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Extraction, Physico-Chemical Analysis and Microbial Activities Evaluation of the Body Lipid of Tripletail (*Lobotes surinamensis*) of the Bay of Bengal

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Abstract: Due to the discernible variation in the marine fish lipids compositions, the direct and indirect outcomes of it on human health physiology and nutrition have emphasized heightened focus. For this, the inquisition was reported with the extraction of the body lipid of Tripletail (*Lobotes surinamensis*) of the Bay of Bengal by Bligh and Dyer extraction method and different analytical parameters were rummaged and assimilate with those of typical fish lipids. The presence of several fatty acids namely myristic acid (C14:0), palmitic acid (C16:0), stearic acid (C18:0), palmitoleic acid (C16:1), oleic acid (C18:1), vaccenic acid (C18:1), linoleic acid (C18:2), linolenic acid (C18:3), arachidonic acid (C20:4), eicosadienoic acid (C20:2), eicosapentaenoic acid (C20:5), docosapentaenoic acid (C22:5) and docosahexaenoic acid (C22:6) etc was confirmed by Gas-Liquid Chromatography (GLC). It revealed that Saturated Fatty Acids (SFA%) were higher than Unsaturated Fatty Acids (UFA%). Moreover total Monounsaturated Fatty Acids (MUFA%) were higher than Polyunsaturated Fatty Acids (PUFA%) i.e. [25.5777% > 12.6850%] and ω-3 Polyunsaturated Fatty Acids (ω-3 PUFA) were higher than ω-6 Polyunsaturated Fatty Acids (ω-6 PUFA) i.e. [8.1113% > 4.5737%] along with higher ω-3/ω-6 ratio (1.77). The muscle of the target specimen was assessed to quantify the minerals content (N, P, K and Ca). Several metals (Fe, Pb, Ni, Co, Cd, Cu, Zn, Mn, Cr, As, Mg) were estimated with the help of Atomic Absorption Spectrophotometric (AAS) method. Additionally, using established techniques, the isolated lipid sample was evaluated for microbial (bacterial and fungal) activity. Thus from the findings, it is resolved with a variety of significant facts relating to nutritional and medicinal aspects.

Keywords: Lipid, Tripletail, Fatty Acid, PUFA, Mineral Content, Microbial Activity

1. Introduction

Bangladesh is encircled by a broad belt of the Bay of Bengal's coastline (13° 31' 53.994"N 87° 32' 22.5096"E) along its southern frontier and the majority of its population relies on marine fish as a source of protein, which accounts for around 75-80% of the country's total animal protein [1]. Tripletail sometimes referred to as Samudra Koi or Koi Koral locally, is a significant component of Bangladesh's fisheries. It is abundant in the Bay of Bengal, but the general public is unaware of its significance, and there is a lack of information regarding its nutritional worth and potential medicinal uses. At this time, the physiological effects of fish lipids on human diet and health [2, 3] have revived focus on the relative variation in fish oil compositions [4]. We all understand that essential fatty acids are necessary precursor in the biosynthesis of a group of fatty acids derivatives called

prostaglandins, hormone like compounds which in trace amounts have profound effects on a number of important physiological activities. Polyunsaturated fatty acids (PUFAs) are mostly found in fish lipids [5], and among PUFAs, eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6) are reported to reduce blood cholesterol levels and platelet aggregation [6]. In addition to their importance in membrane biochemistry, lipids and fatty acids also have a direct impact on human membrane-mediated processes like balance of osmotic pressure, uptaking and transport of nutrients [7]. Recently scientists are focusing more on analytical analysis, microbiological research, and the fatty acid profile of lipids in diverse marine fishes, but the outcomes of these sorts of studies on Tripletail are largely unreported or are reported in a very limited way. The current study is concerned with the isolation of the body lipid of Tripletail with the intention of determining the appearance of PUFAs in it, studying its physico-chemical constants and microbial activities, including quantifying the mineral content, and assimilating the outcomes with the information about pharmacological aspects available in the literature.

