Analytical Characterization and Antimicrobial Studies on Muscle Lipid of Silver Ribbon fish (*Lepturacanthus savala*) of the Bay of Bengal

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Abstract

Muscle (edible portion) lipid of Silver Ribbon fish (*Lepturacanthus savala*) was extracted by solvent extraction method and characterized with respect to various physical and chemical properties and compared those with other lipid samples. Unsaturated fatty acids in this muscle lipid sample were confirmed by refractive index and iodine value. Presence of good amount of sterols, tocopherols, vitamins A & D, is considered with respect to unsaponifiable matter and percentage of F.F.A validated suitability of the oil for edible purpose. Semi drying nature of the fish lipid was pointed out by iodine value and confirmed by Elaiden test. Chromatographic examinations substantiated the presence of Palmitic acid, Mystiric acid, Linoleic acid, and Erucic acid in this muscle lipid. In antibacterial studies of lipid of Ribbon fish showed good growth of inhibition against three pathogenic bacteria (both Gram positive and Gram negative) out of ten human pathogenic bacteria. The mycelial growth of (three out of four) tested phyto-pathogenic fungi was inhibited by this lipid sample. Protein and other important minerals (Ca, P and K) also found with significant values in the muscle of Silver Ribbon fish.

Key words: Lipid, Polyunsaturated fatty acid (PUFA), Antibacterial, Antifungal, Silver Ribbon fish, Protein, Mineral.

1. Introduction

Bangladesh has more than 118,813 sq kms of territorial sea, 200 nautical miles (NM) of exclusive economic zone and all kinds of animal and non-animal resources under the continental shelf up to 354 NM from the Chittagong coast and these areas are constantly supplying a variety of economically important fishes. Out of them Silver Ribbon fish (*Lepturacanthus savala*) is found plenty in the Bay of Bengal but people are not aware about the importance of this fish and sufficient data of the food value and pharmaceutical aspects are not available also. Marine oils are rich in polyunsaturated fatty acids (PUFA), especially those of the ω 3family such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) [1, 2]. Effects of n-3 fatty acids on coronary heart disease have been shown in hundreds of experiments in animals and in humans, tissue culture studies, and clinical trials [3, 4]. On the other hand significant effect of storage-time is observed due to its high content of polyunsaturated fatty acids, including EPA and DHA, fish oils are highly susceptible to oxidative spoilage and the rate of fish oil oxidation is significantly different from that of other oils [5]. Several research groups have studied and reported different antimicrobial properties of marine lipids [6-8].

Now a day's researchers are giving more emphasize on physico-chemical analysis, microbial study and proximate composition of various marine fishes [9, 10]. However, results of such types of studies on Silver Ribbon fish (*Lepturacanthus savala)* are very unknown or less reported but this fish is found plenty in the Bay of Bengal.

Present study is about the analytical (physico-chemical) characterization of the solvent-extracted oil from the muscle of Silver Ribbon fish (*Lepturacanthus savala*) from the Bay of Bengal and comparing the results with the data available in literature about pharmacological aspects of muscle lipid of Silver Ribbon fish. Performance of the muscle lipid of Silver Ribbon fish against some common microbial species is also reported.

2. Experimental

Muscle of fresh Silver Ribbon fish, collected from the local fish supplier, and was treated by solvent extracted method to extract the lipid. The extract was then dried free of solvent by rotary evaporation and finally by blowing a slow stream of nitrogen gas. Total lipid content of Silver Ribbon fish was found to be 4.1 mg per g muscle. Moisture content, crude fat, crude fibre and ash contents of the muscle of Silver Ribbon fish and refractive index of that lipid were determined by standard methods [11]. Saponification value, saponication equivalent value, acid value and percentage of free fatty acid (as oleic acid), iodine value acetyl value [12], peroxide value [13], Thiocyanogen value, Richert Meissl value and Polenske value[11], Henher value, Elaiden test result [14] and quantity of un saponifiable matter [15] of the muscle lipid of Silver Ribbon

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fish were determined by standard methods. Thin layer chromatographic (TLC) investigation of the fatty acids present in the fish oil system was done in various solvent systems [16].

