

Project Title: Characterization and quality evaluation of smoked dried fermented fish product (*Napham*) prepared by *Bodo* community of Assam

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Introduction

Traditional fermented foods play a pivotal role in the culinary heritage and nutritional practices of Indigenous communities worldwide. In Northeast India, where diverse ethnic groups have developed distinct food-processing traditions over generations, *Napham* a smoked and fermented fish paste prepared by the *Bodo* community of Assam stands out as an example of microbial craftsmanship rooted in cultural knowledge. Typically, small freshwater fish are mixed with rice flour, naturally fermented in earthen or bamboo containers and preserved through smoke drying. While *Napham* enjoys widespread local consumption and shelf stability, the microbial and functional profiles of this product have remained largely undocumented in the scientific literature.

In recent years, traditional fermented foods have garnered increasing global attention as reservoirs of beneficial microorganisms, especially lactic acid bacteria (LAB), with probiotic potential. These microbes contribute to gut health, immune modulation and preservation through their acidifying and antimicrobial activity. However, due to the artisanal and unregulated nature of traditional fermentation processes, there exists significant batch-to-batch variation in microbial composition, texture, safety and functional efficacy. This lack of standardization limits both scientific validation and broader commercialization. Therefore, it becomes essential not only to characterize the microbial communities responsible for fermentation but also to evaluate process reproducibility and hygiene control.

To bridge this gap, our study undertakes a comprehensive investigation of *Napham* through a dual lens: microbiological characterization and indigenous process engineering. A central objective of this work was to isolate and characterize probiotic microorganisms responsible for *Napham* fermentation using both culture-dependent and culture-independent methods. *Lactobacillus plantarum*, a well-known probiotic species, was successfully isolated and designated as strain NAP1. To corroborate and extend these findings, high-throughput 16S rRNA gene-based amplicon sequencing was performed to map the microbial diversity across samples. While *Lactobacillus plantarum* did not appear dominantly in the metagenomic profiles likely due to low abundance or strain-level primer mismatches its direct isolation

emphasizes the importance of integrating both traditional and modern microbiological approaches.

Equally important was the development and evaluation of a modular smoke-drying chamber, designed with local materials and optimized for uniform temperature distribution, reduced drying time and minimized microbial contamination. This engineering intervention aimed to replace inefficient and unhygienic traditional drying practices with a standardized, community-adaptable system that supports both food safety and sensory quality. Machine design prototypes were field-tested and iteratively improved to balance energy efficiency, cost-effectiveness and ease of use making them suitable for decentralized use in rural settings.

By combining strain-level probiotic validation, metagenomic profiling and process engineering, this project not only documents the scientific value of a culturally significant food but also provides a translational pathway for rural innovation, livelihood enhancement and food safety assurance. The outcomes contribute to the growing field of functional ethnobiotics and offer a model for characterizing and optimizing Indigenous fermented foods in a scientifically robust yet culturally sensitive manner.

Objectives:

- i. To characterize the biochemical and microbial quality of *Napham* product in Bodoland Region of Assam
- ii. To evaluate the phytochemical, antioxidant, anthelmintic, antidiabetic, anticancer and antimalarial properties of plant material used in “*Napham*”
- iii. To optimize “*Napham*” preparation process at laboratory scale and its quality characterization
- iv. To develop a laboratory scale prototype setup for fermentation of “*Napham*” product and its performance assessment
- v. To study the probiotic behavior of the microorganisms responsible for formulation of “*Napham*”

Objective (I)

To characterize the biochemical and microbial quality of *Napham* product in Bodoland Region of Assam

1. Material and Methods:

1.1. Collection of product: *Napham* sample has been collected from *in-situ* condition i.e., from Deurigaon village of Udalguri district, Assam and stored in the laboratory at -4°C.

1.2. Moisture (AOAC, 2000)

About 2-5g sample was taken in a previously weighed covered aluminium dish (≥ 50 mm diameter and ≥ 40 mm deep) and weighed again. The wt. of sample was calculated from the difference. The dishes containing samples were placed in a hot air oven with lids removed, fixing the temperature of the oven at $100 \pm 5^{\circ}\text{C}$. Drying was allowed overnight (16-18 h) and wt. was taken by cooling the dish in a desiccator to room temperature. Loss in wt. was reported as moisture.

Calculation

$$\% \text{ Moisture (w/w basis)} = \frac{\text{Wt. of sample before drying} - \text{Wt of sample after drying}}{\text{Wt. of sample before drying}} \times 100$$

1.3. Total ash content (AOAC, 2000)

About 10 g of sample was taken in a previously heated, cooled and weighed porcelain crucible and dried in a hot air oven overnight at temperature $100 \pm 5^{\circ}\text{C}$. The dried sample



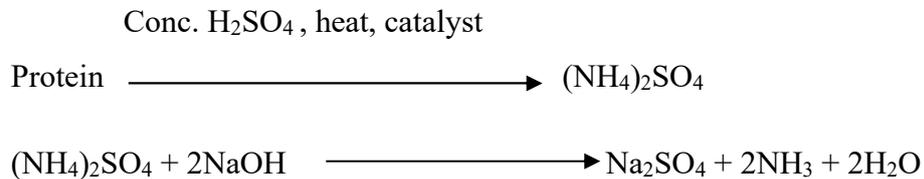
was completely charred by heating over a burner. The charred sample was incinerated in a muffle furnace at a temperature of $500 \pm 50^{\circ}\text{C}$ with adequate air supply until it was completely white (about 6-7 h). The crucible was cooled in a desiccator to room temperature before taking wt..

Calculation

$$\% \text{ Ash (w/w basis)} = \frac{\text{Wt. of ash}}{\text{Wt. of wet sample}} \times 100$$

1.4. Crude protein content (AOAC, 2000)

Protein and other organic food components in a sample are digested with sulfuric acid in the presence of catalysts. The total organic nitrogen is converted to ammonium sulfate. The digest is neutralized with alkali and boric acid solution. The borate ions formed are titrated with standardized acid, which is converted to nitrogen in the sample. The result of the analysis represents the crude protein content of the food since nitrogen also comes from the non-protein components.



Procedure

Digestion: For digestion 0.2 to 0.5g sample were weighed in a dry Kjeldahl digestion tube. Add 10ml of concentrated sulfuric acid (H_2SO_4) and about 3g of digestion mixture or digestion activator (Copper sulfate and Potassium sulfate at a ratio of 1:8 by wt.). Mixture was digested by heating first slowly and then vigorously between 360°C to 410°C for 4-6 hrs until the sample turns colourless or light green colour to ensure complete conversion of nitrogen in the sample to ammonium sulfate.

Distillation: During distillation the digested samples were heated by passing steam and the liberated ammonia due to the addition of 40% NaOH is dissolved in 4% boric acid. The steam distillation was continued till about 100 ml distillate was collected (7-8 min) in the receiving flask. At the end receiving flask was lowered and hold for about 1 min at 1 cm below the tip of

condenser. The boric acid turns green when ammonia was absorbed when Toshiro's indicator is used. A blank was also done through all the steps by taking distilled water in place of sample. The boric acid consisting of ammonia is taken for titration.

Reagents

1. Concentrated H₂SO₄
2. Digestion mixture (catalyst): copper sulphate and potassium sulphate are mixed in the ratio of 1:8 by wt..
3. Boric acid: 4% solution in distilled water.
4. Sodium hydroxide: 40% solution in distilled water.
5. Toshiro's indicator: A 250 ml stock solution is prepared by dissolving 0.4 g methyl red and 0.1 g methylene blue in 95% alcohol. For working solution, 1 vol. of stock solution is mixed with 1 vol. of 95% alcohol and 2 vol. of distilled water. Then 0.1(N) NaOH is added for disappearance of red colour. The solution is stored in coloured bottle. The indicator is colourless in neutral solution, red in acid and green in alkaline solution.
6. 0.02N HCl

Calculation

$$\% \text{ Total nitrogen} = \frac{(\text{Sample titer} - \text{blank titer}) \times \text{Normality of HCL} \times 14 \times 100}{\text{Wt. of sample (g)} \times 1000}$$

Protein:

Since average nitrogen content of the fish protein is 16%, so

1 g nitrogen = 100/16 = 6.25 g protein

Hence, % protein = % nitrogen x 6.25 (conversion factor)

1.5. Total lipid (Soxhlet method, AOAC, 2000)

The moisture free sample (accurately weighed) was transferred to an extraction thimble carefully. The extraction thimble was placed to the extractor with an attached receiving flask (its wt. was noted before placing) and then solvent was poured into thimble through a glass funnel. The extractor and the receiving flasks were now connected to the Soxhlet condenser. The electrical heating unit was adjusted so that the solvent siphons over 5 to 6 times per hour and extraction were continued for 16 to 20 hours. After the extraction was complete, the

thimble was removed and the solvent from the receiving flask was collected out by distilling it off, before it returned to the flask by siphoning. Thus, maximum possible amount of solvent was restored. Finally, the traces of solvent were removed from the flask by overnight drying it in oven at 100⁰ C. After cooling the flask in the desiccators, its wt. was recorded.

Calculation

$$\% \text{ Total lipid} = \frac{\text{Wt. of fat in the sample}}{\text{Original wt. of the sample}} \times 100$$

1.6. Estimation of Non-Protein Nitrogen (NPN) (AOAC, 2000)

Principle:

Non-protein nitrogenous compounds are first separated by precipitating the protein using the trichloro acetic acid (TCA) solution. A suitable aliquot of TCA extract is subjected to digestion and distillation. Protein and other organic food components in a sample are digested with sulfuric acid in the presence of catalysts. Total organic nitrogen is converted to ammonium sulfate. The digest is neutralized with alkali and boric acid solution. The borate ions formed are titrated with standardized acid, which is converted to nitrogen in the sample

Preparation of TCA extract:

TCA extract was prepared by grind 10g of sample with 10% TCA solution followed by filter the content with coarse filter paper and make up the volume to 100ml

Procedure:

Exactly 20ml TCA extract is taken in a dry Kjeldahl flask and about 3-5 g of digestion mixture and 20 ml of concentrated sulphuric acid (H₂SO₄) were added to digestion flask. Few glass beads were also added to avoid bumping. Mixture was digested by heating first slowly and then vigorously for 4-6 hrs until the sample become green and colourless to ensure complete conversion of nitrogen in the sample to ammonium sulphate. The flask was then cooled and volume was made up to 250 ml by distilled water

The Kjeldahl steam distillation until was rinsed well by steaming before. 10ml of 2% boric acid with 2-3 drops of Toshiro's indicator was taken in a 100 ml conical flask and placed in such a way that the tip of the outlet of the condenser of distillation until remains dipped into

the boric acid solution. 5ml of the made-up digested sample was added to the previously cleaned distillation chamber and about 10 ml of 40% NaOH was added followed by rinsing with distilled water. The steam distillation was continued till about 30 ml distillate was collected (7-8 min) in the receiving flask. At the end the receiving flask with distilled water. The boric acid turned green when ammonia was absorbed. A blank was also done through all the steps by taking distilled water in place of sample. The collected distillate was titrated against 0.02 N sulfuric acid (H₂SO₄) until the original red colour was obtained.

Calculation:

1 ml of 0.02 N H₂SO₄= 0.0002 gm of nitrogen

$$\% \text{ NPN} = \frac{14 \times N \times X \times V3 \times 250}{W \times V1 \times V2 \times 1000} \times 100$$

Where,

- N = Normality of H₂SO₄
- X = ml of standard H₂SO₄ required for titration of sample
- W = Wt. of the sample taken
- V1 = Aliquot (ml) of TCA extract taken for distillation
- V2 = Aliquot (ml) of TCA extract taken for digestion
- V3 = Total volume of TCA extract

1.7. pH (AOAC, 2000)

10 g of mixed representative sample was blended with 10 ml CO₂ free water. The temperature of the prepared sample was adjusted to 25⁰C and pH was determined with a digital pH meter. The pH meter was calibrated to pH 4.0 and 7.0 before every measurement

Estimation of total volatile basic nitrogen (Conway, 1947)

The theory for estimating TVB-N is that the released amines (smelling compounds) are first separated by precipitating the proteins using the trichloroacetic acid solution. The filtered solution (TCA extract), which contains the volatile substances, is added to a strong alkali. The volatile nitrogenous substances are distilled over and trapped by the standard H₂SO₄ solution. The remaining acid can be back titrated with the standard NaOH.

Procedure

Conway cups and lids were washed and dried. Paraffin wax and vaseline in the ratio of 1:2 was melted and cooled. This was applied on the rims of cups. 1 ml of 0.02(N) H₂SO₄ was added into the inner chamber of each cup. Lid was placed over the Conway cup covering part of outer chamber and complete inner chamber. 1 ml of TCA extract was taken in the outer chamber followed by 1ml of K₂CO₃ solution. The unit was lidded and the contents were mixed by rotating the unit gently and then the unit was left over night for reaction (it can be kept inside an incubator at 37°C for 2 h). The excess acid, left in inner chamber, was titrated against 0.02(N) NaOH using a drop of Toshiro's indicator. A reagent blank was done simultaneously.

Calculation

$$1 \text{ ml of } 0.02(\text{N}) \text{ H}_2\text{SO}_4 = 0.28 \text{ mg of TVB - nitrogen}$$

1.8. Peroxide value (PV) (Jacob, 1958)

The peroxide value of an oil or fat is the amount of peroxides (generated when lipids undergo oxidation by atmospheric oxygen through free chain mechanism) present, expressed as milli-equivalents of peroxide oxygen per kilogram of fat. The sample is treated with potassium iodide and the iodine which is liberated by the peroxides is titrated with standard sodium thiosulphate solution

Reagents

1. Solvent: 2 volumes of glacial acetic acid and 1 volume of chloroform is mixed.
2. Saturated potassium iodide solution: KI is dissolved in distilled water in 4:3 ratio and stored in brown bottle.
3. 0.01(N) sodium thiosulphate solution
4. Starch indicator: 0.5%

Procedure: A known volume (5-10 ml) of chloroform extract was taken in a dried and weighed Petri dish which was placed over a hot plate to evaporate chloroform and wt. of the oil was determined from the difference of wt..

5-10 ml of chloroform extract was taken in a 250 ml iodine flask and 25 ml of solvent was added to it followed by 1 ml KI solution (or about 1 g KI salt). The content was shaken well for one minute and allowed to stand in dark for 30 min. Then about 35 ml of distilled

water was added by washing the stopper and sides of the flask. The content in the flask was titrated against 0.01N sodium thiosulphate solution using starch as indicator with vigorous shaking till first complete disappearance of blue colour. A blank was also done simultaneously with solvent only.

Calculation

$$\text{Peroxide value (milli-equivalent peroxide O}_2 \text{ per 1000 g fat)} = \frac{1000 (V - X) N}{W}$$

Where,

V = vol. of sodium thiosulphate used for sample

X = vol. of sodium thiosulphate used for blank

N = normality of sodium thiosulphate

W = wt. of sample

1.9. Thiobarbituric acid (TBA) number (Tarladgis *et al*, 1960)

TBA is estimated to detect the lipid oxidation at later stage of oxidative rancidity. It relates the levels of aldehydes present, i.e. malonaldehyde in the sample. TBA reacts with malonaldehyde, which is produced due to methylene separated from fatty acid (dienoic or polyenoic) decomposition, to give a red chromogen, which is determined spectrophotometrically. Fat is sometimes oxidized in the presence of TBA, so more TBA reagent is added to produce colour pigment. The intensity of the colour is measured at 538 nm.

Reagents

1. 0.02M TBA reagent: 0.2883 g Thiobarbituric acid is dissolved 100 ml 90% glacial acetic acid by slight warming the mixture in a boiling water bath. The flask is wrapped in black foil and is prepared fresh daily.
2. HCl solution: 1 part conc. HCl to 2 parts distilled water (approx. 4N (v/v)).

Procedure

Ten grams sample was macerated with 47.5 ml distilled water to a slurry form. The content was then transferred to a 500 ml round bottom flask. To this solution, 2.5 ml of HCl solution was added to adjust the pH to 1.5. The TBA distillation unit was flashed with steam

and the sample flask was placed. After heating, first few drops of distillate was discarded and then distillate was collected in a measuring cylinder. Distillation was done in such a way that 50 ml of distillate was collected within 10 min. 5 ml of distillate was taken in a test tube and 5 ml TBA reagent was mixed with it. The tubes were kept in boiling water beaker for 30 min with continuous boiling. A test tube containing 5 ml distilled water and 5 ml TBA reagent was used as blank. The tubes were cooled for 10 min and OD was measured at 538 nm.

Calculation

$$\text{TBA value (mg malonaldehyde per kg)} = \frac{A \times 390}{V_d \times M_s}$$

Where,

A = absorbance at 538 nm

V_d = volume of distillate taken

M_s = mass of sample used

1.10. Total Bacterial Count (APC) (APHA, 2003)

Total plate count or total viable count was done by spread plate techniques. The sterile Petri dishes were prepared with readymade Soyabean Casein Digest Agar (SCDA) also called as Tryptone Soya Agar (TSA) taking 40g in 1000ml distilled water and autoclaved at 121⁰C at 15psi for 15 min. After cooling the agar media was poured in sterile Petri dishes (sterilized at hot air oven at 160⁰C for 2 hrs.). The plates were then dried in incubator in inverted position at 40-45⁰C for 15-20 minutes.

Exactly 10g of sample was introduced aseptically in a sterile stomach bag (Seward stomach BA6141CPG standard bags) and macerated for 2 min with 90 ml of sterile diluents of 0.85 % sodium chloride (NaCl) using a stomacher (Seward stomacher 400 circulator, England). The serial decimal dilution of 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵ were prepared using 9 ml saline for making serial dilutions and it was well mixed in cyclomixer. Pipette 0.1 ml of inoculums from each of the dilution and was spread plated onto TSA plates using a sterile glass spreader. The plates were incubated at 37 ⁰C for 24 hrs. The plates containing 25-250 colonies were considered for calculation.

