Isolation, Characterization, and GC-MS Analysis of Fatty acid of *Mugil parsia* fish oil

Abstract: This paper reports the isolation, characterization, and GC-MS analysis of *Mugil parsia* fish oil collected from the Rupsha River in Khulna. The oil was extracted from the dried fish by using the Soxhlet apparatus with n-hexane, with an oil yield of 17± 4.0 %. The moisture, ash, and protein content of the raw fish were found to be 75.45 %, 1.258 %, and 20.00 % respectively. The physicochemical compositions of the extracted oil like color, specific gravity, peroxide value, saponification value, free fatty acid (FFA), acid value, and iodine value were determined and were found to be yellowish brown in color, 0.913±0.31, 9.9±0.1 meq/kg, 295.4±3.0 mg KOH/g, 11.9±2.0%, 5.7±0.11mg KOH/g, 5.7± 0.11 and 150.3±0.12 mg/100g respectively. Fatty acid composition was identified by GC-MS analysis of the *Mugil parsia* fish oil, showed the Phthalic acid, mono-(2-ethylhexyl) ester, palmitic acid, oleic acid, stearic acid, icosanoic anhydride, glycidyl stearate were identified as the major constituents.

Keywords: *Mugil parsia,* Soxhlet apparatus, peroxide value, iodine value, GC-MS analysis.

UNDER PEER REVIEW

1. Introduction

Fishes are fascinating creatures that live in water and are known for their unique physical features. They are cold-blooded and have scales that cover their bodies. Fishes also have fins that allow them to swim, and they use their gills to breathe [1]. There are many different types of fish, and they come in all shapes and sizes. Some are brightly colored, while others are more subdued. It's amazing to see how diverse the fish species are [2].

"Mugil parsia (Synonym *Liza parsia* Hamilton 1822), English name 'Gold spot mullet' occurs in the area, along the coasts of west India, Sri Lanka, Pakistan, Bangladesh and the Andaman Islands. In shallow coastal waters, estuaries, lagoons and sometimes entering tidal rivers, there occurs a schooling species. In ponds with 87% salinity, they can survive, Spawning takes place at sea". [33]

Many studies have been conducted on fish flesh and its oil. "Fish oil is currently under intensive scientific research due to its numerous health benefits. This fish oil is receiving a lot of attention because of its health benefits associated with the high levels of the long chain omega-3 polyunsaturated fatty acid (PUFA)" (Wu and Peter, 2008). "Fish flesh is composed of high-quality proteins and lipids (oils) that are high in monounsaturated and polyunsaturated fatty acids" [3]. "The nutritional benefits of fish oil consumption are due to the presence of protein of high biological value, unsaturated essential fatty acid, minerals and vitamins namely vitamin B3 (niacin), vitamin B6 (pyridoxine), vitamin B_{12} (cobalamine), vitamin E (tocopherol) and vitamin D (cholecalciferol) in fish tissues" [4]. "Fish oil has been used to relieve muscular pain as well as arthritis" [5]. Various reports on the study and identification of fish oil having high pharmacological activity potential as a hypoglycemic [6], hypolipidemic agent [7−9], antiarthritic agent [10−12] and preventing an agent renal damage [13−15]. "The most important health benefit of taking fish oil is that it is good for the functional development of infant's

UNDER PEER REVIEW

nervous system (nerve and brain) as well as the retina particularly in premature infants” [16,17]. These uses inspired us to undertake the present study. This investigation deals with the fatty acid and lipid characterization of fish oils from marine fish *Mugil parsia* available in the coastal waters of Bangladesh.

2. Materials and methods

2.1 Sample Preparation

Mugil parsia (Synonym *Liza parsia* Hamilton 1822), samples were collected from the Rupsha River in Khulna at Rajshahi University in May, 2016. The average weights of Gold spot mullets were1.5 kg. The head, scales, bones, fins, viscera and gills were removed and cut the meat in to small pieces and dried for an hour to reduce the moisture content and then crushed well to almost paste form by a hand crusher. All fish samples were frozen and stored at -20° C until use.