Antibacterial activity of the muscle lipid of Silver Ribbon fish against selected bacterial strains and fungal pathogens were checked. Disc diffusion method [17, 18] and poisoned food technique [18-20] were followed for screening the oil sample against the relevant bacteria and fungi, respectively. For the detection of antibacterial activities, Nutrient agar (NA) medium was used as basal medium.

3. Results and discussion

3.1. Physical characteristics

The moisture content of muscle of Silver Ribbon fish was determined and found 74.5% (Table 1). It shows that the value of moisture content is related with the probability of finding the percentage of lipid and other organic or bioactive compounds in the sample.

Table 1: Moisture content, fat, fibre and ash content of the muscle of Silver Ribbon fish (*Lepturacanthus savala*) and some related fishes [21].

Fish species	Moisture (%)	Fat (%)	Ash (%)	Fibre (%)
Herring (spring)	72	8	2	…
Pilchard, South Africa	69	9	3	…
Anchovy, South Africa	70	10	3	…
Herring, Winter	70	11	2	…
Mackerel (spring), North Sea	75	0.50	1.6	…
Dog – fish	70	8.9	2.3	….
Muscle of Silver Ribbon fish	**74.5**	**4.1**	**1.2**	**0.0962**

Table 2 : Physical constants of the muscle lipid of Silver Ribbon fish (*Lepturacanthus savala*).

Name of the sample	Refractive index	Specific gravity	Viscosity, mp
Muscle lipid of Silver Ribbon fish	**1.4731** (at 24 ˚C)	**0.9735** (at 30 ˚C)	**320.665** (at 30 ˚C)

The amount of total fat content of the muscle of Silver Ribbon fish was found 4.1 g/100g (Table 1). Total lipid content was determined gravimetrically from this sample which is much more in comparison to other marine fishes or natural sources. So this muscle lipid may claim valuable demands for edible purpose due to their higher lipid level.

The fibre content of muscle of Silver Ribbon fish was determined and found 0.0962% (Table 1). Animal fibers are natural fibers that consist largely of particular proteins. It also gives an idea of percentage of nitrogen (N).

The ash content of muscle of Silver Ribbon fish was determined and found 1.2% which indicate the compounds that remain after a sample is burned; mainly it comes from the metallic oxides. So it is also an indication of some mineral contents.

The refractive index of the muscle lipid of Silver Ribbon fish was found to be 1.4731 at 24 °C (Table 2). The refractive power of oil or fat varies widely and chiefly governed by the proportion and degree of unsaturation present. It is also an intensive property of any substance. The present result also indicates that the muscle lipid from the specimen contained moderate amounts of unsaturated fatty acids. This was also supported by its iodine value.

The specific gravity of the lipid solution (5% lipid solution was prepared by dichloromethane) was determined and found 0.9735 at 30 ˚C (Table 2). This sample was found in semisolid condition. From the result of this experiment, we got an idea about the specific gravity of the original lipid sample.

The viscosity of the lipid solution of the muscle lipid of Silver Ribbon fish was determined and found 320.325 milipoise at 30 °C with the help of Ostwald's viscometer (Table 2). The energy of activation for viscous flow was also calculated and found 5.7683 Kcal. From the result of this experiment, we got an idea about the intermolecular hydrogen bonding in the lipid sample. The present results suggested that there are a few hydroxyl groups and few free acid molecules present in the lipid. This observation is supported by low acetyl value of the lipid sample.

Table 3: Chemical constants of the muscle lipid of Silver Ribbon fish (*Lepturacanthus savala*) and some related fats and oils [14, 15, 22-24].

Name of the Sample	**S.V.**	**S.E.V.**	**A.V.**	**F.F.A.(%) (as oleic)**	**I.V.**	**P.O.V**	**E.V.**	**Acetyl Value**	**T. V.**	**Titre value (°C)**	**H.V.**	**U.S.M. (%)**	**P.V.**	**R.M.V.**
Sardine oil	189.8-193.8	---	2.2-21.7	---	138.89-177	---	---	---	---	---	---	---	---	---
Whale oil	184-200	---	0.3-51	---	126.9	---	---	---	---	---	---	---	---	---
Muscle lipid of Hilsa	203.25	276.01	3.108	1.56	92.55	55.05	---	10.255	52.54		93.27	0.74	0.764	0.965
Brain lipid of Baghda Chingri	229.255	244.71	1.11	0.56	95.83	194.95	28.14	10.58	43.63	27.2	95.32	0.566	0.796	1.04
Brain lipid of Kerani Chingri	214.11	262.06	1.04	0.52	100.38	192.26	13.07	10.82	45.29	26.7	92.19	0.641	0.694	0.95
Muscle lipid of Flathead mullet	182.5	307.397	2.83	1.26	125.92	68.09	-	11.22	27.6	-	79	1.16	0.36	-
Muscle lipid of Silver Ribbon fish	**203**	**273**	**1.51**	**0.79**	**98**	**30**	**201.5**	**13.64**	**58.61**	**27.2**	**72.68**	**1.24**	**0.693**	**0.946**