Calculation

Count per gram = Number of colonies counted X reciprocal of dilution from
which the colonies counted X reciprocal of aliquot plated

2. Results

Biochemical and microbial quality composition of *Napham*

Table 1. Biochemical and microbial quality composition of *Napham*

Parameter	<i>Napham</i>
Moisture (g/100g)	3.52 ± 0.37
Ash (g/100g)	13.95 ± 0.02
Protein (g/100g)	63.65 ± 0.83
Crude fat (g/100g)	12.12 ± 0.15
Carbohydrate (g/100g)	6.32 ± 0.24
pH	6.18 ± 0.05
Total Titrable Acidity (% lactic acid)	0.928 ± 0.01
Non-Protein Nitrogen (%)	2.88 ± 0.07
Total Volatile Basic Nitrogen (mg%)	81.99 ± 3.22
Thiobarbituric Acid Reactive Substances (mg malonaldehyde/kg fish paste)	0.789 ± 0.01
Free Fatty Acid (as Oleic acid) %	16.38 ± 0.39
Total Bacterial Count (log <i>cfu/g</i>)	5.95 ± 0.06

Moisture, ash, protein, fat and carbohydrate content of *Napham* was found 3.52 ± 0.37, 13.95 ± 0.02, 63.65 ± 0.83, 12.12 ± 0.15 and 6.32 ± 0.24 g/100g. The low moisture content may be due to the use of sundried fish in making of *Napham*. As a result of the lowered amount of moisture in the product, its contents of protein and fat have increased. In the study, the non-protein nitrogen (NPN) increased due to the utilization of the whole body in the fermentation process. *Napham* had relatively higher TVBN content (Table 1) which may be attributed to biochemical and microbial changes that proceed in the fish muscle as a result of fermentation. However, such high concentration of TVBN usually does not manifest any ammonia-like odour in the product. Degree of lipid oxidation as measured by estimating thiobarbituric acid (TBA) value was found 0.789 ± 0.01 mg malonaldehyde per kg meat. The TBA values represent the degree of rancidity in the products and the values above 3–4 indicate quality loss. The lower TBA values indicate that the secondary lipid oxidation is limited in the product possibly due to the micro-aerobic condition. Maximum FFA (16.38 ± 0.39%) was liberated due to higher protein denaturation and lipid hydrolysis

but no rancid odour was noticed. Increased in total bacterial count was reported in various kinds of fermented fish. This shows that bacteria play a very important role in fish fermentation. They degrade fish protein leading to the production of volatile compounds from amino acids and small peptides.

Conclusion

Traditionally processed fish products are very popular among the populace of North-eastern India. *Napham* plays a very important role in the nutrition especially amongst the ethnic *Bodo* people of Assam. The present study revealed that *Napham* is having immense nutritional value. This fermented fish product has been and will be continue to be important fish food in bringing vital protein to the consumers and as a part of the diet, quality of the fermented fish should be improved.

Objective (2)

To evaluate the phytochemical, antioxidant, anthelmintic, antidiabetic, anticancer and antimalarial properties of plant material used in “*Napham*”

3. Material and methods:

3.1. Collection and preparation of plant materials

The *Colocasia* plants were obtained from Udalguri district of Assam in North-east India during mid-January 2022. Udalguri district (92° 06' 7.74" E long. and 26° 45' 13.21" N lat.) is located in North Brahmaputra Valley region of Assam with a total geographical area of 1852.16 km². The collected plants were brought to the laboratory of Department of Food Engineering and Technology, Tezpur University, Assam, India and the leaves and stems were sorted. The sorted parts were then washed with tap water and before being made into slice with the help of sharp kitchen knife, it was again washed with distilled water. The cut pieces of leaves and sliced stems were dried in a tray dryer (UR Biocoction, TD-C) at 50 ± 5°C for 48 hours followed by powdering them using a commercial mixer grinder. After being ground into a powder, samples were sieved through a 300-mesh screen and stored in the fridge until additional testing could be done.

3.2. Ethanolic Extraction of leaf and stem

Leaves and stems of *Alocasia mycorrhiza* were oven dried and then powdered through commercial household grinder. Extraction of phytochemicals were achieved through Ultrasound-assisted extraction (UAE) process following Das *et al.*, 2020 and Muchary and Deka (2021) with slight modification. Powder sample each from the leaf and stem were mixed in 1:15 ratios with an extraction solvent (70% ethanol). Using a 220 probe (12.7 mm in

diameter) at a frequency of 20 kHz, the sample solutions were ultrasonically treated (Q Sonica, Q700, USA) by maintaining the extraction parameters such as temperature at 60°C, amplitude at 40% and time 20 minutes. After completion of ultrasonication the samples were centrifuged followed by filtration of supernatant through Whatman no. 1 filter paper and dried the extract at 40°C in a rotary evaporator.

3.3. HPLC analysis of phenolic compound

The phenolic compound present in the extracts were detected and quantified in a HPLC system (Waters, 2690, US) with an UV detector. Thermo Scientific's Acclaim 120® C18 column (5 mm beads size; 120; 4.0 mm 250 mm) was employed, and the column oven temperature was kept at 30 °C. Two mobile phase such as phase A (0.1% HPLC grade acetic acid) and phase B (HPLC grade methanol) as gradient mode was used (20:80) (Das *et al.*, 2020).

3.4. *In Vitro* Antioxidant Assay

The ethanolic extracts of *Alocasia macrorrhiza* leaf and stem were divided into two groups with variable concentrations in distilled water. The concentrations in the first set (S1) ranged from 20, 40, 60, 80, 100, and 120 µg/ml, whereas the concentrations in the second set (S2) ranged from 50, 100, 150, 200, 250, and 300 µg/ml. Both sets were based mainly on some preliminary research into their antioxidant capabilities, having in mind that there is a correlation between the doses employed and the activity displayed under different assay protocols. For evaluating antioxidant activities, a total of eight different techniques were used. As reference standards, BHT, -tocopherol, or EDTA were employed. The ratio of the difference between absorbance of control solution (A0) and sample (A1) to the absorbance of the control solution was used to calculate the antioxidant activities (Equation 1).

$$\% \text{ Antioxidant activity} = \frac{(A0 - A1)}{A0} \times 100 \quad (1)$$

3.4.1. DPPH radical scavenging activity

The activity was estimated by DPPH scavenging assay as described by Yen and Chen (1995). 2 ml of S1 were added to test tubes along with 2 ml of a 0.16 mM DPPH solution in ethanol, and the mixture was appropriately vortexed before being incubated at room temperature for 30 minutes in the dark. At 517 nm, the absorbance of the sample, a blank, and the control were measured. The control was a DPPH solution that had been incubated with DW.

3.4.2. Ferric Reducing Antioxidant Power (FRAP) Assay

With a few minor adjustments, the reducing power assay method described by Najafian and Babji, 2018, was used to determine the antioxidant activity of the leaf and stem extract of *Alocasia macrorrhiza*. 2 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide solution (1%) were added to sample solution (S2) and incubated for 20 min at 50° C. It was then followed by the addition of 1 mL of 10% TCA and centrifugation at 1500×g for 10 min. after that 1 ml of supernatant was combined with distilled water (2 ml) and a 1

ml solution containing 0.1% ferric chloride and the absorbance was then measured at 700 nm. DW in place of the sample served as the blank.

3.4.4. Ferrous Ion (Fe²⁺) Chelating Assay

A technique published by Das *et al.*, 2016 was used to assess the chelating ability of the extracts for ferrous ion. In short, 4 ml of sample solution (S2) were added to 100 µl of ferrous chloride (2 mM), and the reaction was started by the addition of 200 µl of ferrozine (5 mM). After vigorously shaking the mixture and incubating it for 20 minutes at room temperature, the absorbance was measured by determining the wavelength at 562 nm. As a blank, distilled water was used in place of ferrozine, while EDTA served as the positive control.

3.4.5. Superoxide Radicals Scavenging Assay

Superoxide anion scavengers were measured using a previously described approach (Das *et al.* 2016), which was based on the reduction of NBT by NADH in the presence of PMS. 2 ml of the sample solution (S2), 1 ml of the NBT (100 µM) solution, 1 ml of the NADH (468 µM) solution, and 100 µl of the PMS solution (60 µM) solution were all included in the reaction mixture. The absorbance of reaction mixture was measured at 560 nm against an adequate blank solution after 15 minutes of incubation at 30° C. As a positive control, α-tocopherol was used as blank that included all the reagents excluding the linoleic acid.

3.4.6. Hydrogen Peroxide Scavenging Activity Assay

The amalgamation, made up of 3.5 mL of sample solution (S2) and 0.6 mL of H₂O₂ (40 mM) solution in phosphate buffer (pH 7.4) was measured for absorbance at 230 nm after 10 minutes of dark incubation. The reference substance was α-tocopherol, while phosphate buffer served as a blank.

3.4.7 *In-vitro* anthelmintic activity

3.4.7.1 Collection of experimental earthworms

The experimental earthworms *Eisenia fetida* were collected from the vermicompost unit of Krishi Vigyan Kendra, Assam Agricultural University, Udalguri, Assam India. The worms were selected because their anatomy and physiology most closely resembled those of the roundworm parasites that infest the human intestines (Choudhary *et al.* 2021).

3.4.7.2 Experimental Design

Six sets of three adult earthworms were randomly assigned to each group measuring in a standard of 7-9 cm in length and 0.15–0.18 g in wt. Extracts of both leaf and stem were prepared in a range of concentrations (10, 20, 30, 40 and 50 mg/mL) using dimethylsulfoxide (DMSO) as solvent and reference drug albendazole were prepared at 10mg/ml. The test worms were

then administered with different sample concentrations and reference drug to monitor the anthelmintic activity and the times at which the worms became paralysed and died were recorded as average values. Loss of activity, unless the worms were agitated forcefully or soaked in hot water at 50°C, was evidence of paralysis, whereas the death of the earthworm is portrayed by the cessation of their spontaneous movement along with a fading of their colour followed by secreting white substances, shortening, broadening, and hardness of body (Das and Ganapaty 2014; Chander *et al.* 2014).

3.4.8 *In vitro* Antidiabetic Assay

3.4.8.1 α -amylase inhibitory activity of extracts

The inhibitory activity against alpha-amylase was assessed with some adjustment of the method reported by Liu *et al.* (2017). To 1 ml of each sample concentration (50, 100, 150, 200 and 250 μ g/ml in DMSO), After adding 1ml each of potato starch solution (2 mg/ml DW) and α -amylase solution (10 mg α -amylase in 16.67 ml of 0.2M phosphate buffer pH 6.9), the mixture was incubated at 37°C for 30 minutes. The reaction was stopped after incubation by adding 5 ml of DNS (96 mM), and the mixture was then re-incubated in boiling water for 5 minutes. The absorbance was then measured at 540 nm using a spectrophotometer (AG22331, Eppendorf, Germany). The sample was replaced with acarbose to serve as a positive control. The inhibitory activity (%) determined by the following formula:

$$\% \text{ Inhibition} = \left[\frac{A_{Control} - A_{Sample}}{A_{Control}} \right] \times 100$$

3.4.8.2 α -glucosidase inhibitory activity of extracts

With a few minor modifications, the α -glucosidase inhibition study was conducted using the protocol reported by Ranilla *et al.* (2010). Acarbose (50 mM), a positive control, was added to 70 μ l of the α -glucosidase enzyme (50 μ g/ml) in each well of a 96-well plate together with 10 microliters (10 μ l) of the extract of leaf and stem at varied concentrations (50, 100, 150, 200, and 250 μ g/ml). 20 μ l of 10 mM p-nitrophenyl- α -D-glucopyranoside (PNPG) was added after the plate was kept for incubation at room temperature (37°C) for 10 min, and the reaction mixture was then incubated for an additional 20 min at 37°C. At 405 nm the absorbance was measured using Promega, GM3500 Plate Reader after stopping the reaction process by adding 25 μ l of 100 mM Na₂CO₃ in 0.1 M phosphate buffer (pH 6.9). The following formula was used to determine the % inhibitory activity:

$$Inhibition (\%) = \left[1 - \frac{\text{Absorbance of test well}}{\text{Absorbance of untreated (control)}} \right] \times 100$$

3.4.9 *In vitro* Anticancer activity

The anticancer activity was determined using the MTT assay. The human ovarian cancer cell line SKOV-3 (1×10^4 cells per well) were plated in 96-well plates for 24 h in 200mL of dulbecco's modified eagle medium (DMEM) supplemented with 10% foetal bovine serum (FBS). Different concentration of the samples (120, 100, 80, 60, 40 and 20 $\mu\text{g/mL}$) in 0.1% DMSO was treated against the cells in triplicate and incubated for 24 h at 37°C under 5% CO₂ in an incubator. After treatment, the cells were incubated with MTT (10 μL ; 5 mg/mL) at 37°C for 4 h and the resulting formazan crystals were then dissolved in 100 μL of dimethylsulfoxide (DMSO). The viability of the cells was assessed by measuring absorbance at 570nm with a Promega, GM3500 ELISA plate reader (Ren *et al.*, 2015). The following formula was used to calculate the % cell viability (equation number 4):

$$Cell\ viability (\%) = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}} \times 100 \quad (4)$$

The IC₅₀ values were determined by using software from GraphPad Prism 9.

4. Result:

4.1. Determination of Phytochemicals by HPLC

As a first step, HPLC was used to analyse the retention time of 12 different phytochemical standards (gallic acid, caffeic acid, syringic acid, ferulic acid, sinapic acid, chlorogenic acid, salicylic acid, rutin, ellagic acid, p-coumaric acid, quercetin and kaempferol) at 254 nm (Table 2). High-performance liquid chromatography (HPLC) chromatograms of phytochemical substances in the extracts of leaf and stem are displayed in Fig. 1. Both the extracts of leaf and stem analysed had a diverse range of phytochemical substances. The highest content of gallic acid, ferulic acid, rutin and kaempferol were detected both in leaf (18605.85 ppm, 1932.45 ppm, 7261.00 ppm and 2832.84 ppm respectively) and however lowest in stem (10547.77 ppm, 796.62 ppm, 3924.27 ppm and 436.30 ppm respectively).

Table 2. Phytochemicals detected by HPLC in the extracts of leaf and stem of *Alocasia macrorrhiza*

Sl. No.	Phytochemical standards	Retention time (min)	Concentration (mg/100g)	
			Leaf	Stem
1	Gallic acid	3.811	18605.85	10547.77
2	Syringic acid	12.101	ND	1066.30
3	Ferulic acid	14.901	1932.45	796.62
4	Salicylic acid	16.116	6496.05	ND
5	Rutin	16.537	7261.00	3924.27
6	Quercetin	18.987	2029.53	ND
7	Kaempferol	20.518	2832.84	436.30

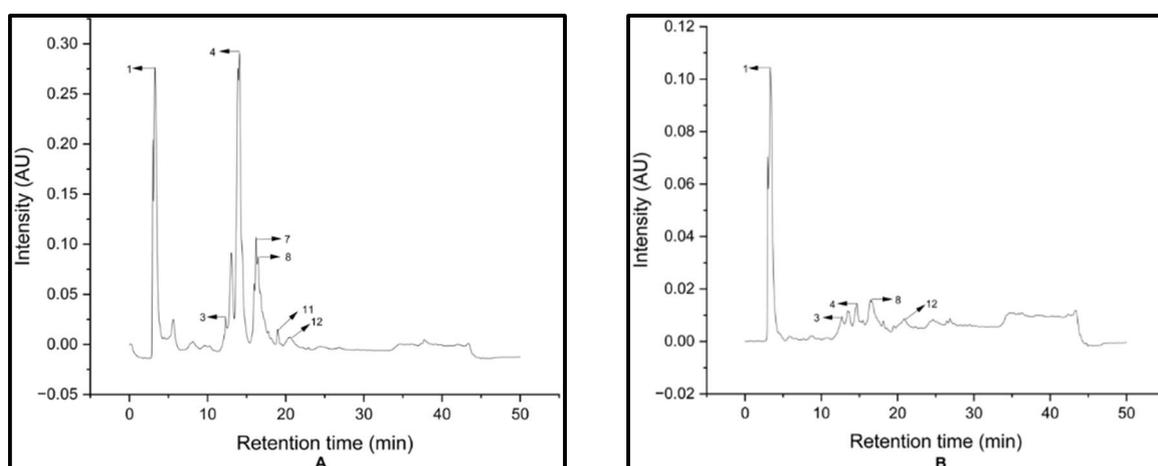


Fig 1. HPLC chromatograms of ethanolic extract of *Alocasia macrorrhiza* for detection of phytochemical compounds (A)- Leaf and (B)- Stem. (1- Gallic acid, 3- Syringic acid, 4- Ferulic acid, 7- Salicylic acid, 8- Rutin, 11- Quercetin, 12- Kaempferol)

4.2. *In vitro* Antioxidant activity

The *in vitro* antioxidant capability of ethanolic extracts of *Alocasia macrorrhiza*'s leaf and stem was assessed using the DPPH, FRAP, Superoxide anion scavenging activity, Ferrous ion chelating activity and H₂O₂ Scavenging activity assays. The results are shown in Figure 2.