2.2 Physico-chemical analyses of fish

2.2.1 Determination of moisture content

UNDER PEER REVIEW

“Moisture was determined by drying the sample at +105^{0} C in an oven” [18]. By subtraction the moisture was calculated. The weight of the aluminium dish was taken into account and the difference between the weight of the sample before and after drying was calculated to determine the moisture

content. The dish with the sample was dried in a controlled oven at 105^0C until a constant weight was achieved.

The percentage moisture content was calculated using the following equation: Moisture (%) = $W_2 – W_3 /W_2 –W_1 \times 100$

Where,

W_1=initial weight of empty crucible

W_2= weight of crucible and sample before drying

W_3= Final weight of crucible and sample after drying

2.2.2 Determination of ash content

Ash was determined by muffling the sample at 6000- 7000^0 C to dry ash. [18]. First, cleaned porcelain crucibles and heated them in a muffle furnace at 6000°C. Once the crucibles reached a constant weight, weighed them with the sample and recorded the weight. Then ignited the sample at 6000°C for about 6 hours until the residue was uniformly grayish to white. Afterward, transferred the crucibles to a desiccator and allowed them to cool at room temperature for a few minutes before recording the final weights.

The following equation was used to determine the ash content of the dry fish samples:

Ash (%) = weight of ash /weight of sample ×100 W_3-W_1/W_2-W_1 × 100

Where: W_1= weight of empty crucible

W_2= weight of crucible + sample before ashing

W_3= weight of crucible + ash

2.2.3 Determination of protein content

Water-soluble protein was determined spectrophotometrically by the Lowry method [19]. The process involved adding 0.2g of sample to a conical flask along with 20 ml of concentrated H2SO4 and 5

UNDER PEER REVIEW

ml of CUSO4 (0.2N). After digesting for thirty minutes, a greenish-blue color was observed and the mixture was diluted by adding 10ml of water. To the mixture, 5 ml of 2% boric acid and 2 drops of phenolphthalein indicator were added. From the digested material, 10ml was obtained and to that was added 20ml of NaOH (40%) and titrated against (0.1N) HCl. The following equation was used to obtain amount of protein

N (%) = (Volume of HCl ×normality HCl × 0.014 × 100) /weight of sample Protein (%) = % Nitrogen × 6.2

2.3 Extraction of oil

100g of dried *Mugil parsia* fish was then weighed into a thimble and plugged with fat-free cotton and then placed into Soxhlet apparatus for approximately 16 h. Anhydrous n-hexane was used for the extraction of fish oil. Then the oil was freeze-dried by freeze dryer and stored at -80° C until use.

2.3.1 Physico-chemical analyses of extracted fish oil

2.3.1.1 Determination of fish oil color

Colour of absolute oil was noted from physical appearance.

2.3.1.2 Determination of Specific gravity

"Specific gravity of fish oil was determined using the specific gravity bottle by following the steps outlined. A clean and dry bottle of 25 mL capacity was weighed (W_0) and then filled with the fish oil, stopper was inserted and reweighed to give (W_1). The oil was substituted with water after washing and drying the bottle and weighed to give (W_2)" [20]. The specific gravity (Sp.gr) was obtained from the expression [20]:

UNDER PEER REVIEW

Specific gravity = $W_1 - W_0 / W_2 - W_0$ = Mass of the substance / Mass of an equal volume of water.

2.3.1.3 Determination of Peroxide values:

The Peroxide values of the fish oil were determined according to AOAC method [21]. "Weighed 5 g of fish oil sample into a 200 ml conical flask and mixed it with 300 ml of glacial acetic acid and chloroform (3:1) thoroughly by swirling the flask. Then, added 0.5 ml of saturated potassium iodide and left the mixture in the dark for 1 minute with occasional swirling. After that, added 30 ml of distilled water and titrated the mixture with 0.1 N sodium thiosulphate solution. Then used 1 ml of 1.0% soluble starch as an indicator and continued titration until the blue color disappeared. Also carried out a blank sample titration in the same manner but with no oil added" [21].

