Borneo Journal of Resource Science and Technology (2024), 14(2): 31-40
DOI: https:doi.org/10.33736/bjrst.6896.2024

Characterizing Fatty Acid Profiles and Evaluating Antibacterial Activity of Edible Yellow Puffer Fish, *Xenopterus naritus*

SAMSUR MOHAMAD*[1], NUR EQMAL DINIE NOR AZMI[1], ZAINI ASSIM[1], AHMAD SYAFIQ AHMAD NASIR[1], JULIAN RANSANGAN[2] & RABUYAH NI[3]

[1] Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia; [2] Borneo Marine Research Institute, Universiti Malaysia Sabah, Jalan UMS, 88400 Kota Kinabalu, Sabah, Malaysia; [3] Faculty of Applied Science, Universiti Teknologi Mara Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia
*Corresponding author: msamsur@unimas.my
Received: 3 April 2024 Accepted: 5 July 2024 Published: 31 December 2024

ABSTRACT

Puffer fish oil extracted from *Xenopterus naritus* represents a beneficial source of bioactive compounds with health-promoting properties. Despite the known benefits of puffer fish oil, there is a lack of detailed information on its fatty acid composition. This study aimed to fill this gap by investigating the fatty acid profiles of puffer fish oil extracted from the liver and muscle tissues. The oil was extracted using the solvent Bligh & Dyer method, and the samples were derivatized into fatty acid methyl esters (FAME) before being analyzed via Shimadzu QP2010 Plus gas chromatography-mass spectrometry (GC-MS). This analysis highlighted the prevalence of omega-3 fatty acids, particularly Docosahexaenoic acid (DHA) (8.28 ± 0.08% in liver, 6.15 ± 0.33% in muscle oil) and Eicosapentaenoic acid (EPA) (3.29 ± 0.12% in liver and 2.16 ± 0.06% in muscle oil), along with the abundance of omega-6 and omega-9 fatty acids, including arachidonic and oleic acid. Additionally, the antimicrobial properties of these fish oils were assessed against Gram-negative and Gram-positive bacteria using the Minimum Inhibitory Concentration (MIC) method, revealing promising inhibitory effects, with liver oil demonstrating greater efficacy. These findings suggest that puffer fish oil is rich in beneficial fatty acids and possesses antimicrobial properties that could find applications in food preservation, medicine, and agriculture, thereby offering a fresh perspective on the functional and nutritional value of *Xenopterus naritus.*

Keywords: Antimicrobial activity, fish oil profiles, omega-3, *Xenopterus naritus*

INTRODUCTION

Omega-3 fatty acids are abundant in fish oil, which is well known for its many health advantages. Fish consumption offers numerous health benefits, including protection against atherosclerotic lesions, liver diseases, and cholestasis. These benefits are due to the presence of fish oil, peptides, hydrolysates, essential vitamins, and various fatty acids (Chen *et al*., 2022). Additionally, fish consumption and omega-3 supplementation have been shown to reduce inflammation and improve outcomes in cognitive health, muscle mass decline, cancer treatment, and critical illness (Troesch *et al*., 2020). Omega-3 and omega-6 fatty acids that obtained from both terrestrial and marine sources provide protection against diseases like osteoarthritis, cancer, and autoimmune disorders, and contribute to various cellular activities including cell signaling, structural integrity, and regulation of blood pressure and glucose levels (Abbott *et al*., 2020). Furthermore, EPA and DHA are omega-3 fatty acids which are rich in fish oil, and have been linked to improved brain structure and function, reduced mortality, lower risk of ischemic stroke, and better cognitive health (von Schacky, 2021). The quality of fish oil can be improved through the sustainable recovery of omega-3 fatty acids from fish waste, which enhances its nutritional value and environmental sustainability (Alfio *et al*., 2021).

Fish oil, rich in polyunsaturated fatty acids (PUFA), has remarkable antibacterial properties (Noutsa *et al*., 2022). These PUFAs disrupt bacterial communication, ATP production, and membrane properties, making them potent antimicrobial agents (Kannan *et al*., 2021). Moreover, they have the potential to hinder bacterial colonization and the expression of

virulence factors. Recent research has highlighted specific omega-3 polyunsaturated fatty acids (ω-3 PUFAs), such as docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), for their significant antimicrobial activity against various bacterial strains, including multi-drug resistant strains isolated from patients with periprosthetic joint infections (PJI) (Coraça-Huber *et al*., 2021). These omega-3 PUFAs have been shown to disrupt bacterial communication, ATP production, and membrane properties, rendering them potent antimicrobial agents. Additionally, they possess the ability to hinder bacterial colonization and the expression of virulence factors, which are crucial for infection establishment. This underscores the importance of exploring marine species, such as fish, as valuable sources of these potent antibacterial compounds (Inguglia *et al*., 2020).