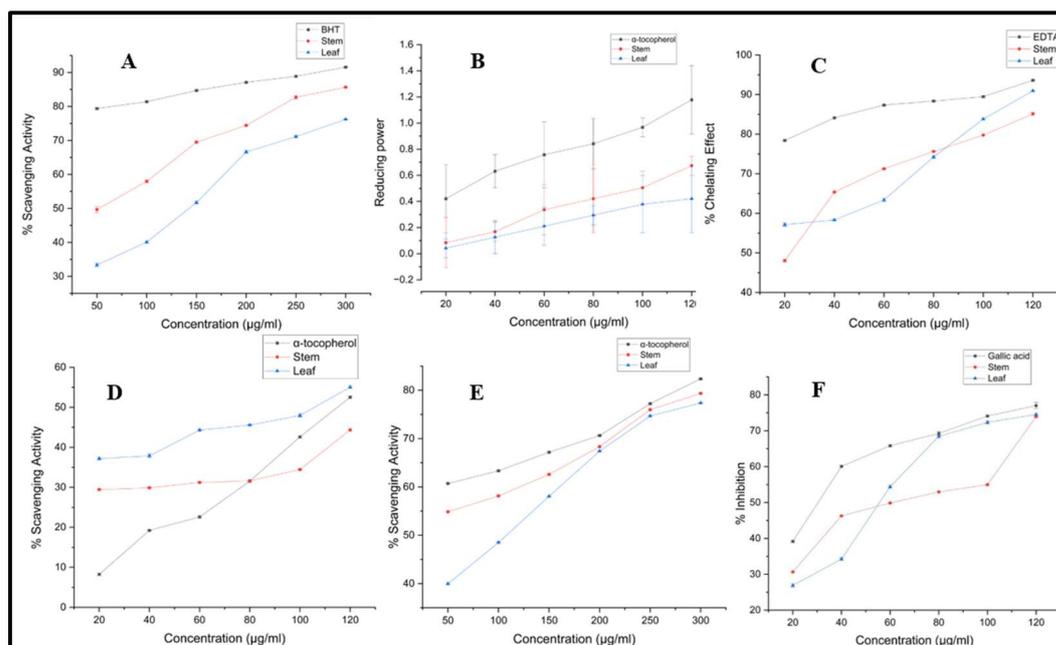


Fig 2. DPPH free radical scavenging activity assay of the extracts; B: Ferric reducing antioxidant power assay of the extracts; C: Ferrous-ion chelating assay of the extracts; D: Superoxide anion scavenging activity assay of the extracts; E: H₂O₂ scavenging activity assay of the extracts; F: Ferric thiocyanate assay. The results are means \pm SD (n = 3)

The scavenging of free radicals' ability of compounds can be studied with the help of the reagent DPPH, which has been used widely in the evaluation of reducing compounds. Leaf extract has an inhibition of 76.20% compared to stem extract 85.70% at the maximum concentration of 300 $\mu\text{g/mL}$, whereas BHT, the reference standard, has a 91.6% inhibition at the same concentration (Fig. 2A). The capacity to scavenge DPPH, a persistent free radical with an unpaired valence electron at one nitrogen atom bridge, is regarded as a key antioxidant characteristic. The findings of this work for DPPH free radical scavenging activity indicate that both extracts have promising prospect. In the reducing-power assay, an antioxidant substance is assessed on its ability to donate either an electron or a hydrogen atom. The reducing ability and the antioxidant activity of leaf and stem of *Alocasia macrorrhiza* is shown in Fig. 2B. The reducing ability rises with the rising concentration of the extract and at the highest concentration (120 $\mu\text{g/mL}$) leaf and stem extract showed reducing power of 0.421% and 0.673% respectively. The extract of stem exhibited a highest ferrous ions chelating activity of 85.1% at 120 $\mu\text{g/mL}$ of concentration and leaf extract exhibited 91% as the highest value at same concentration (Fig. 2C). Whereas, at the same concentration the reference standard EDTA showed maximum Fe^{2+} chelation of 93.6%. These results revealed that Fe^{2+} chelating activity was higher in leaf extract than that of stem but lower than the reference standard EDTA. In the

NBT assay, superoxide anion activity was determined and significant scavenging activity were observed in leaf extract (37.18- 55.03%), stem extract (29.44- 44.34%) and α -tocopherol (8.23- 52.53%) with increasing concentration (10- 120 $\mu\text{g/mL}$) (Fig. 2D). The IC_{50} value of leaf (101.04 $\mu\text{g/mL}$) was found to be lesser compared to stem (200.33 $\mu\text{g/mL}$). H_2O_2 scavenging activity was highest in stem (79.36%) followed by leaf (77.37%) with IC_{50} value of 15.75 and 104.96 $\mu\text{g/mL}$, respectively at a concentration (300 $\mu\text{g/mL}$) (Fig. 2E and Table 3). On the other hand, reference standard α -tocopherol exhibited H_2O_2 scavenging activity of 82.4% at same concentration. According to the findings, extracts of both the leaf and the stem possessed high hydrogen peroxide scavenging capabilities. Fig. 2F presents a visual representation of the percent inhibition of linoleic acid peroxidation that is achieved by extracts of leaf and stem as well as reference standard gallic acid. It is evident from the result that the reference standard (77.03%) and both the extracts (leaf 74.53% and stem 73.93%) had good inhibitory activity at highest concentration of 120 $\mu\text{g/mL}$ with IC_{50} stem (65.86 $\mu\text{g/mL}$), leaf (60.18 $\mu\text{g/mL}$) and gallic acid (27.52 $\mu\text{g/mL}$). The antioxidant properties of the extracts were measured to assess how well it protected the linoleic acid system from lipid peroxidation. Therefore, it is possible to predict that these two extracts would be useful in protecting against the risk of lipid peroxidation, which can occur in the body as well as in the food components.

Table 3. The IC_{50} values of the extracts under various assay conditions

Assay	IC_{50} values ($\mu\text{g/ml}$)	
	Leaf	Stem
DPPH	139.64	39.99
FRAP	42.79	43.22
Fe ²⁺ chelating	11.83	7.27
Superoxide anion scavenging	101.04	200.33
H_2O_2 scavenging	104.96	15.75

4.3. *In vitro* Anthelmintic activity

The anthelmintic activity of the ethanolic extracts of *A. macrorrhiza*'s leaf and stem against live adult *Eisenia fetida* were found to be much higher. The most effective dose of leaf and stem extract was 50 mg/mL and it showed that the time to paralysis was approximately 47 seconds and 2.41 minutes and the time to death was around 1.38 minutes and 3.04 minutes, respectively. On the other hand, the effect of the reference drug albendazole, which was administered at 10 mg/mL, was significantly below that of the leaf and stem extract administered at 50 mg/mL (Table 4). When compared to albendazole, the leaf and stem of *A.*

macrorrhiza were found to be more effective in the killing of worms in a less span of time. This was probably because of the phytochemical ingredients present in the extract of these parts of the plant.

Table 4. Anthelmintic activity of leaf and stem of *A. macrorrhiza* extract

Drug/treatment	Concentration (mg/ml)	Time taken (min)	
		Paralysis	Death
Albendazole	10	4.12 ± 0.04	5.05 ± 0.35
Leaf	10	3.14 ± 0.03	4.47 ± 0.14
Leaf	20	2.49 ± 0.06	4.23 ± 0.42
Leaf	30	2.17 ± 0.05	3.51 ± 0.86
Leaf	40	1.09 ± 0.03	2.04 ± 0.39
Leaf	50	0.47 ± 0.05	1.38 ± 0.06
Stem	10	4.12 ± 0.04	5.05 ± 0.41
Stem	20	3.3 ± 0.03	4.39 ± 0.14
Stem	30	3.21 ± 0.04	4.13 ± 0.32
Stem	40	3.02 ± 0.02	3.54 ± 0.30
Stem	50	2.41 ± 0.01	3.04 ± 0.05

4.4. *In vitro* Antidiabetic activity

Table 5. Inhibitory potential of leaf and stem of *A. macrorrhiza* extract on α -amylase and α -glucosidase. Values are denoted as IC₅₀ (μ g/ml)

Sample	IC ₅₀ (μ g/ml)	
	α -amylase	α -glucosidase
Leaf	71.94	137.27
Stem	208.15	170.36
Acarbose	227.31	263.26

Postprandial hyperglycaemia can be remedied by lowering sugar levels in blood by hindering α -amylase and α -glucosidase enzymes and as a result, these enzymes serve an important role as chemotherapy drugs for noninsulin-dependent diabetic mellitus. The inhibitory activity of leaf and stem of *A. macrorrhiza* extracts against α -amylase and α -glucosidase is demonstrated in Fig. 3A and 3B respectively. The α -amylase activity of both leaf and stem were exhibited in a dose dependent pattern at the concentration of 50, 100, 150, 200 and 250 μ g/mL with corresponding values of 47.35%, 53.21%, 56.78%, 58.41% and 63.47% (leaf) and 41.89%, 44.11%, 46.30%, 50.97% and 51.55% (stem) respectively. For α -glucosidase enzyme, both extracts followed the same trend as of α -amylase enzyme, as shown in Fig. 3B, with the

increment value of 22.97%, 45.97%, 59.21%, 65.64% and 70.91% (leaf) and 44.03%, 47.13%, 49.04%, 49.79% and 55.00% (stem) at the same concentration level. The IC₅₀ values for the inhibition of α -amylase and α -glucosidase are displayed in Table 5. Despite the fact that the IC₅₀ values for inhibiting α -amylase and α -glucosidase were lower than those for acarbose, the results nonetheless indicated the extract's potential hypoglycaemic effect. These results recommended that the active enzymes α -amylase and α -glucosidase, which are crucial for conversion complex carbohydrates into adsorbable simple sugars in food, could be inhibited by extracts of the leaf and stem of *A. macrorrhiza* to lower the postprandial glucose level.

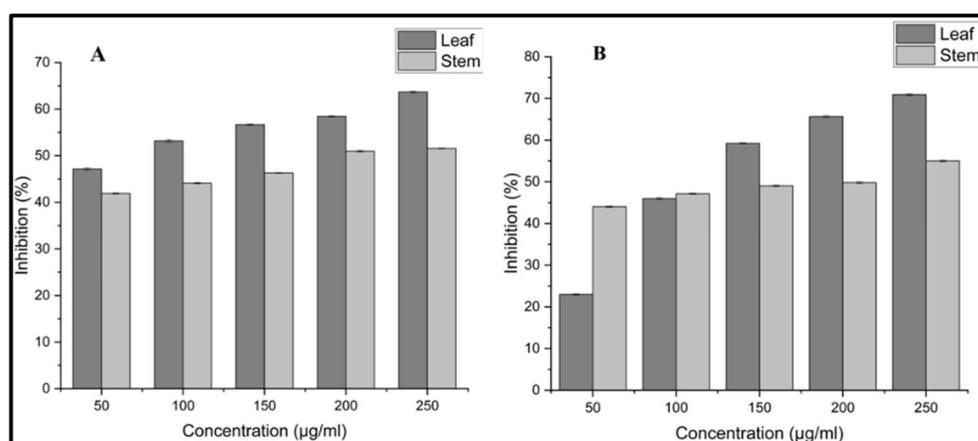


Fig 3. Inhibitory effect of leaf and stem extract of *A. macrorrhiza* on the activity of (A) α -amylase and (B) α -glucosidase. Values are presented as mean \pm SD.

4.5. *In vitro* Anticancer activity

Table 6. IC₅₀ (μ g/mL) of leaf and stem extract of *A. macrorrhiza* on human ovarian cancer cell line (SKOV-3)

Sample	IC ₅₀ (μ g/ml)
Leaf	61.27 \pm 2.25
Stem	46.65 \pm 1.84

***Values are given as means of 3 replicates \pm SD**

The importance of phytochemical compounds in the extracts of *Alocasia macrorrhiza* leaf and stem and their antioxidant capacity in cancer cell damage is clearly demonstrated in this research. In a human ovarian cancer cell line (SKOV-3), both the extracts were able to induce cell death in a dose-dependent manner (Fig. 4). The highest activity was observed in the stem, with an IC_{50} value of $46.65 \pm 1.84 \mu\text{g/mL}$ followed by the leaf ($61.27 \pm 2.25 \mu\text{g/mL}$) (Table 6). Fig 5 depicts the morphological changes brought on in cancer cells by exposure to both extracts. The results show that both extracts produced cytotoxicity in the cells, with the maximum activity observed in stem.

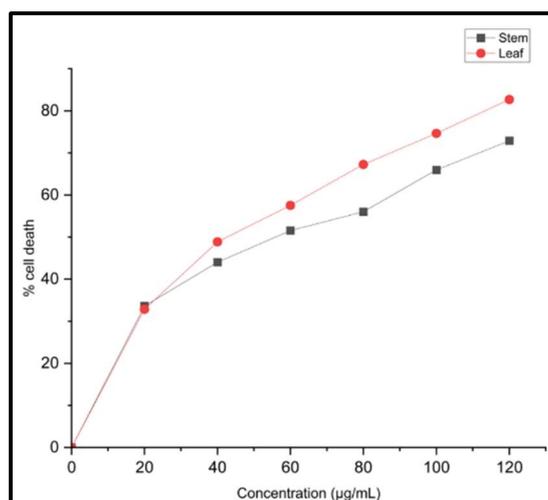


Fig 4. Cytotoxicity evaluation of the extracts leaf and stem of *Alocasia macrorrhiza*, against human ovarian cancer cell according to the MTT assay

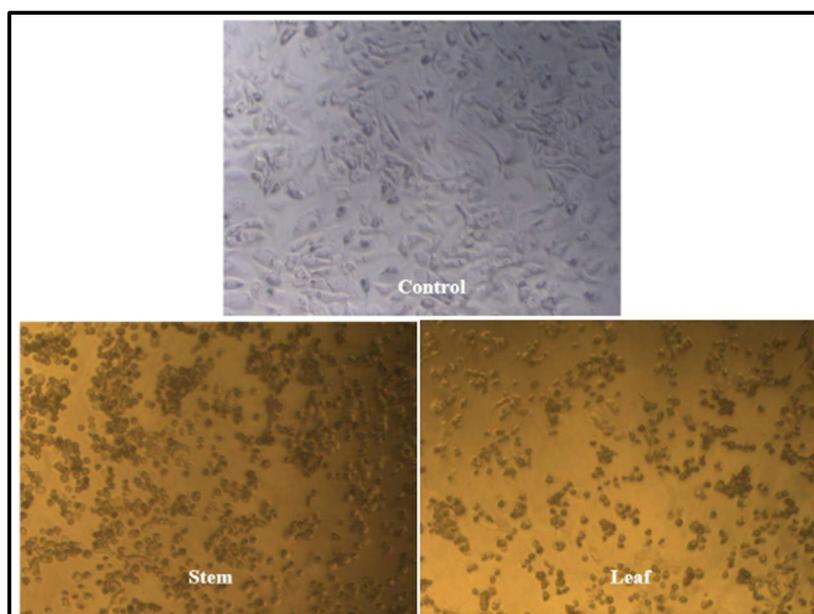


Fig 5. Morphological changes in human ovarian cancer cell (SKOV-3) exposed to the extracts of leaf and stem of *A. macrorrhiza*

Conclusion

In this research, significant amounts of phytochemicals were found in extracts of both the leaf and the stem of the plant. These compounds are the primary contributors to the plant's antioxidant abilities and may also be accountable for a broad spectrum of other therapeutic properties. Both of the extracts considerably reduced the α -amylase and α -glucosidase activities, which is an indication that the extracts have the ability to lower postprandial hyperglycemias by prolonging the carbohydrate digesting process. The experimental results have also showed considerable anthelmintic activity of ethanolic extracts of *A. macrorrhiza* leaf and stem. This is due to the fact that it required less time for these extracts to cause paralysis and death in the earthworms. The results of anticancer activity indicated significantly higher anticancer activities in human ovarian cancer cell for *A. macrorrhiza*, with the highest in stem, in MTT assay system. Therefore, this study may lay the way for future endeavors to focus on the potential of *A. macrorrhiza* leaf and stem as alternative nutraceutical and pharmacological agents.

Objective (3)

To optimize “*Napham*” preparation process at laboratory scale and its quality characterization

5. Material and Methods

5.1. Laboratory scale preparation of “*Napham*”

Napham was prepared with different proportions of arum petioles (Table 7) following the standardized method described in Fig. 6. Single species of fish namely goroi (*Channa punctatus*) was used in the treatments. The traditional *Napham* was prepared with mixed species and kept as control. The fermentation was allowed to continue for 2.5 months. There were 4 treatments with one control, having three replicates for each treatment.



Fig 6. Preparation process of *Napham*

Table 7. Different treatments used in the optimization of *Napham* preparation.

Treatment	Species of Fish used	Arum petiole (%)
T1	Mixed species (60%)	40
T2	<i>Channa punctatus</i> (70%)	30
T3	<i>Channa punctatus</i> (65%)	35
T4	<i>Channa punctatus</i> (60%)	40

Experimental design

Preparation of *Napham* was undertaken in bamboo containers. Periodically five bamboo containers were prepared for biochemical and microbial analysis with a sampling frequency of 15 days. A fermentation period for 1.5 months was allowed to continue and the samplings were continued for this entire period. The data were documented under three categories such as Microbiological, Biochemical and sensory. The detailed experimental schedule is summarized in Fig. 7.

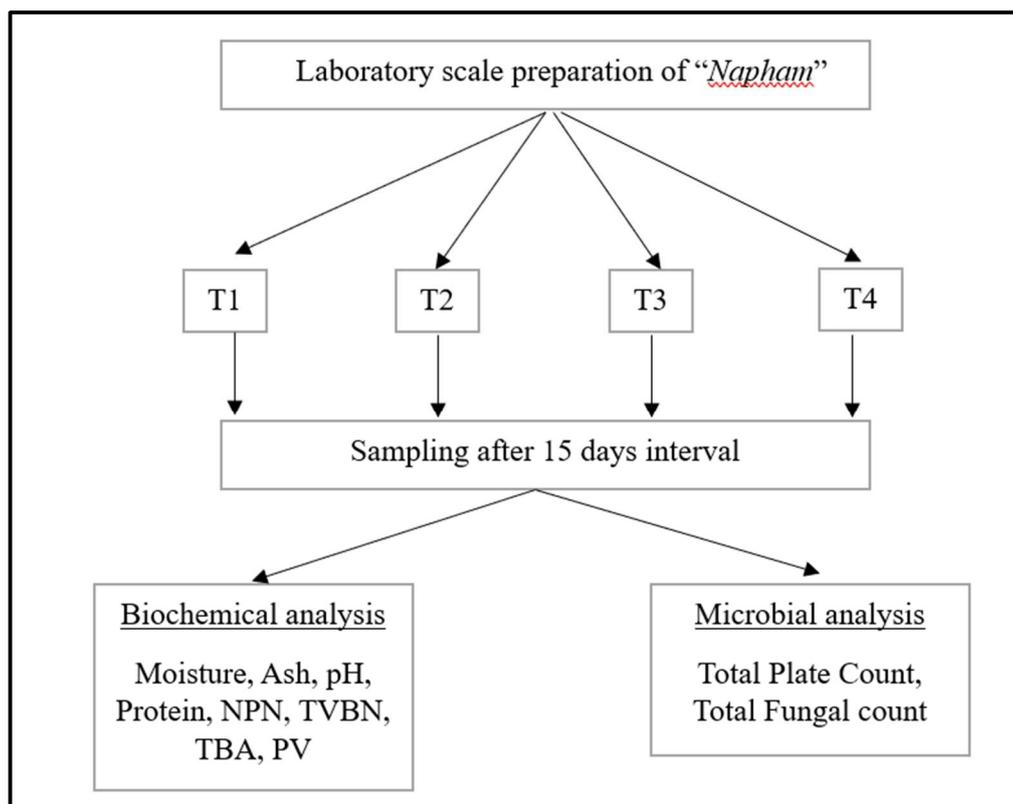


Fig 7. Schematic flow chart of work plan

5.2. Moisture (AOAC, 2000)

About 2-5g sample was taken in a previously weighed covered aluminium dish (≥ 50 mm diameter and ≥ 40 mm deep) and weighed again. The wt. of sample was calculated from the difference. The dishes containing samples were placed in a hot air oven with lids removed, fixing the temperature of the oven at $100 \pm 5^{\circ}\text{C}$. Drying was allowed overnight (16-18 h) and wt. was taken by cooling the dish in a desiccator to room temperature. Loss in wt. was reported as moisture.

Calculation

$$\% \text{ Moisture (w/w basis)} = \frac{\text{Wt. of sample before drying} - \text{Wt. of sample after drying}}{\text{Wt. of sample before drying}} \times 100$$

5.3. Total ash content (AOAC, 2000)

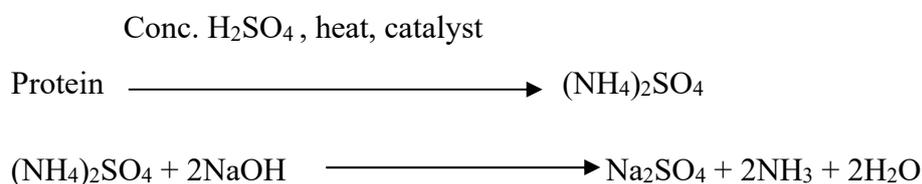
About 10 g of sample was taken in a previously heated, cooled and weighed porcelain crucible and dried in a hot air oven overnight at temperature $100 \pm 5^{\circ} \text{C}$. The dried sample was completely charred by heating over a burner. The charred sample was incinerated in a muffle furnace at a temperature of $500 \pm 50^{\circ} \text{C}$ with adequate air supply until it was completely white (about 6-7 hrs.). The crucible was cooled in a desiccator to room temperature before taking wt.