2.3.1.4 Determination of saponification value:

"The saponification value (SV) of the fish oil was successfully determined using the procedures described in AOCS method" [20]. We dissolved 1 g of the oil sample in 12.5 ml of 0.5 N ethanolic potassium hydroxide and refluxed the mixture for 30 minutes until the oil droplets disappeared. After cooling the mixture to room temperature, we added phenolphthalein indicator and titrated the hot soap solution with 0.5 N HCl until the pink color disappeared. We also carried out a blank titration in the same manner but with no oil added.

2.3.1.5 Determination of FFA value and acid value:

"2 grams of extracted oil was weighed and added in a 250 milliliters conical flask and 100 milliliters of 95% ethanol previously neutralized with 2 millimeters of phenolphthalein indicator was also added to it. The conical flask was placed in a hot water bath until the oil was completely dissolved into the solvent. The hot solution was titrated with 0.1-mole KOH until a pink color appeared which persisted for about 10 seconds". [33] The acid value was calculated using:

Acid value (milligram) = $56.1 \times M \times V / W$

Where V= titre value

UNDER PEER REVIEW

M= molarity of KOH used

W= weight in grams, of sample

From the FFA value, the acid value (AV) was also calculated.

2.3.1.6 Determination of Iodine value:

In the 250 milliliters conical flask, 1 gram of oil was weighed and added, 10millitres of carbon tetrachloride was also added to it. To the mixture, added 13 milliliters of Wij's solution, and the flask was stoppered, Shaked, and allowed to stand in the dark for one hour. After an hour, added 10 milliliters of 15% potassium iodide solution and 50 millimeters of water to the flask. The iodide solution was titrated with standard thiosulphate solution and shaken vigorously, with starch used as an indicator until the blue color disappeared. Also carried out a blank test without the oil under the same conditions.

The iodine value was calculated using:

Iodine value (mg) = $12.69 \times M \times (V_b - V_a)/W$

Where,

V_b = Volume of standard $Na_2S_2O_3$ solution used for blank test

V_a = Volume of standard $Na_2S_2O_3$ solution used for the test sample

M = Molarity of $Na_2S_2O_3$

W = Weight of oil sample (gram)

2.3.1.7 Gas chromatography–mass spectrometry (GC-MS) analysis of *Mugil parsia* fish oil

Gas chromatography–mass spectrometry (GC-MS) is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. GC-Mass spectroscopy was conducted (GCMSQP5050, Shimadzu, Japan) to characterize bioactive compound. A large number of constituents have been identified by GC-

UNDER PEER REVIEW

MS analysis of the crude ethanol extract. The quantification and the identification of compounds in the crude extract and active bands isolated by preparative TLC were accomplished using GC-MS analysis.

2.3.1.8 GC-MS analysis of fatty acid composition of *Mugil parsia* fish

The chemical compositions of fish oil were analyzed by using GC–MS technique and the fragmentation analysis was performed. Fatty acid Compositions of the fish oil were separated and identified by gas chromatography–mass spectrometry (GC–MS) agilent 6890N gas chromatography hooked to agilent 5973N mass selective detector. They equipped with a flame ionization detector and capillary column with HP-5MS (30 m×0.25 mm×0.25μm). The GC settings were as follows: the initial oven temperature was held at 60 °C for 1 min and ramped at 10° C min $^{-1}$to 180° C for 1 min and then ramped at 20° C min $^{-1}$ to 280 °C for 15 min. The injector temperature was maintained at 270° C. The samples (1μL) were injected neat, with a split ratio of 1: 10. The carrier gas was helium at flow rate of 1.0mL min $^{-1}$. Spectra were scanned from 20 to 550 *m/z* at 2 scans s $^{-1}$. Most constituents were identified by gas chromatography by comparison of their retention indices with those of the literature or with those of authentic compounds available in database.