Abbreviations: S.V.= Saponification Value; S.E.V.= Saponification Equivalent Value; A.V.= Acid Value; F.F.A.= Free fatty acid; I.V.= Iodine Value; P.O.V= Peroxide Value; E.V.= Ester Value T.V.= Thicyanoge Value; H.V.= Henher Value; U.S.M.= Unsaponifiable Matter; P.V.= Polenske Value; R.M.V.= Reichert-Meissl Value

3.2. Chemical properties of the lipid sample

The saponification value of the muscle lipid of Silver Ribbon fish was found 203 (Table 3). The saponification equivalent value of this lipid sample was found 273 (Table 3). It is a measure of the average molecular weight (or chain length) of all the fatty acids present. It allows for comparison of the average fatty acid chain length. The long chain fatty acids found in fats have low saponification value because they have a relatively fewer number of carboxylic functional groups per unit mass of the fat as compared to short chain fatty acids. Saponification value is inversely proportional to the average molecular weight or chain length of the fatty acids present in the fat or oil. The results clearly indicate that the lipid sample contains higher proportion of high molecular weight fatty acids.

The acid value of the muscle lipid of Silver Ribbon fish was found 1.51 (Table 3). The percentage of free fatty acid (FFA), as oleic, was calculated from acid value and was found 0.79 for the muscle lipid of Silver Ribbon fish (Table 3). Acid value indicates the proportions of free fatty acid in the oil or fat. The free fatty acid is produced by the hydrolytic decomposition of the oil or fat. Hence, such low acid value is an indication of freshness of the oil or fat and suitability of the lipid for edible purpose.

Iodine value gives an estimation of the degree of unsaturation and the relative amounts of unsaturated fatty acids in the triglyceride molecules of the fat or oil. Iodine value of lipid of Silver Ribbon fish was found 98 (Table 3). This value indicates that the lipid sample contains in moderate proportion of unsaturated fatty acids and semidrying type which also maintain the criteria of edible oil and PUFA containing oil which is beneficial for cardiac health. The peroxide value of an oil or fat is used as a measurement of the extent to which rancidity reactions have occurred during storage. The double bonds found in fats and oils play a role in autoxidation. Peroxide value of muscle lipid of Silver Ribbon fish was found 30 (Table 3). It indicates the freshness and less auto oxidative property of this lipid.

Ester value of muscle lipid of Silver Ribbon fish was found to be 201.5 (Table 3). This value indicates the amount of ester present in the lipid sample.

The acetyl value is a measure of hydroxylated fatty acids present in a fixed oil or fat. The acetyl value of the muscle lipid of Silver Ribbon fish was found 13.64 (Table 3). This result indicates low content of free hydroxyl groups in the lipid sample.

The thiocyanogen value of the muscle lipid of Silver Ribbon fish was found 58.61 (Table 3). This observation also supports its moderate iodine value and peroxide value.

The titre value of the muscle lipid of Silver Ribbon fish was found 27.2°C (Table 3). This value indicates that the lipid sample is of fat types which support their semisolid condition at room temperature.

The Henher value of the muscle lipid of Silver Ribbon fish was found 72.68% (Table 3). This result indicates the higher percentage of water insoluble nonvolatile fatty acids in the lipid sample.

The unsapoinfiable matter in the muscle lipid of Silver Ribbon fish was 1.24% (Table 3) which indicates that the lipid contains a good amount of unsaponifiable sterols, tocopherols, vitamins A & D, hydrocarbons and so on.

