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Full Length Article

Microwave-assisted extraction and characterization of fatty acid from eel fish (*Monopterus albus*)

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ABSTRACT

Eel fish (*Monopterus albus*) is a functional food that has shown remarkable effects on a range of diseases, which include inflammatory diseases, type 2 diabetes and cancer. This study emphasized on the use of microwave-assisted extraction (MAE) technique in studying the effects of three MAE parameters (irradiation time, microwave temperature and microwave power) to obtain extract from eel fish using ethanol as the extracting solvent. The free fatty acid (FFA), acid value, fatty acid contents, and functional groups in the extract were also examined. More so, the maximum extraction yield of 16.13% w/w was obtained at a microwave power of 800 W, FFA and acid value of 1.35 and 2.69 mg KOH/g, respectively confirmed good quality of the obtained extract. In addition, the major fatty acid contents in the extract using gas chromatography-mass spectrometry (GC-MS) analysis were arachidonic acid, linolenic acid, myristic acid, oleic acid, palmitic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Consequently, the Fourier transform infrared spectrometry (FTIR) analysis confirmed the presence of different functional groups in the extract. Therefore, this study divulged that MAE is an efficient and reliable technique for extracting high yields of fatty acids from *M. albus* fish with a notable potential for industrial applications.

1. Introduction

Eel fish is native to south-east of Asia. However, it now exists in the west, central and north of Africa and South America (Razak et al., 2001). It belongs to the family of freshwater eel-like teleost fishes with a facultative air-breathing that possess reduced gills with the scale-less body (Adeoti and Hawboldt, 2014; Liang et al., 2016). Many factors such as oxygen, salinity and temperature which limit the dispersal of other aquatic animals do not affect the Asian swamp eel (Nico et al., 2011). However, the application of large scale of pesticides on fish farming and overfishing has caused a serious decline in the wild population (Liang et al., 2016). In Malaysia and another part of Asia, swamp eel is considered as food enrich in protein. In the United States of America, eel fishes are widely consumed as food and pet meals (Liang et al., 2016; Nico et al., 2011; Razak et al., 2001; Rosli and Sarbon, 2015).

The extract obtained from eel fish has been used to manage autoimmune medical conditions such as arthritis and rheumatoid which often affects the joints and bones. Additionally, it reduces morning stiffness in the body, joint inflammation as well as the potential dependence on the non-steroidal anti-inflammatory medicines or NSAIDs (Liang et al., 2016; Routray and Orsat, 2012). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to be the two major omega-3 fatty acids which reduce the levels of blood pressure and cholesterol (Moffat and McGill, 1993; Wong et al., 2016). The eel has also been investigated not to contain sugar, as its sodium content is very low with higher phosphorus. It is enriched with vitamins such as A, B1, B2, B12, D, and E which are required for body's general wellbeing (Abdul Mudalip et al., 2010; Rosli and Sarbon, 2015; Wong et al., 2016). Veldink et al. (2006) reported that the intake of polyunsaturated fatty acids and vitamin E reduces the risk of developing amyotrophic lateral sclerosis (ALS). In addition, Moffat and McGill (1993) reported that there is a very low risk of getting gastrointestinal upset when consuming about 1 g of the omega-3 fatty acids per day from a dietary fish intake. In a recent study, Lee et al. have confirmed that omega-3 and omega-6 fatty acids are very important structural components of the cell membranes that play the important role as precursors to bioactive lipid mediators which serve as energy sources (Lee et al., 2016).

In the extraction process, the selection of rightful method is imperative. However, microwave-assisted extraction (MAE) method has become one of the most increasing extraction technique employed by researchers in recent years. Its fundamental process is quite different from conventional method due to the fact that extraction takes place

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because of the changes which occur in cell structure extracted using electromagnetic waves (Alara et al., 2018a; Lee et al., 2016; Melgar-Lalanne et al., 2017). MAE technique results in a higher recovery of analytes within a shorter period of extraction with higher reproducibility (Alara et al., 2018a; Lee et al., 2016; Tatke and Jaiswal, 2011). Not only that but also, it protects the constituents of thermolabile compounds and gives a fast and high extraction performances (Alara et al. 2018b; Ahmed, 2003). Therefore, microwave has been chosen as a modern technique of extraction in this study. This study emphasizes the effects of three MAE parameters (irradiation time, microwave temperature and microwave power) on extraction yield from eel fish. In addition, the fatty acid constituents using GC-MS, acid value and FFA, as well as Fourier transform infrared spectrometry (FTIR) analysis were studied.