Figure 1. *Tripletail (Lobotes surinamensis).*

2. Materials and Methods

2.1. Sample Collection and Specification

The target specimen, Tripletail fish (*Lobotes surinamensis*) was collected from Sadarghat (22°20'18.24" N 91°49'54.05" E), one of the largest local fish market in Chattogram which is located in the coast of Bay of Bengal. The fish was titled as export quality and the seller ensured that it was caught from the Exclusive Economic Zone (EEZ) of Bangladesh of the Bay of Bengal. After collection, the specimen was specified at the same time in Zoology department and in the Institute of Marine Science & Fisheries of the University of Chittagong. The muscle was carefully separated and kept frozen until subsequent works.

2.2. Extraction of the Lipid

Using acetone and ethyl acetate as solvents, the Bligh and Dyer method was used to extract the lipid from the Tripletail's muscle. The extract was then dried, solvent-free, first using rotary evaporation and then through the slow-flowing of nitrogen gas. All the chemicals and reagents that are used in this investigation were of analytical grade and these were prepared by following the standard procedures [8-10].

2.3. Analysis of Physical and Chemical Properties

To characterize the physical properties, refractive index was determined by abbe refractometer. Specific gravity bottle was used to measure specific gravity of the sample. The co-efficient of viscosity of the lipid solution at different temperature was determined with Ostwald viscometer. Also by following established procedures, ash content, protein content and fiber content of the de-oiled muscle of the Tripletail fish were determined [11]. Chemical parameters like saponification value and saponification equivalent value were assessed by using potassium hydroxide. Acid value and percentage of free fatty acid (as oleic acid) were determined to evaluate the quality of lipid sample. Iodine value was determined by using Hanus method. To measure the free hydroxyl group in the lipid sample, acetyl value was determined [12]. Peroxide value was determined by using potassium iodide [13]. By following the established methods, thiocyanogen value, Richert Meissl value, Polenske value [11], Henher value, Elaiden test [14] and quantity of unsaponifiable matter [15] of the body lipid of Tripletail were determined.

2.4. Chromatographic Examinations

The methyl esters of the fatty acids were analyzed by GLC, using a packed column. The peak areas were calculated from their height and width at the half height. Individual fatty acids ester was identified by comparing its retention time (RT) with those of reference esterified fatty acid samples. The amount of fatty acids was calculated by comparing with the peak area of benzoic acid used as internal standard [16].

2.5. Estimation of Minerals

To estimate the minerals content (N, P, K and Ca) of lipid containing muscle of Tripletail fish, standard methods were applied [17].

2.6. Metal Analysis

Wet digestion was used to prepare the sample solution, which was then refrigerated until analysis to determine the amount of metal in the fish muscle. An exactly similar blank solution of 100 ml was prepared for the test. A thermoscientific ICE 3000 series Atomic Absorption Flame Emission Spectrophotometer was used to determine the presence and concentration of metals in the sample [16, 18].

2.7. Microbial Analysis

Using five bacteria and five fungi, the microbial activities (antibacterial activities and antifungal activities) of the body lipid of Tripletail were assessed. The disc diffusion method and food poison technique [19] were followed using Nutrient Agar (NA) and Potato Dextrose Agar (PDA) as basal medium for the detection of antibacterial and antifungal activities respectively. Using chloroform as a solvent 10% and 5% solution of the lipid sample were prepared.

