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Extraction, Analytical Analysis and Biological Activities Evaluation of the Body Lipid of Spanish Mackerel (*Scomberomorus guttatus*) of the Bay of Bengal

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Abstract: The outward effects of the detectable diversity in marine fish lipid compositions on human physiology and alimentation have highlighted focus. Considering this, to assess and compare various physical and chemical constants the body lipid of Spanish mackerel (*Scomberomorus guttatus*) from the Bay of Bengal was extracted using a solvent extraction method. Gas-Liquid Chromatography (GLC) marked the presence of (C14:0), (C16:0), (C18:0), (C16:1), (C18:1), (C18:1), (C18:2), (C18:3), (C20:4), (C20:2), (C20:5), (C22:5) and (C22:6) fatty acids. It figured that Saturated Fatty Acids (SFA %) > Unsaturated Fatty Acids (UFA%), Monounsaturated Fatty Acids (MUFA %) >Polyunsaturated Fatty Acids (PUFA %) i.e. [34.8373% > 7.9989%] and ω-3 Polyunsaturated Fatty Acids (ω-3 PUFA) >ω-6 Polyunsaturated Fatty Acids (ω-6 PUFA) i.e. [5.6562% > 2.3427%]. ω-3/ω-6 ratio was mathematically calculated (2.42). Several minerals (N, P, K and Ca) and metal contents (Fe, Pb, Ni, Co, Cd, Cu, Zn, Mn, Cr, As, Mg) were determined quantitatively. Furthermore, the inhibition effect of extracted lipid against few bacteria and fungi was examined using presiding techniques. As a result of the research, it is clarified by a number of noteworthy facts pertaining to nutritional and therapeutic elements.

Keywords: Marine Fish Lipid, Spanish Mackerel, PUFA, Inhibition Effect

1. Introduction

Bangladesh is blessed with three-dimensional water masses covered by the Bay of Bengal (13° 31' 53.994"N 87° 32' 22.5096"E) at the southern part which constitutes a virgin potential of fishery resources that supplies over 70-80% of the nation's animal protein [1]. The Spanish mackerel, often known locally as maitya, is an important part of Bangladesh's fishery. Though it is enormous in the Bay of Bengal but due to scarcity of analytical data general masses do not understand its implications. Nowadays the biochemical response and growth-promoting effects of marine fish oils on human physiology and nutrition [2, 3] have enlivened focus on the discernible difference in fish oil compositions [4]. It is well recognized that the main source of polyunsaturated fatty acids (PUFAs) is fish lipids, specifically eicosapentaenoic acid (EPA; C20:5) and docosahexaenoic acid (DHA; C22:6) [5]. These PUFAs have a physiological impact in the refraining and treatment on a variety of ailments and diseases, such as atherosclerosis, deformity and immobility of joints, and asthmatic bronchitis [6]. Lipids and fatty acids are crucial for membrane biochemistry, but they also directly affect osmotic balance across membranes, nutrients absorption from small intestine and their transportation [7]. Though to reveal various impact of marine fish lipid on human physio-chemical function, researchers are emphasizing much on it recently but such type of work so far

less has been done on Spanish mackerel. For this, present study is engaged with the isolation of lipid from this species and analysis it targeting physic-chemical parameters, inhibition property against micro-organism growth and comparing those with existing data.

Figure 1. *Spanish mackerel (Scomberomorus guttatus).*

2. Materials and Methods

2.1. Sample Collection and Identification

Unprocessed fresh fish, Spanish mackerel (*Scomberomorus guttatus*) was procured from a nearby fish market; Sadarghat (22°20'18.24" N 91°49'54.05" E). Taxonomic study of it was carried out at the Institute of Marine Sciences & Fisheries of the University of Chittagong and confirmed its genus and species. Later its size, color and distinguish characteristics are determined and confirmed.

2.2. Extraction of the Lipid

Only muscle was kept aside separately for extraction by Bligh and Dyer method. The muscle (about 100 g wet weight) was first ground in a pestle. The pulp was transferred into a conical flask (500 ml capacity) and 200 ml of acetone (1:2, w/v) was added and shaken well. For complete extraction it was kept overnight at room temperature, preferably in the dark. After filtration the solution was concentrated by using rotary evaporator. The processes were repeated for three times. The resulting aqueous suspension was extracted with ethyl acetate (EtOAc) to extract crude lipid. The total extract was taken in a vial for complete dryness with nitrogen gas. It was also noted to keep the sample covered with aluminium foil to protect from light. This was because some lipids got polymerized or decomposed in exposure to light, heat and oxygen. Following standard methods, solutions were prepared [8-10].