3. Statistical Analysis: All the experiments were carried out in triplicate and the data is reported as mean ± standard deviation (SD) [22].

4. Result and Discussion

Physico-chemical properties of Mugil parsia fish oil extracts

UNDER PEER REVIEW

To determine the stability and quality of fish oil extracts, some quality assessment was conducted. These results are presented in Table 1, Table 2 and Table 3. Table 1 shows that the moisture, ash and protein content were 56.45± 4.0 %, 1.258± 0.2 % and 20± 0.23 g% respectively. The estimated oil in *Mugil parsia* fish was 17± 4.0 %. The oil obtained from *Mugil parsia* fish had a yellowish-brown in color and showed the specific gravity 0.913±0.31. As shown in Table 2, the PV content was 9.9 ± 0.1 meq/kg, which is well below acceptable limit of 20 meq /kg oil. This suggest that the fish oil extracted had a low lipid oxidation rate [23]. Young [24] had reported that "peroxide value (PV) of crude fish oil was between 3 and 20 meq/kg. The values of acid value (AV) and free fatty acids (FFA) were 5.7 ± 0.11 mg KOH/g and 11.9 ± 2.0 %, respectively". "The acceptable limit for AV was reported to be 7-8 mg KOH/g" [25]. Chantachum [26] had reported that "high heating temperature during oil extraction deactivated the enzyme and the release of free fatty acids by the lipase activity thus lowered the FFA value". Ashraduzzaman et al [27] had reported that acid value of seed oil was between 1.3-1.6 mg KOH/g. "Due to low temperature used during the extraction of oil in this study, enzyme lipase present may not have been deactivated and thus more free fatty acids could be release by lipase activity. Thus, caused high fatty acid value in this study which was also probably due to enhanced oxygen transfer which led to increased lipid oxidation", as propounded by Dauksas et al. [28]. "Saponification is the process of breaking down a neutral fat into glycerol and fatty acids by alkali treatment. The SV of fish oil obtained in this study was higher (295.4 ± 3.0 mg KOH/g) than standard value for fish oil (180-200 mg KOH/g)", given by AOCS [18]. Bimbo &Crowther [25] reported that "crude oil contains minor amount of non-triglyceride substances. Thus, it is possible that high SV was due to impurities present in crude fish oil". "Additionally, higher saponification value may be contributed by the unsaponifiable matter present in the

UNDER PEER REVIEW

leaching wastes materials such as sterols, glyceryl ethers, hydrocarbons, fatty alcohols and some minor quantities of pigments and vitamins". [25]

GC-MS analysis:

The chemical composition of fish oil identified the aid of gas chromatography and decomposition products were characterized by mass analyzer detector GC/MS. The present study was carried out to identify fatty acid composition by GC-MS analysis of *Mugil parsia* fish oil (Fig 1). Phthalic acid, mono-(2-ethylhexyl) ester, palmitic acid, Oleic acid, Stearic acid, Icosanoic anhydride, Glycidyl stearate were identified as the major constituents of *Mugil parsia* fish oil by GC-MS analysis. Oleic acid, a monounsaturated fatty acid. It decreased LDL cholesterol and increased HDL cholesterol [29]. It may also be responsible for the hypotensive (blood pressure reducing) effects. [30]. In previous study, it is revealed that stearic acid lowers LDL cholesterol [31]. It is reported that icosanoic acid (arachidonic acid) should be noted that for persons with chronic inflammatory disorder such as rheumatoid arthritis or inflammatory bowel disease. It is possible that icosanoic acid can exacerbate joint inflammation and pain [32].

5. Conclusion:

The current study's findings revealed that *Mugil parsia* fish contained significant amount of oil and protein. The presence of appreciable level of essential fatty acids and other favorable physiochemical characteristic make the *Mugil parsia* fish oil nutritionally viable for human health. Unsaturated fatty acids content in fish oils may reduce the risk of chronic diseases.