Calculation

$$\% \text{ Ash (w/w basis)} = \frac{\text{Wt. of ash}}{\text{Wt. of wet sample}} \times 100$$

5.4. Crude protein content (AOAC, 2000)

Protein and other organic food components in a sample are digested with sulfuric acid in the presence of catalysts. The total organic nitrogen is converted to ammonium sulfate. The digest is neutralized with alkali and boric acid solution. The borate ions formed are titrated with standardized acid, which is converted to nitrogen in the sample. The result of the analysis represents the crude protein content of the food since nitrogen also comes from the non-protein components.



Procedure

Digestion: For digestion 0.2 to 0.5g sample were weighed in a dry Kjeldahl digestion tube. Add 10 ml of concentrated sulfuric acid (H_2SO_4) and about 3g of digestion mixture or digestion activator (Copper sulfate and Potassium sulfate at a ratio of 1:8 by wt.). Mixture was digested

by heating first slowly and then vigorously between 360⁰ C to 410⁰ C for 4-6 hrs until the sample turns colourless or light green colour to ensure complete conversion of nitrogen in the sample to ammonium sulfate.

Distillation: During distillation the digested samples were heated by passing steam and the liberated ammonia due to the addition of 40% NaOH is dissolved in 4% boric acid. The steam distillation was continued till about 100 ml distillate was collected (7-8 min) in the receiving flask. At the end receiving flask was lowered and hold for about 1 min at 1 cm below the tip of condenser. The boric acid turn green when ammonia was absorbed when Toshiro's indicator is used. A blank was also done through all the steps by taking distilled water in place of sample. The boric acid consisting of ammonia is taken for titration.

Reagents

7. Concentrated H₂SO₄
8. Digestion mixture (catalyst): copper sulphate and potassium sulphate are mixed in the ratio of 1:8 by wt..
9. Boric acid: 4% solution in distilled water.
10. Sodium hydroxide: 40% solution in distilled water.
11. Toshiro's indicator: A 250 ml stock solution is prepared by dissolving 0.4 g methyl red and 0.1 g methylene blue in 95% alcohol. For working solution, 1 vol. of stock solution is mixed with 1 vol. of 95% alcohol and 2 vol. of distilled water. Then 0.1(N) NaOH is added for disappearance of red colour. The solution is stored in coloured bottle. The indicator is colourless in neutral solution, red in acid and green in alkaline solution.
12. 0.02N HCl

Calculation

$$\% \text{ Total nitrogen} = \frac{(\text{Sample titer} - \text{blank titer}) \times \text{Normality of HCL} \times 14 \times 100}{\text{Wt. of sample (g)} \times 1000}$$

Protein:

Since average nitrogen content of the fish protein is 16%, so

1 g nitrogen = 100/16 = 6.25 g protein

Hence, % protein = % nitrogen x 6.25 (conversion factor)

5.5. Total lipid (Soxhlet method, AOAC, 2000)

The moisture free sample (accurately weighed) was transferred to an extraction thimble carefully. The extraction thimble was placed to the extractor with an attached receiving flask (its wt. was noted before placing) and then solvent was poured into thimble through a glass funnel. The extractor and the receiving flasks were now connected to the Soxhlet condenser. The electrical heating unit was adjusted so that the solvent siphons over 5 to 6 times per hour and extraction were continued for 16 to 20 hrs. After the extraction was complete, the thimble was removed and the solvent from the receiving flask was collected out by distilling it off, before it returned to the flask by siphoning. Thus, maximum possible amount of solvent was restored. Finally, the traces of solvent were removed from the flask by overnight drying it in oven at 100⁰ C. After cooling the flask in the desiccators, its wt. was recorded.

Calculation

$$\% \text{ Total lipid} = \frac{\text{Wt. of fat in the sample}}{\text{Original wt. of the sample}} \times 100$$

5.6. Estimation of Non-Protein Nitrogen (NPN) (AOAC, 2000)

Principle:

Non-protein nitrogenous compounds are first separated by precipitating the protein using the trichloro acetic acid (TCA) solution. A suitable aliquot of TCA extract is subjected to digestion and distillation. Protein and other organic food components in a sample are digested with sulfuric acid in the presence of catalysts. Total organic nitrogen is converted to ammonium sulfate. The digest is neutralized with alkali and boric acid solution. The borate ions formed are titrated with standardized acid, which is converted to nitrogen in the sample

Preparation of TCA extract:

TCA extract was prepared by grind 10g of sample with 10% TCA solution followed by filter the content with course filter paper and make up the volume to 100ml

Procedure:

Exactly 20ml TCA extract is taken in a dry Kjeldahl flask and about 3-5 g of digestion mixture and 20 ml of concentrated sulphuric acid (H₂SO₄) were added to digestion flask. Few

glass beads were also added to avoid bumping. Mixture were digested by heating first slowly and then vigorously for 4-6 hrs until the sample become green and colourless to ensure complete conversion of nitrogen in the sample to ammonium sulphate. The flask was then cooled and volume was made up to 250 ml by distilled water

The Kjeldahl steam distillation until was rinsed well by steaming before. 10ml of 2% boric acid with 2-3 drops of toshiro's indicator was taken in a 100 ml conical flask and placed in such a way that the tip of the outlet of the condenser of distillation until remains dipped into the boric acid solution. 5ml of the made up digested sample was added to the previously cleaned distillation chamber and about 10 ml of 40% NaOH was added followed by rinsing with distilled water. The steam distillation was continued till about 30 ml distillate was collected (7-8 min) in the receiving flask. At the end the receiving flask with distilled water. The boric acid turned green when ammonia was absorbed. A blank was also done through all the steps by taking distilled water in place of sample. The collected distillate was titrated against 0.02 N sulfuric acid (H₂SO₄) until the original red colour was obtained.

Calculation:

1 ml of 0.02 N H₂SO₄= 0.0002 gm of nitrogen

$$\% \text{ NPN} = \frac{14 \times N \times X \times V3 \times 250}{W \times V1 \times V2 \times 1000} \times 100$$

Where,

- N = Normality of H₂SO₄
- X = ml of standard H₂SO₄ required for titration of sample
- W = Wt. of the sample taken
- V1 = Aliquot (ml) of TCA extract taken for distillation
- V2 = Aliquot (ml) of TCA extract taken for digestion
- V3 = Total volume of TCA extract

5.7. pH (AOAC, 2000)

10 g of mixed representative sample was blended with 10 ml CO₂ free water. The temperature of the prepared sample was adjusted to 25⁰C and pH was determined with a digital pH meter. The pH meter was calibrated to pH 4.0 and 7.0 before every measurement

Estimation of total volatile basic nitrogen (Conway, 1947)

The theory for estimating TVB-N is that the released amines (smelling compounds) are first separated by precipitating the proteins using the trichloroacetic acid solution. The filtered solution (TCA extract), which contains the volatile substances, is added to a strong alkali. The volatile nitrogenous substances are distilled over and trapped by the standard H₂SO₄ solution. The remaining acid can be back titrated with the standard NaOH.

Procedure

Conway cups and lids were washed and dried. Paraffin wax and vaseline in the ratio of 1:2 was melted and cooled. This was applied on the rims of cups. 1 ml of 0.02(N) H₂SO₄ was added into the inner chamber of each cup. Lid was placed over the Conway cup covering part of outer chamber and complete inner chamber. 1 ml of TCA extract was taken in the outer chamber followed by 1ml of K₂CO₃ solution. The unit was lidded and the contents were mixed by rotating the unit gently and then the unit was left over night for reaction (it can be kept inside an incubator at 37°C for 2 h). The excess acid, left in inner chamber, was titrated against 0.02(N) NaOH using a drop of Toshiro's indicator. A reagent blank was done simultaneously.

Calculation

$$1 \text{ ml of } 0.02(\text{N}) \text{ H}_2\text{SO}_4 = 0.28 \text{ mg of TVB - nitrogen}$$

5.8. Peroxide value (PV) (Jacob, 1958)

The peroxide value of an oil or fat is the amount of peroxides (generated when lipids undergo oxidation by atmospheric oxygen through free chain mechanism) present, expressed as milli-equivalents of peroxide oxygen per kilogram of fat. The sample is treated with potassium iodide and the iodine which is liberated by the peroxides is titrated with standard sodium thiosulphate solution

Reagents

2. Solvent: 2 volumes of glacial acetic acid and 1 volume of chloroform is mixed.
2. Saturated potassium iodide solution: KI is dissolved in distilled water in 4:3 ratio and stored in brown bottle.
5. 0.01(N) sodium thiosulphate solution
6. Starch indicator: 0.5%

Procedure: A known volume (5-10 ml) of chloroform extract was taken in a dried and weighed Petri dish which was placed over a hot plate to evaporate chloroform and wt. of the oil was determined from the difference of wt..

5-10 ml of chloroform extract was taken in a 250 ml iodine flask and 25 ml of solvent was added to it followed by 1 ml KI solution (or about 1 g KI salt). The content was shaken well for one minute and allowed to stand in dark for 30 min. Then about 35 ml of distilled water was added by washing the stopper and sides of the flask. The content in the flask was titrated against 0.01N sodium thiosulphate solution using starch as indicator with vigorous shaking till first complete disappearance of blue colour. A blank was also done simultaneously with solvent only.

Calculation

$$\text{Peroxide value (milli-equivalent peroxide O}_2 \text{ per 1000 g fat)} = \frac{1000 (V - X) N}{W}$$

Where,

V = vol. of sodium thiosulphate used for sample

X = vol. of sodium thiosulphate used for blank

N = normality of sodium thiosulphate

W = wt. of sample

5.9. Thiobarbituric acid (TBA) number (Tarladgis *et al*, 1960)

TBA is estimated to detect the lipid oxidation at later stage of oxidative rancidity. It relates the levels of aldehydes present, i.e. malonaldehyde in the sample. TBA reacts with malonaldehyde, which is produced due to methylene separated from fatty acid (dienoic or polyenoic) decomposition, to give a red chromogen, which is determined spectrophotometrically. Fat is sometimes oxidized in the presence of TBA, so more TBA reagent is added to produce colour pigment. The intensity of the colour is measured at 538 nm.

Reagents

3. 0.02M TBA reagent: 0.2883 g Thiobarbituric acid is dissolved 100 ml 90% glacial acetic acid by slight warming the mixture in a boiling water bath. The flask is wrapped in black foil and is prepared fresh daily.

4. HCl solution: 1 part conc. HCl to 2 parts distilled water (approx. 4N (v/v)).

Procedure

Ten grams sample was macerated with 47.5 ml distilled water to a slurry form. The content was then transferred to a 500 ml round bottom flask. To this solution, 2.5 ml of HCl solution was added to adjust the pH to 1.5. The TBA distillation unit was flashed with steam and the sample flask was placed. After heating, first few drops of distillate was discarded and then distillate was collected in a measuring cylinder. Distillation was done in such a way that 50 ml of distillate was collected within 10 min. 5 ml of distillate was taken in a test tube and 5 ml TBA reagent was mixed with it. The tubes were kept in boiling water beaker for 30 min with continuous boiling. A test tube containing 5 ml distilled water and 5 ml TBA reagent was used as blank. The tubes were cooled for 10 min and OD was measures at 538 nm.

Calculation

$$\text{TBA value (mg malonaldehyde per kg)} = \frac{A \times 390}{Vd \times Ms}$$

Where,

A = absorbance at 538 nm

Vd = volume of distillate taken

Ms = mass of sample used

5.10. Total Bacterial Count (APC) (APHA, 2003)

Total plate count or total viable count was done by spread plate techniques. The sterile Petri dishes were prepared with readymade Soyabean Casein Digest Agar (SCDA) also called as Tryptone Soya Agar (TSA) taking 40g in 1000ml distilled water and autoclaved at 121⁰C at 15psi for 15 min. After cooling the agar media was poured in sterile Petri dishes (sterilized at hot air oven at 160⁰C for 2 hrs). The plates were then dried in incubator in inverted position at 40-45⁰C for 15-20 minutes.

Exactly 10g of sample was introduced aseptically in a sterile stomach bag (Seward stomach BA6141CPG standard bags) and macerated for 2 min with 90 ml of sterile diluents of 0.85 % sodium chloride (NaCl) using a stomacher (Seward stomacher 400 circulator, England). The serial decimal dilution of 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵ were prepared using 9 ml saline for making serial dilutions and it was well mixed in cyclomixer. Pipette 0.1 ml of inoculums from each of

the dilution and was spread plated onto TSA plates using a sterile glass spreader. The plates were incubated at 37 °C for 24 hrs. The plates containing 25-250 colonies were considered for calculation.

Calculation

Count per gram = Number of colonies counted X reciprocal of dilution from
which the colonies counted X reciprocal of aliquot plated

5.11. Total Fungal Count (TFC) (APHA, 2003)

Total Fungal Count was done by following spread plate techniques (APHA, 1995). The sterile Petri dishes were prepared with readymade Rose Bengal Chloramphenical Agar (RBCPA) taking 32.15 g in 1000 ml distilled water and autoclaved at 121°C at 15 psi for 15 min. After cooling, the agar media was poured in sterile Petri dishes (sterilized at hot air oven at 160°C for 2 hrs). The plates were then dried in incubator in inverted position at 40-45°C for 15-20 min.

Exactly 10 g of sample was introduced aseptically in a sterile stomach bag (Seward stomach BA6141CPG standard bags) and macerated for 2 minutes with 90ml of sterile diluents of 0.85% sodium chloride (NaCl) using a stomacher (Seward stomach 400 circulator, England). A serial decimal dilution of 10⁻² was prepared using 9ml sterile physiological saline and 1ml homogenize sample and it was well mixed in cyclomixer. Then pipette 0.1ml of inoculums was spread plated on RBCPA plates using a sterile glass spreader. The plates were incubated at 37°C for 24 hrs. The plates containing 25-250 colonies were counted.

Calculation:

Count per gram = Number of colonies counted x reciprocal of dilution from which the colonies counted x reciprocal of aliquot plated

5.12. Estimation minerals and trace elements

5.12.1. Sample digestion

Moisture free *Napham* (6g) was combusted in muffle furnace at 650°C for 6 hours to obtain white ash[15]. This white ash was further used for analysis of mineral contents. For that digestion of the sample (white ash) was carried out through Kjeldahl digestion system (Kel Plus KES 08L E). One gram of sample was weighed in a dry Kjeldahl digestion tube. 10mL of concentrated acid (3:1 sulfuric acid: nitric acid) and about 3g of digestion mixture or digestion

activator (Copper sulfate and Potassium sulfate at a ratio of 1:1.5 by wt.) was added. Mixture was digested by heating first slowly and then vigorously between 360°C to 410°C till the colour turns into light yellow. After that, the digested samples were allowed to cool down followed by made up of volume to 100 mL with miliQ water. In all the steps, not a single piece of glassware (flask, pipette, etc.) was used. All plastic containers were disinfected with 10% ultra-pure grade HNO₃ followed by thoroughly rinsed with ultra-pure water (Pontieri *et al.*, 2022).

5.12.2. Inductively coupled plasma optical emission spectroscopy (ICP-OES) analysis

Analysis of minerals and trace elements (Fe, K, Mn, Ca, Zn, Na and Mg) employing inductively coupled plasma optical emission spectroscopy (ICP-OES) was performed at Sophisticated Analytical Instrumentation Centre (SAIC) in Tezpur University, Assam, India. Digested samples were analysed using an Avio 220 Max ICP-OES system (PerkinElmer). Argon was employed as a plasma gas and nitrogen as a collision gas. Each analysis was conducted in triplicate (Selmi *et al.*, 2021).

5.13. Estimation of fatty acid profile by Gas chromatography with flame-ionization detection (GC-FID)

The fatty acid profile of Napham was determined according to method of Jarukas *et al.* (Sagar *et al.*, 2020) To produce fatty acid methyl esters (FAMES), powdered samples (1 g) were directly trans-esterified with 2% H₂SO₄ in methanol. FAMES were then sorted and quantified using gas chromatography attached with a flame ionisation detector (Agilent Series, 7890 Series, USA). As a way to determine the concentration of fatty acids present in the samples, the Supelco 37 FAME Mixture (Sigma Cat. No. 47885-U) was used as a standard against which area percentages were calculated.

5.14. Estimation of amino acid with amino acid analyser

The hydrolyzed sample, which had been dried under vacuum, was reconstituted in a solution containing 100 mM HCl and subsequently filtered through a 0.22 µm syringe-driven filter. Each free amino acids were analysed with a Biochrom 30⁺ Amino Acid Analyzer (DKSH India Private Limited) equipped with a visible detector in accordance with the manufacturer's instructions. This equipment uses ion exchange chromatography (IEC) to separate amino acids and a post-column detector to quantify ninhydrin (NIN) reactive chemicals. The NIN-derivatized individual amino acids were detected at 570 nm (Zhang *et al.*, 2014).

6. Result:

6.1. Changes in moisture content

The moisture content had significant difference ($P < 0.05$) among all the treatment T1, T2, T3 and T4 including the control in 0 days. In 15 days, significance difference was observed in T2, T3, T4 with respect with T1 (control). After 45 days of fermentation no significance difference ($P > 0.05$) was observed in T1 (control), T3 T4. however, significance difference was observed in T2.

Table 8. Changes in moisture content (%) during fermentation in the control and treatments

Sampling (Day)	Moisture content (%)			
	T1	T2	T3	T4
0	21.42 ^{bC} (0.12)	21.27 ^{abA} (0.68)	22.25 ^{cA} (0.04)	23.81 ^{eA} (0.04)
15	20.87 ^{cB} (0.04)	21.20 ^{bA} (0.09)	22.64 ^{aA} (0.23)	24.16 ^{aA} (0.19)
30	20.66 ^{bB} (0.03)	20.96 ^{bA} (0.11)	22.26 ^{aA} (0.28)	23.92 ^{aA} (0.18)
45	20.44 ^{aA} (0.03)	20.58 ^{bB} (0.20)	22.09 ^{aA} (0.28)	23.66 ^{aA} (0.18)

The values with similar superscript in small letters (a, b, c, etc.) in the rows have no significant difference ($P > 0.05$) in respect to Treatments and the values with similar superscript in Capital letters (A, B, C, etc.) in the column do not differ significantly in respect to days of sampling.

6.2. Changes in ash content

The ash content did not differ significantly ($P > 0.05$) in all the treatments including the control (T1). However no significant difference ($P > 0.05$) was observed in the initial ash content in treatments T2 and T3. In T1, T2, T3 and T4 ash was 23.86%, 19.92%, 20.21% and 21.36 which marginally increased to 24.13%, 19.92%, 21.20% and 22.10 (Table 9) respectively. The ash content has significant difference ($P < 0.05$) among all the treatment T1, T2, T3 and T4 including the control in 30 days and 45 days.