3. Results and Discussion

3.1. Physical Characteristics

It was discovered that the amount of total lipid content isolated from Tripletail’s body was 21.33 mg/g. This could

result in significant demand for culinary purposes because of its greater lipid content. Tripletail's body lipid was revealed to have a refractive index of 1.4753 at 28°C (Table 1). Oils and fats refractive power varies somewhat and is mostly determined based on the amount and to what extent degree of unsaturation present. Refractive index is a fundamental property of all substances. The current finding suggests that the specimen's body lipid contained a considerable level of unsaturated fatty acids. Its iodine value also provides support for this. From our analysis, we got the specific gravity and viscosity of the lipid solution of target specimen 0.933 and 298.01 milipoise respectively at 28°C. As the sample was found in semisolid condition, so the lipid solution was used to determine specific gravity and viscosity. The result of viscosity indicates that intermolecular hydrogen bonding may exist in the lipid sample. It also suggests that there may exist a few hydroxyl groups and few free acid molecules which is favored by low acetyl value and low acid value.

Table 1. *Physical constants of the body lipid of Tripletail and other fish lipid sample.*

Name of the sample	Refractive index	Specific gravity	Viscosity (mp)
Brain lipid of Baghda Chingri	1.4736	0.941	303.26
Liver lipid of Spanish fish	1.4630	0.919	361.00
Muscle lipid of Cuttle fish	1.4731	0.973	320.32
Body lipid of Vetki fish	1.4745	0.923	290.38
Body lipid of Tripletail fish	1.4753	0.933	298.01

1.65% moisture was detected in the extracted fish lipid sample which is relatively low. The lower the moisture content remains in extracted fish lipid sample, the less likely it is to spoil. The de-oiled muscle of tripletail was analyzed for the determination of ash, protein and crude fiber content and their values found to be 1.27%, 53% and 2.21% respectively.

Table 2. *Moisture content of body lipid; ash, protein and crude fiber content of the oil free muscle of Tripletail.*

Name of the sample	Moisture content (%)	Ash content (%)	Protein content (%)	Crude fiber content (%)
Body lipid of Tripletail	1.65%	1.27	53	2.21

3.2. Chemical Characteristics

Table 3 displays the findings for various chemical constants from the examination of the body lipid of the Bay of Bengal Tripletail. Here, it was found to have a saponification value (S. V.) of 288.01 and saponification equivalent (S. E. V.) of 194.78. The carbon-carbon chain length of the fatty acids which can also be expressed by the average molecular weight is inversely proportional to the saponification value. On the other hand, it is however, directly relates to the saponification equivalent. The data unmistakably show that the extracted fish lipid sample holds a greater proportion of high molecular weight fatty acids.

The acid value (A. V.) of Tripletail's body lipid was found to be 1.57. Additionally, the proportion of free fatty acid (F. F. A.), as oleic, was determined from the acid value and was found to be 0.76%. The quantity of free fatty acids in an oil or fat is measured by its acid value. Therefore, a low acid value of the extracted lipid sample indicates freshness, and a low proportion of free fatty acid indicates that the lipid is suitable for use in edible products. Through calculation, the ester value (E. V.) was determined to be 284.84. This value represents the quantity of ester in the lipid sample.

According to the iodine value (I. V.) of 110.25, the lipid has a considerable number of carbon-carbon double bonded fatty acid components and is of the semidrying type. The outcomes of the physical property test and the Elaiden test also support this.

The peroxide value (P. O. V.) serves as a gauge for the amount of unsaturation in fats and oils. Unsaturated fats and oils that are higher in content absorb more oxygen, produce more unstable hydro peroxide, and have higher peroxide values. Tripletail's body lipid was discovered to have a peroxide value of 112.44. The result indicates that the body lipid under study contains a significant proportion of unsaturated fatty acids. The sample's thiocyanogen value (T. V.) was found to be 56.76. This finding is consistent with the results showing a modest iodine value and peroxide value for the lipid sample.

The acetyl value of 9.20 suggests that the lipid sample has little free hydroxyl group as it is an indicator of the quantity of it in a lipid sample. The solidifying point or the titre value of the body lipid of Tripletail was determined to be 28°C which suggested that the lipid sample is of fat type.

