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Essential fatty acid content in the ethnozoological products used in the folklore medicines by the Tangkhul tribe of the North East India

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Abstract: Tangkhul tribes are main habitant of the Ukhrul district of Manipur, a north eastern state of India and popular for using the products of animal fauna in the folklore medicines for curing certain human ailments. To our knowledge, no investigation has been done on the essential fatty acids content in the animal products used in the traditional medicines by the Tangkhul tribe of Manipur. The objective of the study is to determine the essential fatty acids concentration in the selected ethnozoological products namely, *Periplaneta americana*, *Upupa epops*, *Scolopendra gigantea*, *Pheretima posthuma*, *Hoplobatrachus tigerinus*, *Sus scrofa domesticus*, and *Tylototriton verrucosus* by Fatty Acid Methyl Esters (FAME) methods. The result of the study revealed that unsaturated fatty acids are found to be contained generally higher than saturated fatty acids in the seven-ethnozoological products studied. Among the monounsaturated fatty acids, Oleic Acid (7579.19 ± 70.23 mg/100g) is found to be highest in concentration and is recorded in the *P. americana*. Some of the common essential PUFAs recorded in the samples of the ethnozoological products studied were Linolenic Acid, Linoleic acids, Erucic Acid, Oleic Acid, Elaidic Acid, Palmetoleic Acid, Myristoleic Acid etc. with the highest concentration of linolenic acid (5518.03mg/100g) found in the *P. americana*. The used of seven ethnozoological products mentioned per se by Tangkhul Tribes of Manipur in their folklore medicines for the treatment of various human ailments may be attributed to the presence of various polyunsaturated fatty acids especially high amount of omega-3 fatty acids, palmetoleic acid, ecosapentanoic acid in them.

Keywords: *Bio resources, Ethnozoology, Fatty acid, Traditional knowledge, Tangkhul tribe, Traditional medicinal practices.*

1. Introduction

Ethnozoological practices are traditional in nature among the different ethnic communities for the treatment of various diseases [1]. Some ethnozoological products like hooves, scales, bones, feathers, tusks, etc. were used as special ingredients for the preparation of medicines and drugs for the treatment of diseases [2, 3]. In some countries, a traditional method of healing using ethno zoological products are one of the popular forms of treatment and prevention of diseases and has been a new prospect of medicinal practices [4]. Certain human ailments like hypertension, fever, cough skin and wound diseases, diabetes mellitus, cancer, bone fractures and eyes problems etc. are treated by using ethno-zoological products [5, 6]. In this connection, most of the natives of the North-East India are highly dependent on ethno zoological practices as traditional medicines [7-9] for the treatment of various human ailments [10].

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Besides the above, it has been indicated by certain investigators that human body also needed essential fats in order to keep a good health, for the treatment of various diseases and strengthening our immune system [11]. Fatty acids are the chains of carbon having a methyl and a carboxyl group at one opposite end of the molecule. Saturated fatty acids are found to be present with hydrogen while unsaturated are characterized by the presence of one or more double bonds in the carbon chain and are known as monounsaturated fatty acids (MUFAs) having one double bond and polyunsaturated fatty acids (PUFAs) having more than one double bond in between the carbon-carbon chain [12, 13]. It has been reported that naturally occurring fatty acids specially n-3 fatty acids may help in resolving inflammations, platelet aggregations, improve reproductive functions and may also acts as an antioxidant against harmful chemicals [11]. Numerous studies have reported that n-3 PUFAs administration exert beneficial health effects to prevent and manage type 2 diabetes mellitus [14- 16] as the regular intake of n-3 PUFA rich items increased the level of hormone insulin in human body. Further, it has been reported that by modifying the content of the endogenously synthesis fatty acids present in the bacteria, the presence of the antibacterial fatty acids by the unsaturated fatty acids can be established [17]. The vital roles played by PUFA in managing certain human diseases such as diseases related to bone, functions of kidney are well known now. In above to this, the therapeutic roles of long chain omega fatty acids in the treatment of heart related problems, neuropsychiatric disorders and various health issues in the children has been some of the reported research findings in the last few years and as a result of all these, health professional are advising to have a regular administration of the natural products having appreciable amount of essential fatty acids [18, 19, 20].

Manipur is the North Eastern States of India located in the region of the famous Indo Burma Biodiversity Hotspot rich in variety of flora and fauna. Tangkhul tribe of Manipur are the main inhabitant of the Ukhrul district of Manipur where using various animal products as an ingredients in the preparation of folklore medicines for the treatment and management of the various human diseases is a common practices among the natives of the region [21]. However, the survey of the literature indicates that there is non-existent of data on the identification of various biochemicals especially essential fatty acids present in the ethno zoological products used by them in indigenous medicinal practices. Therefore, the present study on the evaluation of essential fatty acid content in the ethnozoological products used in the folklore medicines by the Tangkhul tribe of Manipur was undertaken to provide a scientific database of the important fatty acids present in these animals products and also to validate the therapeutic uses of such animal products in the human health. The result of the study is given in this comminution.

2. Materials and Methods

2.1. Study Area

Manipur is a small hill state of the Indo-Burma biodiversity hotspot located in the north-eastern part of India and lies between 23.50N and 25.40N and between 93.40E and 94.30E. The total area of the state is of 22,327sq.km with an altitude ranging between 1,500 to 3,000 m above the mean sea level (amsl). Tangkhul Naga tribe are the main native of the Ukhrul District, a district bounded by Myanmar in the east, Nagaland state in the [22]. The district is characterized by hilly in terrain with varying heights of 913 to 3114m amsl with temperature ranging from 3°C to 33°C with an average annual rainfall about 85.32cm.

2.2. Sample Collection

A survey was conducted for a period of one year in the year 2021- 2022 at the Ukhrul district of Manipur, India (Figure-1) and data were collected through semi-structural questionnaires administered to the local indigenous people as well as an interaction with local medicinal practitioners. They were asked simple questions such as different animals or animal products used in the treatment of certain human ailments and any other traditional practices which is related with the animal in questions. When the whole animal body parts or its products were used, the specimen was shown to us by interviewee

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and from the pictorial illustrations; we are able to identify the species in question. When some species of animals belongs to a protected species, we could not collect specimen samples; however, we collected local name and common name [21]. The animal species used in the indigenous folklore practices were identified by using relevant and standard literature. Seven ethnozoological products i.e. *Periplaneta americana*, *Upupa epops*, *Scolopendra gigantea*, *Pheretima posthuma*, *Hoplobatrachus tigerinus*, *Sus scrofa domesticus*, and *Tylototriton verrucosus*, (Photograph -1) were collected for the evaluation of fatty acids content based on the abundant availability of the samples as well as frequent used by local people.