2. Materials and methods

2.1. Raw materials

Fresh eel fish (*M. albus*) was purchased from local market in Kuantan, Malaysia (Latitude: N3°43′25.183″; Longitude: E102°6′8.972″) in March 2017. Ethanol, hexane, methanol, and anhydrous potassium hydroxide were purchased from Sigma Aldrich chemicals company (M) Sdn Bhd, Selangor. All the reagents and chemicals used were of analytical grade.

2.2. Sample preparation

The fish was cut into small pieces prior to proper separation of fillets from the bones, both head and internal organs were removed. Thereafter, it was washed with a running tap to remove blood and the fillets were oven-dry at a temperature of 70 °C for 15 h. The dried fillets were then ground into fine powder form using an E8150 warring blender and sieved with a plastic sieve to homogenize the sample. The powdery form sample was weighed and stored in a dark container at −4°C to avoid degradation. The moisture content was obtained using the Eq. (1).

$$\%Moisture\ Content = \frac{Wet\ weight - Dry\ weight\ (g)}{Wet\ weight\ (g)} * 100\% \quad (1)$$

2.3. Microwave-assisted extraction process

Milestone ATC-FO 300 microwave laboratory system was used for the extraction process. The powdered sample of 10 g was weighed and mixed with 75 mL of ethanol in a round bottom flask of the microwave extractor and then shock for 1 min to allow uniformity as well as expansion of the surface area of powder particles. The effects of different extraction time of 10, 20, 30, 40, and 50 min were first examined each sequentially by fixing other factors, microwave power was set at 600 W and temperature at 50 °C. Subsequently, the effects of different temperatures of 50, 60, 70, 80, and 90 °C and different microwave powers at 600, 700, 800, 900, and 1000 W were also examined. The extraction was carried out in a closed vessel with a solvent system of ethanol and no evaporation was observed. Extracts obtained from each run were filtered with a Whatman N°1 filter paper on a Buchner funnel under Buchi Vac V-500 pump, Switzerland to remove some of the residues which are left behind after the extraction. Evaporation of the filtered extract was then achieved in a 100 mL round-bottom flask by means of Buchi Rotavapor R-2000 rotary evaporator at 35 °C to remove the excess solvent in filtered extract and obtain the concentrated extract. The mass of concentrated yield generated was measured and kept in a refrigerator at −4 °C for analysis. Eq. (2) was used to calculate yields of extract from the eel fish.

$$\%\ Yield = \frac{Weight\ of\ oil\ obtained\ (w)}{Weight\ of\ sample\ used\ (w)} * 100\% \quad (2)$$

2.4. Determination of free fatty acid content and acid value

Determinations of Free Fatty Acid (FFA) and Acid Value were carried out on the extracted eel extract yields using 785 DMP Titrino Metrohm to know the quality of the extract (Abdul Mudalip et al., 2010). A 5.5 g of potassium hydroxide (KOH) and 1 L of absolute ethanol was used to prepare a titrant solution of 0.1 mol/L KOH in ethanol. Then, 2 g of the extract was diluted with 20 mL of absolute ethanol in a 50 mL beaker for each run. The beaker was placed on the 728 metrohm stirrer with a magnetic stirrer and a potentiometric sensor immersed in the diluted solution for detection of fatty acids value. The 785 DMP titrino analyzer was set ON and put in 'USER' mode, the parameter FFA was entered. It took about 10 min to dilute the solution used in the analysis in order to break out the molecules in the sample. After 10 min, the 785 DMP titrino screen displayed a titration curve and gives values that correspond to the marked point by the cursor. Finally, the free fatty acid and acid value data were determined from the displayed values. This process was repeated for all other samples.