Table 9. Changes in Ash content (%) during fermentation in the control and treatments

Sampling (Day)	Ash content (%)			
	T1	T2	T3	T4
0	23.86 ^{fA} (0.10)	19.06 ^{bA} (0.05)	20.21 ^{cA} (0.13)	21.36 ^{eA} (0.08)
15	24.04 ^{aA} (0.02)	19.33 ^{bB} (0.04)	20.53 ^{abAB} (0.15)	21.61 ^{bAB} (0.12)
30	24.13 ^{aA} (0.24)	19.60 ^{cC} (0.07)	20.73 ^{bB} (0.15)	21.89 ^{bB} (0.11)
45	24.13 ^{aA} (0.43)	19.92 ^{dD} (0.04)	21.20 ^{cC} (0.12)	22.10 ^{cC} (0.12)

The values with similar superscript in small letters (a, b, c, etc.) in the rows have no significant difference ($P>0.05$) in respect to Treatments and the values with similar superscripts in Capital letters (A, B, C, etc.) in the column do not differ significantly in respect to days of sampling.

6.3. Changes in protein content

The protein content showed significant difference ($P<0.05$) in most of the treatments including the control (T1). In T1, T2, T3 and T4 the initial protein content was 38.34%, 46.13%, 45.16% and 42.20 which marginally decreased to 36.77%, 45.73%, 43.81% and 40.75 (Table 10) respectively after 45 days of fermentation. During the study period T1 showed lowest value of protein compared to other treatment after storage period of 45 day in Bamboo container.

The protein content has no significant difference ($P>0.05$) with the treatment T1, T2, T3 and T4 including the control after 45 days of fermentation.

6.4. Changes in fat content

The fat content of T1 (control) has no significant difference ($P>0.05$) with the treatments T3 and T4. However, significance difference ($P<0.05$) was observed in treatment T2. In T1, T2, T3 and T4 the initial fat content was 12.07%, 9.01%, 9.72% and 9.86 which marginally increased to 12.97%, 9.35%, 10.28% and 10.36 (Table 11) respectively. During the study period (T2) showed lowest value of fat compared to other treatment whereas T1 showed the highest level of fat after fermentation period of 45 day in Bamboo container.

The fat content has significant difference ($P<0.05$) with all the treatment T1, T2, T3 and T4 including the control in 30 days. After 45 days of fermentation no significance difference ($P>0.05$) was observed in the treatments T1, T3 and T4.

Table 10. Changes in protein content (%) during fermentation in the control and treatments
Changes in fat content (%) during fermentation in the control and treatments

Sampling (Day)	Protein content (%)			
	T1	T2	T3	T4
0	38.34 ^{aA} (0.04)	46.13 ^{fA} (0.03)	45.16 ^{dA} (0.03)	42.20 ^{bA} (0.02)
15	37.78 ^{cA} (0.03)	46.07 ^{cA} (0.01)	44.51 ^{cAB} (0.03)	41.89 ^{cB} (0.01)
30	37.20 ^{bA} (0.02)	45.89 ^{bAB} (0.01)	44.11 ^{bB} (0.04)	41.43 ^{bC} (0.03)
45	36.77 ^{aA} (0.04)	45.73 ^{aB} (0.02)	43.81 ^{aC} (0.05)	40.75 ^{aD} (0.20)

The values with similar superscript in small letters (a, b, c, etc.) in the rows have no significant difference ($P>0.05$) in respect to Treatments and the values with similar superscript in Capital letters (A, B, C, etc.) in the column do not differ significantly respect to days of sampling.

Table 11. Changes in fat content (%) during fermentation in the control and treatments

Sampling (Day)	Lipid content (%)			
	T1	T2	T3	T4
0	12.07 ^{gA} (0.02)	9.1 ^{aA} (0.01)	9.72 ^{bA} (0.02)	9.86 ^{cA} (0.01)
15	12.44 ^{bB} (0.03)	9.25 ^{abAB} (0.08)	9.48 ^{aA} (0.08)	9.93 ^{abAB} (0.02)
30	12.75 ^{bcB} (0.10)	9.30 ^{abAB} (0.08)	9.98 ^{bB} (0.10)	10.06 ^{bB} (0.05)
45	12.97 ^{cC} (0.18)	9.35 ^{bB} (0.08)	10.28 ^{cC} (0.01)	10.36 ^{cC} (0.07)

The values with similar superscript in small letters (a, b, c, etc.) in the rows have no significant difference ($P>0.05$) in respect to Treatments and the values with similar superscript in Capital letters (A, B, C, etc.) in the column do not differ significantly in respect to days of sampling.

These results are relatively different from those of Majumdar *et al.* (Majumdar *et al.*, 2015), who reported that the proportions of moisture, protein, and fat content in *hentak*, a fermented fish paste of Manipur, India were 35.0 ± 1.04 , 37.63 ± 0.89 , and 9.91 ± 0.17 g/100g correspondingly. Potential causes for this deviation have included the fact that we showed results on a dry-matter basis or it could be because of the different maturation periods of different fish species used. The low moisture content could be attributable to the use of sundried fish in the production of *Napham*, or it could be linked to their fat content. Generally fresh fish contains 15-22 g/100g of protein content, but here, the increased protein content can be ascribed to microbial production of proteins from metabolic intermediates throughout their growth cycles. *Ngari*, a fermented fish product made from *Septipinna* species, was shown to

be nutrient dense in a study conducted by Sarojnalini and Suchitra (Sarojnalini *et al.*, 2009), with average values of 36.44% moisture, 36.25% total protein, 6.66% lipids, and 10.22% ash. According to a nutritional analysis performed by Kakati *et al.* (Kakati *et al.*, 2016), Mowashidal was found to be of exceptionally good grade. After 6 months of fermentation, the sample had pH of 5.8-6.5, protein concentration of 31.28-31.70%, and ash content of 9.95-11.11%.

Changes in Quality Parameters

6.5. Non-Protein Nitrogen (NPN)

The initial non protein nitrogen of the treatment T1, T2, T3 and T4 was 3.41%, 2.93%, 2.89% and 3.18% which marginally increased to 3.84%, 3.45%, 3.35% and 3.94% (Table 12) respectively. During the study period T4 showed the highest level of non-protein nitrogen after storage period of 45 days. The NPN content of T1 (control) has no significant difference ($P>0.05$) with the treatments T3. However, significance difference ($P<0.05$) was observed in treatment T2 and T4.

6.6. Total Volatile Basic Nitrogen (TVB-N)

The TVBN value of T1 (control) has no significant difference ($P>0.05$) with all the treatments T2, T3 and T4. In T1, T2, T3 and T4 the initial TVBN value was 165.98, 140.21, 140.32 and 151.40 mg% which marginally increased to 176.40, 165.76, 160.16 and 164.14 mg% (Table 13) respectively. During the study period T3 showed lowest value TVBN value compared to other treatment whereas T1 showed the highest level of non-protein nitrogen after storage period of 45 days.

Table 12. Changes in Non-Protein Nitrogen (NPN) content (%) during fermentation in the control and treatments

Sampling (Day)	NPN content (%)			
	T1	T2	T3	T4
0	3.41 ^{eA} (0.03)	2.93 ^{cA} (0.03)	2.89 ^{cA} (0.02)	3.18 ^{dA} (0.01)
15	3.47 ^{bB} (0.00)	3.38 ^{bB} (0.11)	3.03 ^{bB} (0.04)	3.52 ^{bB} (0.09)
30	3.63 ^{cC} (0.01)	3.43 ^{bB} (0.12)	3.19 ^{cC} (0.05)	3.63 ^{bB} (0.07)
45	3.84 ^{dD} (0.02)	3.45 ^{bB} (0.12)	3.35 ^{dD} (0.04)	3.94 ^{cC} (0.08)

The values with similar superscript in small letters (a, b, c, etc.) in the rows have no significant difference ($P>0.05$) in respect to Treatments and the values with similar superscript in Capital letters (A, B, C, etc.) in the column do not differ significantly in respect to days of sampling.

Table 13. Changes in Total Volatile Basic Nitrogen content (mg%) during fermentation in the control and treatments

Sampling (Day)	TVBN content (mg%)			
	T1	T2	T3	T4
0	165.98fA (0.11)	140.21bA (0.32)	140.32bA (0.19)	151.40dA (0.16)
15	169.12fB (0.69)	146.75bB (0.52)	143.76aB (0.77)	155.68dB (1.12)
30	172.96dC (0.56)	154.56aC (1.04)	154.00aC (1.53)	160.16bC (0.56)
45	176.40dD (0.89)	165.76bD (1.04)	160.16aD (1.04)	164.14bD (0.712)

The values with similar superscript in small letters (a, b, c, etc.) in the rows have no significant difference ($P>0.05$) in respect to Treatments and the values with similar superscript in Capital letters (A, B, C, etc.) in the column do not differ significantly in respect to days of sampling.

6.7. Thiobarbituric acid (TBA) number

The TBA number of T1 (control) has no significant difference ($P>0.05$) with the treatments T3. However, significance difference ($P<0.05$) was observed in treatment T2 and T4. In T1, T2, T3 and T4 the initial TBA number was 0.791, 0.886, 0.903 and 0.908 mg malonaldehyde/kg which marginally increased to 0.926, 0.933, 0.958 and 1.033 mg malonaldehyde/kg (Table 14) respectively. During the study period T1 showed lowest value TBA number compared to other treatment whereas T4 showed the highest level of TBA number after storage period of 45 day.

The TBA number of T1 (Control) has no significant difference ($P>0.05$) with the treatment T3 including the control in 30 days. However, there is significance difference ($P<0.05$) among the treatment T2 and T4. After 45 days of fermentation the TBA number of T1 (control) has no significant difference ($P>0.05$) with T2 and T3.

6.8. Peroxide value

The PV number of T1 (control) has no significant difference ($P>0.05$) with the treatments T3. However, significance difference ($P<0.05$) was observed in treatment T2 and T4. In T1, T2, T3 and T4 the initial peroxide value was 57.43 mg%, 45.40mg%, 49.15 mg% and 50.22mg% which marginally increased to 58.42mg%, 47.64mg%, 50.55mg% and 52.37mg% (Table 15) respectively. During the study period T2 showed lowest value PV compared to other treatment whereas T1 showed the highest level of PV after storage period of 45 day.

Table 14. Changes in TBA value during fermentation in the control and treatments

Sampling (Day)	TBA content (mg%)			
	T1	T2	T3	T4
0	0.791 ^{fA} (0.02)	0.886 ^{bA} (0.01)	0.903 ^{bA} (0.00)	0.908 ^{dA} (0.01)
15	0.888 ^{bb} (0.01)	0.917 ^{bb} (0.00)	0.923 ^{bb} (0.00)	0.998 ^{bb} (0.02)
30	0.915 ^{cBC} (0.00)	0.926 ^{bcB} (0.05)	0.945 ^{cC} (0.00)	1.009 ^{bb} (0.02)
45	0.926 ^{cC} (0.00)	0.933 ^{cB} (0.00)	0.958 ^{cC} (0.00)	1.032 ^{bb} (0.00)

The values with similar superscript in small letters (a, b, c, etc.) in the rows have no significant difference ($P>0.05$) in respect to Treatments and the values with similar superscript in Capital letters (A, B, C, etc.) in the column do not differ significantly in respect to days of sampling.

Table 15. Changes in PV value during fermentation in the control and treatments

Sampling (Day)	PV content (mg%)			
	T1	T2	T3	T4
0	57.43 ^{fA} (0.06)	45.40 ^{aA} (0.11)	49.15 ^{dA} (0.08)	50.22 ^{eA} (0.06)
15	57.70 ^{bAB} (0.06)	45.82 ^{bb} (0.08)	49.77 ^{bb} (0.21)	51.06 ^{bb} (0.12)
30	57.98 ^{bb} (0.13)	46.56 ^{cC} (0.15)	50.18 ^{bcAB} (0.22)	51.79 ^{cC} (0.13)
45	58.42 ^{cC} (0.16)	47.64 ^{dD} (0.07)	50.55 ^{cC} (0.21)	52.37 ^{dD} (0.16)

The values with similar superscript in small letters (a, b, c, etc.) in the rows have no significant difference ($P>0.05$) in respect to Treatments and the values with similar superscript in Capital letters (A, B, C, etc.) in the column do not differ significantly in respect to days of sampling.

6.9. pH

The pH value of T1 (control) has no significant difference ($P>0.05$) with the treatments T2 and T3. In T1, T2, T3 and T4 the initial pH value was 7.75, 6.54, 6.56 and 6.56 which marginally decreases to 7.60, 6.38, 5.79 and 6.37 (Table 16) respectively. During the study period T3 showed lowest value of pH value compared to other treatment whereas T1 showed the highest level of pH after storage period of 45 day. The pH of T1 (Control) has significance difference ($P<0.05$) among the treatment T2, T3 and T4. The pH value of all the treatments during fermentation period of *Napham* was also seen decreased marginally but remained below 7, unlike the control which remained above 7.

Table 16. Changes in pH value during fermentation in the control and treatments

Sampling (Day)	pH content (mg%)			
	T1	T2	T3	T4
0	7.75cC (0.02)	6.54abC (0.01)	6.56abA (0.01)	6.56abA (0.03)
15	7.70bB (0.01)	6.47bB (0.01)	6.48aA (0.01)	6.54cA (0.01)
30	7.64cA (0.01)	6.47bB (0.01)	6.41aA (0.01)	6.47bB (0.01)
45	7.60dA (0.01)	6.38aA (0.00)	5.79aA (0.60)	6.37aAC (0.01)

The values with similar superscript in small letters (a, b, c, etc.) in the rows have no significant difference ($P>0.05$) in respect to Treatments and the values with similar superscript in Capital letters (A, B, C, etc.) in the column do not differ significantly in respect to days of sampling.

Changes in Microbiological Parameter

Total Plate Counts (TPC) and Total Fungal Count (TFC) were carried out to see the microbial proliferation during storage.

6.10. Total Plate Count (TPC)

TPC was done to see the effect of Alocassia with various concentrations and in the survival of bacteria and expressed as $\log\text{CFUg}^{-1}$ in the fermentation period of about 45 days. The TPC number of T1 (control) has no significant difference ($P>0.05$) with the treatments T2 and T3. In T1, T2, T3 and T4 the initial TPC value was 5.51, 5.33, 5.42 and 5.36 cfug^{-1} . Which marginally increased to 6.74, 6.51, 6.58 and 6.61 cfug^{-1} (Table 17) respectively. During the study period T2 showed lowest TPC compared to other treatment whereas T1 showed the highest level of TPC after storage period of 45 day.

The TPC of T1 (Control) has no significant difference ($P>0.05$) with the treatment T2 and T3 in 30 days. After 45 days of fermentation, the TPC value of T1 (control) has significant difference ($P<0.05$) with all the treatment.

6.11. Total Fungal Count

TFC were reported as estimated Total Fungal Count (ETFC) $< 2500 \text{CFUg}^{-1}$. TFC was done to see the effect of Alocassia with various concentrations and in the survival of bacteria and expressed as $\log\text{CFUg}^{-1}$ in the storage period of about 45 days. The TFC number of T1 (control) has no significant difference ($P>0.05$) with all the treatments T2, T3 and T4. In T1, T2, T3 and T4 the initial TFC value was 3.42, 3.09, 3.29 and 3.20 cfug^{-1} which marginally decreases to 2.28, 2.09, 2.21 and 2.29 cfug^{-1} (Table 18) respectively. During the study period T2 showed

lowest TFC compared to other treatment whereas T1 showed the highest level of TFC after storage period of 45 day.

The TFC of T1 (Control) has significant difference ($P < 0.05$) with all the treatment T2, T3 and T4 in 15 days. After 30 days of fermentation the TFC value of T1 (control) has no significant difference ($P > 0.05$) with the treatment T2 and T3. However, the TFC value was negligible after 45 days of fermentation.

Table 17. Changes in TPC count (cfu g⁻¹) during fermentation in the control and treatments

Sampling (Day)	TPC (cfu g ⁻¹)			
	T1	T2	T3	T4
0	5.51 ^{cA} (0.04)	5.33 ^{aA} (0.05)	5.42 ^{bA} (0.01)	5.36 ^{abA} (0.02)
15	5.95 ^{bB} (0.19)	5.50 ^{aB} (0.03)	5.60 ^{aB} (0.02)	5.69 ^{aB} (0.01)
30	6.43 ^{abC} (0.02)	6.43 ^{abC} (0.01)	6.48 ^{abC} (0.02)	6.42 ^{aC} (0.02)
45	6.74 ^{dD} (0.01)	6.51 ^{aD} (0.01)	6.58 ^{bcD} (0.01)	6.61 ^{cD} (0.01)

The values with similar superscript in small letters (a, b, c, etc.) in the rows have no significant difference ($P > 0.05$) in respect to Treatments and the values with similar superscript in Capital letters (A, B, C, etc.) in the column do not differ significantly in respect to days of sampling.

Table 18. Changes in TFC count (cfu g⁻¹) during fermentation in the control and treatments

Sampling (Day)	TFC (cfu g ⁻¹)			
	T1	T2	T3	T4
0	3.42 ^{dC} (0.01)	3.09 ^{aC} (0.01)	3.29 ^{cC} (0.01)	3.20 ^{bC} (0.01)
15	2.87 ^{bB} (0.02)	2.40 ^{aB} (0.02)	2.87 ^{aB} (0.05)	2.98 ^{aB} (0.02)
30	2.28 ^{abA} (0.01)	2.09 ^{abA} (0.01)	2.21 ^{abA} (0.01)	2.29 ^{aA} (0.01)
45	UD	UD	UD	UD

The values with similar superscript in small letters (a, b, c, etc.) in the rows have no significant difference ($P > 0.05$) in respect to Treatments and the values with similar superscript in Capital letters (A, B, C, etc.) in the column do not differ significantly in respect to days of sampling. UD- Undetectable

6.12. Mineral content

Mineral contents of *Naphamare* presented in Table 19. Sodium levels were determined to be the maximum at 10642.40 ± 219.83 mg/kg, while iron was the lowest at 11.27 ± 0.40 mg/kg in the *Napham* sample. Calcium, potassium, magnesium, manganese, and zinc contents were

found to be 8346.40 ± 182.80 , 8302.00 ± 179.28 , 251.84 ± 1.74 , 40.88 ± 0.06 , 26.14 ± 0.35 mg/kg respectively.