The unsaponfiable matter (U. S. M.) in the lipid of Tripletail was found to be 0.898%. All substances that cannot be saponified by an alkali but are soluble in ether or petroleum ether are referred to as unsaponifiable matter. A fixed oil or fat should generally be considered adulterated if it contains unsaponifiable materials in excess of 2%. The outcome suggests that a minor quantity of unsaponifiable material, such as sterols, vitamins A and D, hydrocarbons, etc., may be present in the lipid sample.

The Reichert-Meissl value (R. M. V.) indicates the amount of volatile, water-soluble acid constituents of the lipid whereas the Polenske value (P. V.) is a measure of steam distillable, water-insoluble acid constituents of the lipid sample. The Reichert-Meissl value and Polenske value was found to be 1.65 and 1.60 respectively which are the evidence that there are relatively few volatile water-soluble and volatile water-insoluble but alcohol soluble fatty acids may present.

It was discovered that the Tripletail's muscle lipid has a Henher value (H. V.) of 88.25%. This finding shows that a higher concentration of non-volatile, water-insoluble fatty acids may present in the extracted lipid sample.

The body lipid of the Tripletail has a Kirschner value (K. V.) of 0.334. This outcome shows that the Reichert-Meissl distillate contains a small quantity of fatty acid, which results in the formation of soluble silver salt. During the experiment, it was obtained that the body lipid of the Tripletail formed cloudy solution with bromine and a precipitate formed due to the insoluble bromide. The lipid is therefore marine oil (fish oil).

Throughout the test, it was found that after 24 hours, the body lipid of the Tripletail had a treacle-like consistency when exposed to the mercuric nitrate, $Hg(NO_3)_2$ solution. As a result, the lipid is of the semi-drying type. It was discovered that Tripletail had 23.95 mg/g of cholesterol in its body lipid. In the body lipid of Tripletail, less cholesterol is found. According to its cholesterol content, it can be inferred that Tripletail fish has lipids that are more acceptable for eating.

The Tripletail's body lipid revealed a substantial fluctuation in different values as a result of storage duration. As storage time rose, the value of the acid and peroxide grew, but the value of the R-M, thiocyanogen, titre, and iodine declined. This means that as storage time increased, the quality of the lipids degraded.

Table 3. *Chemical constants of the body lipid of Tripletail and some related fish lipid sample.*

Name of the Sample	S. V.	S. E. V.	A. V.	F. F. A. (%) (as oleic)	E. V.	I. V.	P. O. V.
Body lipid of Hilsa	203.25	276.01	3.10	1.56	---	92.55	55.05
Brain lipid of Baghda Chingri	229.255	244.71	1.11	0.56	28.14	95.83	194.95
Brain lipid of Kerani Chingri	214.11	262.06	1.04	0.52	13.07	100.38	192.26
Muscle lipid of Cuttle fish	260.87	215.05	1.78	0.89	258.77	106.82	109.45
Body lipid of Indian mackerel	208.12	269.556	1.18	0.594	206.94	112.36	122.72
Body lipid of Tripletail	288.01	194.78	1.57	0.76	280.84	110.25	112.44

Table 3. *Continued.*

Name of the Sample	Acetyl Value (%)	T. V.	Titre value (°C)	H. V.	U. S. M. (%)	P. V.	R. M. V.
Body lipid of Hilsa	10.255	52.54	---	93.27	0.74	0.764	0.96
Brain lipid of Baghda Chingri	10.58	43.63	27.2	95.32	0.566	0.796	1.04
Brain lipid of Kerani Chingri	10.82	45.29	26.7	92.19	0.641	0.694	0.95
Muscle lipid of Cuttle fish	12.95	54.82	27.5	77.98	1.10	0.72	0.91
Body lipid of Indian mackerel	10.75	58.82	26.2	92.63	0.622	0.86	1.06
Body lipid of Tripletail	9.20	56.76	28	88.25	0.898	1.60	1.65

