CHAPTER V

Fatty Acid Composition of Fish Species

V.1. Materials and Methods

V.1.1. Sample preparation

The sample of nine fish species was prepared as per the procedure mentioned in the **Section IV.1.1** (**Page no. 58**).

V.1.2. Determination of fatty acid composition

Fish fat of selected fish species was obtained as per the procedure mentioned in the **Section IV.1.6** (Page no.). Derivatisation of the obtained fish fat (0.5 g) was carried out using 5 mL of 14% BF_3 in methanol by vortexing at 60^{o}C for 20 min. The mixture was then cooled in ice and was extracted with chloroform within 5 min. The extraction with chloroform was repeated thrice. The chloroform layer was separated, collected and then it was reduced using a vacuum to get dry mass. The dry sample obtained was re-dissolved again in chloroform. Then 1 µL solution was injected into the column of gas chromatography-mass spectrometry (GC-MS). Analysis of fatty acid methyl esters was executed using GC-MS (Shimadzu, GC-MS, QP 2010 Plus). The column of GC used was DB-5MS, 30 m × 0.25 mm with a 0.25 µm of film thickness. Initially, temperature of the oven was held at 100°C for 4 min and then increased at 5°C/min to 250°C, held for 10 min, and the temperature was raised gradually at 5°C/min to 280°C. It was then held for 4 min. The ion source was adjusted at 200^{o}C, the injection temperature used was 200^{o}C, and the interface was set at 280^{o}C. The carrier gas was Helium and was set at the constant flow rate of 1 mL/min. EI mass spectra were taken with a mass-to-charge ratio of 50–600 scanning range and the library search was performed for identification purposes. The methyl esters of fatty acids identified were presented as percentage (%) of the total area of fatty acids.

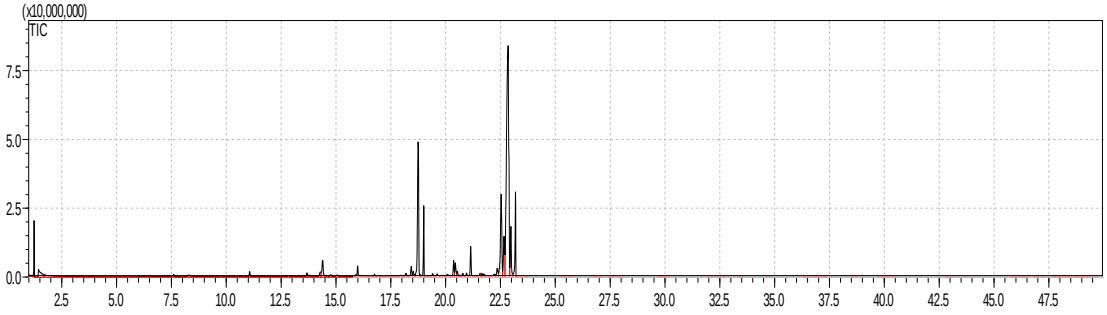

Fig. V.1. Gas chromatogram of *Barilius bendelisis*.

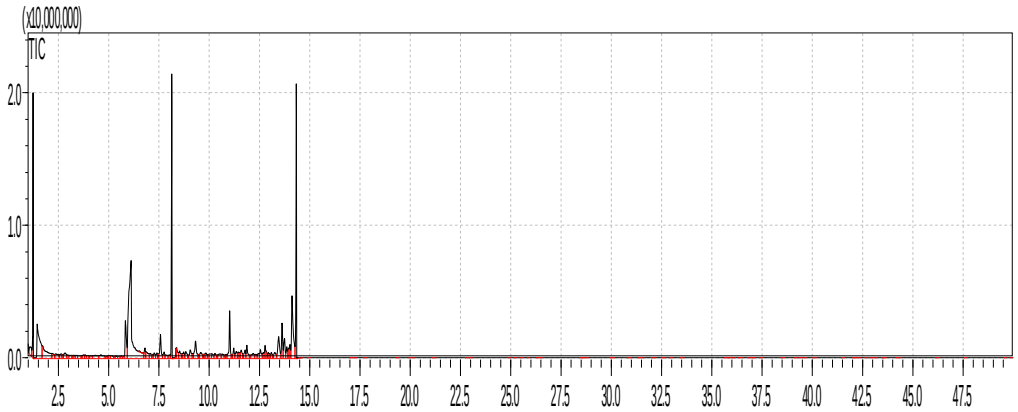

Fig. V.2. Gas chromatogram of *Chagunius chagunio*.