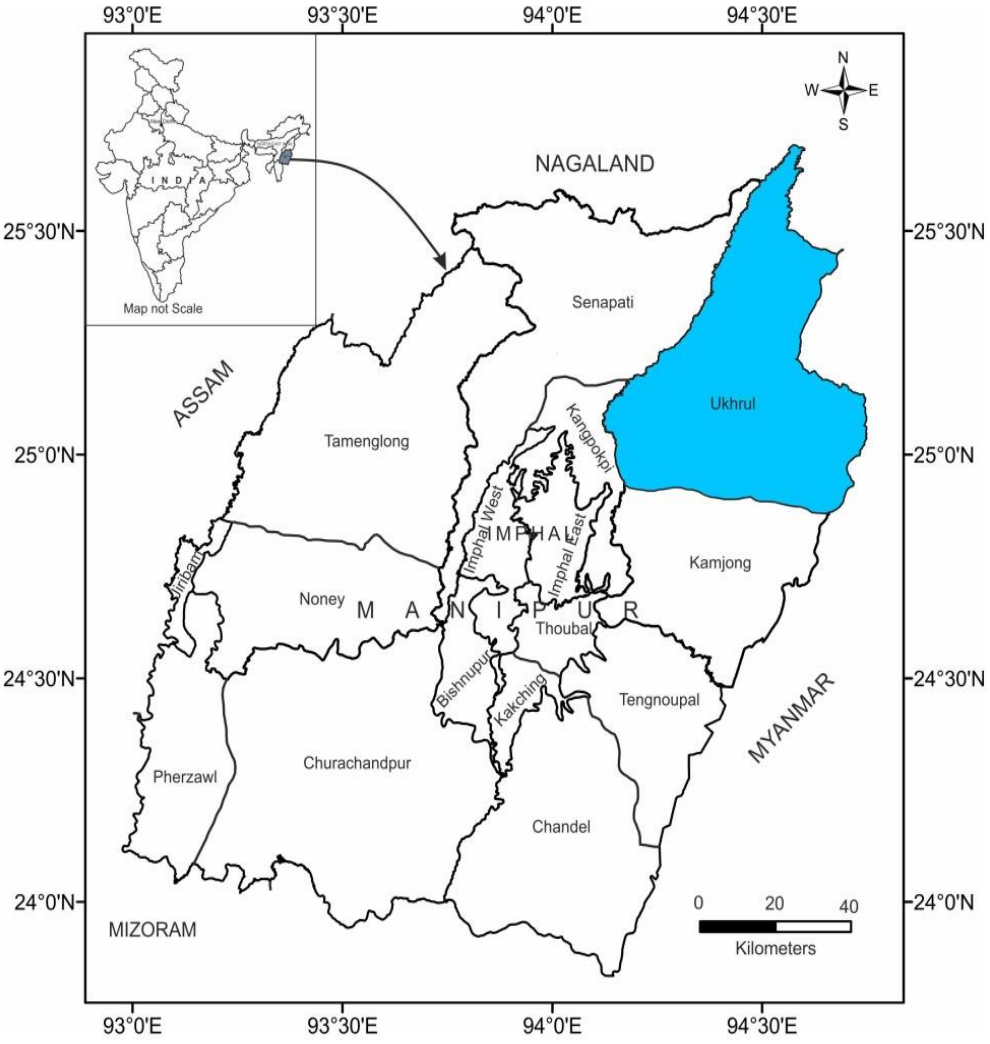

Figure 1.
Map of Manipur State, India showing Ukhrul district (Blue shaded are)

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Periplaneta americana

Epopa epops

Scolopendra gigantea

Pheretima posthuma

Hoplobatrachus tigerinus

Sus scrofa domesticus

Tylototriton verrucosus

Photograph 1.
Photographs showing selected seven Ethnozoological Animals or body parts.

Fatty Acid Methyl Ester (FAME) analysis for Essential fatty acids Concentration: The FAME analysis in the seven selected ethnozoological products i.e. *P. americana*, *U. epops*, *S. gigantea*, *P. posthuma*, *H. tigerinus*, *S.domesticus*, and *T. verrucosus*, were carried out as per standard method of AOAC Official Method 996.06-Hydrolytic Extraction Gas Chromatographic Method [23]. Percentage of conversion to FAME in all experiments was determined by using the gas chromatography mass spectrometry (GCMS; Shimadzu Co., Kyoto). Sample (2 µl) was injected into the capillary column CBPI-M25-05 with diameter of 0.25mm and length of 25 m. Helium was used as carrier gas. The column head pressure was regulated at 43.0 kPa, and the flow rate at 1 ml/min. Initial temperature was programmed at 600 C. The temperature was then increased to 230 C at 150 C/min. Injection of samples was performed at the set detector temperature of 2300 C. Chromatograms generated from mass spectra were compared to

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authentic peaks obtained from standard compounds from the National Biology Society (NVS) & National Institute of Standards and Technology (NIST) Mass Spectra Library, Japan.

Results: 7 (seven) ethno zoological products i.e. *P. americana*, *U. epops*, *S. gigantea*, *P. posthuma*, *H. tigerinus*, *S. scrofa domesticus*, and *T. verrucosus*, were selected based on their abundant availability of the samples to be studied as well as commonly uses by the Tangkhul Tribe of Manipur in their medicinal practices for the analysis of essential fatty acids content. The indigenous way of uses of seven animal products in the folklore practices by Tangkhul tribe of Manipur has been given in the Table 1.

Table 1.
Indigenous way of uses of Seven Ethnozoological Products in the folklore practices by Tangkhul tribe of Manipur.

Sl. no.	Common name	Scientific name	Local name	Part used	Preparation/Way of uses	Type of ailment/Disease
1.	Cockroach	*Periplaneta americana*	Pharao (T) Kharambi (M)	Whole Body Part without intestine	Body part of the animal roasted properly and eaten	Treatment of Diabetes mellitus to reduce sugar level.
2.	Hoopoe	*Upupa epops*	Kazeihar (T) Chongaraba (M)	Thorax Part	Flesh with bone is cooked or boiled slowly for more than one hour with water and used two times a day	Treatment of kidney stone cases, urinating problem and white discharge in women
3.	Centipede	*Scolopendra gigantea*	Katei nakhui (T) Ching Nachal (M)	Whole body parts except tail part	Centipede is put in a glass with wine for few hours. The wine is mixed with its secretion and then prescribed one glass of mixture twice a day.	Treatment of Diabetes mellitus to reduce sugar level and cancer
4.	Earthworm	*Pheretima posthuma*	Ringdak (T) Tinthrok (M)	Whole body parts without alimentary canal.	Whole body part of the earthworm is crushed and prepared a decoction with water and drink for about 10 ml, thrice a day.	Treatment of typhoid, jaundice, bladder stone and antidote in snake and spider bites.
5.	Frog	*Hoplobatrachus tigerinus*	Khaifa (T) Hangoi (M)	Meat portion of hind leg	The flesh part of the hind legs of frog is slowly boiled with water for more than an hour and filtered the concentrated liquid portion obtained.	Used for the treatment of cough.
6.	Pig	*Sus scrofa domesticus*	Hok (T) Okk (M)	Fermented pork	The fermented pork after properly cut in to small	Administered to the woman after the delivery of child and

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					pieces were slowly boiled in water for one to one and half hours and liquid portion obtained after boiled is remove in a spate container.	lactating mothers for health and protection from anaemia.
7.	Salamander	*Tylototriton verrucosus*	Lengba (T)	Body muscle	Body muscle of the animal is roasted properly and mixed with salts at least once a week for the treatment of cancer.	Consumed at least once a week for the treatment of cancer

Note: T = Tangkhul dialek, M = Meiteilon dialek.