Xenopterus naritus belongs to the family Tetraodontidae and is a marine fish that can inhabit both saltwater and freshwater environments, including Malaysian waters (Mohd Nor Azman *et al*., 2014). In Malaysia, puffer fish including species such as *Lagocephalus lunaris*, *L. sceleratus* and *L. spadiceus* are caught and become a local delicacy (Mohd Nor Azman *et al*., 2014). Among these, *X. naritus* stands out as a migratory species inhabiting the South China Sea's coastline waters off Sarawak (Ahmad Nasir *et al*., 2017). In Sarawak, *X. naritus* also known as rebellious “Buntal Kuning” or yellow pufferfish, is celebrated as a culinary delight (Mohd Nor Azman *et al*., 2014). The Yellow puffer fish exhibits a torpedo-shaped body with striking yellow or golden coloration, particularly on its lower body portion. These migratory fish return to rivers for spawning with juveniles found in coastal waters during the non-spawning season (Mohd Nor Azman & Wan Norhana, 2013).

However, puffer fish widely recognized for its toxicity attributed to the presence of tetrodotoxin (TTX) leading to lack essential nutritional information specifically regarding the fatty acid profile of *X. naritus*. This study aims to address the lack of knowledge regarding the nutritional value of *X. naritus* by comprehensively analyzing its fatty acid composition from liver and muscle tissues. The findings highlight the significant presence of omega-3 fatty acids (DHA and EPA) alongside omega-6 and omega-9 fatty acids, emphasizing the potential nutritional benefits for human health. Additionally, this research promotes *X. naritus* fish oils as a promising supplement with antimicrobial properties against gram-positive and gram-negative bacteria, aiming to contribute to informed consumption and utilization of this species.

MATERIALS AND METHODS

Collection of Puffer Fish Samples and Extraction of Fish Oils

Puffer fish specimens (Figure 1) were purchased from Kubah Ria wet fish markets in Kuching, Sarawak. The fish were selected for their marketable size with an average weight of between 1 and 1.2 kilograms each. The fish samples were carefully brought to the lab and kept there at -20 °C. The fish samples were prepared to separate liver and muscle tissue. The liver and muscle tissue, each weighing 100 grams were then brought together separately. According to Iverson *et al*. (2001), oil extraction from the pooled samples was done using the procedure outlined in the method of Bligh and Dyer (1959). The solvent mixture consisted of chloroform, methanol, and water in a 1:2:1 ratio (v/v/v). The homogenized samples were centrifuged at 3000 rpm for 10 minutes to separate the chloroform phase, which contained the fish oil. The chloroform phase was collected and subjected to solvent evaporation using a rotary evaporator to remove the chloroform. The concentrated fish oils were then blown with nitrogen gas to create an inert atmosphere and prevent oxidation. The oil refining process was following Nazir *et al.* (2017) with minor adjustments that involved three stages: degumming, neutralization, and bleaching. For degumming, the oil was heated and stirred at 70 °C, then centrifuged with hot water to separate oil, gum, and water layers, repeating until neutral pH was achieved. In neutralization, the degummed oil was heated and stirred at 80 °C, treated with a 20% KOH solution, and centrifuged to obtain oil, soap stock, and water layers, repeating until neutral pH. Finally, in bleaching, the neutralized oil was heated (80 – 100 °C), stirred with 1% activated charcoal, filtered, completing the process.

Figure 1. Yellow Puffer Fish, *Xenopterus naritus*

Fatty Acids Profiling in *Xenopterus naritus* by GC/MS Analysis

The extracted fish oil was derivatized following the procedure by Ichihara *et al*. (1996) with minor changes. In a centrifuge tube, a 20 mg oil sample was dissolved in 2 ml of n-heptane. The tube was then filled with 4 ml of 2 M methanolic potassium hydroxide, which was vortexed for 2 minutes at room temperature before being centrifuged for 10 minutes at 4000 rpm. After centrifugation and standing for 10 minutes, a clear solution of fatty acid methyl ester (FAME) separated on the cloudy aqueous layer. The top FAME layer, dissolved in n-heptane, was collected and analyzed directly using a Shimadzu gas chromatograph (GC-MS), model QP2010plus. The methylated oil samples were injected directly into the GC-MS column. The column used was a DB5 column measuring 30 m x 0.25 mm x 250 µm with an autosampler. The test was started at 50 °C for 10 minutes, followed by a temperature increase to 350 °C at a rate of 4.5 °C/min to maintain final temperature for 10 minutes. The carrier gas was helium flowing at a rate of 1.0 ml/min. The retention times were compared to an external standard solution composed of a mixture of 37 FAME components, known as FAME 37, to determine the peaks.