2.5. Determination of chemical composition

Gas Chromatography-Mass Spectrometer Agilent 6890 instrument coupled to an Agilent 5973 mass spectrometer and an Agilent Chem were employed to determine and identify the fatty acids content of the extract. Initially, the extract samples were converted to their constituent fatty acid methyl esters (FAME) (Razak et al., 2001). In brief, 100 mg sample was weighed in a 20 mL reaction vial. Then, the sample was dissolved in 10 mL hexane, 100 µL of 2 N potassium hydroxide was subsequently added in methanol (11.2 g in 100 mL), followed by vortex for 30 s. Thereafter, the sample was centrifuged for 10 min at 448 g. The obtained clear supernatant was then transferred into 2 mL autosampler vial bottle. The GC-MS analysis was carried out on polyethene glycols DB-Wax column (30 m × 0.250 mm × 0.250 µm) and helium gas was used as the carrier. About 1 µL of the sample was injected and the conditions of operation were oven temperature of 35 °C; an inlet temperature of 250 °C and a flame ionization detector of 300 °C. Identification of the fatty acid methyl esters was done by comparing the retention time with those of the NIST 05a library database.

2.6. Fourier transform infrared spectrometry analysis

For the purpose of detection of functional groups and their characteristics peak values, the eel fish extract was characterized using the Fourier transform infrared spectrometry (FTIR). The analysis was carried out using FTIR spectrometer (Nicolet iS5 iD7 ATR; Thermo Scientific, Germany) equipped with OMNIC software. The standard procedure of KBr was employed for the analysis of the samples so as to obtain the IR spectra in the scanning wave number ranging from 500 to 4000 cm^{-1} with a resolution of 4 cm^{-1} (Alara et al., 2018c). The expected absorption bands table for various molecule groups and bonds were used for comparing the spectra obtained from the extract.

3. Results and discussion

Generally, the extraction time, microwave temperature and microwave power are considered to play a very significant role in the extraction of eel fish extract using MAE (Zhang et al., 2011). A constant extraction time of 30 min and microwave temperature of 60 °C obtained from the effect of both time and temperature studied was used along with different ranges of microwave power (600–1000 W) to determine a maximum yield of 16.13% extract as discussed below.

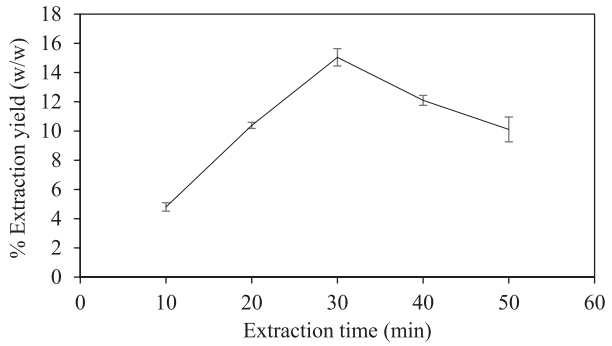

Fig. 1. Effect of extraction time on the recovery of extract from eel fish.

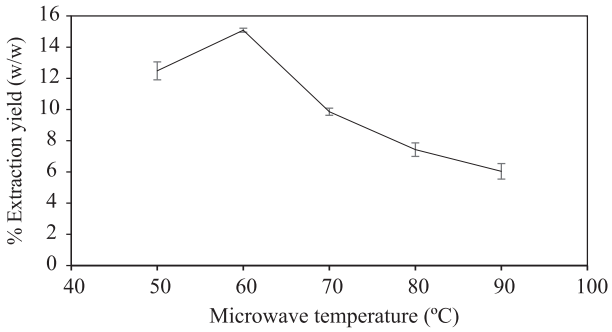

Fig. 2. Effect of microwave temperature on the recovery of extract from eel fish.