2.3. Physico-Chemical Parameters Evaluation

Refractive index was determined using Abbe refractometer. Water at 4°C is chosen as the standard substance during determination of specific gravity. Co-efficient of viscosity of the lipid solution was determined at different temperature. Moisture content of muscle lipid was calculated by standard method [11]. Saponification value (S.V.) and saponication equivalent value (S.E.V.) was determined and calculated by following the procedure described by Kolles Dorfer. Titrating against potassium hydroxide in rectified sprit, the acid value (A.V.) of fish lipid was determined and percentage of free fatty acid (F.F.A.) was calculated. Standard techniques were used to assess the Spanish mackerel's body lipid's iodine value (I.V.), acetyl value (A.V.) [12], peroxide value (P.V.) [13], thiocyanogen value (T.V.), Richert Meissl value (R.M.V.), Polenske value (P.V.) [11], Henher value, Elaiden test [14], and the amount of unsaponifiable materials (U.S.M.) [15].

2.4. Chromatographic Analysis

Gas-liquid-chromatographic (GLC) separation of the lipid sample was carried out by partitioning the sample between a mobile gas phase and a thin layer of nonvolatile liquid phase on an inert support. The sample was first converted to methyl esters, and then it was injected into a heated column constructed from a coiled tube. On injection the sample was vaporized and mixed with an inert gas. Under pressure the gas mixture then moved through the column, and substances were partitioned by virtue of their different solubility in the stationary liquid phase [16].

2.5. Minerals Quantifications

Minerals (N, P, K, and Ca) of Spanish mackerel muscle that included lipid were assessed using the conventional techniques [17].

2.6. Analysis for Metals

The wet digested sample solution as well as the blank solution was analyzed by using air acetylene flame with combination as well as single element hollow cathode lamps into an atomic absorption spectrophotometer (ICE 3000 series). The sample was injected by automatic sampler and the absorbance and concentration data were automatically recorded. A number of standard solutions were made for each metal and bracketed the expected metal concentration [16, 18].

2.7. Microbial Analysis

Antibacterial activity against five common bacteria namely *Salmonella typhi*, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus cereus*, and *Proteus vulgaris* of the lipid sample were performed by following disc diffusion method using Nutrient Agar (NA) medium [19]. Antifungal activity of the sample against five general fungi namely *Fusarium equiseti*, *Aspergillus fumigateus*, *Alternaria alternate*, *Curvularia lunata*, and *Aspergillus flavus* were also evaluated by imitating food poison technique using Potato Dextrose Agar (PDA) medium. To make intended solution (5% and 10%), chloroform was initially used.

3. Results and Discussion

3.1. Physical Constants

Yield of total lipid content of Spanish mackerel was determined 19.00 mg/g which indicates that it has a good

demand for cooking purpose with higher health impact as fish lipids are valuable for lowering the blood cholesterol level. Refractive index as well as iodine value of a lipid indirectly reveals the level of unsaturation in it. Here refractive index of sample was determined 1.4744 which also indicates the presence of endurable amount of unsaturation in the structure of its fatty acid which also confirmed by the result of iodine value. The specific gravity of 5% lipid solution in chloroform of Spanish mackerel was determined 0.920 (At 30°C) which lies in between 0.90 to 0.95, the general range of specific gravity of any lipid. The viscosity and the energy of activation of the sample lipid solution were calculated 335.25 milipoise and 6.91 kcal respectively at 30°C. This high value of viscosity imparts their intermolecular attractions of the long chains of glyceride molecule. Also, this notifies that considerable amount of hydroxyl groups along with free acid molecules may lead there. These observations are also consistent with the outcomes of few chemical parameters like acetyl value and acid value.