Minimum Inhibitory Concentration (MIC) evaluation

According to the procedure outlined by Simplice *et al.* (2018), the antibacterial effectiveness of fish oil was evaluated using a broth microdilution technique in 96-well microtitre plates. Inoculum preparation was performed using the broth culture method, in which bacterial colonies were transferred to Mueller-Hinton broth (MHB) using a loop and then incubated at 35 – 37 °C for 24 h. To obtain turbidity corresponding to a McFarland standard of 0.5, the culture was adjusted with MHB. Turbidity adjustment was assessed photometrically at 625 nm (UV-VIS spectrophotometer, UV-1900i, SHIMADZU) in the desired absorbance range of 0.08 – 0.10 using EUCAST guidelines (2003). The fish oil stock solution was prepared by dissolving the oil in a 5% Tween 20 solution. This stock solution was then serially diluted to achieve concentrations ranging from 7.8 to 500 mg/ml, with each well containing a total volume of 100 µl. Each well was then filled with 100 µl of bacterial inoculum. The growth was then observed on the microtitre plates using P-iodotetrazolium chloride (INT; 0.2 mg/ml) and incubated at 35 °C for 18 hours. Iodonitrotetrazolium, a yellow dye, became pink in response to the living bacteria. The MIC was determined as the lowest oil concentration at which no obvious color change was observed. As a positive control, chloramphenicol was utilized at doses ranging from 3.9 to 250 g/ml.

RESULTS

Puffer Fish Oils Characterization

By using GC/MS, the fish oil fatty acid profiles (Figure 2) that were extracted from the liver and muscle of *X. naritus* were qualitatively

described. The analysis shows in Table 1 determines differences in the composition of saturated fatty acids (SFAs) and unsaturated fatty acids (UFAs) between liver and muscle tissues. In the liver, SFAs comprised 38.91% of the total fatty acids while UFAs made up 61.09%. This indicates a higher prevalence of UFAs in liver tissue. Conversely, muscle tissue showed a lower proportion of SFAs at 32.19% and a higher proportion of UFAs at 67.81% suggesting a greater abundance of UFAs in muscle tissue.

The study identified key fatty acids that were the primary contributors to each category in both liver and muscle tissues. Palmitic acid (C16:0) emerged as the most predominant SFA in both liver and muscle tissues which underscoring its significance in the fatty acid composition of these tissues. Oleic acid (C18:1n-9) was recognized as the primary monounsaturated fatty acid (MUFA) in both liver and muscle tissues highlighting its prominent role in these tissues. Docosahexaenoic acid or DHA (C22:6n-3) stood out as the dominant polyunsaturated fatty acid (PUFA) in both liver and muscle tissues.

In addition, liver tissue exhibited a higher concentration of specific MUFAs including Vaccenic acid, Cis-10-heptadecenoic acid, Eruric acid and Gondoic acid as compared to muscle tissue. Conversely, liver tissue displayed a greater abundance of certain PUFAs, such as Adrenic acid, Arachidonic acid, Eicosapentaenoic acid and Docosapentaenoic acid when contrasted with muscle tissue.

Although the current study did not analysed toxicity, previous research has detected tetrodotoxin (TTX) in both muscle and liver tissues of *X. naritus*, with variations depending on individual specimens, seasons, and tissues (Mohd Nor Azman *et al*., 2014). For instance, samples from Betong, Sarawak, showed TTX levels of 17.8 μg/g in liver tissue and 11.1 μg/g in muscle tissue (Mohd Nor Azman *et al*., 2014). These concentrations are considered weakly toxic (Noguchi *et al*., 2006) but still exceed the Japanese safety limit for human consumption (>2 μg/g), as Malaysia does not have specific regulations for TTX. Nevertheless, with proper preparation, the fish can be made safe to eat.

Overall, liver tissue contains a higher percentage of SFAs, while muscle tissue has a higher percentage of UFAs. Palmitic acid, oleic acid, and docosahexaenoic acid play pivotal roles as major constituents in these tissues.

Table 1. Fatty acid profile of *Xenopterus naritus* in liver and muscle oil

Name	IUPAC	Relative % ± D.S	
		Liver	Muscle
Myristic acid	C14:0	2.1 ± 0.07	2.46 ± 0.06
Pentadecanoic acid	C15:0	1.53 ± 0.38	2.29 ± 0.06
Palmitic acid	C16:0	17.37 ± 0.28	15.67 ± 0.51
Heptadecanoic acid	C17:0	2.86 ± 0.06	2.73 ± 0.05
Stearic acid	C18:0	9.62 ± 0.27	6.87 ± 0.53
Heneicosanoic acid	C21:0	2.24 ± 0.09	2.17 ± 0.14
Tricosanoic acid	C23:0	1.92 ± 0.29	0 ± 0
Lignoceric acid	C24:0	0.26 ± 0.05	0 ± 0
Palmitoleic acid	C16:1n-7	8.53 ± 0.28	9.19 ± 0.22
Cis-10-heptadecenoic acid	C17:1n-7	0.53 ± 0.22	2.62 ± 0.45
Vaccenic acid	C18:1n-7	0.95 ± 0.09	2.08 ± 0.43
Oleic acid	C18:1n-9	15.2 ± 0.19	20.05 ± 0.39
Paullinic acid	C20:1n-7	0.86 ± 0.11	0 ± 0
Gondoic acid	C20:1n-9	1.35 ± 0.44	5.05 ± 0.91
Eruric acid	C22:1n-9	0.79 ± 0.34	3.25 ± 0.64
Linoleic acid	C18:2n-6	2.46 ± 0.34	2.27 ± 0.07
Arachidonic acid	C20:4n-6	3.25 ± 0.47	2.02 ± 0.44
Adrenic acid	C22:4n-6	7.57 ± 0.43	5.97 ± 0.13
Docosapentaenoic acid	C22:5n-6	2.9 ± 0.35	1.91 ± 0.02
Eicosapentaenoic acid	C20:5n-3	3.29 ± 0.12	2.16 ± 0.06
Docosapentaenoic acid	C22:5n-3	5.84 ± 0.25	4.93 ± 0.14
Docosahexaenoic acid	C22.:6n-3	8.28 ± 0.08	6.15 ± 0.33