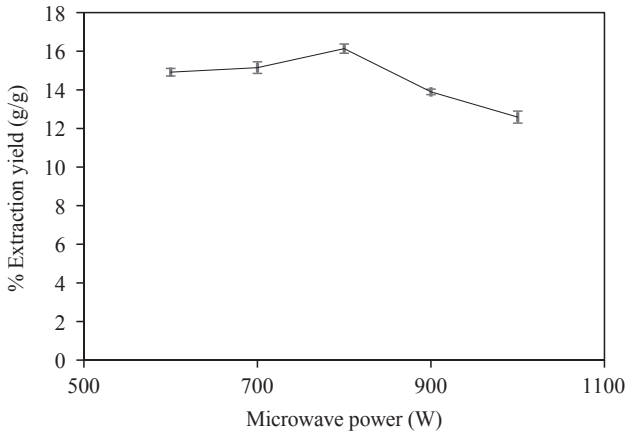

Fig. 3. Effect of microwave power on the recovery of extract from eel fish.

Table 1
Summary of thie fatty acid components identified in the eel fish extract.

Fatty acids	Peak area (%)	Retention time (min)
Myristic acid, C14:0	3.63	39.998
Palmitic acid, C16:0	4.43	42.041
Myristic acid, C14:0	4.43	42.041
Oleic acid, C18:1n-9	26.61	43.608
Linolenic acid, C28:2n6c	4.12	44.298
Arachidonic acid, C20:4n-6	15.54	44.876
Arachidonic acid, C20:4n-6	4.73	46.465
EPA	11.99	51.615
DHA	1.03	55.049

3.1. Effect of microwave irradiation time on the recovery of eel extract

The effect of different microwave extraction time on the yield of eel fish extract in which an increase in the irradiation time (10, 20 and 30 min) leads to increase in amount of yields recovered and any further increment in the extraction time beyond 30 min resulted in a declined extract yield (Fig. 1). Veggi et al. (2013) had reported that any increment in microwave extraction time of sample increases the yield of extract rapidly up to a maximum value where any further prolong irradiation time reduces the yield. The increase in yield might be due to increase in the penetrating power of solvent towards sample matrix when the irradiation time was prolonged while the later reduction in extraction yield might be as a result of over-exposure or overheating of the sample matrix leading to thermal degradation of effective chemical constituents in the sample (Routray and Orsat, 2012; Camel, 2000). The yield (15.04%) obtained at 30 min was the highest, however, the one recovered at 40 min was also significantly high (12.1%) followed by the yield at 20 min (10.39%), 50 min (10.11%) and 10 min (4.8%). This decline in extraction yield with extraction time was also reported for polyunsaturated fatty acids of peanut extract, soybean, and sunflower (Hassanein et al., 2003).

Table 2
The free fatty acid (%) and acid value of the extract at different microwave power.

Power (W)	Acid value (mg KOH/g)	Free fatty acid (%)
600	2.14	1.08
700	2.49	1.25
800	2.69	1.35
900	2.6	1.31
1000	2.24	1.13

3.2. Effect of microwave temperature on the recovery of eel extract

Temperature is one of the most investigated parameters in microwave-assisted extraction technique since it is a key factor that contributes to the increase in yields of the process. The yield of eel fish extract obtained at different microwave temperatures is illustrated in Fig. 2. As seen, the percentage of yield at temperature 50, 60, 70, 80, and 90 °C were 12.48, 15.15, 9.86, 7.43, and 6.04%, respectively. This implies that the highest yield was obtained at a temperature of 60 °C (15.15%), followed by 50 °C (12.48%) while the lowest yield was at a temperature of 90 °C (6.04%). The amount of yield obtained increase from 12.48 to 15.15% when the temperature was raised initially from 50 to 60 °C and further increase in temperature to 70, 80 and 90 °C reduced the amount of yield drastically to 9.86, 7.43 and 6.04%, respectively. The microwave extraction efficiency increases with increase in the temperature until it reaches a certain optimum temperature where it started to decline with further increment in temperature (Routray and Orsat, 2012). The increase in yield could be associated to the fact that during heating there is a migration of dissolved ions which properly allows the solubilization of solutes and henceforth facilitating the higher collection of target compounds (Routray and Orsat, 2012; Eskilsson and Bjorklund, 2000). In addition, the decrease or low yield obtained at high temperature is most probably due to the analytes decomposition (Eskilsson and Bjorklund, 2000). However, temperature of the microwave is being controlled by the incident microwave power which influences the sum of energy needed in sample matrix (Mandal et al., 2007).