Aknowledgement:

UNDER PEER REVIEW

I would like to acknowledge the University Grand Commission (UGC) Faculty of Science, University of Rajshahi, Bangladesh (Project No.: A-132-5/52/BMOK/Science- 5/ 2015-2016) for providing financial assistance to carry out the present work.

REFERENCES:

1. Helfman, (1997). Microbiological and Physiochemical Level of Fresh Catfish(Arius hendolitic) fromdifferent Markets in Akwa-Ibom State, Nigeria. New York Science Journal.5(4):46-52 .
2. Aryee,A.,Simpson, B.(2009).Comparative Studies on Yield and Quality of solvent-extracted oil from Salmon skin. Journal of Food Engineering. 92(3):353-358.
3. Exler, J. Composition of foods: Finfish and Shellfish Products. United state Department of Agriculture, Human Nutrition Information Service, Agriculture Handbook 8-15 (Updated 1992) Washington, DC. 1987, pp: 192.
4. Sidhu,K.S.(2003).Health Benefits and Potential Risks Related to Consumption of Fish Oil or Fish. Journal of Regulatory Toxicology andPharmacology.38: 336-344.
5. https://www.verywellhealth.com/fish-oil-for-arthritis-5092351 Arthritis .Fish Oil for Arthritis and Joint Pain: Can It Help? The omega-3 in fish oil may block some sources of inflammation. By Yvelette Stines ,Updated on May 09, 2023.Medically reviewed by Mohamad Hassan, PT

6. Mahmud, I, Anwar H, Shahdat H, Abdul H, Liaquat A and Hashimoto M. Effects of Hilsa ilisa fish oil on the atherogenic lipid profile and glycaemic status of streptozotocin-treated type 1 diabetic rats.2004, Clinical and Experimental Pharmacology and Physiology. **31:** 76–81.
7. Castro R, Queirós J, Fonseca I, Pimentel JP, Henriques AC, Sarmento AM, Guimarães S, Pereira MC. Therapy of post-renal transplantation hyperlipidaemia: comparative study

UNDER PEER REVIEW

with simvastatin and fish oil. Nephrol Dial Transplant. 1997 Oct;12(10):2140-3. doi: 10.1093/ndt/12.10.2140. PMID: 9351079.

8. Anil K, Jayadeep A, Sudhakaran PR. Effect of n-3 fatty acids on VLDL production by hepatocytes is mediated through prostaglandins. Biochem Mol Biol Int. 1997 Dec;43(5):1071-80. doi: 10.1080/15216549700204891. PMID: 9415816.
9. B.J. Van Vlijmen, R.P. Mensink, H.B. Van't-Hof, R.F. O_ermans, M.H. Hofker, L. M. Havekes, (1998). J. Lipid. Res., 39, 1181.
10. Ariza-Ariza R, Mestanza-Peralta M, Cardiel MH. Omega-3 fatty acids in rheumatoid arthritis: an overview. Semin Arthritis Rheum. 1998 Jun;27(6):366-70. doi: 10.1016/s0049-0172(98)80016-4. PMID: 9662755.
11. James MJ, Cleland LG. Dietary n-3 fatty acids and therapy for rheumatoid arthritis. Semin Arthritis Rheum. 1997 Oct;27(2):85-97. doi: 10.1016/s0049-0172(97)80009-1. PMID: 9355207.
12. W.A. Gonnerman, R.F. Mortensen, J.M. Tebo, J.M. Conte, C.A. Leslie, S. Edgar, J. Immunol., 140, 796 (1988).
13. Manitius J, Sulikowska B, Fox J, Jakubowski Z, Ludwiczak E, Lysiak-Szydłowska W, Rutkowski B. The effect of dietary enrichment with fish-oil on urinary excretion of N-acetyl-beta-D-glucosaminidase and renal function in proteinuric patients with primary glomerulopathies. Int Urol Nephrol. 1997;29(4):489-95. doi: 10.1007/BF02551118. PMID: 9406009.
14. Dillon JJ. Fish oil therapy for IgA nephropathy: efficacy and interstudy variability. J Am Soc Nephrol. 1997 Nov;8(11):1739-44. doi: 10.1681/ASN.V8111739. PMID: 9355077.

