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Analytical characterization and biological evaluation of the muscle lipid of Bombay duck (*Harpadon nehereus*) of the Bay of Bengal

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Abstract
The lipid of muscle of the Bombay Duck (*Harpadon neherus*), collected from the economic zone Bay of Bengal, was extracted using a solvent extraction method and then characterized with regard to various physical and chemical properties and contrasted with those of standard lipids because marine fish lipids have grown in importance due to their positive effects on human health. In accordance with Gas Liquid Chromatography (GLC), the lipid sample contained 42.3412% Saturated Fatty Acids, 34.4958% Monounsaturated Fatty Acids (MUFA), and 23.2229% Polyunsaturated Fatty Acids (PUFA). Among PUFAs, percentages of ω-3 PUFAs (19.5245%) were higher than that of ω-6 PUFAs (3.6984%). The lipid sample's microbial activity was assessed (four bacteria were used for bacterial activity; four fungi were used for fungal activity). The titled specimen's lipid-containing muscle had undergone quantitative analysis to determine the percentages of its mineral contents (N, P, K, and Ca). Thus, it comes to a conclusion with a number of significant findings pertaining to industrial, pharmacological, and nutritional importance.

Keywords: Marine fish lipids, Bombay duck, glc, pufa, microbial activity, pharmacology

1. Introduction
Bangladesh is rich in sufficient varieties of marine fishes in the Bay of Bengal which covered southern region of this country. Most of the people of this country depend completely on fish as the major source of protein. According to estimates, fish alone provides around 80% of the animal protein in human diets [1]. The biochemistry of membranes is significantly influenced by lipids and fatty acids, which also have a direct impact on human membrane-mediated processes as osmoregulation, nutrition absorption, and transport [2]. Marine algae and marine phytoplanktons are able to synthesizew-3 PUFAs (e.g. eicosapentaenoic acid and docosahexaenoic acid). As marine fishes eat these plants, they accumulate these w-3 PUFAs in their body [3]. The high levels of ω-3 Polyunsaturated Fatty Acids (PUFAs) found in marine fish lipids make them important because these PUFAs have been proven to reduce blood pressure, plasma triacylglycerol levels, and the risk of coronary heart disease [4,5]. Moreover, they can lessen the signs and symptoms of a number of other physical conditions, including diabetes [6]. The fishing resources of Bangladesh include the important species of Bombay duck, locally known as Loitta fish. It is abundantly found in the Bay of Bengal, but the general public is unaware of the nutritional and therapeutic potential of this fish, and there is also a lack of information regarding its dietary value and pharmacological implications. These days, scientific researchers are paying more attention to physico-chemical assessment, microbial investigation, and the close-proximate composition of various marine fish species [7, 8]. However, the outcomes of these kinds of studies on Bombay duck fish are mostly unreported or unreliable, despite the abundance of this fish in the Bay of Bengal. The goal of the current study is physico-chemical characterization of the muscle lipid of Bombay duck fish that was extracted using solvent, and to compare the findings to information on physico-chemical characteristics that are already accessible in the literature. The lipid sample has also been evaluated on the basis of microbial assays (bacterial and fungal) and nutritional analyses for the pharmacological and nutritional aspects, respectively.

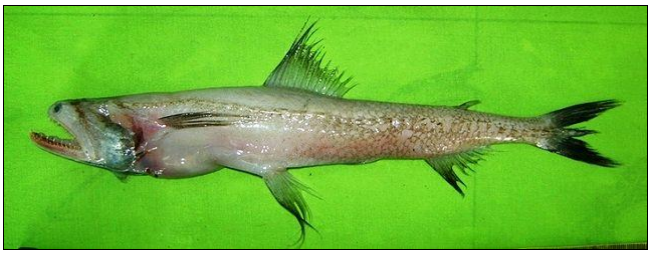

Fig 1: Bombay duck (*Harpadon nehereus*)

2. Materials and Methods

2.1 The marine species collection

The fish market in Sadarghat, Chattogram (22°20'18.24" N 91°49'54.05 E), provided the export-quality Bombay duck fish, which was procured, transported to the laboratory. After identification, the fish sample was stored in a deep freezer (-20 °C) for further work. The muscle from the fish was carefully separated and preserved until extraction.

2.2 Extraction of lipid from muscle

Acetone and ethyl acetate were used as solvents during the solvent extraction method used to isolate lipids from the specimen's muscle. The mixed extract was recovered using a rotary evaporator at 45 °C, dried, and the remaining solvent was flushed out using nitrogen gas.