3.3. Effect of microwave power on the recovery of eel extract

The optimum extraction time and microwave temperature were used to study the effect of microwave power on the recovery yield of extract from eel fish as shown in Fig. 3. It can be clearly seen that the extraction yield ranged from 12.59 to 16.13%. An increase in microwave power from 600 to 800 W causes an increase in the percentage yield. Afterward, there was a declination in yield as the microwave power was set beyond 800 W. Thus, the recovery yield decreases in the

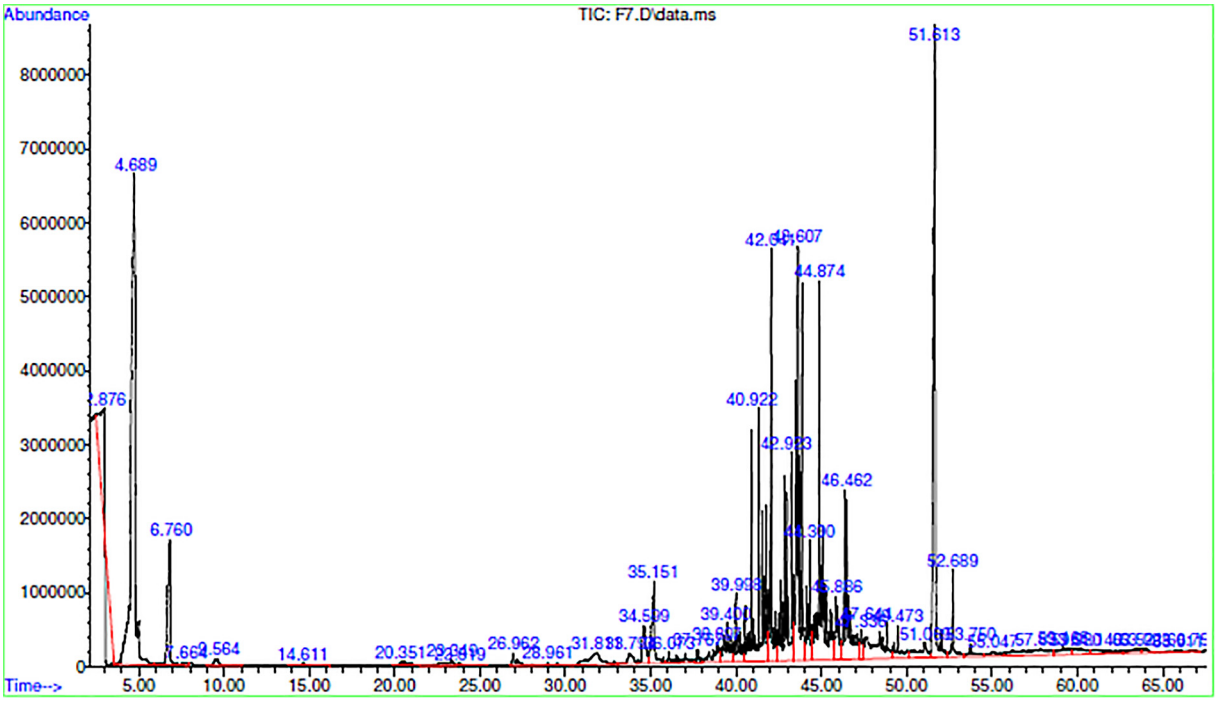

Fig. 4. The chromatogram of the eel fish extract.