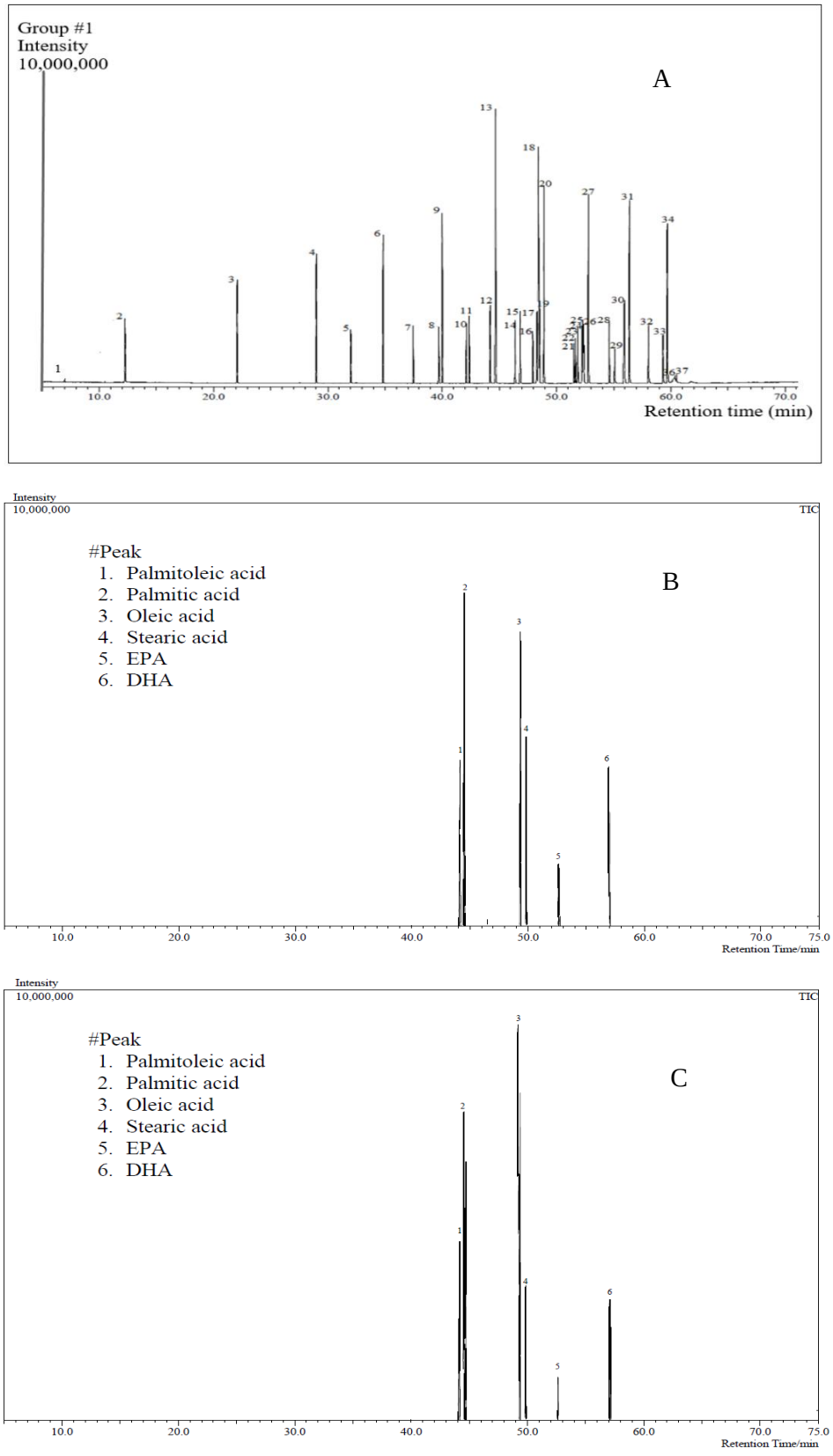

Figure 2. GC-MS Chromatogram of (A) standard solution containing 37 FAMEs, (B) fatty acid profiles in *X. naritus* liver oil, and (C) muscle oil by Bligh & Dyer method

Antibacterial Activity of the *X. naritus* Fish Oils

The study assessed the antimicrobial effectiveness of oils extracted from both the liver and muscle tissue of puffer fish. In order to undertake this study, the MIC values against Gram-positive and Gram-negative reference strains of bacteria were evaluated. Table 2 provides an overview of the findings to present the antimicrobial activity of these fish oils. The liver oil displayed superior antimicrobial activity against several Gram-positive bacteria, including *S. aureus*, *B. cereus*, and *S. saprophyticus* with MIC values of 125 mg/ml as compared to the muscle oil with higher MICs (250-500% v/v). On the other hand, liver oil demonstrated increased potency against Gram-negative bacteria with *K. pneumonia* (MIC 62.5 mg/ml), while both oils were equally effective against *E. coli* and *E. cloacae* (MIC 125 mg/ml).