Table 1. *Outcomes of physical constants of the body lipid of Spanish mackerel.*

Lipid sample	Refractive index	Specific gravity	Viscosity (mp)
Lipid of*Penaeus monodon*	1.4735	0.940	313.26
Lipid of *Epinephelus megachir*	1.4770	0.943	337.23
Lipid of *Cyprinus carpio*	1.4741	0.963	329.32
Lipid of *Latus calcarifer*	1.4645	0.933	298.38
Body lipid of *Scomberomorus guttatus*	1.4744	0.920	335.25

It was discovered that the sample lipid had a moisture content of 1.56%. Fish lipids have low moisture content hence are less differentiated from fixed oils or fats. After removal of lipid, the residue had ash, protein and crude fiber content 1.53%, 59% and 2.00% individually.

Table 2. *Moisture content of muscle lipid; ash, protein and crude fiber content of the de-oiled muscle of Spanish mackerel.*

Name of the sample	Moisture content (%)	Ash content (%)	Protein content (%)	Crude fiber content (%)
Body lipid of Spanish Mackerel	1.56%	1.53	59	2.00

3.2. Chemical Characteristics

Information on the chemical properties of oils, fats and lipids sample is a very important part of lipid analysis as they are more capable than the physical constants about giving valuable data on the studied lipid sample. The results of chemical analysis of various chemical parameters of targeted lipid sample are shown in table 3. Saponification and saponification equivalent value was calculated reacting with KOH and was determined 215.57 and 260.24 respectively. Since fats or oils are mixtures of glycerides and the glycerides in turn contain fatty acids of various chain lengths, the saponification value is an index of the average molecular size of the fatty acids present. It gives an estimate of the non fatty impurities and tells the number of alkalis that would be actually required by the fat or oil for its conversion into soap. These values clearly allude that there exist comparatively higher proportion of long chain fatty carboxylic acid molecules. The acid value and percentage of free fatty acid of the body lipid of Spanish mackerel were determined 1.44 and 0.69 respectively. The hydrolysis reaction, accompanied of shorter- chain fatty acids through oxidation of double bonds, can give a high acid value as in the case of rancidity in the fat or oil. Thus, the outcomes as, it is relatively low; impart freshness and good for use in esculent purpose. Ester value, however rarely used, is obtained by subtracting the acid value from the saponification value and was calculated 208.13 which signify the amount of ester present in the lipid sample. Iodine value of the extracted lipid of Spanish mackerel was calculated 109.55 which is the measure of unsaturation present in the long chain of the fatty acid molecules by absorbing iodine there. It also allows the classification of lipids in drying, semidrying and nondrying types and the outcome surely indicates that it should have moderate amount of unsaturation present. Values of physical parameters and Elaiden test also approved this. Lipids having higher unsaturation absorb more oxygen from air via autoxidation at the carbon-carbon double bond and thus consequently form unstable hydroperoxides. Peroxidee value of the studied sample was evaluated 104.04 which is the amount of iodine liberated from potassium iodideby the peroxides present there. Also, the thiocyanogen value was calculated 35.86. This outcome is in line with the lipid sample's low iodine and peroxide values, as shown by the results. Acetyl value of the investigated sample was determined 10.06 which is a measure of the number of hydroxyl groups present in the lipid sample. It lies in between 3 and 15 which is a range for most fats and oils. The solidifying point of the mixed fatty acids or the titre value was determined 30°C which characterize its hardness and semisolid condition at ambient temperature. To be sure to take idea about a lipid regarding adulteration, percentage of unsaponifiable matters should be determined. If its result exceeds the limit of 2%, then it can be said adulterated. Here the lipid sample exhibited 0.602% unsaponifiable matters that means it was not adulterated and trace amount of steroids, vitamins and hydrocarbons may be exist there. It was unfolded that the lipid sample possess low amount of volatile water soluble and volatile water insoluble but alcohol soluble fatty acids by the results of Reichert-Meissl value and

Polenske value which figured 1.72 and 1.48 respectively. Henher value of the lipid sample of Spanish mackerel was calculated 90.72% which is a measure of non-volatile fatty acids and was determined by the remaining materials in the flask after removal of the distillate during previous two tests. The Kirschner value of the extracted lipid of Spanish mackerel was determined 0.37 which allude the appearance of little amount of long chain carboxylic acid forming soluble silver salt. The lipid sample was tested with bromine and exhibited obscure solution due to the infusible bromide hence confirmed marine oil. To reveal drying property Elaiden test was performed using $Hg(NO_3)_2$ solution which acquainted semi-drying type of extracted sample.