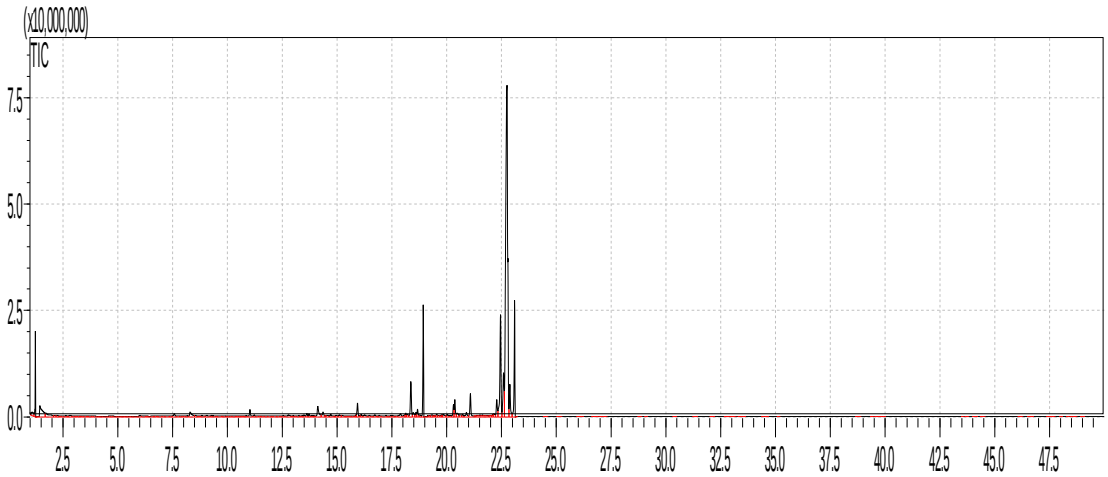

Fig. V.3. Gas chromatogram of *Garra gotyla*.