DISCUSSION

Due to its toxicity, puffer fish is always regarded as trash fish by trawlers. The huge mass of caught puffer fish may be a significant source of bioactive chemicals for supplementation in diet. *X. naritus* is a good protein resources and can be taken into account for human diet (Mohd Nor Azman *et al*., 2015) PUFAs is another significant source of high quality bioactive molecules that can be extracted from puffer fish oil.

The male *X. naritus* can represent up to 22.5 cm in total length and 274.05 g in body weight, while female *X. naritus* can up to 33.9 cm in total length and 711.00 g in body weight (Mohd Nor Azman & Wan Norhana, 2013).

This study has confirmed that PUFAs are present in the oil extracted from the *X. naritus* liver and muscle tissues and have demonstrated how these PUFAs are characterize by the presence of omega-3 fatty acids (Table 2). The most abundant omega-3 found in the fish oil was the DHA, which represent 8.28±0.08% in liver oil and 6.15±0.33% in muscle oil. Besides EPA represent 3.29±0.12% in liver and 2.16±0.06% in muscle also have health beneficial. The DHA and EPA is really important in the human diet such as proper fetal and infant development (Carver *et al*.,2001; Ramakrishnan *et al.*, 2010), cardiovascular function (Kromhout *et al*., 2010; Bernasconi *et al.*, 2021), Alzheimer's disease (Quinn *et al*., 2010), immune response (Krauss-Etschmann *et al*., 2008), cognitive function (Titova *et al*., 2013) eye health (Cortina & Bazan, 2011) and prebiotics (Fu *et al*., 2021).

Table 2. MIC of *Xenopterus naritus* from liver and muscle oil against gram-positive and gram-negative strains

		MIC (mg/ml)	
	Bacteria strains	Liver	Muscle
Gram-positive	*S. aureus*	125	250
	B. cereus	125	500
	S. saprophyticus	125	500
Gram-negative	*E. coli*	125	250
	K. pneumonia	62.5	500
	E. clocae	125	250

The omega-6 linoleic acids are also the major precursor of the eicosanoids, including thromboxane, prostaglandins, prostacyclin, anandamides, and leukotrienes which regulate a wide range of physiological processes (Innes & Calder, 2018). Arachidonic acid (ARA) is a crucial component of cell structure and is required for growth and development as well as in the event of severe or pervasive cell injury (Tallima & Ridi, 2018). The liver (15.20 ± 19%) and muscles (20.050 ± 39%) contained the highest concentrations of omega-9 oleic acid. This molecule is MUFA has shown to exert many biological function. Incorporating oleic acid into diets could be a valuable strategy in the context of high-lipid dietary trends with potential benefits for growth performance, feed utilization and overall health (Martins *et al*., 2023).

Fatty acids play a significant role in the fight against microbial infections. Fatty acids may exert their antimicrobial effects by altering the hydrophobicity, charge, and integrity of cell membranes, which causes electron leakage and subsequent cell death (Inguglia *et al*., 2020). A number of microorganisms have been shown to be resistant to omega-6, -7, and -9 fatty acids, such as arachidonic, linoleic, oleic, and palmitoleic acid, as well as their methyl esters and ethyl esters (Huang *et al*., 2010). A number of microorganisms have been shown to be resistant to omega-6, -7, and -9 fatty acids, such as arachidonic, linoleic, oleic, and palmitoleic acid, as well as their methyl esters and ethyl esters (Huang *et al*., 2010). Fatty acids are appealing for a variety of applications in medicine, agriculture, and food preservation, especially where convents are involved. This is because of their mechanism of antibacterial activity involves disruption of the bacterial cell membrane, interference with the electron transport chain and oxidative phosphorylation, impairment of nutrient uptake, inhibition of enzyme activity, auto-oxidation degradation, generation of peroxidation products and direct lysis of bacterial cells (Desbois & Smith, 2010).

In this study, the liver and muscle of puffers were tested for their antimicrobial abilities against Gram-positive and Gram-negative pathogens, and the results revealed a range of MIC values. Liver oil inhibit the growth of Gram-positive and Gram-negative bacteria with concentration at 125 mg/ml except, *K. pneumonia* (62.5 mg/ml). On the other hand, muscle oil inhibits the growth of bacteria with higher MIC than liver oil. This difference in effectiveness could be attributed to the fatty acid compositions of the oils.