2.3 Characterization of Physical Properties

The amount of total extracted lipid was measured using gravimetry. Standard techniques were used to determine the moisture content, fat, fiber and ash contents of the muscle of the Bombay duck, and the refractive index of lipid sample [9].

2.4 Characterization of Chemical Properties

The specific conditions of the conventional procedures were used to determine a number of chemical characteristics of the lipid sample. Standard procedures were used to calculate the lipid's saponification value, equivalent value for saponification, acid value and percentage of free fatty acid, ester value, iodine value, thiocyanogen value, peroxide value, amount of unsaponifiable matter, acetyl value, Henher value, Reichert-Meissl value, Polenske value, marine oil test and Elaiden test [10, 14].

2.5 Chromatographic Examination

The opportunities for extracting compositional information from biological matter have been greatly altered by the chromatographic technique, particularly gas liquid chromatography (GLC). Chromatographic separations have become a potent analytical tool as a result of constant improvements in their precision and refinement. Lots of information on lipids' fatty acid composition are now available due to their simplicity and speed. The extracted lipid was esterified with methanolic sulphuric acid (85:15, v/v) [15]. The reaction mixture was placed in vials and heated in an oven for two hours at 80 °C. After cooling, it was diluted with water, extracted with diethyl ether, and then subjected to gas liquid chromatography analysis. GLC examined the methylated esters of fatty acids. Quantitative analysis of the fatty acid content of the lipid sample was performed using gas liquid chromatography [16].

2.6 Microbial Evaluation

Microbial activity of the muscle lipid of Bombay duck against disease-causing bacteria and fungal pathogens was checked. The relevant bacteria were screened by the disc diffusion method using the lipid sample [17]. The foundational medium for the bacterial test was nutrient agar (NA). The lipid was again tested against the pertinent fungi using the poisoned food technique. For the fungal test, potato dextrose agar (PDA) was utilized as the basic medium. The lipid sample was prepared into the desired solution (10% and 5%) using chloroform as a solvent. Chloroform was used to keep things under control.

2.7 Quantification of Minerals

Determination of the mineral contents (N, P, K, Ca and Na) of the Bombay duck's lipid-containing muscle was done by using well-known techniques [18].

3. Results and Discussion

In order to ascertain its nature and evaluate its suitability for a particular application, the muscle lipid of Bombay duck (*Harpadon nehereus*) fish has been examined for several physical and chemical features as well as microbial activities.

3.1 Physical Characteristics

The results on physical characteristics of extracted muscle lipid of Bombay duck are shown in Table 1. At 25 °C, the lipid's refractive index was determined to be 1.4730, which is a relatively high value and an indication of moderate concentrations of unsaturated fatty acid in the fatty acid constituents. The specific gravity of the lipid sample was measured to be 0.934 at 25 °C. At 30 °C, the lipid solution's viscosity was measured to be 301.56 milipoise. From the viscosity data, we deduced something concerning the intermolecular hydrogen bonding in the lipid sample. The current study revealed that the lipid sample might contain a few free acid molecules and hydroxyl groups. Not only low acetyl value but also low acid value of the lipid sample confirms this observation.

Table 1: Physical parameters of the muscle lipid of Bombay duck and different lipid samples

Name of the sample	Refractive index (25 °C)	Specific gravity (25 °C)	Viscosity (mp) (30 °C)
Brain lipid of Baghda Chingri	1.4736	0.941	303.260
Liver lipid of Blue Spotted Fantial Ray	1.4760	0.9575	325.325
Muscle lipid of Vetki fish	1.4745	0.923	290.38
Muscle lipid of Bombay duck	1.4730	0.934	301.56

It was determined that 73% of the muscle in Bombay duck fish had moisture (Table 2). It demonstrates how the percentage of fat and other organic or bioactive components In the sample is connected to the value of moisture content. The lipid-containing muscle of the Bombay duck contained 2.03% fat, 2.15% fiber and 0.94% ash respectively (Table 2).