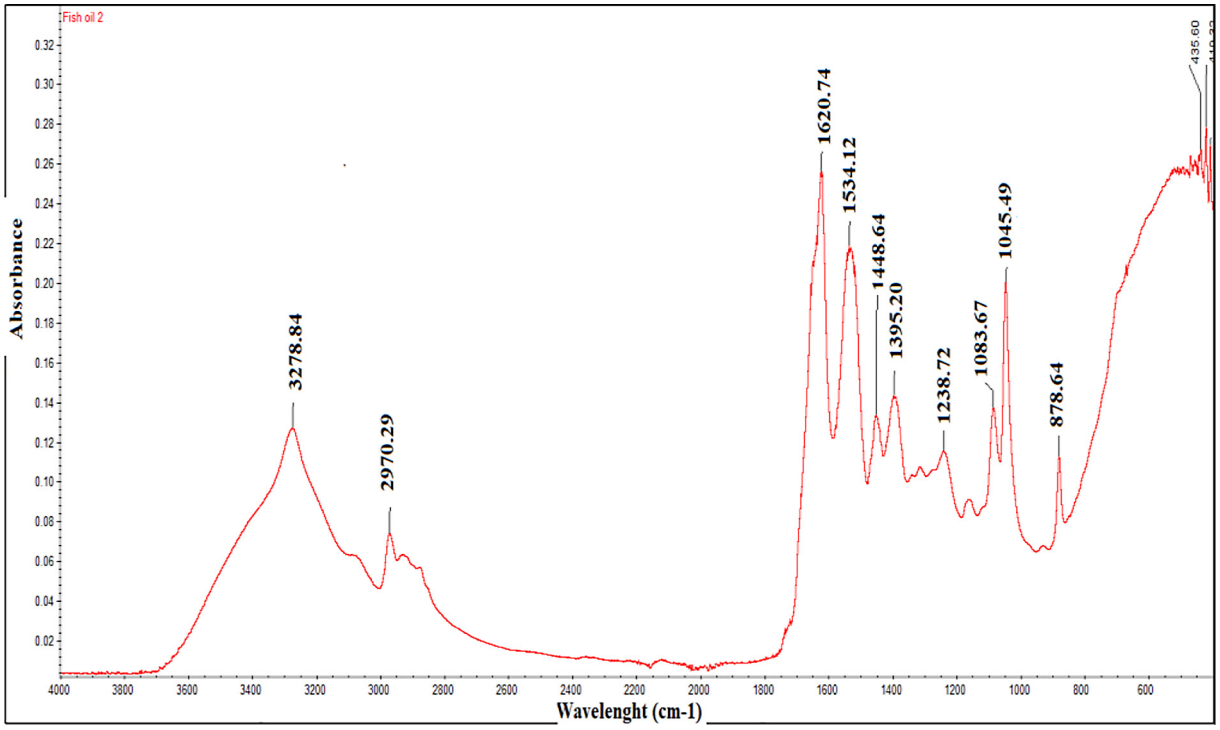

Fig. 5. IR spectra of the eel fish extract.

following order: 800 W > 700 W > 600 W > 900 W > 1000 W. Briefly, it can be observed that 800 W of microwave power gave the highest yield, while 1000 W gave the lowest yield. This may be attributed to the fact that excessive microwave power tends to degrade solute that causes over-pressure in fish sample which lowers the yield (Young, 1995). Previous studies revealed that generally when microwave power is increased, the yield of an extract increases (Routray and Orsat, 2012; Veggi et al., 2013). It should be noted that increase in the yield can be attributed to the increase in the solvent power leading to a drop in surface tension and viscosity which facilitate the solvent to solubilize solutes, and improve the matrix wettings and penetration (Eskilsson and Bjorklund, 2000; Mandal et al., 2007; Veggi et al., 2013).