Table 3. *Comparison of Chemical constants of Spanish mackerel with some others lipids.*

Name of the Sample	S.V.	S.E.V.	A.V.	F.F.A. (%) (as oleic)	E.V.	I.V.	P.O.V.
Lipid of *Tenualosa ilisha*	203.35	275.01	3.12	1.36	---	93.55	56.05
Lipid of*Penaeus monodon*	229.266	254.71	1.21	0.66	27.14	94.83	184.95
Lipid of *Macrobrachium rosenbergii*	213.11	264.06	1.14	0.55	14.07	100.44	190.26
Lipid of *Cyprinus carpio*	262.87	225.05	1.88	0.88	248.77	105.82	108.45
Lipid of *Scomber scombrus*	218.12	268.556	1.38	0.584	216.94	110.36	112.72
Body lipid of Spanish mackerel	215.57	260.24	1.44	0.68	208.13	109.55	104.04

Table 3. *Continued.*

Name of the Sample	Acetyl Value (%)	T. V.	Titre value (°C)	H.V.	U.S.M. (%)	P.V.	R.M.V.
Lipid of *Tenualosa ilisha*	10.245	52.88	---	94.27	0.84	0.774	0.95
Lipid of*Penaeus monodon*	10.49	43.44	28.2	96.32	0.556	0.895	1.14
Lipid of *Macrobrachium rosenbergii*	10.72	44.29	27.7	93.19	0.632	0.794	0.96
Lipid of *Cyprinus carpio*	12.85	55.82	29.5	84.98	1.112	0.832	0.92
Lipid of *Scomber scombrus*	10.77	42.82	28.2	89.63	0.582	0.782	1.16
Body lipid of Spanish mackerel	10.146	35.86	30	90.72	0.602	1.48	1.72

3.3. Gas-Liquid Chromatographic Analysis

To obtainrelative amount of different long chain fatty acids, gas-liquid chromatography was performed. Analysis of chromatogram mentioned the presence of (C14:0), (C16:0), (C18:0), (C16:1), (C18:1), (C18:1), (C18:2), (C18:3), (C20:4), (C20:2), (C20:5), (C22:5) and (C22:6) fatty acids. From chromatogram (C16:0) namely palmitic acid being present 32.1843% is the highest among all other saturated fatty acids. From there it becomes more evident that the significant amount of myristic acid11.3303% and stearic acid 12.1336% were also present there. Significant amount of monounsaturated fatty acids (34.8373%) was quantified by GLC. Though the amount of PUFA were relatively low in comparison with MUFA but this specimen possessed high amount of it (7.9989%) comparing with others. Among ω-3 PUFA, eicosapentaenoic acid (EPA, C20:5) was quantified 2.3294% which was the highest among all others and among ω-6 PUFA, linoleic acid (C18:2) was quantified 1.1286%which was the highest among others. Relative amount of ω-3 PUFA was higher than ω-6 PUFA (5.6562% > 2.3427%) and the ratio of them was calculated 2.42.

Table 4. *Relative amount of fatty acids in the body lipid of Spanish mackerel.*

Types	Formula of fatty acid		Relative amount (%)	Total amount (%)	
Saturated fatty acid (SFA)	$C_{13}H_{27}COOH$, (C14:0)		11.3303	57.1638	
	$C_{14}H_{29}COOH$, (C15:0)		0.7769		
	$C_{15}H_{31}COOH$, (C16:0)		32.1843		
	$C_{16}H_{33}COOH$, (C17:0)		0.4505		
	$C_{17}H_{35}COOH$, (C18:0)		12.1336		
	$C_{19}H_{39}COOH$, (C20:0)		0.2882		
Monounsaturated fatty acid (MUFA)	$C_{15}H_{29}COOH$, (C16:1)		17.1871	34.8373	
	$C_{17}H_{33}COOH$ (C17:1)		---		
	$C_{18}H_{35}COOH$, (C18:1)		14.7100		
	$C_{18}H_{35}COOH$, (C18:1)		2.7730		
	$C_{20}H_{39}COOH$, (C20:1)		0.1672		
Polyunsaturated fatty acid (PUFA)	ω-3 PUFA	$C_{16}H_{26}COOH$, (C16:3)	1.1404	5.6562	7.9989
		$C_{18}H_{30}COOH$, (C18:3)	0.1183		
		$C_{20}H_{30}COOH$, (C20:5)	2.3294		
		$C_{20}H_{34}COOH$, (C22:5)	0.5109		
		$C_{20}H_{32}COOH$, (C22:6)	1.5572		
	ω-6 PUFA	$C_{18}H_{32}COOH$, (C18:2)	1.1286	2.3427	
		$C_{20}H_{36}COOH$, (C20:2)	0.4353		
		$C_{20}H_{32}COOH$, (C20:4)	0.7788		