UNDER PEER REVIEW

15. FOLCH J, LEES M, SLOANE STANLEY GH. A simple method for the isolation and purification of total lipides from animal tissues. J Biol Chem. 1957 May;226(1):497-509. PMID: 13428781.
16. Alexandre,L.Craig,L.J.(2009).Reevalution of the DHA requirement for premature infant.Prostanglandins,Leukotrienes and Essential Fatty Acids 81:143-150.
17. Dexuan,M., Minmin,Z.& Christain, P. (2010). DHAPromotes the Neuronal Differentiation of rat Neural Stem Cells Transfected with GPR40 gene. Brain Research
18. AOCS In: AOCS (ed) Official methods & recommended practices of the American Oil Chemists Society, 4th edn. Champaign, IL,Official Method Ai 275 (1993).
19. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. (1951). Protein measurement with the Folin-Ciocalteu's reagent. J. Biol.Chem. 193, 265-275.
20. AOCS. (1992). Official methods and recommended practices of the American Oil Chemists Society (4th ed.), Champaign, American Oil Chemists' Society, USA.
21. AOAC. (2002). Official methods of analysis of the Association of Official Analytical Chemists (14th ed.), Arlington.
22. Steel, R.G.D., J.H. Torrie and D.A. Dickey. (1997). Principals and procedure of statistics. McGraw Hill Book Co. Inc., New York.
23. Pike O.A. (1998). Fat characterization in food analysis, (3/e), Aspen Pub.Inc., pp.219-233.
24. Young, S., Sarda, X., & Rosenberg, M. (1993). Microencapsulating Properties of Whey Proteins. 1. Microencapsulation of Anhydrous Milk Fat. Journal of Dairy Science, 76(10), 2868-2877. https://doi.org/10.3168/jds.S0022-0302(93)77625-0
25. Bimbo, A. P., & Crowther, J. B. (1991). Fish oil: processing beyond crude oil. INFOFISH International, 6(91), 20-25.

UNDER PEER REVIEW

26. Chantachum, S., Benjakul, S., &Sriwirat, N. Separation and quality of fish oil from precooked and non-precooked tuna heads. Food Chemistry. 2000, 69, 289-294.

27. Ashraduzzaman M, Alam MA, **Khatun S**, Bari L. and Absar N. Extraction and characterization of Vigna unguiculata Linn. walp. seed oil, Journal of Science and technology.2013, **3(2):**431-438.

28. Dauksas, E., Falch, E., Slizyte, R. &Rustad, T. Composition of fatty acids and lipid classes in bulk products generated during enzymic hydrolysis of cod (Gadusmorhua) by-products, Process Biochemistry. 2005, 40, 2659-2670.

29. Merck & Co "You Can Control Your Cholesterol: A Guide to Low-Cholesterol Living". Inc. Retrieved 2009-03-14.

30. Teres, S.; Barcelo-Coblijn, G.; Benet, M.; Alvarez, R.; Bressani, R.; Halver, J. E.; Escriba, P. V. (2008). "Oleic acid content is responsible for the reduction in blood pressure induced by olive oil". Proceedings of the National Academy of Sciences. **105** (37): 13811–6. Bibcode:2008PNAS..10513811T. doi:10.1073/pnas.0807500105. JSTOR 25464133. PMC 2544536. PMID 18772370.

31. Mensink R.P. (2005). Effects of stearic acid in plasma lipid and lipoproteins in human. Lipids. 2005; 40 (12):1201-5.

32. Das UN. Arachidonic acid in health and disease with focus on hypertension and diabetes mellitus: A review. J Adv Res. 2018 Jan 4;11:43-55. doi: 10.1016/j.jare.2018.01.002. PMID: 30034875; PMCID: PMC6052660.