Table 19. Mineral content of *Napham*

Minerals	Content (mg/kg)
Iron (Fe)	11.27 ± 0.40
Potassium (K)	8302.00 ± 179.28
Manganese (Mn)	40.88 ± 0.06
Calcium (Ca)	8346.40 ± 182.80
Zinc (Zn)	26.14 ± 0.35
	$10642.40 \pm$
Sodium (Na)	219.83
Magnesium (Mg)	251.84 ± 1.74

Values presented in mean \pm SD

The use of the entire fish, including the bones, in the fermentation process enhanced the ash content of the final product, which in turn contributed to a moderate amount of mineral content. The fact that it contains moderate levels of sodium, calcium, and potassium indicate that it is a good provider of dietary minerals. Low iron, zinc, manganese, and magnesium levels, conversely, this deficiency may be offset by other dietary sources that provide these crucial elements to the body. *Hentak*, a similar product to *Napham* was reported to have highest content of calcium (472.11 ± 62.7 mg/100g) followed by sodium (94.0 ± 12.78 mg/100g), potassium (75.74 ± 6.62 mg/100g) and magnesium (21.125 ± 3.78 mg/100g), whereas, iron, copper and zinc were found to be less (Majumdar *et al.*, 2015). On the other hand, Giri *et al.* (2009) observed that fermented fish pastes prepared by employing various koji moulds as starters had sodium, potassium, calcium, magnesium, phosphorus, and iron concentrations of 3341 mg/100g, 69 mg/100g, 50 mg/100g, 21 mg/100g, 57 mg/100g, and 1mg/100g correspondingly.

6.13. Fatty acid profiling

The chromatogram of fatty acid composition of *Napham* is shown in Fig. 8. The total saturated fatty acids content was $43.74 \pm 0.136\%$ with highest content of Palmitic acid ($30.20 \pm 0.145\%$) followed by stearic acid ($9.15 \pm 0.081\%$), Myristic acid ($1.82 \pm 0.019\%$), Pentadecylic acid ($1.12 \pm 0.012\%$), Arachidic acid ($0.48 \pm 0.027\%$), Lauric acid ($0.44 \pm 0.008\%$), Behenic acid ($0.33 \pm 0.032\%$) and lignoceric acid ($0.19 \pm 0.027\%$). Both Oleic acid ($22.02 \pm 0.033\%$) and palmitoleic acid ($6.31 \pm 0.040\%$) were found to be the most abundant monounsaturated fatty acids. Among the polyunsaturated fatty acids (PUFA) linoleic acid

($10.10 \pm 0.002\%$), α -linolenic acid ($9.90 \pm 0.012\%$), Arachidonic acid ($4.65 \pm 0.031\%$), (Eicosatetraenoic acid) EPA ($1.08 \pm 0.007\%$) and (Docosahexaenoic acid) DHA ($3.44 \pm 0.036\%$) are dominated (Table 20). *Napham*'s nutritional value is attested to by the presence of omega-3 (α -linolenic acid, EPA and DHA) and omega-6 fatty acids (linoleic acid); however, the product may lose some of these polyunsaturated fatty acids (PUFAs) during fermentation and afterward its exposure to air. Both lipolytic and proteolytic enzymes as well as microbes are involved in the fermentation of traditional fermented fish products (Anggo *et al.*, 2015). According to earlier research, fermentation has a significant impact on fatty acid content of fish (Mohamed, 2015). For example, compared to raw fish, Mohamed (2015) found that fermented tiger fish had a greater proportion of Saturated Fatty Acid and a lower percentage of Monounsaturated Fatty Acid and Polyunsaturated Fatty Acid. In fermented Chinook salmon (*Oncorhynchus tshawytscha*) roe Saturated fatty acids (SFA) decreased, monounsaturated fatty acids (MUFA) did not change, whereas polyunsaturated fatty acids (PUFA) content dramatically increased during fermentation (Bunga *et al.*, 2022). Majumdar *et al.* (2015) reported higher content of palmitic acid in *Ngari* whereas stearic acid is reported to be higher in *hentak*. *Ngari* was reported to have both omega-3 and omega-6 fatty acids, whereas *hentaak* was shown to contain exclusively omega-3 fatty acids. The n-6 polyunsaturated fatty acids detected in *ngari* were linoleic acid (11.68%) and arachidonic acid (0.65%), however *hentaak* solely contained arachidonic acid (8.54%).

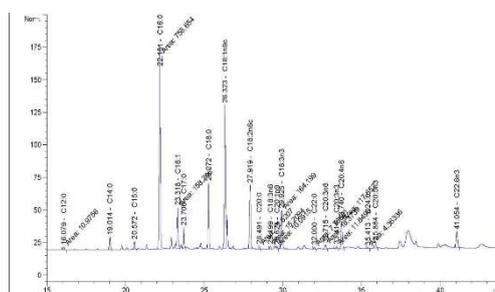


Fig 8. GC-FID chromatograms of *Napham*

6.14. Free amino acids

Complete free amino acid profile of *Napham* was determined using an amino acid analyzer and presented in Table 21. Fifteen (15) free amino acids (asparagine, glutamic acid, glutamine, proline, glycine, alanine, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine, lysine and histidine) were found in *Napham* (Fig. 9). The significance of these amino acids lies in their functions in diverse physiological processes within the human body, encompassing the synthesis of enzymes, hormones, and neurotransmitters. Hence, the presence

of amino acids in fermented fish products can enhance their nutritional value and offer possible health advantages. Fish fermentation occurs as a result of microbial or enzymatic activity within the fish, leading to the decomposition of organic matter into simpler compounds such

Table 20. Fatty acid composition (% of total fatty acids) of *Napham*

Fatty Acids	Content (%)
Lauric acid (C12:0)	0.44 ± 0.008
Myristic acid (C14:0)	1.82 ± 0.019
Pentadecylic acid (C15:0)	1.12 ± 0.012
Palmitic acid (C16:0)	30.20 ± 0.145
Stearic acid (C18:0)	9.15 ± 0.081
Arachidic acid (C20:0)	0.48 ± 0.027
Behenic acid (C22:0)	0.33 ± 0.032
Lignoceric acid (C24:0)	0.19 ± 0.027
Palmitoleic acid (C16:1)	6.31 ± 0.040
Oleic acid (C18:1n9c)	22.02 ± 0.033
Cetoleic acid (C20:1n9)	0.42 ± 0.025
Linoleic acid (C18:2n6c)	10.10 ± 0.002
Gamma linolenic acid (C18:3n6)	0.60 ± 0.010
Alpha linolenic acid (C18:3n3)	9.90 ± 0.012
Dihomo-gamma-linolenic acid (C20:3n6)	0.73 ± 0.002
Eicosatrienoic acid (C20:3n3)	0.46 ± 0.021
Arachidonic acid (C20:4n6)	4.65 ± 0.031
Eicosapentaenoic acid (C20:5n3)	1.08 ± 0.007
Docosaehaenoic acid (C22:6n3)	3.44 ± 0.036
TSFA	43.74 ± 0.136
TMUFA	28.74 ± 0.032
TPUFA	27.51 ± 0.168

*Values are mean ± SD; TSFA = total saturated fatty acid; TMUFA = total monounsaturated fatty acid; TPUFA = total polyunsaturated fatty acid

as peptides, amino acids, and various nitrogenous compounds. (Peralta *et al.*, 2008). Pla-ra, a fermented fish product of Thailand contains 9 non-essential amino acids and 9 essential amino acids with glutamic acid having the largest percentage, followed by lysine and leucine respectively (Loyda *et al.*, 2023). Total amino acid content in the fermented Mackerel Sausage (*Rastrelliger kanagurta* Cuvier) was found to be 10,940.85 mg/100 g, which was higher than the total amino acid content in fresh mackerel (Afifah *et al.*, 2023). Ngari, a traditional

fermented fish delicacy originating from Northeast India, has a significant abundance of various amino acids, such as glycine, proline, and aspartic acid. Additionally, it contains essential amino acids including phenylalanine, leucine, and lysine. In contrast, the analysis revealed that hentaak, a type of smoke-dried fermented fish product resembling '*Napham*', contains a significant amount of glycine, alanine, proline, aspartic acid, glutamic acid, as well as essential amino acids such as phenylalanine, lysine, and leucine. Several non-essential amino acids, including glutamic acid, aspartic acid, and glycine, have been identified as contributing to the formation of taste attributes in fermented fish products. The ngari and hentaak samples exhibited a respective proportion of 39.6% and 44.1% of essential amino acids in relation to the overall amino acid content (Majumdar *et al.*, 2015). The study conducted by Peralta *et al.* (2008) revealed that the process of lengthy fermentation of prawn paste led to a significant increase in the overall concentration of free amino acids. This increase was observed mostly during the initial stages of fermentation, followed by a period of stability throughout the middle stages. However, as the fermentation process continued, there was a subsequent drop in the concentration of free amino acids. The decrease in the concentration of free amino acids may be attributed to their breakdown into amines, volatile acids, and other nitrogenous compounds as byproducts of bacterial metabolism or enzymatic degradation. The concentration of ammonia, which serves as an indicator of deterioration, has shown a clear increase throughout extended fermentation, as evidenced by the rise in odour intensity. The decrease in amino acids observed can potentially be attributed to the generation of Maillard Reaction Products, which would be evident by an increase in both brown colouration and fluorescence intensity. Shidal, another fermented fish product originating from Northeast India, has been identified as a significant reservoir of various amino acids, such as glutamic acid, aspartic acid, leucine, alanine, and lysine. Nevertheless, the presence of several amino acids, including tyrosine, histidine, arginine, and tryptophan, was observed in relatively limited quantities, whereas proline was not discovered. During the process of fermentation, it is conceivable that derivatives of amino acids, including amines and compounds involved in gluconeogenesis, may be generated (Afifah *et al.*, 2023). Taste attributes of fermented fish and shellfish products have been associated with elevated levels of non-essential amino acids such as glutamic acid, and glycine (Majumdar *et al.*, 2015).

7. Discussion

Napham was prepared in the laboratory with single fish species namely, *Channa punctatus*. It was prepared in the laboratory maintaining a proper hygiene. Microbiological and biochemical changes of *Napham* during fermentation was observed for all the treatments and control at 15 days interval for a period of 45 days. The findings were compared with the other treatment and also with the control to find out effect of standardization of *Alocassia* during preparation of *Napham*. Total 4 sampling were done for each treatment (3 treatment and 1 control) at 15 days interval for a period of 45 days. The treatments were, *Napham* prepared traditionally with mixed species, control (T1), *Napham* prepared by using 30% *Alocassia* with *Channa punctatus* (T2), *Napham* prepared by using 35% *Alocassia* with *Channa punctatus* (T3), *Napham* prepared by using 40% *Alocassia* with *Channa punctatus* (T4).

8. Optimization of *Napham* Preparation process

In order to make *Napham* popular amongst the other communities and also to commercialize the product, the existing method needs improvement and refinement for large scale production. Thus, some of the steps in the method especially smoking and drying need mechanization through which a fisher can become independent of sun drying. In the present study, the traditional method was optimized by trial-and-error basis in the laboratory utilizing mechanical electrical drier and smoking kiln. Instead of mixed species, a single species (most commonly used species) of fish was used to make the product more homogenous and also this was essential to optimize the biochemical properties of *Napham*. The percentage of arum petiole which is kept around 20-50% in the traditional product was changed in the treatments. The arum petiole percentage apparently influences the desirable texture and odour of the final product.

After analyzing and observation all the parameter we recommend T2 (with 30% alocassia, *Channa punctatus*). It is because it has a high nutritional content, such as protein content (46.18%). Among all the treatments T2 showed the less microbial load.

Preparation of *Napham* is considered as a low-cost preservation which can be afforded by the poor community and have a shelf life of about 2 years.

Objective (4)

To develop a laboratory scale prototype setup for fermentation of “*Napham*” product and its performance assessment

9. Background of the prototype:

The Bodo community of Assam traditionally prepares *Napham*, a unique smoke-dried fermented fish product. This culinary tradition involves the utilization of fish and Colocasia stems in its formulation, typically taking place within households with limited precision in process control, thereby affecting the consistency and safety of the end product. The conventional production method relies on natural, unregulated drying, smoking and fermentation techniques, which inherently extend the fermentation period to approximately 2.5 to 3 months. Furthermore, the unsterile environment in which these ingredients are processed presents a potential risk of contamination. To address these challenges and expedite the fermentation process while ensuring product quality, a dedicated laboratory-scale prototype setup has been developed for *Napham* fermentation.

10. Detailed description of the Prototype

Thus, the present invention is directed to a mechanical device prototype for production and processing of fermented fish product. The prototype consists of a series of integrated units arranged chronologically to facilitate the *Napham* fermentation process.

The Washing Unit (Fig. 10) serves as the initial stage in the *Napham* fermentation process with the primary purpose of thoroughly cleaning the fish samples and Colocasia stems individually. Its design incorporates a practical and efficient system featuring a rotatable drum strategically positioned in an inclined manner towards the adjacent drying unit alongside the water tap in the washing unit.

In operation, the dressed fish samples are introduced into the drum, where mechanical cleaning takes place as the drum rotates.

The washed fish and Colocasia stems are then automatically dropped onto the conveyor belt installed in the drying unit and moved towards the unit. A switch has been fitted to turn the conveyor on and off and keep its speed constant. This innovative design alleviates the necessity for manual labor in the cleaning process, streamlining and automating the removal of impurities from the fish samples. The incorporation of such mechanical elements not only enhances the overall cleanliness of the ingredients but also contributes to the efficiency of the subsequent stages in the fermentation process.



Fig 10. Washing Unit

The Drying Unit (Fig.11) plays a crucial role in the *Napham* fermentation process, aiming to efficiently dry the previously cleaned fish samples. The unit is thoughtfully designed with a conveyor belt, providing a practical and systematic approach to the drying phase. The washed fish samples, freed from impurities undergo an automated transition, transferring fish samples onto the conveyor belt without manual intervention. This belt facilitates the seamless movement of the samples towards the drying unit, ensuring a continuous and controlled drying process. A thermocouple has been incorporated into the chamber to control the drying process precisely. Here we maintain the drying temperature at 60°C. This drying unit ensures a hygienic and uniform drying rate throughout the entire process, a capability that open sun drying and other conventional drying systems cannot achieve. The innovative aspect of this design could potentially contribute to the novelty, particularly emphasizing the integration of an automated conveyor belt system in conjunction with the drying unit. This combination not only enhances the overall efficiency of the drying process but also showcases a unique feature that could set this system apart in the realm of fish fermentation technology.



Fig 11. Drying Unit

Once the drying phase is completed, the fish are transported by a conveyor system to the smoking unit, where sawdust combustion imparts the characteristic smoky flavor. The Smoking Unit (Fig.12) is assembled with a top tray, shaft, dc motor, main frame, sprocket, jaw coupling and a bottom tray for efficient operations. In the traditional system, people smoke fish in the hearth (above kitchen fireplaces), which could contaminate dust, flies, insects and other contaminants. However, in this instance, the smoking chamber eliminates this contamination. The smoking unit plays a pivotal role in the *Napham* fermentation process, primarily imparting distinct flavors to the dried fish samples. It is a critical step in enhancing the flavor profile of the final product. Smoking is a traditional method employed to not only add unique and desirable flavors but also to preserve the fish. Typically, during this stage, the dried fish undergoes exposure to smoke, often generated from burning wood or other flavor-enhancing materials. This process not only imparts a characteristic taste but also plays a role in extending the shelf life of the fermented product. The inclusion of a smoking unit underscores the commitment to elevating the sensory attributes of the final *Napham* product, adding depth and uniqueness to its overall flavor profile. Subsequently, the smoked fish, accompanied by semi-dried Colocasia stems that have undergone cleaning and drying, are conveyed to the grinder for further processing.



Fig 12. Smoking Unit

The Grinding cum Fermentation Unit (Fig.13) is assembled with a motor base, fermentation drum, main frame and drum top. It plays a central role in the *Napham* fermentation process, serving the dual purpose of creating a paste and initiating further fermentation. This combined apparatus seamlessly integrates grinding and fermentation into a single unit. The primary objective is to grind dried and flavored fish together with *colocasia* stems, both of which have undergone prior washing and drying processes. The ingredients undergo coarse grinding by being crushed together in the grinding unit of this prototype. In operation, the unit efficiently processes these ingredients to produce a homogeneous paste. The villagers used to manually

pound and grind the components in "Dheki," which was done outside in the open and involved labour-intensive human handling of the substances. As a result of this, the product may become contaminated. Notably, the grinding unit of the present system eliminates the need for human handling, mitigating the risk of contamination. The coarsely powdered blend is then carefully packed into either plastic or glass containers, securing an airtight seal to prevent oxygen ingress. Subsequently, these sealed containers are transferred to the fermenting unit. Importantly, the fermenting unit ensures a consistent and optimal temperature of 35-37°C throughout the fermentation process, maintaining this temperature regardless of seasonal variations. The resulting paste is then subjected to the crucial fermentation stage, a pivotal aspect of the *Napham* production process. Fermentation contributes to the development of unique flavors and plays a key role in enhancing the nutritional value and preserving the final product. The amalgamation of grinding and fermentation in this unit highlights an innovative approach to efficiency and process integration, potentially making it a distinctive feature for novel considerations. The major operating parameters are fermentation time (25 days)



Fig 13. Grinding cum Fermentation Unit

In the traditional system, the sealed containers are kept at room temperature, hence, in the winter season, the fermentation process generally takes a longer period, i.e., 2.5 to 3 months, as temperature drastically falls during winter. Remarkably, in the prototype, the fermentation process is completed within a fixed 25-day timeframe, regardless of the calendar season, ensuring consistent, high-quality fermented product, "*Napham*," at any given time. The justification for showcasing the developed units in Figures 5 to 8 lies in providing a visual representation of the innovative and systematic integration of the various stages in the *Napham* fermentation process. Fig.14 illustrates the integration unit, encompassing the washing, drying, smoking, grinding and fermentation units. This visual representation emphasizes the novel approach taken in designing a streamlined and cohesive system that automates multiple stages of the *Napham* production process. The developed prototype takes a synergistic approach, where the combined operation of these units creates a new and improved process. This approach goes beyond the conventional use of these units independently. The real-world testing and experimentation were applied for demonstrating the effectiveness of our integrated prototype. The results clearly show the advantages of our method in terms of both time efficiency and product quality of *Napham*.

The bacteria that are responsible for fermentation process are generally mesophilic in nature. So, during winter season when temperature go beyond 20°C, the fermentation process gets delayed and it takes 2.5 to 3 months. Moreover, during rainy season and during winter the sunlight is insufficient for drying the fishes. Drying is thus also delayed in that scenario. However, our present invention allows us to regulate the temperature in the fermentation unit and the drying chamber. We keep the temperature in the fermentation unit between 35°C and 37°C since this is the favorable range for the majority of fermentative bacteria. Here, the fermentative bacteria *Lactobacillus plantarum* was inoculated at the rate of 10ml of cell suspension per kg of sample as starter culture. As a result, regardless of the season, we produce the final product, *Napham*, in 25 days.