Fish oil that contain PUFA inhibits bacterial growth by disrupting cell membrane hydrophobicity, charge, and integrity, leading to electron leakage and cell death (Calo *et al*., 2015). Fatty acids play a crucial role in combating microorganism infections, as pathogens produce virulence factors and form biofilms (Schroeder *et al*., 2017). Both omega-3 fatty acids, particularly linolenic acid, and omega-6, -7, -9 fatty acids, including γ-linolenic, linoleic, arachidonic, palmitoleic, and oleic acids, demonstrate antimicrobial properties (Chanda *et al*., 2018). These fatty acids contribute to bacterial death through mechanisms such as cell lysis, enzyme activity inhibition, and the production of lethal oxidation products (Desbois & Smith, 2010). Our findings suggest that fish oil from liver and muscle is effective against tested microorganisms regardless of the bacterial wall type, with liver oil showing greater efficacy, possibly due to differences in fatty acid compositions (Inguglia *et al*., 2020). Hence, PUFA specifically accounts for the antibacterial activity, emphasizing the importance of preventing PUFA oxidation (Simplice *et al*., 2018).

CONCLUSION

This study highlights the nutritional value of puffer fish oil from *X. naritus*, countering its negative reputation due to toxicity concerns. The fatty acid profile revealed high levels of beneficial omega-3, omega-6, and omega-9 fatty acids, important for fetal development, cardiovascular health, cognitive function, and immune response. Additionally, the antimicrobial properties of the oil, particularly from the liver, showed significant potency against both Gram-positive and Gram-negative bacteria. Although this study did not measure tetrodotoxin (TTX) levels, previous research indicates weakly toxic TTX concentrations in *X. naritus*, exceeding the safe limit for consumption. However, proper preparation can make the fish safe to eat. Overall, this study provides valuable insights into the nutritional and antimicrobial benefits of puffer fish oil, while also addressing safety considerations related to TTX.

ACKNOWLEDGMENT

The authors would like to acknowledge the valuable contributions of their colleagues, Mr. Rajuna Tahir and Mr. Benedict Samling, from the Faculty of Resource Science and Technology at Universiti Malaysia Sarawak. They also express gratitude to the Malaysia Comprehensive University Network-MCUN (UNIMAS, UiTM, and UMS) for the financial assistance provided through the grant program [GL/F07/MCUN/09/2020].

REFERENCES

Abbott, K.A., Burrows, T.L., Acharya, S., Thota, R. N., & Garg, M.L. (2020). DHA-enriched fish oil reduces insulin resistance in overweight and obese adults. *Prostaglandins, Leukotrienes and*

Essential Fatty Acids, 159, 102154. DOI: 10.1016/j.plefa.2020.102154.

Abedi, E., & Sahari, M.A. (2014). Long-chain polyunsaturated fatty acid sources and evaluation of their nutritional and functional properties. *Food Science and Nutrition*, 2(5): 443-463. DOI: 10.1002/fsn3.121.

Ahmad Nasir, A.S., Mohamad, S., & Mohidin, M. (2017). The first reported artificial propagation of yellow puffer, *Xenopterus naritus* (Richardson, 1848) from Sarawak, Northwestern Borneo. *Aquaculture Research*, 48(8): 4582-4589. DOI: 10.1111/are.13103.

Alfio, V.G., Manzo, C., & Micillo, R. (2021). From fish waste to value: an overview of the sustainable recovery of omega-3 for food supplements. *Molecules*, 26(4): 1002. DOI: 10.3390/molecules26041002.

Bernasconi, A.A., Wiest, M.M., Lavie, C.J., Milani, R.V., & Laukkanen, J.A. (2021). Effect of Omega-3 dosage on cardiovascular outcomes: An updated meta-analysis and meta-regression of interventional trials. *Mayo Clinic Proceedings*, 96(2): 304–313. DOI: 10.1016/j.mayocp.2020.08.034.

Calo, J.R., Crandall, P.G., O'Bryan, C.A., & Ricke, S.C. (2015). Essential oils as antimicrobials in food systems–A review. *Food Control*, 54: 111-119. DOI: 10.1016/j.foodcont.2014.12.040.

Carver, J.D., Benford, V.J., Han, B., & Cantor, A.B. (2001). The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects. *Brain Research Bulletin*, 56(2): 79–85. DOI: 10.1016/s0361-9230(01)00551-2.

Chanda, W., Joseph, T.P., Guo, X.F., Wang, W.D., Liu, M., Vuai, M. S., Padhiar, A.A., & Zhong, M.T. (2018). Effectiveness of omega-3 polyunsaturated fatty acids against microbial pathogens. *Journal of Zhejiang University-SCIENCE B*, 19(4), 253-262. DOI: 10.1631/jzus.B1700063.

Chen, J., Jayachandran, M., Bai, W., & Xu, B. (2022). A critical review on the health benefits of fish consumption and its bioactive constituents. *Food Chemistry*, 369: 130874. DOI: 10.1016/j.foodchem.2021.130874.