The Polenske value of the muscle lipid of Silver Ribbon fish was 0.693 (Table 3). The Polenske value represents a measure of volatile water insoluble but alcohol soluble fatty acids in the lipid sample.

The Reichert-Meissl value of the muscle lipid of Silver Ribbon fish was 0.946 (Table 3). Since the Reichert-Meissl value is a measure of the volatile water soluble lower fatty acids present in the fat or oil, so the lower R-M value of the lipid sample is indication of low content of volatile water soluble fatty acids. The muscle lipid of Silver Ribbon fish was found to form cloudy solution with bromine and a precipitate appeared due to the insoluble bromide during the experiment. Hence, the lipid is marine oil (fish oil).

The muscle lipid of Silver Ribbon fish was found to form treacle-like consistency with mercuric nitrate, $Hg(NO_3)_2$ solution after 24 hours during the experiment. Hence, the lipid is of semi-drying type [14]. Semidrying oils absorb oxygen from air slowly and thicken after keeping exposed to air for some time but do not dry up.

The Kirschner value of the muscle lipid of Silver Ribbon fish as determined was found 0.432. This indicates the presence of trace amount of fatty acids in the Reichert-Meissl distillate which form soluble silver salt.

In order to fulfill the expectations of consumers, good quality control of oil should be assured in the course of production and storage time. During storage of the oil/fat, the hydrolysis,

esterification and oxidation originate changes in the minor constituents.

3.3 Effect of storage time

Table 4: The effects of storage time on muscle lipid of Silver Ribbon fish (Lepturacanthus savala)

Observation	Fresh lipid	Lipid after three months storage
Acid value	1.51	4.96
Peroxide value	30	57
Iodine value	98	81
R-M value	0.956	0.831
Thiocianogen value	58.61	51.82

The effect of storage time on the muscle lipid of Silver Ribbon fish showed a significant variation in different values just after 90 days at room temperature (Table 4). Acid value, peroxide value increased with increasing time of storage and R-M value, thiocyanogen value, titre value and iodine value decreased with increasing time of storage. That means, the quality of the lipid deteriorated with increasing time of storage. In addition to being affected by temperature and the degree of unsaturation, oxidation may be accelerated or retarded by various catalytic agents. Certain metals, visible light and light of shorter wavelengths, some oxidative enzymes, and other biological substances, such as hemoglobin, markedly accelerate this type of oxidative deterioration. For safe consumption of fish oil without antioxidant, 90 days shelf life is not suggested under storage conditions for domestic consumption.

3.4. Antibacterial and antifungal activities of the lipid sample

In the present study, the muscle lipid of Silver Ribbon fish was selected and screened for antibacterial activities against ten selected bacteria and antifungal activities against four phytopathogenic fungi.

3.4.1. Antibacterial test

The antibacterial activities of lipid sample were studied against four Gram positive and six Gram negative bacteria. The results of antibacterial activity of muscle lipid of *Lepturacanthus savala* is given in the list (Table 5). Where ciprofloxacin (standard) showed a good inhibition of growth against all tested bacterial species at the concentration of 50 µg/ml whereas lipid of *Lepturacanthus savala* showed good growth of inhibition against 3 tested bacteria and there was no growth of inhibition against remaining 7 bacterial strains. Among them the highest performance was observed against *Bacillus subtilis* and the lowest performance was found against *Vibrio cholerae*. It was also observed that the lipid sample had positive activity against both Gram positive and Gram negative bacteria. It is considered as an important phenomenon of any antibiotic. From these results it is concluded that this lipid sample bears high importance for their antibiotic property against these bacteria.

Table 5: Antibacterial activities of muscle lipid of Silver Ribbon fish (*Lepturacanthus savala*).

