151: 71-78.
33. Mohapatra, S. B., & Patra, A. K. (2013). Utilization of water hyacinth (*Eichhornia crassipes*) meal as partial replacement for fish meal on the growth performance of Cyprinus carpio fry. *International Research Journal of Biological Sciences*, *2*(12): 85-89
34. Najmudeen, T. M., ArakkalFebna, M. A., Rojith, G., & Zacharia, P. U. (2019). Characterisation of biochar from water hyacinth *Eichhornia crassipes* and the effects of biochar on the growth of fish and paddy in integrated culture systems. *Journal of Coastal Research*, *86*(SI): 225-234.
35. Nakajima, M., & Taniguchi, N. (2002). Genetic control of growth in the guppy (*Poecilia reticulata*). *Aquaculture*, *204*(3-4): 393-405.
36. New, M.B. (1989). Formulated aquaculture feeds in Asia: Some thoughts on comparative economics, industrial potential, problems and research needs in relation to smallscale farmer. In: Report of the Workshop on Shrimps and Fin Fish Feed Development (Bahru, J. Ed.). ASEAN/SF/89/GEN/11.
37. Okoye F, Daddy F, & Ilesanmi B. The nutritive value of water hyacinth (*Eichhornia crassipes*) and its utilisation in fish feed. Proceedings of the International Conference on Water Hyacinth, 27 Nov - 1 Dec 2000, new Bussa, Nigeria, 2002; 65-70.
38. Olvera-Novoa, M. A., Campos, S., Sabido, M., Pereira-Pacheco, F., Tudor, K. W., Rosati, R. R., & Wu, Y. Fermented feed ingredients as fish meal replacer in aquafeed production. *Technology*, *39*: 253-261.
39. Pandey, A. (2020). Water hyacinth as potential feed and compost in aquaculture: A review. *Journal of Entomology and Zoology Studies*, 8(5): 1434-1437.
40. Poddar K, Mandal L, Banerjee GC. Studies on water hyacinth (*Eichhornia crassipes*)— Chemical composition of the plant and water from different habitats. Indian Vet. J., 1991; 68: 833–837.
41. Polprasert, C., Kongsricharoern, N., & Kanjanaprapin, W. (1994). Production of feed and fertilizer from water hyacinth plants in the tropics. *Waste management & research*, *12*(1): 3-11.
42. Prasetyo, S., Anggoro, S., & Soeprobowati, T. R. (2021, July). Potential of water hyacinth (*Eichhornia crassipes* (Mart.) Solms) in Rawapening lake as raw material for fish feed. In *Journal of Physics: Conference Series* (Vol. 1943; No. 1, p. 012072). IOP Publishing.
43. Pratiwi, D. Y., & Andhikawati, A. (2021). Utilization of Water Hyacinth (*Eichhornia crassipes*) as Fish Feed Ingredient. *Asian Journal of Fisheries and Aquatic Research*, 13(3): 35-42.
44. Pratiwi, D. Y., & Pratiwy, F. M. (2018). Effect of fermented water hyacinth (*Eichhornia crassipes*) on growth performance of fish. *Fish farming*, *2*: 139-141.
45. Priya, P., Nikhitha, S. O., Anand, C., Nath, R. D., & Krishnakumar, B. (2018). Biomethanation of water hyacinth biomass. *Bioresource Technology*, *255*: 288-292.
46. Reznick, D. N. (1980). *Life history evolution in the guppy (Poecilia reticulata)*. University of Pennsylvania.

47. Rumsey, G.L. (1993). Fish meal and alternative sources of Protein. Fisheries, 18: 14-19.
48. Sakthika, T., & Sornalaksmi, V. (2019). Nutrients analysis of vermicompost of water hyacinth supplemented with probiotics. *Acta Scientific Agriculture*, *3*(10): 10-13.
49. Samaddar, A. (2018). A review of fish meal replacement with fermented biodegradable organic wastes in aquaculture. *International Journal of Fisheries and Aquatic Studies*, *6*(6): 203-208.
50. .Sarker, M. A. A., & Aziz, I. (2017). Incorporation of water hyacinth (*Eichhornia crassipes*) in feed for developing eco-friendly low-cost feed of mirror carp, Cyprinus carpio var. specularis (Linnaeus, 1758). *Journal of Agroecology and Natural Resource Management*, *4*(1): 5-9.
51. Sarker, M. A. A., Nahar, K., Banu, H., & Nesa, T. (2020). Incorporation of Water Hyacinth, *Eichhornia crassipes* Meal in Aqua-feed and its Efficacy on Growth Performance of roholabeo, Labeorohita (Hamilton, 1822) Reared in Cagev. *International Journal of Aquaculture and Fishery Sciences*, *6*(2): 043-049.
52. Sayed-Lafi, R. M., Al-Tameemi, R. A., &Gowdet, A. I. (2018). Evaluation of raw and fermented water hyacinth (*Eichhornia crassipes*) incorporated diets on growth and feed efficiency of young grass carp (Ctenopharyngodonidella). *Basrah Journal of Agricultural Sciences*, *31*(1): 31-39.

A. Shimul, T. J. T., Kallem, P., Nandi, S. K., Afrin, S., Suma, A. Y., Kari, Z. A., ... & Kabir, M. (2024). Fermented aquatic weed meal (FAWM) as a protein source in Asian Catfish Clarias batrachus diets: Impacts on growth, blood chemistry profile, liver and gut morphology and economic efficiency. *Aquaculture Reports*, *38*: 102305.

53. Sipaúba-Tavares, L. H., & Braga, F. D. S. (2007). The feeding activity of Colossomamacropomum larvae (tambaqui) in fishponds with water hyacinth (*Eichhornia crassipes*) fertilizer. *Brazilian Journal of Biology*, *67*: 459-466.
54. Sotolu AO, Sule O. Digestibility and Performance Of Water Hyacinth Meal In The Diets Of African Catfish (Clarias gariepinus; Burchell, 1822). Tropical and Subtropical Agroecosystems, 2011; 14: 245-250.
55. Sotolu, A. O., Ansa, E. J., Fashina-Bombatta, H., & Fisheries Society of Nigeria. (2010). Management and utilization of water hyacinth(*Eichhornia crassipes*) for improved aquatic resources. In *Proceedings of the 25th annual conference of the Fisheries Society of Nigeria(FISON)*. Fisheries Society of Nigeria, Lagos(Nigeria).
56. Suharman, I., Lukistyowati, I., Ramayani, S., Caipang, C. M. A., Adelina, A., & Aryani, N. (2021, November). Quality improvement of water hyacinth (*Eichhornia crassipes*) leaf meal fermented with Aspergillus niger as fish feed ingredient. In *IOP Conference Series: Earth and Environmental Science* (Vol. 934, No. 1, p. 012007). IOP Publishing.
57. Sulieman, H. A., & Lado, E. F. (2011). Nutritional effectiveness of water hyacinth leaves combined with wheat bran and cotton seed cake on the performance of Nile tilapia (*Oreochromis Niloticus*). *Online Journal of Animal and Feed Research*, *1*(6): 306309.
58. Sullivan, P. R., & Wood, R. (2012). Water hyacinth (*Eichhornia crassipes* (Mart.) Solms) seed longevity and the implications for management.
59. Terry, P. J. (1995). The water hyacinth problem in mala_i and foreseen methods of control.
60. Strategies for Water Hyacinth Control, 57.