Coraça-Huber, D.C., Steixner, S., Wurm, A., & Nogler, M. (2021). Antibacterial and anti-biofilm activity of omega-3 polyunsaturated fatty acids against periprosthetic joint infections-isolated multi-drug resistant strains. *Biomedicines*, 9(4): 334. DOI: 10.3390/biomedicines9040334.

Cortina, M.S., & Bazan, H.E. (2011). Docosahexaenoic acid, protectins and dry eye. *Current Opinion in Clinical Nutrition and Metabolic Care*, 14(2): 132–137. DOI: 10.1097/mco.0b013e328342bb1a.

Desbois, A.P., & Smith, V.J. (2010). Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology*, 85: 1629-1642. DOI: 10.1007/s00253-009-2355-3.

European Committee for Antimicrobial Susceptibility Testing (EUCAST). (2003). EUCAST of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID): Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. *Clinical Microbiology and Infection*, 9: 1-7. DOI: 10.1046/j.1469-0691.2000.00149.x.

Fu, Y.W., Wang, Y.D., Gao, H., Li, D.H., Jiang, R.R., Ge, L.R., Tong, C., & Xu, K. (2021). Associations among dietary omega-3 polyunsaturated fatty acids, the gut microbiota, and intestinal immunity. *Mediators of Inflammation*, 11. DOI: 10.1155/2021/8879227.

Guil-Guerrero, J.L., & Belarbi, E.H. (2001). Purification process for cod liver oil polyunsaturated fatty acids. *Journal of the American Oil Chemists' Society*, 78: 477-484. DOI: 10.1007/s11746-001-0289-9.

Holub, D.J., & Holub, B.J. (2004). Omega-3 fatty acids from fish oils and cardiovascular disease. *Molecular and Cellular Biochemistry*, 263: 217-225.

Huang, C.B., George, B., & Ebersole, J.L. (2010). Antimicrobial activity of n-6, n-7 and n-9 fatty acids and their esters for oral microorganisms. *Archives of Oral Biology*, 55(8): 555-560. DOI: 10.1016/j.archoralbio.2010.05.009.

Ichihara, K. I., Shibahara, A., Yamamoto, K., & Nakayama, T. (1996). An improved method for rapid analysis of the fatty acids of glycerolipids. *Lipids*, 31(5): 535-539. DOI: 10.1007/BF02522648.

Inguglia, L., Chiaramonte, M., Di Stefano, V., Schillaci, D., Cammilleri, G., Pantano, L., Mauro, M., Vazzana, M., Ferrantelli, V., Nicolosi, R., & Arizza, V. (2020). *Salmo salar* fish waste oil:

Fatty acids composition and antibacterial activity. *PeerJ, 8*, e9299. DOI: 10.7717/peerj.9299.

Innes, J.K., & Calder, P.C. (2018). Omega-6 fatty acids and inflammation. *Prostaglandins, Leukotrienes and Essential Fatty Acids*, 132: 41-48. DOI: 10.1016/j.plefa.2018.03.004.

Iverson, S.J., Lang, S.L.C., & Cooper, M.H. (2001). Comparison of the Bligh and Dyer and Folch methods for total lipid determination in a broad range of marine tissue. *Lipids*, 36(11): 1283–1287. DOI: 10.1007/s11745-001-0843-0.

Kannan, N., Rao, A.S., & Nair, A. (2021). Microbial production of omega-3 fatty acids: an overview. *Journal of Applied Microbiology*, 131(5): 2114-2130. DOI: 10.1111/jam.15034.

Krauss-Etschmann, S., Hartl, D., Rzehak, P., Heinrich, J., Shadid, R., del Carmen Ramírez-Tortosa, M., Campoy, C., Pardillo, S., Schendel, D.J., Decsi, T. and Demmelmair, H., 2008. Decreased cord blood IL-4, IL-13, and CCR4 and increased TGF-β levels after fish oil supplementation of pregnant women. *Journal of Allergy and Clinical Immunology*, 121(2): pp.464-470. DOI: 10.1016/j.jaci.2007.09.018.

Kromhout, D., Giltay, E.J., & Geleijnse, J.M. (2010). n–3 Fatty acids and cardiovascular events after myocardial infarction. *New England Journal of Medicine*, *363*(21): 2015-2026. DOI: 10.1056/NEJMoa1003603.

Martins, N., Magalhães, R., Vieria, L., Couto, A., Serra, C.R., Maia, M.R., Fonseca, A.J., Cabrita, A.R., Pousao-Ferreira, P., Castro, C., Peres, H., & Oliva-Teles, A. (2023). Dietary oleic acid supplementation improves feed efficiency and modulates fatty acid profile and cell signaling pathway in European sea bass (*Dicentrarchus labrax)* juveniles fed high-lipid diets. *Aquaculture*, 576: 739870. DOI: 10.1016/j.aquaculture.2023.739870.