Name of the Organism (Bacterial species)	Types	Zone of inhibition (mm) for Ciprofloxacin 50 µg/ml	Zone of inhibition for muscle lipid (mm)
Escherichia coli (ATCC 10798)	Gram negative	20.58	-
Salmonella typhi (ATCC 13311)	Gram negative	30.37	-
Bacillus subtilis (ATCC 6051)	Gram positive	29.21	19.14
Bacillus cereus (ATCC 10987)	Gram positive	32.19	-
Shigella sonnei (ATCC 29930)	Gram negative	28.18	-
Vibrio colerae (ATCC 39315)	Gram negative	23.78	10.12
Staphylococcus saprophyticus (ATCC 15305)	Gram positive	35.12	-
Shigella dysenteriae (ATCC 13313)	Gram negative	21.32	-
Bacillus megaterium (ATCC 14581)	Gram positive	27.68	15.39
Pseaudomons septica	Gram negative	22.89	-

(ATCC 14545)			

3.4.2. Antifungal activities

The antifungal activities of the lipid sample were studied against four phyto-pathogenic fungi. It is evident from the chart (Table 6) that the muscle lipid of Silver Ribbon fish (*Lepturacanthus savala*) did not show any inhibition on mycelial growth of *Alternaria alternate*. Except these the mycelial growth of three test fungi out of four was inhibited by the lipid sample. *Fusarium equiseti* showed highest zone of inhibition (15.38 mm) for this sample. From the result we can conclude that this work will provide valuable information about the prospect of derivation of pesticides and pharmaceuticals from the muscle lipid of Silver Ribbon fish (*Lepturacanthus savala*).

Table 6: Percent growth inhibition of test fungi by the muscle lipid of Silver Ribbon fish (*Lepturacanthus savala*).

Name of the fungi	Type of sample (**10%** lipid solution)	% Inhibition after 5 days
		Muscle lipid of Silver Ribbon fish
Fusarium equiseti (ATCC 62721)	1ml	15.38
Aspergillus fumigateus (ATCC 13073)	1ml	4.21
Alternaria alternate (ATCC 56836)	1ml	NI
Curvularia lunata (ATCC 12017)	1ml	5.23

3.5. Estimation of N, P, K and Ca in lipid containing muscle of Silver Ribbon fish.

From the chart (Table 8) it is evident that, muscle of Silver Ribbon fish contains a good amount (2.9%) of nitrogen as well as protein (proteineous nitrogen.) In the current investigation protein has been found 17.93 gm (percentage of protein = % of N × 6.25) in 100 gm.

The percentage of phosphorus in the muscle of Silver Ribbon fish was found 2.45 (Table 7). The result indicates that phospholipids may present in this extracted lipid.

The percentage of potassium in the muscle of Silver Ribbon fish was found 1.108 (Table 7).

The percentages of calcium the muscle of Silver Ribbon fish was found 0.464 (Table 7).

Table 7: Percent of N, P, K and Ca in muscle of Silver Ribbon fish (*Lepturacanthus savala*) with other samples [22-24].

Name of the Sample	**N (%)**	**P (%)**	**K (%)**	**Ca (%)**
Brain of Kerani Chingri	3.090	0.5506	1.061	0.798
Brain of Baghda Chingri	3.540	0.7262	1.123	0.914
Muscle lipid of Flathead mullet	9.95	0.4363	1.132	0.9
Muscle of Silver Ribbon fish	**2.9**	**2.40**	**1.108**	**0.464**

3.6. Chromatographic examination (TLC analysis)

The muscle lipid of Silver Ribbon fish was subjected to TLC examination and their fatty acid composition were identified by comparing the R_f values of different spots of chromatograms with those of standard fatty acids as reported (Table 8) in different solvent systems. It was found from the chromatograms that the lipids gave about 3-5 spots. Among the spots, three spots were identified as Palmitic Acid, Mystiric Acid, Linoleic Acid, and Erucic Acid in the muscle lipid of Silver Ribbon fish.

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Table 8: The R_f values (most related) of thin layer chromatographic examination of the muscle lipid of Ribbon fish (*Lepturacanthus savala*).

Solvent System	R_f values of standard fatty acids				R_f values of standard fatty acids			
	PA	MA	LA	EA	PA	MA	LA	EA
P:E (80:20)	0.941	0.472	0.933	0.361	0.940	0.942	0.933	0.365
P:E:A (80:20:1)	0.822	0.419	0.893	0.479	0.824	0.419	0.478	0.421
H:E (80:20)	0.823	0.191	0.641	0.201	0.823	0.195	0.639	0.201

Note; P : Petroleum ether, E: Ether, A: Acetic acid, H : Hexane
PA- Palmitic Acid, MA-Mystiric Acid, LA-Linoleic Acid, EA- Erucic Acid

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