Fatty Acids Content (mg/100g) in *Periplaneta americana:* The result of the study reveals that the concentrations of saturated fatty acids recorded *P. americana* were Caproic Acid (12.14 ± 0.02 mg/100g), Caprylic Acid (36.94 ± 0.06 mg/100g), Lauric Acid (9.67 ± 0.01 mg/100g), Myristic Acid (188.28 ± 1.64 mg/100g) Palmitic Acid (4847.61 ± 41.06 mg/100g), Stearic Acid (129.53 ± 1. 16 mg/100g), Arachidic Acid (353.44 ± 3.25 mg/100g), Pentadecanoic Acid (77.52 ± 0.12 mg/100g), Heptadecanoic Acid (86.17 ± 0. 13 mg/100g), Heneicodenoic Acid (113.96 ± 1. 08 mg/100g), Behenic Acid (52.38 ± 0.08 mg/100g). While the unsaturated fatty acids recorded in *P. americana* were Myristoleic Acid (16.30 ± 0.03), Oleic Acid (7579.19 ± 70.23 mg/100g) , Palmetoleic Acid (690.02 ± 6.43 mg/100g), Elaidic Acid (224.08 ± 2.20 mg/100g), Cis-10-Heptadecanoic Acid (42.33 ± 0.07 mg/100g), Cis-11-Ecosenic Acid (76.40 ± 0.12 mg/100g), Erucic Acid (129.54 ± 1.16 mg/100g), Linolelaidic Acid (38.29 ± 0.06 mg/100g), Linolenic Acid (5518.03 ± 51.06 mg/100g), Gama Linolenic Acid (23.70 ± 0.04 mg/100g), 8,11,14-Eicosadienoic Acid (21.07 ± 0.03 mg/100g), Ecosapantanoic acid (Epa) (28.20 ± 0.04 mg/100g), Cis-11,14- Eicosadienoic Acid (537.70 ± 5.21 mg/100g), and Cis-11,14,17-eicosatrienoic Acid (140.36 ± 1.18 mg/100g) with the highest concentration of unsaturated fatty acid recorded was Oleic Acid (7579.19 ± 70.23 mg/100g) and lowest concentration was Myristoleic Acid (16.30 ± 0.03 mg/100g) (Table No-2). Among the unsaturated fatty acids Myristoleic acid, Oleic acid, Palmetoleic acid, Elaidic acid, Cis-10-Heptadecanoic acid, Cis-11-Ecosenic acid and Erucic acid were MUFA (Mono Unsaturated Fatty Acids) while the fatty acids like Linolelaidic acid, Linolenic acid, Gama Linolenic acid, 8, 11, 14-Eicosadienoic acid, Ecosapantanoic acid (EPA), Cis-11, 14-Eicosadienoic acid and Cis-11, 14, 17-eicosatrienoic acid were PUFA (Polyunsaturated Fatty Acids). Table 2.

Table 2.
Saturated and Unsaturated Fatty Acids content (mg/100g) in thorax part of *Periplaneta americana*. Values are mean ± SEM, 3 observations each.

Sl. No.	Name of Fatty acid	Concentration	Type of fatty acids
1.	Caproic Acid (C6:0)	12.14 ± 0.02	**Saturated**
2.	Caprylic Acid (C8: 0)	36.94 ± 0.06	
3.	Lauric Acid(C12: 0)	9.67 ± 0.01	
4.	Myristic Acid (C14:0)	188.28 ± 1.64	
5.	Palmitic Acid (C16:0)	4847.61 ± 41.06	
6.	Stearic Acid (C18:0)	129.53 ± 1. 16	
7.	Arachidic Acid (C20:0)	353.44 ± 3.25	

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8.	Pentadecanoic Acid(C15:0)	77.52 ± 0.12	
9.	Heptadecanoic Acid (C17:0)	86.17 ± 0. 13	
10.	Heneicodenoic Acid (C21:0)	113.96 ± 1. 08	
11.	Behenic Acid (C22:0)	52.38 ± 0.08	
			Unsaturated
1.	Myristoleic Acid (C14:1)	16.30 ± 0.03	MUFA; Omega-5
2.	Oleic Acid (C18:1)	7579.19 ± 70.23	MUFA; Omega 9
3.	Palmetoleic Acid (C16:1)	690.02 ± 6.43	MUFA; Omega 7
4.	Elaidic Acid (C18:1)	224.08 ± 2.20	MUFA
5.	Cis-10-Heptadecanoic Acid (C17:1)	42.33 ± 0.07	MUFA
6.	Cis-11-Ecosenic Acid (C20:1)	76.40 ± 0.12	MUFA; Omega 9
7.	Erucic Acid (C22:1)	129.54 ± 1.16	MUFA; Omega 9
8.	Linolelaidic Acid (C18:2)	38.29 ± 0.06	PUFA; Omega 6
9.	Linolenic Acid (C18:3)	5518.03 ± 51.06	PUFA; Omega 3
10.	Gama Linolenic Acid (C18:3)	23.70 ± 0.04	PUFA; Omega 6
11.	8,11,14-Eicosadienoic Acid (C20:3)	21.07 ± 0.03	PUFA; Omega 6
12.	Ecosapantanoic acid (Epa) (C20:5)	28.20 ± 0.04	PUFA; Omega 3
13.	Cis-11,14-Eicosadienoic Acid (C20:2)	537.70 ± 5.21	PUFA; Omega 6
14.	Cis-11,14,17-eicosatrienoic Acid (C20:3)	140.36 ± 1.18	PUFA; Omega 3

Note: MUFA: Monounsaturated fatty acid; PUFA: Polyunsaturated fatty acid.

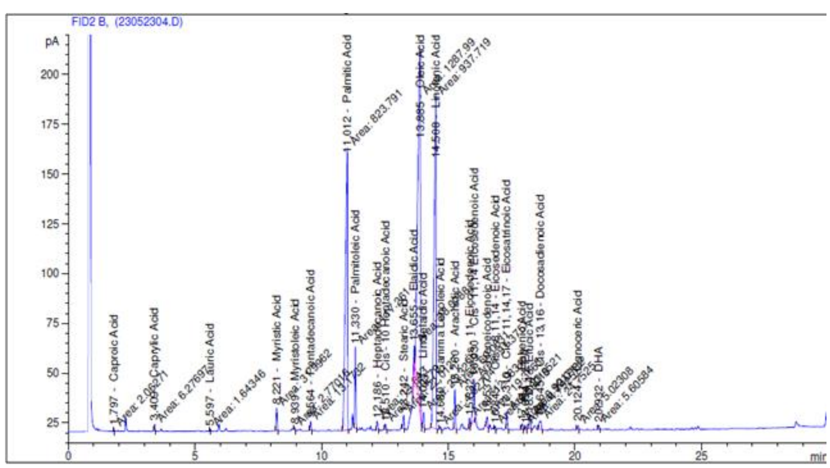

Figure 2.
Chromatogram of FAME Analysis in the *P. americana*.

Fatty Acids Content (mg/100g) in *Upupa epops*: The result of the study reveals that the concentrations of saturated fatty acids recorded were Caproic Acid (3.73 ± 0.01 mg/100g), Caprylic Acid (8.74 ± 0.09 mg/100g), Lauric Acid (6.30 ± 0.05 mg/100g), Myristic Acid (60.34 ± 0.50 mg/100g) Palmitic Acid (1337.62 ± 12. 37 mg/100g), Stearic Acid (751.24 ± 6. 51 mg/100g), Arachidic acid (176.68 ± 1.65 mg/100g), Pentadecanoic Acid (19.65 ± 0.13 mg/100g), Heptadacanoic Acid (29.58 ± 0.21 mg/100g), Heneicodenoic acid (6.50 ± 0.05 mg/100g), Cis- 10- Heptadecanoic Acid (39.27 ± 0. 31 mg/100g) and Lingnoceric Acid (18.58 ± 0.12 mg/100g). (Table 3). While the unsaturated fatty acids recorded in *U. epops* were Myristoleic Acid (24.49 ± 0.20 mg/100g), Oleic Acid (2779.22 ± 26.79 mg/100g), Elaidic Acid (71.62 ± 0. 65 mg/100g), Linolelaidic Acid (3.52 ± 0.01 mg/100g), Nervonic Acid (28.32 ± 0.21 mg/100g), Cis-11,14,17-eicosatrienoic Acid (138.28 ± 1.26 mg/100g) Palmetoleic

Acid (881.43 ± 7.67 mg/100g), Arachidonic Acid (13.71 ± 0.10 mg/100g), Linolenic Acid (1506.41 ± 14.06 mg/100g), Gama Linolenic Acid (25.74 ± 0.20 mg/100g), Cis-11-Ecosenic Acid (52.77 ± 0.42 mg/100g), Cis-11, 14-Eicosadienoic Acid (26.86 ± 0.21 mg/100g), 8, 11, 14- Eicosadienoic Acid (16.95 ± 0.11 mg/100g) and Erucic Acid (14.26 ± 0.10 mg/100g), and with the highest concentration of unsaturated fatty acid was Oleic Acid (2779.22 ± 26. 79 mg/100g) and lowest concentration was Linolelaidic Acid (3.52 ± 0.01 mg/100g). (Table 3).