Mohd Nor Azman, A., Samsur, M., & Othman, M. (2014). Distribution of tetrodotoxin among tissues of puffer fish from Sabah and Sarawak waters. *Sains Malaysiana*, 43(7): 1003-1011.

Mohd Nor Azman, A., Samsur, M., Mohammed, M., Shabdin, M.L., & Fasihuddin, B.A. (2015). Assessment of proximate composition and tetrodotoxin content in the muscle of Yellow puffer fish, *Xenopterus naritus* (Richardson 1848) from Sarawak, Malaysia. *International Food Research Journal*, 22(6).

Mohd Nor Azman. A. & Wan Norhana, M.N. (2013). Detection of tetrodotoxin and soxitoxin in dried salted yellow puffer fish (*Xenopterus naritus*) eggs from Satok Market, Kuching, Sarawak. *International Food Research Journal*, 20(5): 2963-2966.

Nazir, N., Diana, A. & Sayuti, K. (2017). Physicochemical and fatty acid profile of fish lipid from head of tuna (*Thunnus albacares*) extracted from various extraction method. *International Journal on Advanced Science, Engineering and Information Technology*, 7(2): 709-715. DOI: 10.18517/ijaseit.7.2.2339.

Noguchi, T., Arakawa, O., & Takatani, T. (2006). TTX accumulation in puffer fish. *Comparative Biochemistry and Physiology*, Part D 1:145-152. DOI: 10.1016/j.cbd.2005.10.006.

Noutsa, B.S., Tchabong, S.R., Djitieu, A.D.D., Dongmo, F.F.D., Ngamga, F.H.N., Zokou, R., Tamgue, O., Ngane, R.A.N., & Tchoumbougnang, F. (2022). Chemical characterization and antibacterial properties of *Fontitrygon margarita* (Günther, 1870) liver oil. *Journal of Lipids*, 2022(1): 9369387. DOI: 10.1155/2022/9369387.

Quinn, J. F., Raman, R., Thomas, R. G., Yurko-Mauro, K., Nelson, E. B., Van Dyck, C., ... & Aisen, P. S. (2010). Docosahexaenoic acid supplementation and cognitive decline in Alzheimer disease: a randomized trial. *Jama*, 304(17): 1903-1911. DOI: 10.1001/jama.2010.1510.

Ramakrishnan, U., Stein, A.D., Parra-Cabrera, S., Wang, M., Imhoff-Kunsch, B., Juárez-Márquez, S., Rivera, J. and Martorell, R., 2010. Effects of docosahexaenoic acid supplementation during pregnancy on gestational age and size at birth: randomized, double-blind, placebo-controlled trial in Mexico. *Food and nutrition bulletin*, *31*(2_suppl2): pp.S108-S116. DOI: 10.1177/15648265100312S203.

Schroeder, M., Brooks, B.D., & Brooks, A.E. (2017). The complex relationship between virulence and antibiotic resistance. *Genes*, 8(1): 39. DOI: 10.3390/genes8010039.

Simplice, M.R., Macaire, W.H., Hervé, N.N.F., Fabrice, T.D., Justin, D.D., François, T., & Jules-Roger, K. (2018). Chemical composition and antibacterial activity of oils from *Chrysicthys nigrodigitatus* and *Hepsetus odoe*, two freshwater fishes from Yabassi, Cameroon. *Lipids in Health and Disease*, 17(1): 1-7. DOI:10.1186/s12944-018-0690-z.

Tallima, H., & El Ridi, R. (2018). Arachidonic acid: physiological roles and potential health benefits–a review. *Journal of Advanced Research*, 11: 33-41. DOI: 10.1016/j.jare.2017.11.004.

Titova, O.E., Sjögren, P., Brooks, S.J., Kullberg, J., Ax, E., Kilander, L., Riserus, U., Cederholm, T., Larsson, E. M., Johansson, L., Ahlström, H., Lind, L., Schiöth, H.B., & Benedict, C. (2013). Dietary intake of eicosapentaenoic and docosahexaenoic acids is linked to gray matter volume and cognitive function in elderly. *Age*, 35(4): 1495–1505. DOI: 10.1007/s11357-012-9453-3.

Troesch, B., Eggersdorfer, M., Laviano, A., Rolland, Y., Smith, A.D., Warnke, I., Weimann, A., & Calder, P.C. (2020). Expert opinion on benefits of long-chain omega-3 fatty acids (DHA and EPA) in aging and clinical nutrition. *Nutrients*, 12(9): 2555. DOI: 10.3390/nu12092555.

von Schacky, C. (2021). Importance of EPA and DHA blood levels in brain structure and function. *Nutrients*, 13(4): 1074. DOI: 10.3390/nu13041074.

Zaidul, I.S.M., Norulaini, N.N., Omar, A.M., Sato, Y., & Smith Jr, R.L. (2007). Separation of palm kernel oil from palm kernel with supercritical carbon dioxide using pressure swing technique. *Journal of Food Engineering*, 81(2): 419-428. DOI: 10.1016/j.jfoodeng.2006.11.019