Table 2: Moisture content, fat, fibre and ash content of the lipid containing muscle of Bombay duck and some related fishes

Fish species	Moisture (%)	Fat (%)	Fibre (%)	Ash (%)
Herring (spring)	72	8		2
Pilchard	69	9		3
Mackerel (spring)	75	0.50		1.6
Bombay duck	73	2.03	2.15	0.94

3.2 Chemical Characteristics

The results on chemical characteristics of extracted muscle lipid of Bombay duck are shown in Table 3. The muscle lipids contain a higher proportion of fatty acids with high molecular weight, as indicated by the relatively high values of 189.71 and 295.71 for saponification and saponification equivalent, respectively. It was found that the acid value and percentage of free fatty acid (as oleic) were 1.78 and 0.89%, respectively. The hydrolytic breakdown of the oil or fat yields the free fatty acid. Therefore, the low levels of acid value and percentage of free fatty acid (as oleic) are a sign that the lipid is acceptable for consumption. The sample's ester value was determined to be 187.93. This number represents the quantity of ester in the lipid sample. The Elaiden Test and the iodine value of 108.23 both confirm that the lipid is semidrying type and has a moderate number of unsaturated fatty acid components. A moderate quantity of unsaturated fatty acid components is also indicated by the peroxide value of 19.63 and the thiocyanate value of 66.25. The low value of unsaponifiable matter (0.72%) indicates that the muscle lipid holds only a minimal percentage of unsaponifiable sterols, vitamins A and D, tocopherols, hydrocarbons and other substances. The acetyl value of 11.60 indicates that the lipid contains few free hydroxyl groups. The higher Henher value of 91.35% indicates that the lipid contains a substantial amount of fatty acid components that are nonvolatile and insoluble in water. The Reichert-Meissl and Polenske values 1.26 and 0.80 respectively, show that the volatile water soluble and volatile water insoluble but alcohol soluble fatty acid components are both present in low concentrations in the lipid sample. A study on the effects of storage time found that while iodine value, thiocyanogen value and R-M value decrease with time, acid value and peroxide value increase. This indicates that as lipids are stored, their quality degrades with time.

Table 3: Chemical parameters of the muscle lipid of the Bombay duck (*H. nehereus*) and relevant fats and oils.

Name of sample	S.V.	S.E.V.	A.V.	F.F.A.% as oleic acid	E.V.	I.V.	T.V.	P.O.V.	U.S.M.	Acetyl Value	H.V.	R.M.V.	P.V.
Sardine oil	190-194	----	2.2-21.7	----	----	139	----	----	----	----	----	----	----
Whale oil	184-200	----	0.3-51.4	----	----	127	----	----	----	----	----	----	----
Muscle lipid of Cuttle fish	260.9	215.1	1.78	0.89	----	106.8	54.8	----	1.10	12.95	77.9	0.91	0.72
Hilsha fish oil	203.25	276.01	3.11	1.56	----	92.55	52.5	55.05	0.74	10.25	93.23	0.965	0.76
Brain lipid of Baghda chingri	229.3	244.7	1.11	0.56	----	95.8	43.6	----	0.57	10.58	95.3	1.04	0.79
Muscle lipid of Bombay duck	189.71	295.71	1.78	0.89	187.93	108.23	66.25	19.63	0.72	11.60	91.35	1.26	0.80

S.V.= Saponification value; S.E.V.= Saponification equivalent value; A.V.= Acid value; F.F.A.= Free fatty acid; E.V.= Ester Value; I.V.= Iodine Value; T.V.= Thiocyanogen value; P.O.V.= Peroxide value; U.S.M.= Unsaponifiable matter; H.V.= Henher value; R.M.V.= Reichert-Meissl value; P.V.=Polenske Value.

3.3 Chromatographic Examination

The fatty acids in the muscle lipid of the Bombay duck were identified and quantified by GLC, and the results are presented in Table 4. According to the analysis, the investigational lipid had a high concentration of polyunsaturated fatty acids (ω-3 &ω-6). The amount of omega-3 fatty acids in the muscle lipid of the Bombay duck was 19.5245%, with docosahexaenoic acid (13.1954%) coming in higher than eicosapentaenoic acid (4.4751%) and linolenic acid (1.8536%).Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) have biochemical impacts on the prevention and treatment of many disorders and diseases including diabetes, cancer, rheumatoid arthritis, asthma, and coronary heart disease [19]. However, the lipid sample contains 3.6984% omega-6 fatty acids, with Arachidonic acid (2.0950%) outpacing Linoleic acid (1.6034%).