Table 3.
Saturated and Unsaturated Fatty Acids content (mg/100g) in *Upupa epops*. Values are mean ± SEM, 3 observations each.

Sl. No.	Name of fatty acid	Concentration	Type of fatty acids
1.	Caproic acid (C6:0)	3.73 ± 0.01	**Saturated**
2.	Caprylic acid (C8: 0)	8.74 ± 0.09	
3.	Lauric acid (C12: 0)	6.30 ± 0.05	
4.	Myristic acid (C14:0)	60.34 ± 0.50	
5.	Palmitic acid (C16:0)	1337.62 ± 12. 37	
6.	Stearic acid (C18:0)	751.24 ± 6. 51	
7.	Arachidic acid (C20:0)	176.68 ± 1.65	
8.	Pentadecanoic acid (C15:0)	19.65 ± 0.13	
9.	Heptadacanoic acid (C17:0)	29.58 ± 0.21	
10.	Heneicodenoic acid(C21:0)	6.50 ± 0.05	
11.	Cis- 10- Heptadecanoic acid	39.27 ± 0. 31	
12.	Lingnoceric Acid (C21:0)	18.58 ± 0.12	
			Unsaturated fatty acids
1.	Myristoleic acid (C14:1)	24.49 ± 0.20	MUFA
2.	Oleic acid (C18:1)	2779.22 ± 26. 79	MUFA
3.	Elaidic acid (C18:1)	71.62 ± 0. 65	MUFA
4.	Linolelaidic acid(C18:2)	3.52 ± 0.01	PUFA
5.	Nervonic acid (C24:0)	28.32 ± 0.21	PUFA
6.	Eicosatrienoic acid (C20:3)	138.28 ± 1. 26	PUFA; Omega 3
7.	Palmetoleic acid(C16:1)	881.43 ± 7.67	PUFA; Omega 7
8.	Arachidonic acid(C20:4)	13.71 ± 0.10	PUFA; Omega 6
9.	Linolenic acid(C18:3)	1506.41 ± 14.06	PUFA; Omega 3
10.	Gama linolenic acid (C18:3)	25.74 ± 0.20	PUFA; Omega 6
11.	Cis-11-ecosenic acid (C20:1)	52.77 ± 0.42	PUFA; Omega 9
12.	Eicosadienoic acid(C20:2)	26.86 ± 0.21	PUFA Omega 6
13.	8,11,14-Eicosadienoic acid (C20:3)	16.95 ± 0.11	PUFA; Omega 6
14.	Erucic acid (C22:1)	14.26 ± 0.10	PUFA; US Omega 9

Note: MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid.

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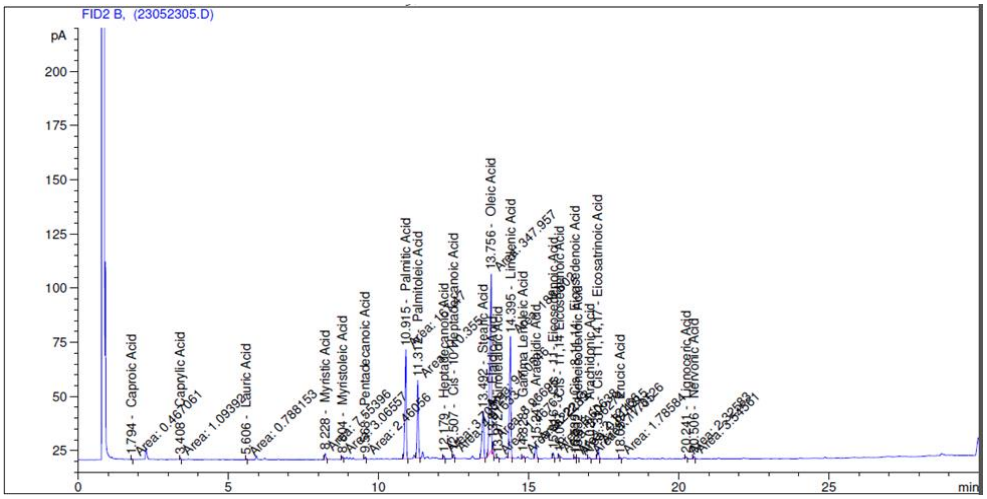

Figure 3.
Chromatogram of FAME Analysis in the *Upupa epops*.

Fatty Acids Content (mg/100g) in *Scolopendra gigantea*: The result of the study reveals that the concentrations of saturated fatty acids recorded were Caproic Acid (15.54 ± 0.12 mg/100g), Caprylic Acid (63.17 ± 0.56 mg/100g), Lauric Acid (43.81 ± 0.37 mg/100g), Myristic Acid (210.63 ± 2.96 mg/100g), Palmitic Acid (3484.18 ± 13.25 mg/100g), Stearic Acid (1170.80 ± 10.23 mg/100g), Arachidic acid (350.95 ± 3.96 mg/100g), Tridecanoic acid (10.20 ± 0.10 mg/100g), Cis-10-Pentadecanoic acid (13.20 ± 0.11 mg/100g), Pentadecanoic acid (53.20 ± 0.48 mg/100g), Heptadacanoic acid (115.92 ± 1. 23 mg/100g), Cis10- Heptadecanoic acid (20.99 ± 0.21 mg/100g), Heneicodenoic acid (151.79 ± 1.59 mg/100g), and Behenic acid (25.82 ± 0. 28 mg/100g). (Table 4). While the unsaturated fatty acids recorded in *S. gigantea* were Myristoleic acid (40.47 ± 0.30 mg/100g), Oleic acid (3605.01± 14. 25 mg/100g), Palmetoleic acid (688.69 ± 5.52 mg/100g), Arachidonic acid (15.07 ± 0.12 mg/100g), Elaidic acid (71.62 ± 0. 65 mg/100g), Cis-11-Ecosenic acid (78.75 ±0.63 mg/100g), Erucic Acid (14.26 ± 0.10 mg/100g), Linolelaidic Acid (7.79 ± 0.06 mg/100g), Linolenic Acid (3363.72 ± 12.69 mg/100g), Linoleic Acid (9.73± 0.09 mg/100g), Gama Linolenic Acid (41.59 ± 0.31 mg/100g), 8,11,14-Eicosadienoic Acid (41.38 ± 0.31 mg/100g), Cis-11,14-Eicosadienoic Acid (36.92 ± 0. 28 mg/100g), and Cis-11,14,17-eicosatrienoic Acid (366.50 ± 2.56 mg/100g) with the highest concentration of unsaturated fatty acid was Oleic Acid (3605.01± 14. 25 mg/100g) and lowest concentration was Linolelaidic Acid (7.79 ± 0.06 mg/100g). (Table 4).