3.4. Free fatty acid content and acid value

Fish extract in comparison with all other vegetable extract and terrestrial animals has been characterized by naturally complex poly-unsaturated, saturated and unsaturated fatty acids; hence, there is the

need for the production of pure and high-grade fish extract which is of greater importance to humanity (Adeniyi and Bawa, 2006). Free fatty acids are referred to as the long chain acids which are not attached or conjugated to any other thing else, which implies that they are free and not bound. However, acid value is the amount in milligram of KOH required to neutralize the free fatty acid present in 1 g of fat, it determines the extract stability during storage (Abdul Mudalip et al., 2010; Adeniyi and Bawa, 2006). Both the free fatty acids (FFAs) and acid value serves as the standard used to determine the eel fish extract quality (Abdul Mudalip et al., 2010; Adeniyi and Bawa, 2006; Khajeh et al., 2010). Table 2 shows the FFA and acid value of fish extract based on different microwave power with ethanol as the extracting solvent. The FFAs and acid values content are found highest at 800 W before it tends to decrease with the increase of microwave power. The FFA and acid value at the optimum yield of 16.13% was 1.35 and 2.69 mg KOH/g, respectively. However, the FFA and acid value does not exceed the acceptable limit of 7–8 mg KOH/g has reported (Abdul Mudalip et al., 2010; Adeniyi and Bawa, 2006). This signifies that there is a low or no enzymatic and bacterial activity from biological tissues or microorganisms, yet which can degrade the quality and protein value of the extract (Moffat and McGill, 1993). Hence, testifying that the extract is of good quality and safe for consumption.

3.5. Chemical composition of the extract

The composition of fatty acid in the eel fish extract using GC-MS analysis is shown in Table 1. The detection of the fatty acid methyl esters was found to be within the range of 39.998–55.049 min at this optimum condition as illustrated in Fig. 4 by the chromatogram. Identification of the FAMEs along with their retention time and peak area was through the GC-MS library (Razak et al., 2001). The FAME result shows that 33.33% of the fatty acids were saturated fatty acid (SFA), 55.56% were polyunsaturated fatty acid (PUFA) and 11.11% were monounsaturated fatty acid (MUFA). The predominant fatty acid was myristic acid and arachidonic acid available in the same proportion of 22.22% each while oleic acid, palmitic acid, linolenic acid, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) were all present in the same quantity of 11.11%. The identified fatty acids in this study were similar to those reported (Razak et al., 2001). However, both EPA and DHA present identified are very important nutrients required by every human for the maintenance of health function of the cardiovascular system, intellectual development and growth (Pike and Jackson, 2010).

3.6. FTIR spectra analysis of essential extract from eel fish

The spectra analysis of eel fish extract is shown in Fig. 5. The peaks at 1045.49, 1083.67 and 1238.72 cm^{-1} revealed the presence of C—O stretch group which indicate the presence of ethers, carboxylic, esters, and alcoholic compounds. The presence of these compounds shows that *M. albus* contains carboxylic ester group. The stretch C—H absorption band at 1395.20 and 1448.64 cm^{-1} show the presence of alkane compounds, whereas the peak at 1534.12 cm^{-1} indicates stretched C=C absorption band. More so, the sharp peak at 1620.74 cm^{-1} could be assigned to C=C conjugated stretching indicating the presence of methylene while the peak at 2970.29 cm^{-1} shows the presence of stretched C—H absorption band. In addition, the presence of a weak broad band at 3278.84 cm^{-1} can be attributed to the O—H stretching vibration. However, it has been reported that presence of O—H group in the extract can enhance antimicrobial activities in the extract (Alara et al., 2018a).

4. Conclusion

This study investigated the effects of different irradiation time, extraction temperature and microwave power on the recovery of extract from eel fish. A microwave power of 800 W, 30 min of irradiation and 60 °C of microwave temperature gave the highest extraction yields of 16.13%. This findings showed that the extract obtained was of good quality and safe for consumption; the major fatty acids in the extract were arachidonic acid, linolenic acid, myristic acid, oleic acid, palmitic acid, DHA, and EPA. FTIR spectra confirmed the presence of functional groups which can enhance antimicrobial activities in the extract. Therefore, these findings also highlight the efficiency and importance of microwave-assisted extraction as an alternative technique of extraction to the conventional methods.

Conflict of interest

The authors declare no conflict of interest.

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