Table 4: Fatty acid composition of the muscle lipid of Bombay duck by GLC

Types of fatty acid	Name of fatty acid		Relative percentage (%)	Total (%)	
Saturated fatty acid	Myristeric acid (C14:0)		5.7543	42.3412	
	Palmitic acid (C16:0)		29.6246		
	Stearic acid (C18:0)		4.9802		
	Behenic acid (C22:0)		-----		
	Lignoceric acid (C24:0)		1.9821		
Unsaturated fatty acid (Mono unsaturated fatty acids MUFA)	Myristoleic acid (C14:1)		-----	34.4958	
	Palmitoleic acid (C16:1)		14.1460		
	Oleic acid (C18:1)		20.2898		
Unsaturated fatty acid (Poly unsaturated fatty acids- PUFA)	ω-3 PUFA	Linolenic acid (C18:3)	1.8536	19.5245	23.2229
		Eicosapentaenoic Acid (EPA) (C20:5)	4.4751		
		Docosahexaenoic Acid (DHA) (C22:6)	13.1954		
	ω-6 PUFA	Linoleic acid (C18:2)	1.6034	3.6984	
		Arachidonic acid (C20:4)	2.0950		

3.4 Microbial activities of the muscle lipid of Bombay duck

The lipid extracted from the muscle of Bombay duck, was used in this study to test its antibacterial and antifungal properties against four pathogenic bacteria and four phytopathogenic fungi, respectively.

3.4.1 Bacterial activity test

The antibacterial effects of the lipid sample were examined using two gram positive and two gram negative bacteria. We used paper discs that had been dipped in lipid solutions (10% and 5%). The lipid sample solution was found to have antimicrobial activity against *Salmonella typhi*, *Escherichia*

coli, and Bacillus cereus (Table 5). Compared to other test bacteria, the sample lipid displayed a higher (18 mm) inhibitory zone against *Salmonella typhi*. There is no inhibitory action of Bombay duck muscle lipid against *Staphylococcus aureus*.

Table 5: Antibacterial activity of the muscle lipid of Bombay duck

Name of bacteria	Type of sample	After 48 hours, Inhibitory zone (diameter in mm)		
		Treatment	Control	Differences
Salmonella typhi	10%	18	0	18
	5%	9	0	9
Staphylococcus aureus	10%	0	0	0
	5%	0	0	0
Escherichia coli	10%	15	0	15
	5%	7	0	7
Bacillus cereus	10%	14	0	14
	5%	6	0	6

3.4.2 Fungal activity test

The lipid sample's antifungal effects on four phyto-pathogenic fungi were investigated. Table 6 demonstrates clearly that the muscle lipid of Bombay duck stimulated themycelial growth of *Aspergillus fumigateus*. Except for this, the lipid sample inhibited the mycelial development of practically all test fungus (i.e. *Fusarium equiseti*, *Alternaria alternate* and *Curvularia lunata*). Compared to other test fungi, the Bombay duck's muscle lipid displayed a higher inhibitory zone against *Curvularia lunata* (19.9920 mm).

Table 6: Bombay duck muscle lipid's percentage-based ability to inhibit the growth of test fungi

Name of fungi	Type of sample	% inhibition of Bombay duck muscle lipid after 5 days
Fusarium equiseti	10%	12.1823
Aspergillus fumigateus	10%	-10.1012
Alternaria alternata	10%	9.8780
Curvularia lunata	10%	19.9920

Further investigation into the lipid sample's antibacterial and antifungal properties was not possible due to lack of laboratory resources and facilities. We can infer from the findings that this investigation will offer useful information about the possibility of medications and insecticides being derived from the muscle lipid of Bombay duck.

3.5 Quantification of N, P, K and Ca in lipid containing muscle of Bombay duck

Percent of N, P, K and Ca in lipid containing muscle of Bombay duck was measured by using standard methods. The majority of people of Bangladesh have severe protein deficiency problems. It is clear from Table 7 that Bombay duck has a sufficient amount (3.460%) of protein (proteineous nitrogen) and is perfectly balanced in terms of essential amino acids. It was encountered that the phosphorus content in the lipid-containing muscle of the Bombay duck was 0.750 percent. According to the experimental findings, Bombay duck muscles that contain lipids may contain phospholipid. It was observed that the potassium content in the lipid-containing muscle of the Bombay duck was 0.499 percent. Eating this marine species may help people with low blood pressure raise their readings. As a result, this might be used to treat low blood pressure. The amount of calcium was determined to be 3.033 percent in the lipid-containing muscle of the Bombay duck. Children that consume this marine species in their growing years may benefit from more solid bone structure.