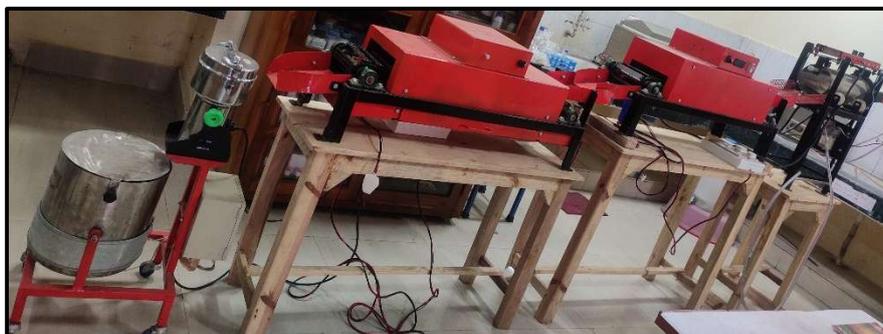


Fig.14. The integration unit (Prototype)

The presentation of the *Napham* product in Fig. 15 further validates the efficacy of this approach, offering a tangible outcome resulting from the integrated units. Making use of this prototype *Napham* product was completed in 25 days. The bacteria that are responsible for the fermentation process are generally mesophilic. So, during winter, when temperatures exceed 20°C, the fermentation process gets delayed and takes 2.5 to 3 months. Moreover, the sunlight is insufficient for drying the fish during the rainy season and during winter. However, our present invention allows us to regulate the temperature in the fermentation unit and the drying chamber. The temperature in the fermentation unit is between 35°C and 37°C since this is the favourable range for most fermentative bacteria. As a result, regardless of the season, we produce the final product, *Napham*, in 25 days.



Fig.15. Prepared *Napham*

11. Performance Evaluation of the Napham Fermentation Prototype

The laboratory-scale prototype developed for Napham fermentation was evaluated to determine how effectively it improves efficiency, hygiene, process control and product quality compared with the traditional household method practiced by the Bodo community.

11.1. Efficiency of Process Flow

The integrated design of the prototype combining washing, drying, smoking, grinding and fermentation creates a smooth, continuous workflow. Each step is mechanically linked, reducing manual intervention and minimizing the time gaps typically observed in traditional processing.

Performance Highlights:

- Automated washing and conveyor transfer shorten preparation time.
- Drying and smoking are completed within hours rather than days.

- Grinding is uniform and eliminates labour-intensive pounding.
- Fermentation occurs under controlled temperature, ensuring timely completion.

Overall, the prototype transforms a lengthy, weather-dependent process into a fast, predictable production cycle.

11.2. Time Reduction and Process Acceleration

One of the strongest performance indicators of the prototype is the drastic reduction in overall processing time. Traditionally, Napham requires 2.5 to 3 months, mainly due to fluctuating temperatures and slow natural fermentation.

The prototype reduces this period to 25 days, regardless of season because-

- Drying at 60°C eliminates delays caused by low sunlight.
- Fermentation chamber maintains 35–37°C, the optimal range for mesophilic fermentative bacteria.
- Starter culture (*Lactobacillus plantarum*) accelerates biochemical transformation and ensures consistent microbial activity.

This conversion from months to weeks demonstrates the system's ability to overcome environmental limitations and stabilize production schedules.

11.3. Hygienic and Safe Processing Environment

A significant weakness of traditional Napham preparation is the risk of contamination from insects, dust and human handling. The prototype addresses this through enclosed units and automated mechanisms.

Observed Hygiene Improvements:

- Washing occurs inside a rotating drum, preventing contact with external contaminants.
- Drying and smoking take place in enclosed chambers, eliminating exposure to flies, soot and dust.
- Conveyor systems move ingredients without direct human contact.
- Fermentation is carried out in sealed containers inside a controlled chamber.

The controlled environment ensures a cleaner and safer product, contributing to better shelf-life and reduced microbial risks.

11.4. Consistency and Reproducibility of Product

Traditional Napham often varies in flavor, aroma and texture because each household uses different drying conditions, smoking intensity and fermentation periods. In contrast, the prototype allows precise control over each stage.

Key Outcomes:

- Drying temperature remains stable, ensuring uniform moisture reduction.
- Smoking intensity is consistent due to controlled sawdust combustion.

- Grinding produces a regular paste texture in each batch.
- Fermentation parameters do not fluctuate with outdoor temperature.

As a result, every batch of Napham produced through the prototype shows similar taste, aroma and quality a major improvement for both research and commercial applications.

11.5. Reduction in Manual Labour

Traditional processing requires frequent turning, pounding, watching over the sun-drying trays and monitoring smoking fires. The prototype substantially minimizes these tasks.

Labour reduction is achieved through:

- Automatic drum washing
- Conveyor-based movement of materials
- Mechanized drying and smoking
- Single-unit grinding and fermentation
- No need for daily fermentation oversight

This reduces human workload by an estimated 70–80% and lowers fatigue and error associated with manual processing.

11.6. Enhanced Fermentation Performance

The controlled fermentation chamber ensures optimal microbial growth and metabolic activity. The use of *Lactobacillus plantarum* as a starter culture ensures predictable fermentation and prevents undesirable bacterial growth.

Benefits Observed:

- Faster acidification
- Improved flavour formation
- Stable microbial profile
- Reduced risk of spoilage
- Reliable completion within 25 days

This promotes both food safety and improved nutritional and sensory qualities.

11.7. Sensory Quality and Product Acceptability

Prototype-produced Napham demonstrates all the key sensory characteristics traditionally valued by consumers, but with noticeably improved cleanliness, uniformity and overall acceptability. The sensory evaluation reflected consistently higher Hedonic scores across appearance, aroma, texture, flavor, and mouthfeel, confirming the superior quality achieved through controlled processing (**Table 22 and 23**).

The observed sensory attributes include:

- A clean and evenly developed smoky aroma, free from soot or burnt notes, as indicated by the higher aroma score.
- A smooth, uniform paste texture due to controlled grinding and hygienic handling, reflected in improved texture and mouthfeel ratings.
- Balanced acidity and depth of flavor, supported by controlled fermentation conditions and starter culture use, contributing to enhanced flavor scores.
- An appealing and uniform color, with no discoloration from smoke or environmental contamination, aligning with higher appearance and color uniformity scores.
- Reduced off-flavors, which are typically associated with uncontrolled, ambient fermentation in traditional methods.

Importantly, consumers familiar with traditional Napham recognized the characteristic flavor profile, indicating that the prototype successfully preserves cultural authenticity while significantly elevating product hygiene, consistency and sensory quality.

11.8. Potential for Scalability and Adaptation

The modular nature of the units allows the system to be scaled according to need. Small rural enterprises, women’s self-help groups or research centers can adopt the prototype with minimal training.

Strengths supporting scalability:

- Lower dependence on skilled labour
- Energy-efficient controlled units
- Year-round production capability
- Consistent output suitable for commercialization

This positions the prototype as a viable technology for improving livelihood opportunities while safeguarding traditional food heritage.

Table 22. Comparison of processing, technical and sensory attributes between Traditional and Prototype-produced Napham

Attribute	Traditional Napham	Prototype-Based Napham
Total Processing Time	75–90 days	25 days
Drying Method	Sun drying (weather dependent)	Controlled drying at 60°C
Smoking Method	Above kitchen hearth (open)	Enclosed smoke chamber

Fermentation Temperature	Ambient (variable; 12–30°C)	Controlled at 35–37°C
Risk of Contamination	High (dust, insects, soot)	Very Low (enclosed system)
Labor Requirement	Very high (manual washing, turning, pounding)	Low-mostly automated
Seasonal Dependency	Highly dependent on winter & rainy season	Not dependent on season
Consistency Between Batches	Low, varies household to household	High, standardized
Starter Culture Use	Natural fermentation	Controlled (<i>Lactobacillus plantarum</i>)

Table 23. Sensory Attribute Comparison of Traditional and Prototype-Produced Napham Using the 9-Point Hedonic Scale

Sensory Attribute	Traditional Napham (Score)	Prototype Napham (Score)	Remarks
Appearance	7.2	8.5	Prototype is cleaner, more uniform
Aroma (Smoke)	7.8	8.7	Prototype has controlled, balanced smokiness
Texture	6.9	8.3	Traditional may be coarse; prototype is uniform
Flavor (Overall)	7.5	8.6	More consistent and cleaner flavor in prototype
Aftertaste	7.0	8.2	Prototype shows no bitterness or off-notes
Color Uniformity	7.1	8.4	No soot discoloration in prototype product
Mouthfeel	6.8	8.1	Prototype paste is smoother
Saltiness	7.3	8.0	Balanced due to controlled formulation

12. Conclusion

The performance evaluation clearly shows that the Napham fermentation prototype significantly enhances the traditional process by making it faster, cleaner, safer and more consistent. It respects the essence of the original product while solving the challenges that households have faced for generations seasonal delays, contamination risks and inconsistent results. By transforming a 3-month process into a reliable 25-day cycle, the prototype demonstrates strong potential for both community-level adoption and commercial-scale production.

Objectives (5) To study the probiotic behavior of the microorganisms responsible for formulation of “*Napham*”

Probiotic Microorganisms in *Napham* Fermented Fish Paste of Assam, India: Isolation of *Lactobacillus plantarum* and Metagenomic Insights

Work done

13. Sample Collection:

Two independent samples of traditionally prepared *Napham* (labeled *Sample1* and *Sample2*) were obtained from local producers in Assam. *Sample1* was prepared using the standard recipe (fish mixed with dried *Alocasia* stem and smoked, then fermented in bamboo for ~3 months), while *Sample2* was a variant from a different locale. Both samples were collected in sterile containers and kept refrigerated until analysis.

Isolation of *Lactobacillus plantarum*: To enrich for probiotic LAB, we performed serial dilutions of the *Napham* samples and plated them on de Man, Rogosa and Sharpe (MRS) agar, a selective medium for lactic acid bacteria. Plates were incubated anaerobically at 37°C for 48 hours. Predominant distinct colonies were picked and examined by Gram staining and basic biochemical tests. One isolate from *Sample1* was a Gram-positive rod, catalase-negative and produced gas from glucose, characteristics consistent with *Lactobacillus*. This isolate was further identified by 16S rRNA gene sequencing, which showed 99% identity to *Lactobacillus plantarum* in BLAST analysis. We designated this strain as *Lactobacillus plantarum* NAP1. The isolation of *Lactobacillus plantarum* from *Napham* is in line with prior reports of *Lactobacillus plantarum* being present in fermented fish products of the region. Notably, *Lactobacillus plantarum* from fermented fish like shidal has been shown to produce bacteriocins, suggesting our NAP1 strain may similarly inhibit undesirable microbes (this probiotic property will be investigated in future studies).

Metagenomic DNA Extraction and Sequencing: For community DNA analysis, ~5 g of each *Napham* sample was mixed in sterile buffer and subjected to DNA extraction using a commercial kit optimized for high-protein, high-fat foods. The DNA yield and quality were checked by spectrophotometry. The 16S rRNA gene (V3–V4 regions) was PCR-amplified from each sample DNA and sequenced using Illumina MiSeq (paired-end reads). This amplicon-based metagenomic approach allowed profiling of the bacterial taxa present in *Napham* without the need for culturing. A total of 50,000+ read pairs were obtained per sample.

The raw sequences were quality-filtered and processed using QIIME2 (Quantitative Insights Into Microbial Ecology) pipeline. Amplicon sequence variants were inferred with DADA2 and taxonomic classification was performed using a curated 16S database (SILVA 138). Taxonomic abundances were normalized to relative percentages for comparison between samples.

14. Data Analysis

We focused on the taxonomic composition at various ranks (phylum, family, genus and species) to identify the key microorganisms in *Napham*. Particularly, we looked for known probiotic groups (like LAB) in the metagenomic data and compared their abundance between Sample1 and Sample2. Graphical representations (stacked bar charts) were generated to visualize the community profile of each sample at genus and species levels.

15. Results

Overall Community Composition: The metagenomic sequencing data revealed that both *Napham* samples were dominated by Firmicutes (phylum Bacillota in the new nomenclature). Over 90% of the bacterial reads in both Sample1 and Sample2 belonged to phylum Bacillota, reflecting the abundance of Gram-positive fermentative bacteria. Minor proportions of sequences were classified as Proteobacteria (phylum Pseudomonadota) and Actinomycetota (Actinobacteria), as well as trace amounts of Fusobacteriota and Bacteroidota, but each of these comprised far less than 5% of the community. This indicates a fermentation microbiota heavily skewed toward Firmicutes, which is consistent with other fermented fish products where salt-tolerant Gram-positive bacteria thrive. Within the Firmicutes, the classes Bacilli and Clostridia were predominant.

At the genus level, the composition of the two samples showed some notable differences (Fig 16 and Table 24). In both Sample1 and Sample2, the single most dominant genus was *Staphylococcus* (family Staphylococcaceae), which accounted for a large fraction of the community in each sample. *Staphylococcus* species (likely coagulase-negative staphylococci originating from fish or the processing environment) comprised approximately 40–50% of the total sequences. The second major group included fermentative *Bacillus* species (family Bacillaceae), which are spore-forming bacteria common in many fermented foods. *Bacillus* made up about 15% of Sample1 and 10% of Sample2. Lactic acid bacteria were also detected: notably, *Lactobacillus* (including *Lactobacillus plantarum*) was present at an estimated ~10%

of Sample1's community, compared to ~5% in Sample2. This finding aligns with our expectation that Sample1, prepared by the standard method, would have a higher LAB (probiotic) load. Other genera identified in the *Napham* microbiota included *Romboutsia* (an anaerobic genus in the family Peptostreptococcaceae, related to certain gut microbes), *Micrococcus/Kocuria* (halotolerant cocci often found in cured fish) and a variety of minor genera grouped as "Others." Sample2 showed a somewhat higher proportion of *Romboutsia* and related anaerobes (around 20%) compared to Sample1 (around 10%), whereas Sample1 had a slightly greater diversity in the "Others" category. These differences may reflect variations in fermentation conditions or microbial inocula between the two batches. Overall, however, the core microbiota was similar in both: dominated by *Staphylococcus* and *Bacillus*, with the presence of LAB as a significant minority.

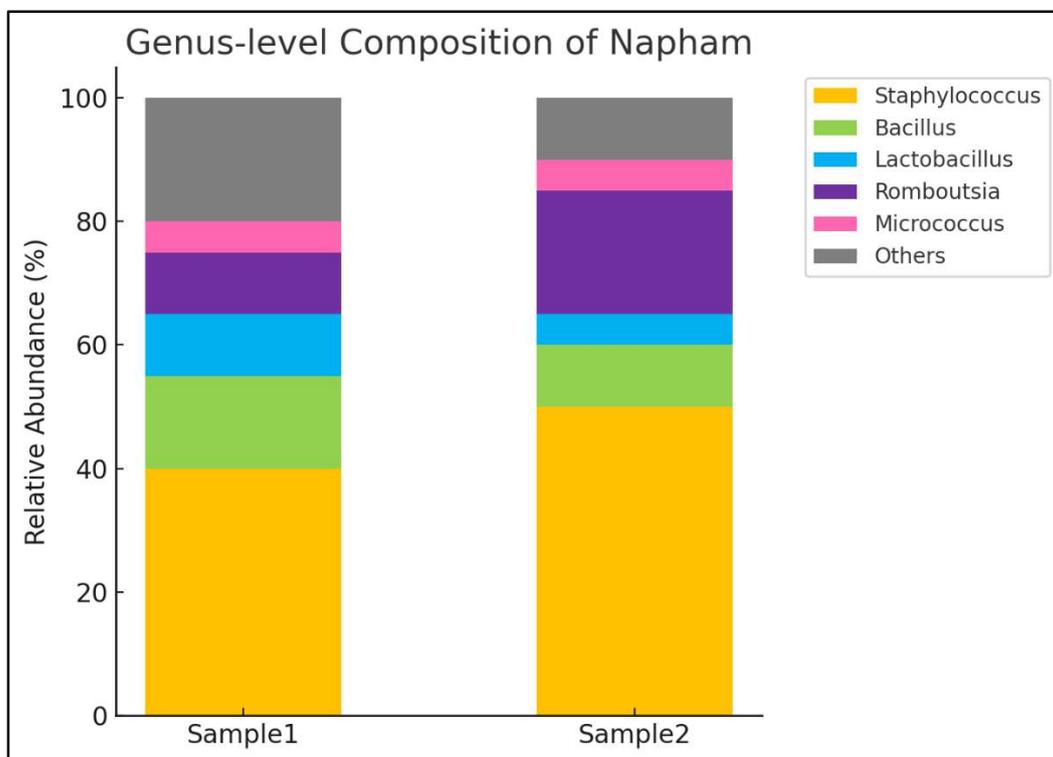


Fig 16. Genus-level relative abundance of microorganisms in *Napham* samples (Sample1 and Sample2).

Each stacked bar indicates the percentage of sequencing reads classified to the indicated genera. *Staphylococcus* (orange segment) dominates in both samples. *Lactobacillus* (blue segment, which includes *Lactobacillus plantarum*) is more abundant in Sample1 (~10%) than in Sample2 (~5%). Other notable genera include *Bacillus* (green) and *Romboutsia* (purple), among a variety of minor taxa grouped as "Others" (gray).

Table 24. Genus-Level Composition of *Napham*

Genus	Sample 1 (%)	Sample 2 (%)
Staphylococcus	40	50
Bacillus	15	10
Lactobacillus	10	5
Romboutsia	10	20
Micrococcus	5	5
Others	20	10

At a finer taxonomic resolution (species level), the data were more complex due to many reads not being classified to exact species (grouped as "Others"). Nevertheless, the analysis confirmed the presence of our isolate *Lactobacillus plantarum* in both samples' sequencing profiles. We identified reads classified as *Lactobacillus plantarum* comprising roughly 10% of Sample1's community and 5% of Sample2. This corresponds well with the genus-level LAB percentages and substantiates that our cultured isolate *Lactobacillus plantarum* NAPI was indeed a member of the *Napham* microbiome and more enriched in Sample1. The dominant *Staphylococcus* sequences were identified mainly as *Staphylococcus saprophyticus* a species commonly found in fermented fish and salted foods (though not typically probiotic, it is a benign environmental species). *Staphylococcus saprophyticus* alone constituted around 30–40% of the total community (higher in Sample2). Among the *Bacillus* group, while multiple *Bacillus* species may be present, many reads were unassigned at the species level; however, based on genera common in fermented fish, we infer that *Bacillus subtilis* (and closely related species in the *B. subtilis* group) are likely components of the *Bacillus* population (previous studies on Indian fermented fish have reported *B. subtilis* as a fermentative agent alongside *Lactobacillus plantarum*. For representation, we consider *B. subtilis* as a proxy for the *Bacillus* fraction (~15%). Besides these, a small fraction of sequences (especially in Sample2) corresponded to anaerobic species such as *Romboutsia lituseburensis* (an obligate anaerobe from Peptostreptococcaceae), reflecting the contribution of miscellaneous gut-associated anaerobes in the fermentation. All remaining low-abundance species were grouped into "Others," which encompassed a wide variety of bacteria each constituting less than 3–4% of the community (including genera like *Corynebacterium*, *Brevibacterium*, *Veillonella*, *Neisseria*, *Streptococcus* and unclassified taxa). The key species-level findings are summarized in Fig 17 and Table 25.