Table 4.
Fatty acids content (mg/100g) in the *Scolopendra gigantea*. Values are mean ± SEM, 3 observations each.

Sl. No.	Name of Fatty acid	Concentration	Type of fatty acids
1.	Caproic acid (C6:0)	15.54 ± 0.12	Saturated
2.	Caprylic acid (C8: 0)	63.17 ± 0 .56	
3.	Pentadecanoic Acid (C15:0)	53.20 ± 0. 48	
4.	Lauric acid (C12: 0)	43.81 ± 0.37	
5.	Myristic acid (C14:0)	210.63 ± 2.96	
6.	Palmitic acid (C16:0)	3484.18 ± 13. 25	
7.	Stearic acid (C18:0)	1170.80 ± 10. 23	

Edelweiss Applied Science and Technology
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8.	Arachidic acid (C20:0)	350.95 ± 3.96	
9.	Tridecanoic acid (C13:0)	10.20 ± 0.10	
10.	Cis-10-pentadecanoic acid (C15:1)	13.20 ± 0.11	
11.	Heptadacanoic Acid (C17:0)	115.92 ± 1. 23	
12.	Cis- 10- heptadecanoic acid (C17:1)	20.99 ± 0.21	
13.	Heneicodenoic acid (C21:0)	151.79 ± 1.59	
14.	Behenic acid (C22:0)	25.82 ± 0. 28	
			Unsaturated fatty acids
1.	Myristoleic acid (C14:1)	40.47 ± 0.30	MUFA; US
2.	Oleic acid (C18:1)	3605.01± 14. 25	MUFA; Omega 9
3.	Palmetoleic acid (C16:1)	688.69 ± 5.52	MUFA; Omega 7
4.	Arachidonic acid (C20:4)	15.07 ± 0.12	PUFA
5.	Linolelaidic acid (C18:2)	7.79 ± 0.06	PUFA
6.	Linolenic acid (C18:3)	3363.72 ± 12.69	PUFA; Omega 3
7.	Linoleic acid (C18:2)	9.73± 0.09	PUFA; Omega 6
8.	Gama linoleic acid (C18:3)	41.59 ± 0.31	PUFA; Omega 6
9.	Cis-11-ecosenic acid (C20:1)	78.75 ± 0.63	PUFA; Omega 9
10.	8,11,14-eicosadienoic acid	41.38 ± 0.31	PUFA; Omega 6
11.	Cis-11,14 – eicosadienoic acid	36.92 ± 0. 28	PUFA Omega 6
12.	Cis-11,14,17-eicosatrienoic acid (C20:3)	366.50 ± 2.56	PUFA; Omega 3
13.	Erucic Acid (C17:0) (C22:1)	189.24 ± 1.74	PUFA; Omega 9
14.	Ecosapantanoic (Epa) acid (C20:5)	33.24 ± 0.26	PUFA; Omega 3

Note: MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid.

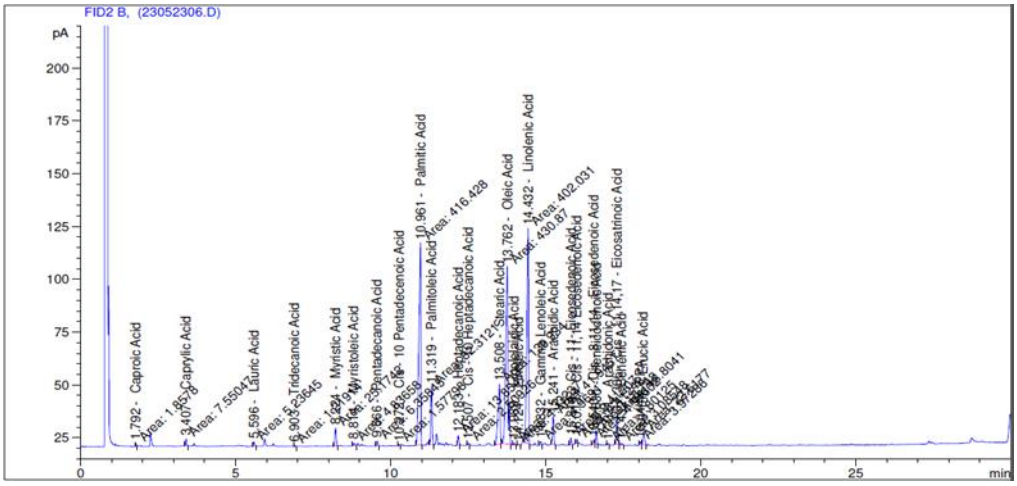

Figure 4.
Chromatogram of FAME analysis in *Scolopendra gigantea.*

Fatty Acids Content (mg/100g) in *Pheretima posthuma*: The result of the study reveals that the concentrations of saturated fatty acids recorded were Caproic Acid (9.23 ± 0.09 mg/100g), Caprylic Acid (19.47 ± 0.18 mg/100g), Myristic Acid (30.39 ± 0. 28 mg/100g), Cis-10-Pentadecanoic Acid (16.64 ± 0. 15 mg/100g), Palmitic Acid (991.32 ± 8.78 mg/100g), Stearic Acid (252.84 ± 1.47 mg/100g), Arachidic acid (22.04 ± 0. 21 mg/100g) and Heptadacanoic Acid (14.30 ± 0.12 mg/100g).

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(Table 4). While the unsaturated fatty acids recorded in *P. posthuma* were Myristoleic acid (41.44 ± 0.34 mg/100g), Oleic acid (1198.19 ± 10.97 mg/100g), Palmetoleic acid (586.68 ± 4. 32 mg/100g), and Linolenic acid (287.45 ± 1.98 mg/100g) with the highest concentration of unsaturated fatty acid was Oleic Acid (1198.19 ± 10.97 mg/100g) and lowest concentration was Myristoleic acid (41.44 ± 0.34 mg/100g). (Table 4).

Table 5.
Fatty acids content (mg/100g) in the *Pheretima posthuma*. Values are mean ± SEM, 3 observations each.

Sl. no.	Name of fatty acid	Concentration	Type of fatty acids
1.	Caproic acid (C6:0)	9.23 ± 0.09	Saturated
2.	Caprylic acid (C8:0)	19.47 ± 0.18	
3.	Myristic acid (C14:0)	30.39 ± 0. 28	
4.	Cis-10-pentadecanoic acid (C15:1)	16.64 ± 0. 15	
5.	Palmitic acid (C16:0)	991.32 ± 8.78	
6.	Stearic acid (C18:0)	252.84 ± 1.47	
7.	Arachidic acid (C20:0)	22.04 ± 0. 21	
8.	Heptadecanoic acid (C17:0)	14.30 ± 0.12	
			Unsaturated
1.	Myristoleic acid (C14:1)	41.44 ± 0.34	MUFA
2.	Oleic acid (C18:1)	1198.19 ± 10.97	MUFA; Omega 9
3.	Palmetoleic acid (C16:1)	586.68 ± 4. 32	MUFA; Omega 7
4.	Linolenic acid (C18:0)	287.45 ± 1.98	PUFA; Omega 3

Note: MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid.