Table 7: Percent of N, P, K and Ca in lipid containing muscle of Bombay duck

Name of the sample	N (%)	P (%)	K (%)	% Ca (%)
Brain lipid of Kerani Chingri	3.090	0.551	1.061	0.798
Brain lipid of Baghda Chingri	3.540	0.726	1.130	0.914
Liver lipid of Blue Spotted Fantail ray	4.099	2.750	1.180	0.641
Muscle of Bombay duck	3.460	0.750	0.499	3.033

4. Conclusion

In the current study, the muscle lipid of Bombay duck (*Harpadon nehereus*) was characterized physico-chemically and microbiologically. R.I., T.V., and I.V. confirmed that the lipid sample contained a reasonable quantity of unsaturated fatty acids% of F. F. A. verified the fish lipid's suitability for consumption. By I.V. and Elaiden test, the semidrying character of the muscle lipid in the Bombay duck was identified. The existence of some significant PUFAs in the fish lipid was confirmed by gas liquid chromatographic analysis. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) which play therapeutic roles in lowering blood triglycerides and thereby effectively preventing cardiovascular disease and obesity, were found in higher concentrations in the extracted lipid's fatty acid composition. We learned from the current study that the extracted lipid has some bacterial and fungal inhibitory properties. The extracted lipid may therefore be used to make topical medications such as germicides, antibacterial creams, antifungal ointments etc. It should be encouraged to catch as much Bombay duck as possible and to use it as a readily available source of vital nutrients as revealed from mineral analysis.

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6. References

1. Begum M, Ahmed ATA, Das MS, Parveen SA. comparative microbiological assessment of five types of selected fishes collected from two different markets. J Advan. Biol. Res. 2010;4:259-265.
2. Ibrahim HI, Bayir A, Sirkecioglu AN, Aras NM, Atamanalp M. Comparison of fatty acid composition in some tissue of rainbow trout (*oncorhychusmy kiss*) living in seawater and freshwater. J. Food Chem. 2004;86:55-59.
3. Stansby ME. Nutritional properties of fish Oils. World review of nutrition and dietetics. 1969;11:46-105.
4. Erkkila AT, Lehto S, Pyorala K, Uusitupa MI. N-3 Fatty acids and 5-y risks of death and cardiovascular disease events in patients with coronary artery disease. Amer. J Clinical Nutr. 2003;78:65-71.
5. Dallongeville J, Yarnell J, Ducimetiere P, Arveiler D, Ferriers J, Montaye M, *et al*. Fish consumption is associated with lower heart rates. Circulation. 2003;108(7):820-825.
6. James MJ, Gibson RA, Cleland LG. Dietary polyunsaturated fatty acids and inflammatory mediator production. Amer. J Clinical Nutr. 2000;71:343S-348S.
7. Rajagopalan V, Abinaya G, Balasubramanian U. A study on biochemical and molecular characterization of a marine fish Lutjanuscampechanus from Nagapattinam, Tamilnadu. J. of Sc. & Inn. Res. 2013;2:303-319.
8. Sankar S, Sujith P, Jayalakshmi S. Microbial study and proximate compositionof six marine fish species in mudasaladai costal region. Int. J Pharm. and Bio. Sc. 2013;3:398-404.
9. Ranganna S. Handbook of Analysis and Quality Control for Fruit and vegetable Products. Edn 2, Tata McGraw-Hill Publishing Company Ltd, New Delhi, India; c1991. p. 3-226.
10. Griffin RC. Technical Method of Analysis, Edn 2, McGraw-Hill Book Company Inc, New York. 1972;309:319-342.
11. Morris BJ. The Chemical Analysis of Foods and Food Products, D. Van Nostrand Company Inc, New York. 1965;375:382.
12. Ranganna S. Handbook of Analysis and Quality Control for Fruit and Vegetable Products, Edn 2, Tata McGraw-Hill Publishing Company Ltd, New Delhi, India; c1991. p. 3-226.
13. Das RK. Industrial Chemistry, Part II, Kalyani Publishers, New Delhi, India; c1989. p. 250-259.
14. Williams KA. Oils, Fats and Fatty Foods, Edn 4, J. & A. Churchill Ltd. 1966;124:275,329,334,356,391.
15. Nelson GJ. The lipid composition of normal mouse liver. J Lipid Res. 1962;3:71.
16. Horwing EC, Ahrens EH, Lipsky SR. Quantitative analysis of fatty acids by gas- liquid chromatography. J Lipid Research. 1964;27:5.
17. Ahmed S, Rahman MS, Chowdhury JU, Begum J, Anwar MN. Antimicrobial activities of seed extracts and crude alkaloids of algae *marmelos* (L) Corr. Chittagong Univ. J Sci. 1998;22:77-81.
18. Basett J, Denney RC, Heffery GH, Mendham J Vogel's Textbook of Quantitative Inorganic Analysis. Edn 4, Longman Group UK Ltd; c1978. p. 837.
19. Pamela M. Fish oil Nutrition. The Pharmaceutical Journal. 2001;265:720-724.