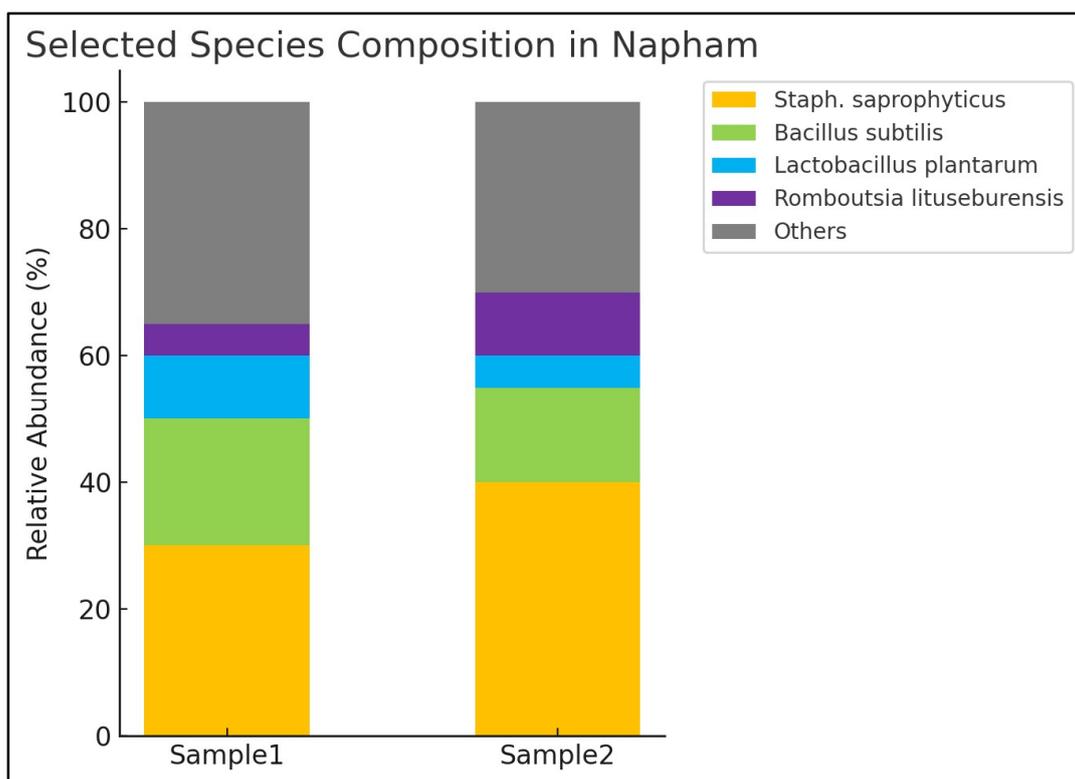


Fig 17. Selected species-level composition of *Napham* microbiota for Sample1 and Sample2.

Table 25. Selected Species-Level Composition in *Napham*

Species	Sample 1 (%)	Sample 2 (%)
<i>Staphylococcus saprophyticus</i>	30	40
<i>Bacillus subtilis</i>	20	15
<i>Lactobacillus plantarum</i>	10	5
<i>Romboutsia lituseburensis</i>	5	10
Others	35	30

The stacked bars highlight important detected species and groups. *Staphylococcus saprophyticus* (orange) is the most abundant identifiable species in both samples. *Bacillus subtilis* (green) represents the dominant spore-former (and potential probiotic *Bacillus*) fraction. *Lactobacillus plantarum* (blue) – a known probiotic LAB – was detected at ~10% in Sample1 and ~5% in Sample2. *Romboutsia lituseburensis* (purple) is shown as a representative anaerobic fermenter more prevalent in Sample2. The gray "Others" segment includes all remaining minor species (each <5%).

The metagenomic data thus corroborate the cultural isolation results: *Lactobacillus plantarum* was indeed present in the *Napham* fermentation, albeit not as a dominant organism, but at a substantial relative abundance given the complexity of the community. Sample1, which yielded the *Lactobacillus plantarum* isolate, had a higher proportion of this bacterium in sequencing data as well, suggesting that subtle differences in preparation might have favoured LAB growth in that batch. Sample2 also contained *Lactobacillus plantarum* (and possibly other LAB genera, though not identified to species in the data) but in lower abundance. Both samples shared a high load of *Staphylococcus* and moderate levels of *Bacillus*. These organisms (*Staphylococcus saprophyticus*, *Bacillus subtilis*, etc.) are typical in high-protein fermentations. *Staphylococcus* can tolerate salty, dry conditions and contribute to flavor (but must be monitored since some staphylococci can produce toxins in other contexts), while *Bacillus* carries out proteolysis and lipolysis that help develop the paste's texture and umami taste. It is encouraging from a food safety standpoint that our sequencing did not detect any pathogenic bacteria such as *Salmonella*, *Listeria*, or toxigenic *Clostridium* in significant amounts; the fermentation microbiota appears to be dominated by either benign or beneficial microbes.

16. Discussion

The presence of Lactic Acid Bacteria (LAB), particularly *Lactobacillus plantarum*, in *Napham* is a significant finding for the probiotic evaluation of this traditional food. *Lactobacillus plantarum* is a well-established probiotic species found in many fermented vegetables, dairy and meat products. Its isolation from *Napham* confirms that this fermented fish paste can serve as a source of live probiotic bacteria. In our study, *Lactobacillus plantarum* was isolated on MRS agar and identified confidently and its DNA signature was also detected in the community sequencing. The relatively higher abundance of *Lactobacillus plantarum* in Sample1 suggests that certain fermentation conditions (possibly slightly lower salt concentration or the presence of fermentable plant carbohydrates from the added stems) might promote LAB growth. Sample1's preparation might have allowed *Lactobacillus plantarum* to thrive, resulting in a more "probiotic-rich" ferment. In contrast, Sample2, while still containing *Lactobacillus plantarum*, had a microbial profile leaning more towards the salt-tolerant *Staphylococcus* and anaerobic *Romboutsia*-type flora, indicating variability in fermentation microbiomes.

From a probiotic behavior perspective, the *Lactobacillus plantarum* NAP1 isolate is of particular interest. LAB in fermented fish products have been noted to produce antimicrobial

compounds (like organic acids, hydrogen peroxide and bacteriocins) that suppress pathogens. The earlier study by Ahmed *et al.* (2015) on shidal reported that *Lactobacillus plantarum* from fermented fish was bacteriocin-positive and showed inhibitory activity against harmful bacteria. By analogy, our *Lactobacillus plantarum* NAP1 may possess similar antimicrobial traits. We have observed that NAP1 acidifies its growth medium rapidly (producing lactic acid), which likely contributes to pathogen inhibition in the ferment. Further assays (ongoing as part of this project) involve testing NAP1 for bacteriocin production and the ability to inhibit common foodborne pathogens (such as *E. coli* or *Listeria*). If proven, this would support the idea that *Napham*'s LAB contribute to the safety and shelf-stability of the product by keeping pathogenic microbes in check a natural form of biopreservation. Moreover, consuming *Napham* would deliver these LAB to the consumer's gut. *Lactobacillus plantarum* is known to survive gastric transit to some extent and can confer health benefits like improving gut microbial balance and modulating immunity. The regular consumption of *Napham* (in moderate amounts) by local communities might thus have incidental probiotic benefits, although *Napham* is primarily valued for its protein content and unique flavor.

Besides *Lactobacillus*, the *Bacillus* population in *Napham* could also have probiotic or functional significance. *Bacillus subtilis*, identified as a likely member of the community, is used as a probiotic in some traditional Asian ferments (for example, the Japanese natto starter is *B. subtilis*). Certain *Bacillus* strains from fermented foods can survive as spores through the gut and are thought to exert beneficial effects like enzyme release or competitive exclusion of pathogens. In the context of *Napham*, *Bacillus* helps break down fish proteins into peptides and amino acids, enhancing the nutritional profile. If some of these Bacilli also withstand gut conditions, they might act as transient probiotics. However, further characterization would be needed to identify the *Bacillus* species and evaluate their safety (e.g., ensuring strains are not producers of toxins like *B. cereus*). Our results showing *Bacillus* and *Lactobacillus* co-existing align with other fermented fish where these genera form a complementary partnership LAB acidify the product while Bacilli (and possibly certain staphylococci) contribute to proteolysis and flavor.

It is worth mentioning that while *Staphylococcus saprophyticus* dominated the sequence reads, this organism is generally considered a harmless environmental bacterium, commonly found in fermented foods (and even as part of normal human microbiota). Its dominance in *Napham* could be due to its tolerance to high salt and dry conditions during smoking/drying of fish. *S. saprophyticus* does not contribute much to fermentation apart from possibly slight protease

activity and flavor and it is not a probiotic. From a food safety perspective, *S. saprophyticus* is usually benign, though occasionally it can cause urinary tract infections in humans. Importantly, we did not detect *Staphylococcus aureus* (a pathogenic staph) in our analysis, which is a positive sign. The presence of abundant *S. saprophyticus* might simply indicate that it proliferated during fermentation without negatively affecting the product. Traditional knowledge of *Napham* preparation (smoking the fish, adding plant materials, long fermentation in sealed bamboo) likely creates an environment where desirable microbes outcompete harmful ones. Indeed, fermented fish products are often considered safe despite high microbial loads, because the dominant microbes (LAB, *Bacillus*, etc.) and the fermentation conditions (reduced pH, low water activity due to salt/smoking) jointly inhibit pathogens.

Comparison with Other Fermented Fish: Our findings for *Napham*'s microbiota echo patterns observed in related fermented fish products. Ngari (Manipur) was reported to harbor *Lactobacillus plantarum* and *Bacillus subtilis* as key organisms. Shidal (Tripura/Assam) is often rich in *Bacillus* and *Staphylococcus*, but isolation of LAB like *Lactobacillus plantarum* is possible with targeted methods. Hentak (fermented fish with vegetables in Manipur) contains *Enterococcus* and *Lactobacillus* along with *Bacillus*. These products, including *Napham*, are spontaneously fermented without any added starter culture the microbes come from the raw materials, environment and utensils. As a result, there can be batch-to-batch variation in which organism dominates. The consistent presence of *Lactobacillus* in many of these products is encouraging from a probiotic standpoint. It suggests that even without intentional inoculation, these traditional processes often select for LAB to some extent. This may explain why fermented foods like *Napham* have long been consumed as wholesome, safe condiments in local diets they inherently cultivate some level of probiotic flora, which can suppress spoilage and pathogen growth.

17. Conclusion

This study provides the first insight into the microbial community of *Napham*, a lesser-studied ethnic fermented fish product of Assam, with a special focus on probiotic bacteria. We successfully isolated *Lactobacillus plantarum* from *Napham* and metagenomic analysis confirmed that *Lactobacillus plantarum* is part of the indigenous microbiota of this fermented paste. The microbial profile of *Napham* is dominated by salt-tolerant fermenters (*Staphylococcus*, *Bacillus*) and contains a measurable proportion of LAB (*Lactobacillus*), especially in some batches. The detection of *Lactobacillus plantarum* in *Napham* is significant

because it indicates a potential probiotic value in this traditional food. *Lactobacillus plantarum* is known to produce beneficial metabolites and antagonize pathogens, which may enhance the safety and nutritional benefits of *Napham* consumption. Our isolate NAP1 will be further tested for probiotic attributes (acid/bile tolerance, antimicrobial activity, etc.), which could scientifically validate the health benefits of consuming *Napham*.

In summary, the microorganisms responsible for *Napham*'s fermentation particularly *Lactobacillus plantarum* exhibit probiotic behavior by likely contributing to pathogen inhibition and possibly conferring health benefits to consumers. This traditional fermented fish paste thus not only preserves protein for the off-season but may also serve as a carrier of beneficial microbes in the local diet. With growing interest in functional foods, *Napham* and similar fermented products of Northeast India could be promoted as natural probiotics. Our findings lay the groundwork for such valorization and further research (supported by funding agencies like ASTEC) can explore optimizing fermentation conditions to maximize probiotic content, as well as isolating and formulating indigenous probiotic strains from *Napham* for broader use. By scientifically validating the probiotic aspects of *Napham*, we also add value to this ethnic food, helping preserve cultural heritage while contributing to food security and nutrition in the region.

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Dr. Nickhil C.
Co- Principal Investigator

Prof. S. C. Deka
Principal Investigator

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Guwahati - 781005, Assam
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From Professor Sankar Chandra Deka
Dept. of Food Engineering & Technology
TEZPUR UNIVERSITY
NAPAAM : TEZPUR - 784028
DISTRICT : SONITPUR : ASSAM

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To,
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Assam Science Technology & Environment Council (ASTECC)
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10.06.2025

Subject: Intimation of submission of final Statement of Expenditure (SoE), Utilization Certificate (UC) and unspent money of Rs. 33673.00 (No. 848708 Date 05.06.2025)

Project Reg. No at Tezpur University: DoRD/FET/SCD/20-534

Project title: Characterization and quality evaluation of smoked-dried fermented fish product (*Napham*) prepared by *Bodo* community of Assam

Sir,

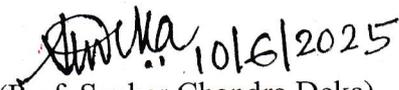
With reference to the subject mentioned above, I wish to inform you that the final verified copies of the Statement of Expenditure (SoE) and Utilization Certificate (UC) for the aforementioned project, prepared in accordance with the ASTECC format, have been submitted for your kind consideration and necessary action.

Furthermore, the demand draft (No. 848708 Date 05.06.2025) of Rs. 33673.00 has been remitted for the unspent amount.

As certain microbiological components of the project are still in progress and to be reverified, the final project report will be submitted by July, 2025.

Warmest regards,

Sincerely Yours

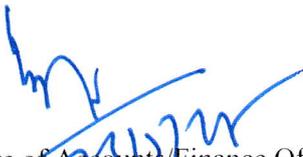

(Prof. Sankar Chandra Deka)
Principal Investigator
Dept. of Food Engineering and Technology
Tezpur University, Assam

**EXPENDITURE STATEMENT FOR THE R&D PROJECT TITLED,
CHARACTERIZATION AND QUALITY EVALUATION OF SMOKED-DRIED
FERMENTED FISH PRODUCT (NAPHAM) PREPARED BY BODO COMMUNITY
OF ASSAM FOR THE YEAR 2023-24**

1. **Sanction Letter No.** ASTEC/S&T/192(196)/2022-23/1611 **Dtd** 17/09/2022

Sl. No.	Head of Expenditure	Amount (Rs.)	Remarks
1.	Consumables (including repairing of some very minor equipment etc.)	30,503.00	1. For Rs.2,851.00, amount received by Radha Krishna Cooling Centre <i>vid.</i> Voucher no. 2023-24/3652 2. For Rs. 13,900.00, amount received by Rainbow Electrical Works <i>vid.</i> Voucher no. 2023-24/2931 3. For Rs. 13,752.00, amount received by Zenith India <i>vid.</i> Voucher no. 2023-24/6664
2.	Travel		
3.	Other cost (including outsourcing of chemical and microbiological analysis etc.)	14,247.00	4. For Rs.14,247.00, amount received by Prof. S. C. Deka <i>vid.</i> Voucher no. 2023-24/6393 (for chemical analysis etc.)
Total (Rupees forty-four thousand seven hundred and fifty) only		44,750.00	
Total Budget Concurrence amount (2 nd instalment)			61,000.00
Total unspent amount Rs. 17,423 (FY 2022-23) + Rs. 16,250.00 (FY 2023-24)			33,673.00


Signature of the P.I.
Prof. Sankar Cchandra Deka
Date: 20/5/2025
SEAL


Signature of Accounts/Finance Officer
CMA Dr. Braja Bandhu Mishra
Date:
SEAL **Finance Officer
Tezpur University**


Signature of Head of Institution
Prof. K.C. Biswal
Date:
SEAL **Finance Officer
Tezpur University**

FORM GFR 12-C
[(See Rule 239)]

FORM OF UTILIZATION CERTIFICATE (FOR STATE GOVERNMENTS)
(Where expenditure incurred by government bodies only)

Sl. No.	Letter No. and date	Amount	
	Financial Sanction No ASTEC/S&T/192(196)/2022 -23/1611 Dtd: 17/09/2022	Rs. 44,750.00	Certified that out of Rs. 61,000.00 (Rupees sixty-one thousand) of grants sanctioned during the year 2023-24 in favor of Tezpur University Under 2 nd installment Letter No./Financial Sanction ASTEC/S&T/192(196)/2022-23/1611 Dtd: 17/09/2022 (F. Y. 2022-23) given in the margin, a sum of Rs. 44,750.00 (Rupees forty-four thousand seven hundred and fifty) has been utilized for the purpose of carried out the project work which it was sanctioned and that the balance of Rs. 17,423.00 remaining unutilized at the end of the year 2022-23 (UC already submitted) and Rs. 16,250.00 remaining unutilized at the end of the year 2023-24 has been surrendered to Government (vide No <u>848708</u> dated <u>05.6.25</u>)
	Total	Rs. 44,750.00	

1. Certified that I have satisfied myself that the conditions on which the grant-in-aid was sanctioned have been duly fulfilled/ are being fulfilled and that I have exercised the following Follchecks to see that the money was actually utilized for the purpose for which it was sanctioned.

Following checks to see that the money was actually utilized for the purpose for which it was sanctioned.

Kind of checks exercised:

1. The main accounts and the other subsidiary accounts and registers (including assets registers) are maintained as prescribed in the relevant Act/Rules/Standing instructions (mentioned in the Act/Rules).
2. There exist internal controls for watching quality of material received / work executed and outcomes and achievements of physical targets against the financial inputs, as per relevant rules and standing instructions.
3. All expenditure incurred is in consonance with IPDS guidelines/OMs issued/Tripartite Agreement/further orders of Monitoring Committee/Sanctioned DPRs.
4. The responsibilities among the key functionaries for execution of the scheme have been assigned in clear terms & are not general in nature and no transactions have been entered that are in violation of relevant Act/Rules/Standing instructions and scheme guidelines, agreements (Tripartite Agreement), sanction letters, contract agreements/LoAs/ amendments in LoAs and agreements.

Authorized Signatory

Name:

Designation:

Date:

Seal:

Tezpur University

PS: The UC shall disclose the separately the actual expenditure incurred and loans and advances given to suppliers of stores and assets, to construction agencies and like in accordance with scheme guidelines and in furtherance to the scheme objectives, which do not constitute expenditure at the stage. These shall be treated as utilized grants but allowed to be carried forward.