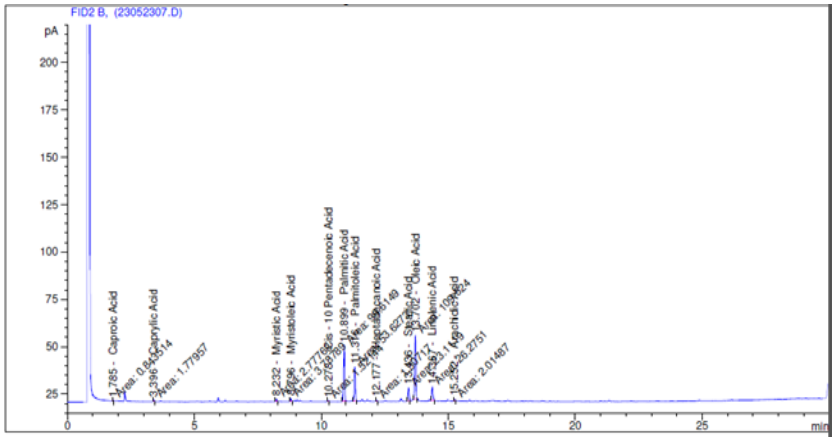

Figure 5.
Chromatogram of FAME Analysis of *P. posthuma*

Fatty Acids Content (mg/100g) in *Hoplobatrachus tigerinus*: In *H. tigerinus* different types of fatty acid was recorded. The result of the study reveals that the concentrations of saturated fatty acids recorded were Myristic acid (110.87 ± 1.12 mg/100g), Palmitic Acid (2251.13 ± 21.45 mg/100g), Stearic Acid (718.74 ± 6.67 mg/100g), Arachidic acid (408.80 ± 3.69 mg/100g) (Table 5). While the unsaturated fatty acids recorded in *H. tigerinus* were Palmetoleic acid (1039.28 ± 10. 46 mg/100g), Oleic acid (2438.92 ± 20.57 mg/100g), Linolenic acid (1377.34 ± 10.56 mg/100g), Cis-11,14,17- Eicsatrienoic (234.93 ± 1.99 mg/100g) with the highest concentration of unsaturated fatty acid was Oleic Acid

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Vol. 8, No. 6: 207-224, 2024
DOI: 10.55214/25768484.v8i6.2045

(2438.92 ± 20.57 mg/100g), and lowest concentration was Cis11,14,17- Eicsatrienoic (234.93 ± 1.99 mg/100g) (Table 6).

Table 6.
Fatty Acids content (mg/100g) in the *Hoplobatrachus tigerinus* Values are mean ± SEM; 3 observations each.

Sl. No.	Name of Fatty acid	Concentration	Type of fatty acids
1.	Myristic acid (C14:0)	110.87 ± 1.12	**Saturated**
2.	Palmitic acid (C16:0)	2251.13 ± 21.45	
3.	Stearic acid (C18:0)	718.74 ± 6.67	
4.	Arachidic acid (C20:0)	408.80 ± 3.69	
			Unsaturated
1.	Palmetoleic acid(C16:1)	1039.28 ± 10. 46	MUFA; Omega 7
2.	Oleic acid(C18:1)	2438.92 ± 20.57	MUFA; Omega 9
3.	Linoleic acid(C18:3)	1377.34 ± 10.56	PUFA; Omega 3
4.	Eicsatrienoic Acid(C20:3)	234.93 ± 1.99	PUFA

Note: MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid.

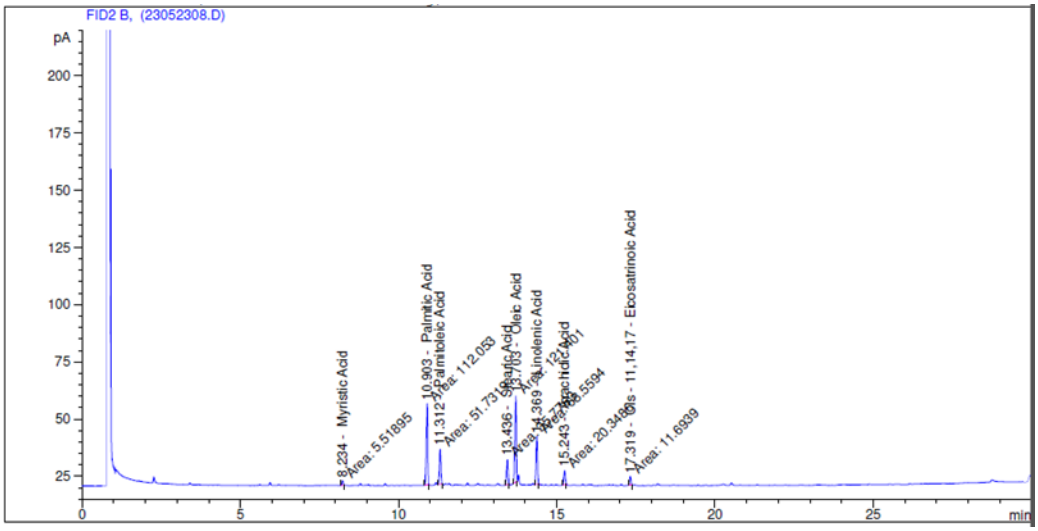

Figure 6.
Chromatogram of FAME Analysis of *H. tigerinus*.

Fatty Acids Content (mg/100g) in *Sus scrofa domesticus*: The result of the study reveals that the concentrations of saturated fatty acids recorded were Caproic Acid (9.38 ± 0.09 mg/100g), Caprylic Acid (42.19 ± 0.38 mg/100g), Lauric Acid (30.41 ± 0.28 mg/100g), Myristic Acid (386.09 ± 2.76 mg/100g), Palmitic Acid (6266.72 ± 56.90 mg/100g), Stearic Acid (703.89 ± 6.58 mg/100g), Arachidic acid (124.83 ± 1.45 mg/100g), Pentadecanoic Acid (21.55 ± 0.21 mg/100g), Heptadacanoic Acid (41.56 ± 0.38 mg/100g), Heneicodenoic acid (137.85 ± 1.46 mg/100g), and Cis-10-Heptadecanoic Acid (38.34 ± 0.21 mg/100g) (Table 7). While the unsaturated fatty acids recorded in *S. scrofa domesticus* were Oleic acid (7894.36 ± 67. 87 mg/100g) , Cis-11-Ecosenic acid (68.58 ± 0.56 mg/100g), Erucic acid (90.44 ± 0.81 mg/100g), Elaidic acid (563.78 ± 4.89 mg/100g), Linolelaidic acid (92.16 ± 0,89 mg/100g), Nervonic acid (48.60 ± 0.39 mg/100g), Cis-11,14,17-eicosatrienoic acid (84.67 ± 0.78 mg/100g), Cis-11,14- Eicosadienoic acid (36.92 ± 0.28 mg/100g), Linolenic acid (3412.14 ± 24.78 mg/100g), Palmetoleic acid (804.76 ± 7.99 mg/100g), Dha acid (1539.74 ± 14. 35 mg/100g), and 13,16-

Docosadienoic acid (130.08 ± 1.98 mg/100g), with the highest concentration of unsaturated fatty acid was Oleic acid (7894.36 ± 67. 87 mg/100g) and lowest concentration was Cis-11,14-Eicosadienoic acid (36.92 ± 0.28 mg/100g). (Table 7.).

Table 7.
Fatty Acids content (mg/100g) in *Sus scrofa domesticus* Value are mean ± SEM; 3 observations each.