3.3. Chromatographic Analysis

GLC analysis was used to obtain qualitative and quantitative data about the presence of myristic acid, palmitic acid, stearic acid, lignoceric acid, palmitoleic acid, oleic acid, vaccenic acid, linoleic acid, linolenic acid, arachidonic acid, eicosadienoic acid, eicosapentaenoic acid, docosapentaenoic acid and docosahexaenoic acid (Table 4). The saturated fatty acid content of the body lipid of the Tripletail was found to be 61.7373%, with palmitic acid (41.8165%) being maximum than other saturated fatty acids such as myristic acid (7.6346%) and stearic acid (10.3323%). It also revealed that there remained monounstaturated palmitoleic acid (8.5077%) and oleic acid (12.7521%). The fatty acids profile obtained from GLC test expressed that the Tripletail's body lipid possessed a large proportion of PUFA, polyunsaturated fatty acid (ω-3 & ω-6). The amount of ω-3 fatty acids in the body lipid of the Tripletail is 8.1113%, with docosapentaenoic acid (3.0622%) being higher than docosahexaenoic acid (2.3351%) and eicosapentaenoic acid (1.5785%). While the lipid contained 4.5737% ω-6 fatty acid in which eicosadienoic acid (1.9678%) is higher than linoleic acid (1.4545%) and arachidonic acid (1.1514%). From the experimental results and calculation the ω-3/ω-6 ratio was found to be (1.77).

Table 4. *Fatty acid composition of the extracted body lipid of Tripletail.*

Types of fatty acid	Name of fatty acid	Relative percentage (%)	Total (%)
Saturated fatty acid (SFA)	Myristic acid (C14:0)	7.6346	61.7373
	Pentadecyclic acid (C15:0)	0.8249	
	Palmitic acid (C16:0)	41.8165	
	Margaric acid (C17:0)	0.8857	
	Stearic acid (C18:0)	10.3323	
	Arachidic acid (C20:0)	0.2433	
Monounsaturated fatty acid (MUFA)	Palmitoleic acid (C16:1)	8.5077	25.5777
	Heptadecenoic acid (C17:1)	0.5138	
	Oleic acid (C18:1)	12.7521	
	Vaccenic acid (C18:1)	3.6540	
	Eicosenoic acid (C20:1)	0.1501	

Types of fatty acid	Name of fatty acid		Relative percentage (%)	Total (%)	
Polyunsaturated fatty acid (PUFA)	ω-3 PUFA	Hexadecatrienoic acid (C16:3)	0.7961	8.1113	12.6850
		Linolenic acid (C18:3)	0.3394		
		Eicosapentaenoic Acid (C20:5)	1.5785		
		Docosapentaenoic acid (C22:5)	3.0622		
		Docosahexaenoic Acid (C22:6)	2.3351		
	ω-6 PUFA	Linoleic acid (C18:2)	1.4545	4.5737	
		Eicosadienoic acid (C20:2)	1.9678		
		Arachidonic acid (C20:4)	1.1514		