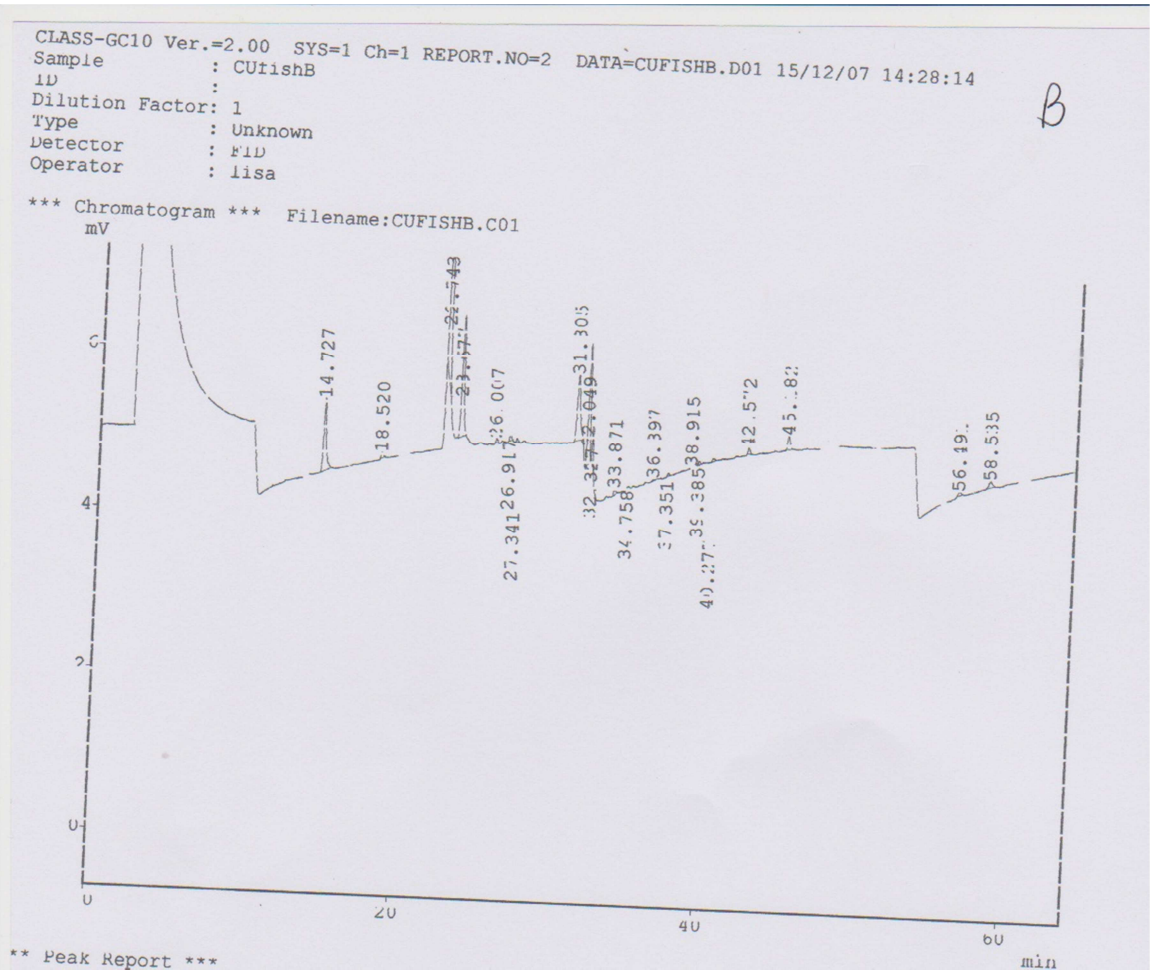

Figure 2. *Chromatogram of body lipid analysis of Spanish mackerel.*

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

The lipid containing muscle of the Spanish mackerel was analyzed for several minerals namely N, P, K and Ca. 9.440% nitrogen was quantified which may be the proteineous and thus introduce Spanish mackerel importance for edible purpose. It also reveals the presence of high amount of P (1.1736%), K (0.150%) and Ca (0.135%). All these minerals are good for health specially to reduce blood pressure and for the formation of bone and teeth. Hence adults and children that take this species may favor in their health condition.