Sl. No.	Name of fatty acid	Concentration	Type of fatty acids
1.	Caproic acid(C6:0)	9.38 ± 0.09	
2.	Caprylic acid(C8:0)	42.19 ± 0,38	
3.	Lauric acid(C12:0)	30.41 ± 0.28	
4.	Myristic acid(C14:0)	386.09 ± 2.76	
5.	Palmitic acid(C16:0)	6266.72 ± 56.90	
6.	Stearic acid(C18:0)	703.89 ± 6.58	Saturated
7.	Arachidic acid(C20:0)	124.83 ± 1.45	
8.	Pentadecanoic acid(C15:0)	21.55 ± 0.21	
9.	Heptadecanoic acid(C17:0)	41.56 ± 0.38	
10.	Heneicodenoic acid(C21:0)	137.85 ± 1.46	
11	Cis-10-heptadecanoic acid(C17:1)	38.34 ± 0.21	
			Unsaturated
1.	Oleic acid(C18:1)	7894.36 ± 67. 87	MUFA Omega 9
2.	Cis-11-ecosenic acid(C20:1)	68.58 ± 0.56	MUFA Omega 9
3.	Erucic acid(C22:1)	90.44 ± 0.81	MUFA Omega 9
4.	Elaidic acid (C18:1)	563.78 ± 4.89	MUFA
5.	Linolelaidic acid(C18:2)	92.16 ± 0,89	PUFA
6.	Nervonic acid(C24:0)	48.60 ± 0.39	PUFA
7.	Cis-11,14,17-eicsatrienoic acid	84.67 ± 0.78	PUFA
8.	Cis-11,14 – eicosadienoic acid	36.92 ± 0.28	PUFA Omega 6
9.	Linolenic acid(C18:3)	3412.14 ± 24.78	PUFA Omega 3
10	Palmetoleic acid(c16:1)	804.76 ± 7.99	PUFA; Omega 7
11	Dha acid(c22:6)	1539.74 ± 14. 35	PUFA; Omega 3
12.	Docosadienoic acid(C22:2)	130.08 ± 1.98	PUFA Omega 6

Note: MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid.

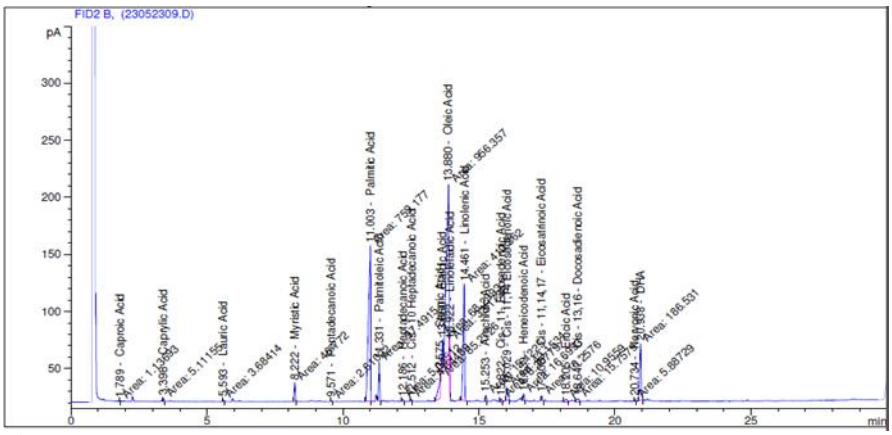

Figure 7.
Chromatogram of FAME Analysis of *S. scrofa domesticus.*

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Vol. 8, No. 6: 207-224, 2024
DOI: 10.55214/25768484.v8i6.2045

Fatty Acids Content (mg/100g) in *Tylototriton verrucosus*: The result of the study reveals that the concentrations of saturated fatty acids recorded were Myristic acid (41.96 ± 0. 38 mg/100g), Palmitic acid (1008.67 ± 10.67 mg/100g), Stearic acid (386.78 ± 2.76 mg/100g), Heneicodenoic acid (30.38 ± 0. 28 mg/100g) and Lingnoceric acid (58.35 ± 0.45 mg/100g). While the unsaturated fatty acids recorded in *T. verrucosus* were Myristoleic acid (55.82 ± 0.44 mg/100g), Palmetoleic acid (633.48 ± 6.76 mg/100g), Elaidic acid (1.78 ± 0.01 mg/100g), Oleic acid (855.22 ± 7.44 mg/100g), Arachidonic Acid (24.27 ± 0. 23 mg/100g), Linolenic acid (264.16 ± 1.89 mg/100g), Cis-11,14-Eicosadienoic acid (36.35 ± 0.31 mg/100g) and Cis11,14,17-eicosatrienoic acid (84.67 ± 0.78 mg/100g) with the highest concentration of unsaturated fatty acid was Oleic acid (855.22 ± 7.44 mg/100g) and lowest concentration was Elaidic acid (1.78 ± 0.01 mg/100g). (Table No.8).

Table 8.
Saturated and unsaturated fatty acids (mg/100g) in *Tylototriton verrucosus* Value are mean ± SEM; No, of 3 observations each.

SL. no.	Name of fatty acid	Concentration	Type of fatty acids
1.	Myristic acid (C14:0)	41.96 ± 0. 38	
2.	Palmitic acid (C16:0)	1008.67 ± 10.67	
3.	Stearic acid (C18:0)	386.78 ± 2.76	Saturated
4.	Heneicodenoic acid (C21:0)	30.38 ± 0. 28	
5.	Lingnoceric acid (C24:1)	58.35 ± 0.45	
			Unsaturated
1.	Myristoleic acid (C14:1)	55.82 ± 0.44	MUFA
2.	Palmetoleic acid (C16:1)	633.48 ± 6.76	MUFA; Omega 7
3.	Elaidic acid (C18:1)	1.78 ± 0.01	MUFA
4.	Oleic acid (C18:1)	855.22 ± 7.44	MUFA
5.	Arachidonic acid (C20:4)	24.27 ± 0. 23	PUFA
6.	Linolenic acid (C18:3)	264.16 ± 1.89	PUFA; Omega 3
7.	Cis-11,14 – eicosadienoic acid (C20:2)	36.35 ± 0.31	PUFA Omega 6
8.	Eicsatrienoic acid (C20:3)	84.67 ± 0.76	PUFA

Note: MUFA: Monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid.

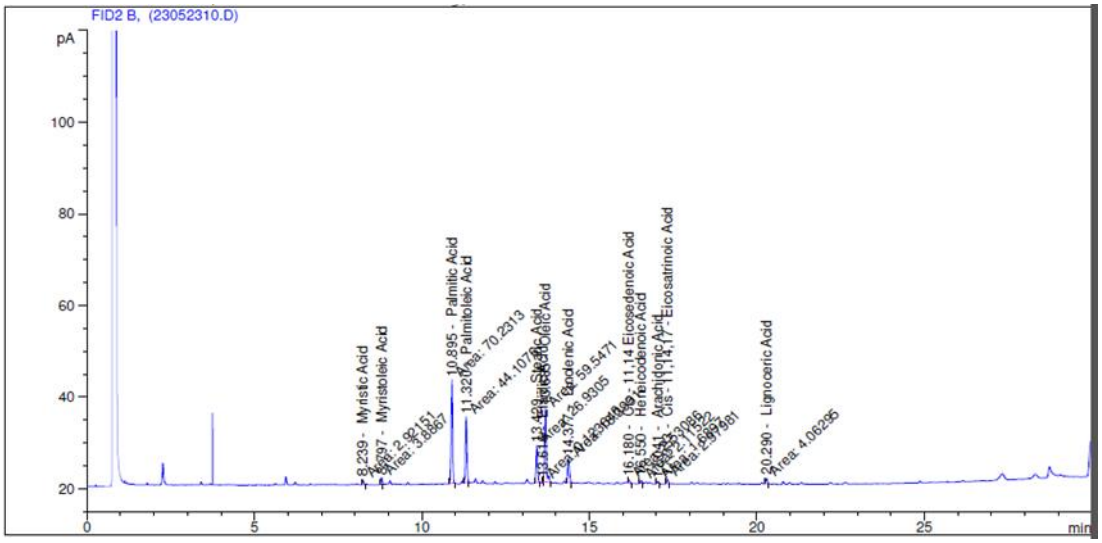

Figure 8.
Chromatogram of FAME analysis of *T. verrucosus*.