33. Ndidiamaka NC, Ifeanyi OE. Proximate and physicochemical analysis of oil obtained from two fish species (fresh and frozen). International Journal of Advanced Research in Biological Sciences. 2018;5(4):167-77.

UNDER PEER REVIEW

Table 1: Proximate composition of *Mugil parsia* fish.

Analysis	Fish oil
Moisture (%)	75.45± 4.0
Ash (%)	1.258± 0.2
Protein (g%)	20± 0.23
Oil (%)	17± 4.0
Color	yellowish brown
Specific gravity	0.913±0.31

Table 2: Physico-chemical analysis of extracted *Mugil parsia* fish oil.

Analysis	Fish oil
Peroxide value (meq/kg)	9.9 ± 0.1
Saponification value (mg KOH/g)	295.4 ± 3.0
Free fatty acid (%)	11.9± 2.0
Acid value (mg KOH/g)	5.7± 0.11
Iodine value(mg/100g)	150.3±0.12

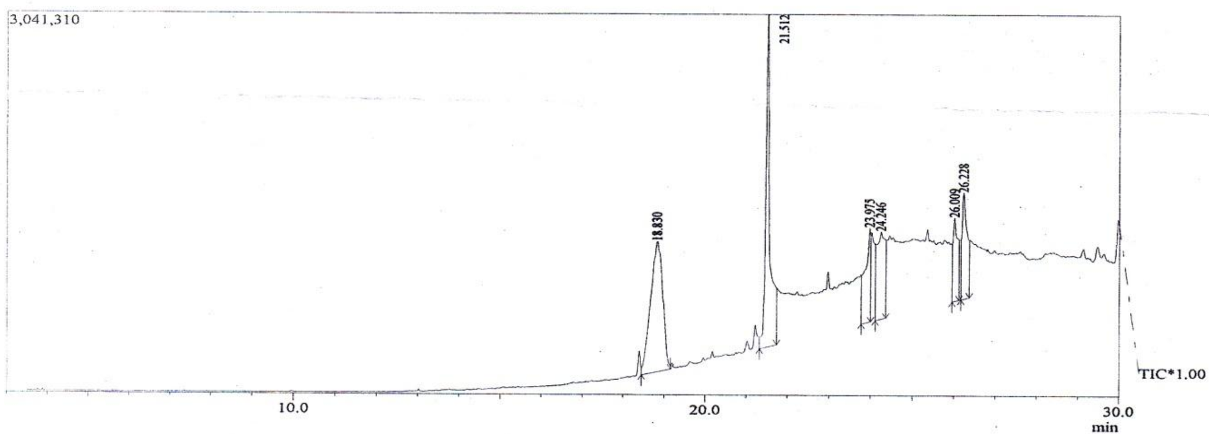
UNDER PEER REVIEW

Table 3: Following compound are identified by GC-MS from *Mugil parsia* fish oil,

SI No	Name of the compound	Molecular weight	Concentration (Area %)
1	Phthalic acid, mono-(2-ethylhexyl) ester	278	30.42
2	Palmitic acid	256	27.24
3	Oleic acid,	282	9.72
4	Stearic acid	284	14.10
5	Icosanoic anhydride	606	7.67
6	Glycidyl stearate	340	10.85

UNDER PEER REVIEW

Fig 1: Chromatogram of *Mugil parsia* Fish oil



Peak Report TIC

Peak#	Name	R.Time	Height	Area	Area%
1	Phthalic acid, mono-(2-ethylhexyl) ester	18.830	1024097	21047781	30.42
2	Palmitinic acid	21.512	2660523	18848497	27.24
3	Oleic acid	23.975	741328	6726926	9.72
4	Stearic acid	24.246	690768	9754487	14.10
5	Icosanoic anhydride	26.009	661952	5307467	7.67
6	Glycidyl stearate	26.228	845740	7511237	10.85
			6624408	69196395	100.00