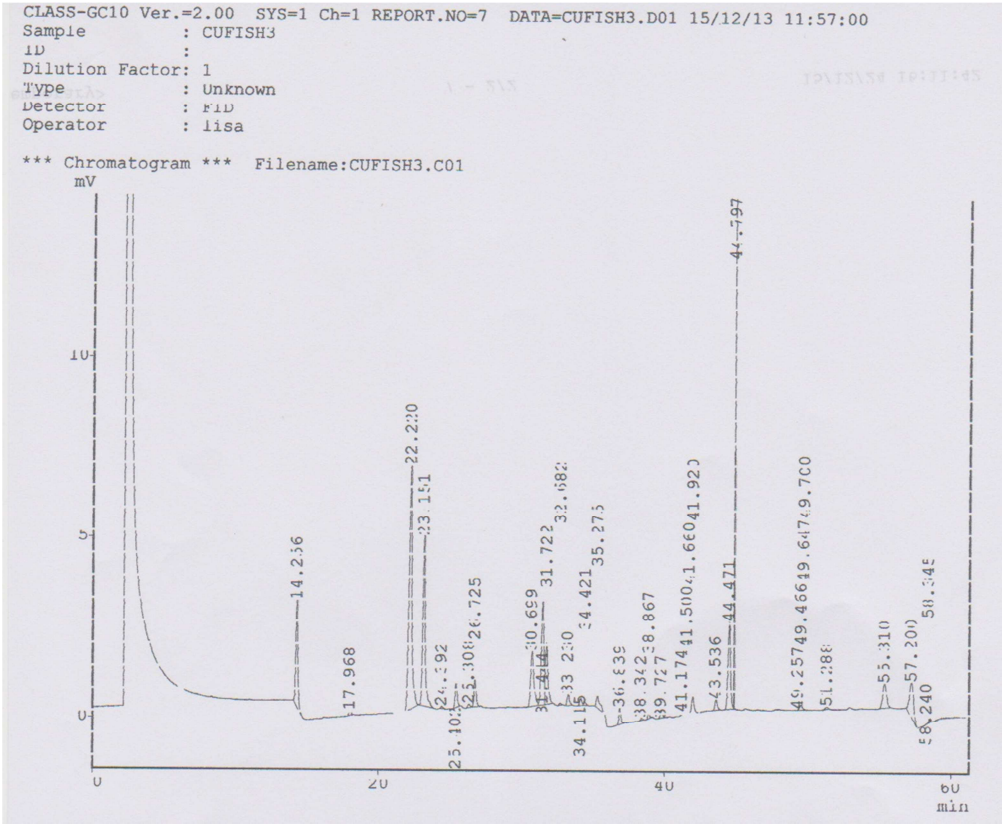

Figure 2. *Chromatogram of body lipid analysis of Tripletail.*

3.4. Estimation of Minerals (N, P, K and Ca)

The majority of people in our country have been severely suffering from protein deficiency. Tripletail contains a good amount of nitrogen (8.480%) as well as protein (proteineous nitrogen) which is well balanced in respect of essential amino acids (Table 5). The presence of phospholipid in the lipid sample is suggested by the amount of phosphorus (1.182). For those who have low blood pressure, the potassium content (1.133%) of the Tripletail muscle may assist to raise blood pressure. It was found that the tripletail fish's muscle had 0.645% calcium (Table 5). Children that consume this marine species in their growing years may benefit from more solid bone structure.

Table 5. *Percentage of minerals (N, P, K and Ca) in the muscle of Tripletail.*

Name of the sample	N (%)	P (%)	K (%)	% of Ca (%)
Brain lipid of Kerani Chingri	3.090	0.550	1.061	0.798
Brain lipid of Baghda Chingri	3.540	0.726	1.123	0.914
Muscle lipid of Hilsa	4.099	2.750	1.180	0.641
Muscle of Cuttle fish	6.533	1.237	1.118	0.450
Muscle of Tripletail	8.480	1.182	1.133	0.645

3.5. Metal Analysis of the of Tripletail

The muscle of selected specimen was analyzed for several metals. From the results (as shown in Table 6) the lipid containing muscle of Tripletail contains 106.19 ppm Iron (Fe), 269.54 ppm Magnesium (Mg) and 31.93 ppm Zinc (Zn). So, this specimen may be a good source of Fe, Mg and Zn. Cadmium (Cd), Lead (Pb), Manganese (Mn) and Arsenic (As) content were 0.53 ppm, 4.46 ppm, 1.14

ppm and 0.19 ppm respectively which is within permissible limit prescribed by World Health Organization (WHO). Cobalt (Co), Nickel (Ni) and Chromium (Cr) were not found in the selected specimen.