Table 5. *Percent of N, P, K and Ca in the muscle of Tripletail.*

Name of the sample	N (%)	P (%)	K (%)	% of Ca (%)
Lipid of *Macrobrachium rosenbergii*	3.090	0.550	1.061	0.798
Lipid of*Penaeus monodon*	3.540	0.726	1.123	0.914
Lipid of *Tenualosa ilisha*	4.099	2.750	1.180	0.641
Lipid of *Cyprinus carpio*	6.533	1.237	1.118	0.450
Body lipid of *Scomberomorus guttatus*	9.440	1.1736	0.150	0.135

3.5. Metal Analysis of the Muscle of Spanish Mackerel

The lipid containing muscle of Spanish mackerel was analyzed for few metals namely Fe, Mg, Zn, Cd, Pb, Mn, As, Co, Ni and Cr etc. Iron (Fe), Magnesium (Mg) and Zinc (Zn) were found 18.26 ppm, 367.65ppm and 25.63ppm respectively. Cadmium (Cd), Lead (Pb), Manganese (Mn) and Arsenic (As) content were 0.48 ppm, 1.77 ppm, 1.08 ppm and 0.23 ppm respectively which falls within the World Health Organization's (WHO) permitted range. Co, Ni and Cr were below the detection limit.

Table 6. *Metal concentration in the lipid containing muscle of Spanish mackerel.*

Metals	Concentration (ppm)	Metals	Concentration (ppm)
Cd	0.48	Zn	25.63
Pb	1.77	Cr	-
Co	-	Cu	12.34
Fe	18.26	As	0.23
Mn	1.08	Mg	367.65
Ni	-		

3.6. Microbial Activities of the Lipid Sample

To reveal pharmacological importance of this specimen, the lipid sample was also analyzed for microbial activities against few harmful bacteria for men and against few harmful fungi for plants.

3.6.1. Bacterial Activity Test

It was evident that the lipid sample was active against all the tested bacteria both gram positive and gram negative except *Proteus vulgaris*. From the result, we got the higher area of inhibition against *Staphylococcus aureus* (19.5 mm). So, it is possible to use this lipid in the production of antibacterial dosage forms.

Table 7. *Antibacterial activity evaluation of the Spanish mackerel lipid.*

Name of bacteria	Type of sample	Zone of inhibition (diameter in mm) after 48 hours		
		Treatment	Control	Differences
Salmonella typhi	10%	18	0	18
	5%	9.5	0	9.5
Staphylococcus aureus	10%	19.5	0	19.5
	5%	10	0	10
Escherichia coli	10%	17.5	0	17.5
	5%	8.5	0	8.5
Bacillus cereus	10%	16	0	16
	5%	8.5	0	8.5
Proteus vulgaris	10%	0	0	0
	5%	0	0	0

3.6.2. Fungal Activity Test

During antifungal activity evaluation, the sample lipid showed maximum inhibitory activity against *Aspergillus fumigateus* (13.50). It also directed inhibitory activity against other tested fungi except *Aspergillus flavus*. In these circumstances the sample lipid flourished fungal growth rather than restrain.

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

Name of the fungi	Type of sample	% inhibition after 5 days
		Muscle lipid of Tripletail
Fusarium equiseti	10%	12.73
Aspergillus fumigateus	10%	13.50
Alternaria alternata	10%	11.45
Curvularia lunata	10%	10.77
Aspergillus flavus	10%	-11.25

(-) means no inhibition

4. Conclusions

In this investigation, there exists coherence in the physical constants with the chemical parameters about the presence of large amount of unsaturation in the structure of long chain fatty acids. It is revealed that Spanish mackerel fish contain higher amount of PUFAs (ω-3 and ω-6) which is obligatory in reducing blood triglycerides and hence in decreasing cardiovascular problems and overweight. Moreover, it has relatively higher amount of essential elements which is helpful for human sound health. So, from this investigation, people can be encouraged to eat this fish because of its health benefits. It also opened the door of possibility for the production of topical medicaments as it shows inhibitory activity against several bacteria and fungi.

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Conflicts of Interest

The authors declare no conflicts of interest.

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