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3. Discussion

The results of the study reveals that 7 (seven) ethno zoological products i.e. *P. americana*, *U. epops*, *S. gigantea*, *P. posthuma*, *H. tigerinus*, *S. scrofa domesticus*, and *T. verrucosus*, were commonly uses by the Tangkhul Tribe of Manipur in their medicinal practices for treatment of various human diseases such as Diabetes mellitus, Cancer, Cough, Typhoid, Kidney problems, Anemia etc. (Table-1). There are various published reports of using animal products in the indigenous traditional medicines by several ethnic tribes, communities and people of Northeast state of India and other parts of the world [21, 24, 25, 26, 27]. The way of uses and parts of the animals used as a component in the traditional medicinal practices for the treatment and management of certain human diseases are different among the different ethnic communities across the countries. In recent times, application of zootherapy is taken into account to be the foremost reliable primary alternative among many other known therapeutic practices in the world [28]. F.H. Jafrin and H. Tysong [29] reported traditional knowledge of animal based medicine for the treatment of various human ailments and also mention the important to conserve our rich biodiversity. H. Gaonkar *et al.*,[30] also recently reported that fat of *Sus scrofa domesticus* are used for burn wound, fracture, flesh of *Hoplobatrachus tigerinus* are also used for the treatment of Asthma and Cough, *Pheretima posthuma* used for snake bite by the tribal communities in Goa,

The FAME study reveals that seven ethnozoological products namely *P. americana*, *E. epops*, *S. gigantea*, *P. posthuma*, *H. tigerinus*, *S. scrofa domesticus*, and *T. verrucosus*, were found to be contained varied concentration of saturated and unsaturated fatty acids. Among the commonly recorded saturated fatty acid were Caproic Acid, Caprylic Acid, Lauric Acid, Myristic Acid, Palmitic Acid, Stearic Acid, Arachidic Acid, Pentadecanoic Acid, Heptadecanoic Acid, Heneicodenoic Acid, Behenic Acid. While Myristoleic Acid, Oleic Acid, Palmetoleic Acid, Elaidic, Cis-10-Heptadecanoic Acid, Cis-11-Ecosenic Acid. Erucic Acid, Linolelaidic Acid, Linolenic Acid, Gama Linolenic Acid, 8, 11, 14-Eicosadienoic Acid, Ecosapantanoic acid, Cis-11, 14-Eicosadienoic Acid, and Cis-11, 14, 17-eicosatrienoic Acid were the unsaturated fatty acids found in the ethnozoological products studied. Generally, unsaturated fatty acids are recorded more higher in concentration than saturated fatty acids in all the ethnozoological products studied (Table-2-8; Figures 2-8). Among the monounsaturated fatty acids, Oleic Acid (7579.19 ± 70.23 mg/100g) is found to be highest in concentration and is recorded in the *P. americana*. Some of the common essential PUFAs recorded in the samples of the ethnozoological products studied were Linolenic Acid, Erucic Acid, Oleic Acid, Elaidic Acid, Palmetoleic Acid, Myristoleic Acid etc. with the highest concentration of linolenic acid (5518.03mg/100g) found in the *P. americana*. Our studies is in corroboration with those of the studies reported in other ethnozoological products such as Green Anaconda fat [31], *Podocnemis expansa* [32] bovine adipose tissue [33] and edible African termites [34] where these ethnozoological products mentioned *per se* were used as antinociceptive, anti-inflammatory activities and for the treatment of the treatment of heart and bone diseases, skin diseases, and for the management of the symptoms of psychological diseases like dementia, Alzheimer's disease and any others related diseases and pathologies. It has been reported that naturally occurring fatty acids specially PUFA like Linol8enic Acid, Erucic Acid, Oleic Acid, Elaidic Acid, Palmetoleic Acid, Myristoleic Acid assisted in prevention of the inflammations, platelet aggregations, improve reproductive functions in animals and humans and may also acts as an antioxidant against harmful free radicals produced in our body during disease conditions [11]. Among PUFAs, omega-3 and omega-6 fatty acids are known as important fatty acids as these cannot be synthesized in our body and need to be supplemented through diet [35].

It has been reported that requirement of essential fatty acids especially omega-3 fatty acids in the body can be covered by taking many natural products including those animal sources. In this regards, it has been advised by the health professionals that omega-3 fatty acids like Eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) and common omega-6 fatty acids such as linoleic acids (LA) and arachidonic acid (ARA) should be administered routinely specially from the natural sources for overall maintenance of the good health and for the prevention of various human ailments. Further, regular taking of MUFA namely oleic acid is found to be correlated with the reduction in the harmful

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cholesterol in the body such as LDL-c, increased the level of good cholesterol in the body i.e. HDL-c and have hypotensive effects including the beneficial effects in the management of body weight [36, 37, 38]. The used of *P. americana*, *E. epops*, *S. gigantea*, *P. posthuma*, *H. tigerinus*, *S. scrofa domesticus*, and *T. verrucosus*, in the treatment of diseases like blood sugar, typhoid, jaundice, bladder stone, cancer, cough etc. by the Tangkhul tribe of the Ukhrul district of Manipur may be due to presence of the notable amount of essential fatty acids specially Oleic acid, ALA, EPA, DHA and Palmetoleic Acid in them.

In addition to that, oleic acid is the main type of MUFAs forming more than 89% of MUFA and popular for its hypocholeterolemic potential besides maintaining good level of HDL-cholesterol in the body and thus acting as one of the protector from the cardiovascular related diseases[39]. Moreover, fatty acids belongs to n-6 series plays a pivotal role in the development of the central nervous system, increasing lifespan of the cell and the protection of skin [40, 41, 42]. And it is worth to mention here that more supplementation of the n-6 series can also affect the body negatively by inducing premature aging changes in the cell membranes integrity, defect in the cell division process and induction of the carcinoma cells [40, 41, 42, 43]. This is also necessary to acknowledged that administration of the extra amount of saturated fatty acids may result in elevation of the level of LDL –c in the blood plasma inducing hypercholesteremia while on the other hand, MUFA such as such as 18:1c9, and PUFA, like Linoleic acid and Linolenic acid help in decreasing the LDL generation by increasing the liver LDL-receptors which ultimately decrease the amount of LDL level in the blood and thus reduce the chances of the heart related diseases in our body [44,45].

4. Conclusions

The results of the study also reveal the presence notable content of essential fatty acids especially Linolenic Acid, Erucic Acid, Oleic Acid, Elaidic Acid, Palmetoleic Acid, Myristoleic Acid etc. in the 7(seven) selected ethnozzological products namely *P. americana*, *E. epops*, *S. gigantea*, *P. posthuma*, *H. tigerinus*, *S. scrofa domesticus*, and *T. verrucosus*, which may infer beneficial health effects to the patients or consumers. These ethnozoological products are very good source of MUFA and PUFA which are very important for people health and can be further explore for the presence other notable bioactive compounds for future drug discoveries and integration of the these ethno zoological products in the modern based medicines.

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