Table 6. *Concentration of different metals in the lipid containing muscle of Tripletail.*

Metals	Concentration (ppm)	Metals	Concentration (ppm)
Cd	0.53	Zn	31.93
Pb	4.46	Cr	-
Co	-	Cu	13.58
Fe	106.19	As	0.19
Mn	1.14	Mg	269.54
Ni	-		

3.6. Microbial Activities of the Lipid Sample

To evaluate the microbial activities, the extracted lipid solution was tested against five human disease causing bacteria and against five plant disease causing fungi.

3.6.1. Bacterial Activity Test

Two gram positive and three gram negative bacteria were used to test the antibacterial activity of the lipid sample. Paper disc of 10 mm soaked in extracted lipid solution (10% and 5%) and Petri plate of 70 cm in diameter were used throughout the experiment. From the evaluation, we got the inhibitory action of the lipid solution against *Salmonella typhi, Staphylococcus aureus* and *Escherichia coli* but there was no such action found against *Bacillus cereus* and *Proteus vulgaris* (Table 7). The Tripletail lipid exhibited higher zone of inhibition (20 mm in diameter) against *Salmonella typhi* at 10% sample solution. It also exhibited 19 mm and 17 mm zone of inhibition against *Staphylococcus aureus* and *Escherichia coli* respectively at 10% sample solution. Regarding *Bacillus cereus* and *Proteus vulgaris* bacteria, no zone of inhibition was seen.

Table 7. *Antibacterial activity of the body lipid of Tripletail.*

Name of bacteria	Type of sample	Zone of inhibition (diameter in mm) after 48 hours		
		Treatment	Control	Differences
Salmonella typhi	10%	20	0	20
	5%	9.5	0	9.5
Staphylococcus aureus	10%	19	0	19
	5%	9	0	9
Escherichia coli	10%	17	0	17
	5%	8	0	8
Bacillus cereus	10%	0	0	0
	5%	0	0	0
Proteus vulgaris	10%	0	0	0
	5%	0	0	0

3.6.2. Fungal Activity Test

Five phytopathogenic fungi were used to assess the antifungal activities of the extracted lipid sample. It is observed that the muscle lipid of Tripletail strongly promoted rather than inhibited *Curvularia lunata*'s mycelial proliferation (Table 8). The lipid sample exhibited maximum inhibition (12.954 mm) after 5 days against *Alternaria alternate*. It also exhibited 10.354 mm, 10.124 mm and 9.467 mm zone of inhibition against *Aspergillus flavus, Fusarium equiseti* and *Aspergillus fumigateus*.

Table 8. *Percent growth inhibition of test fungi by the body lipid of Tripletail.*

Name of the fungi	Type ofs sample	% inhibition after 5 days
		Muscle lipid of Tripletail
Fusarium equiseti	10%	10.124
Aspergillus fumigateus	10%	9.467
Alternaria alternata	10%	12.954
Curvularia lunata	10%	-11.465
Aspergillus flavus	10%	10.354

(-) means no inhibition

4. Conclusions

The current inquisition can be taken into account as an effort to assess the total lipid and lipid types in the nearby marine resources, particularly for PUFAs (polyunsaturated fatty acids, or ω-3 and ω-6 fatty acids), which are crucial for lowering cardiovascular complications and obesity. Here, the findings show that the extracted lipid holds a modest amount of unsaturated fatty acids, which was supported by R. I., I. V., and T. V. Percentage of F. F. A. affirmed decency of the fish lipid for consumption. By I. V. and Elaiden test, the semidrying character of the body lipid of the Tripletail was identified. The existence of some significant ω-3 PUFAs, including eicosapentaenoic acid, docosapentaenoic acid, and docosahexaenoic acid, which have a therapeutic role in lowering blood triglycerides, was confirmed by Gas-Liquid Chromatographic (GLC) analysis. It may be said that the concentration of metals in the fish sample are below the WHO's recommended limit for fish and safe for human consumption. The extracted lipid may also be used to make topical medications as antifungal ointments, antibacterial creams, germicides, etc. due to its inhibitory properties against a few bacteria and fungi.

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