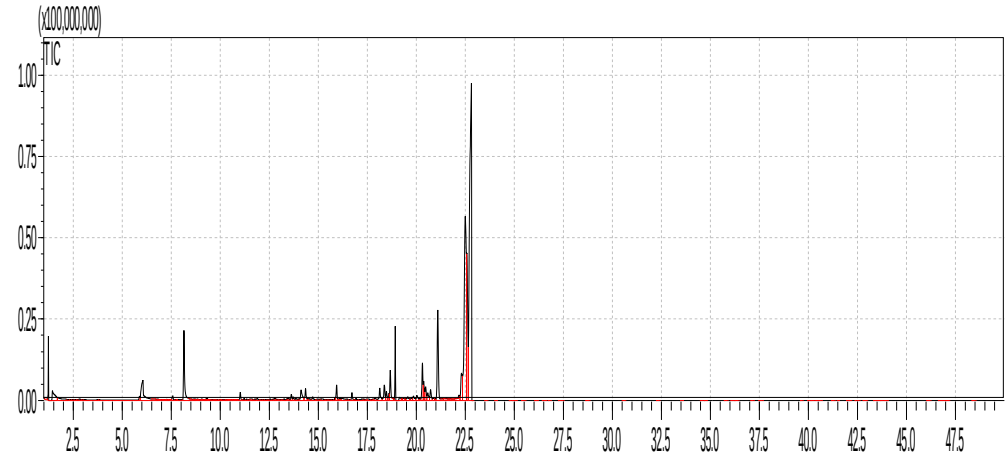

Fig. V.4. Gas chromatogram of *Labeo pangusia.*

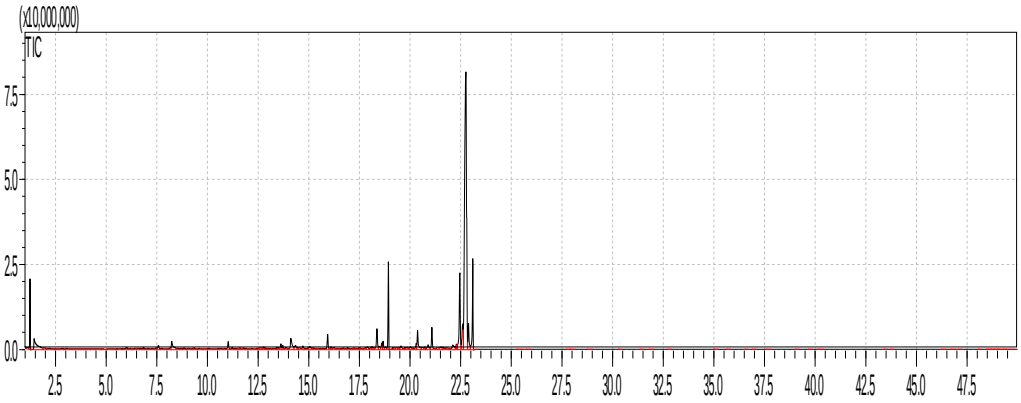

Fig. V.5. Gas chromatogram of *Neolissochilus hexagonolepis.*

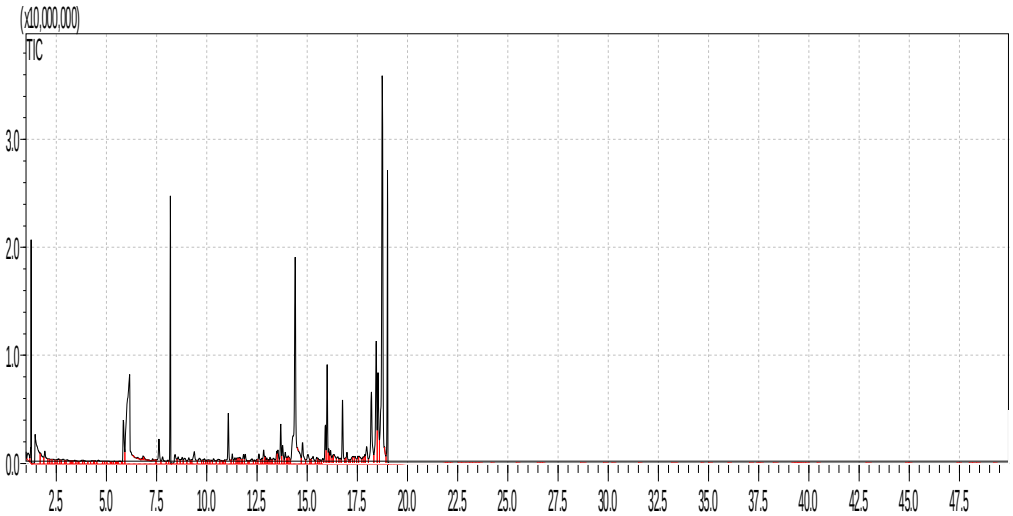

Fig. V.6. Gas chromatogram of *Raiamas bola.*

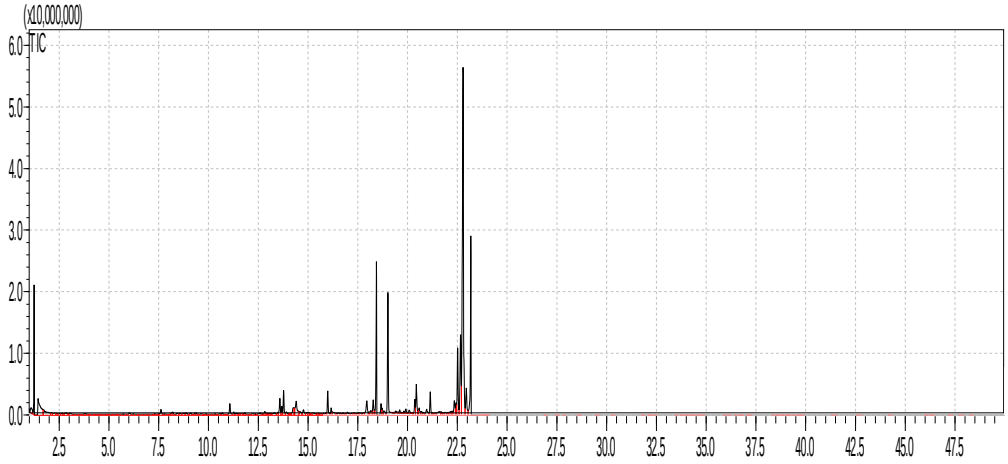

Fig. V.7. Gas chromatogram of *Tor putitora.*

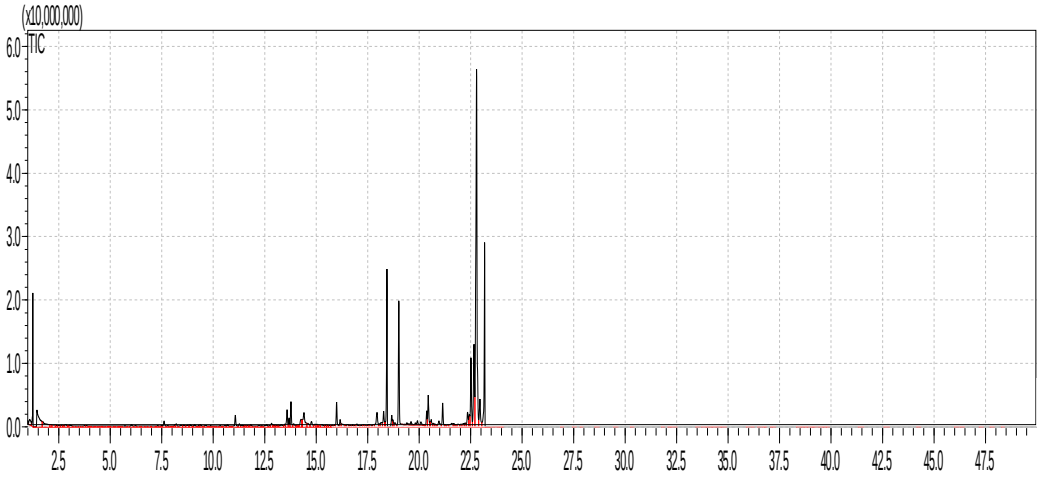

Fig. V.8. Gas chromatogram of *Cyprinion semiplotum.*

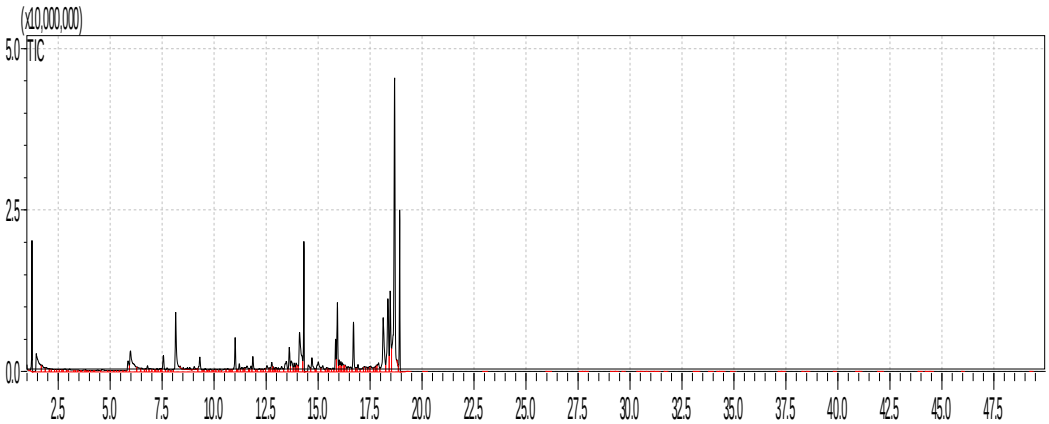

Fig. V.9. Gas chromatogram of *Barilius barna.*

Table V.1. Fatty acid composition of *Barilius bendelisis*

Methyl esters of fatty acids	RT	Composition (%)
Lauric (dodecanoic) acid [C12:0]	13.674	0.863
Myristic (tetradecanoic) acid [C14:0]	14.392	0.898
Palmitic (hexadecanoic) acid C16:0]	15.989	1.137
Palmitoleic (*cis*-9-hexadecenoic) acid [C16:1]	16.759	0.735
Stearic (octadecanoic) acid [C18:0]	18.433	0.535
Oleic (*cis*-9-octadecenoic) acid [C18:1]	18.75	17.795
Linoleic (9,12-octadecadienoic) acid [C18:2]	19.001	14.130
Arachidic (eicosanoic) acid [C20:0]	20.365	4.314
Gondoic (*cis*-11-eicosenoic) acid [C20:1]	20.445	1.149
Eicosapentaenoic acid (C20:5), C20;5n3 (EPA)	21.144	8.887
Behenic (docosanoic) acid [C22:0]	22.848	45.116

Table V.2. Fatty acid composition of *Chagunius chagunio*

Methyl esters of fatty acids	RT	Composition (%)
Lauric (dodecanoic) acid [C12:0]	5.835	8.159
Myristic (tetradecanoic) acid [C14:0]	6.114	37.751
Palmitic (hexadecanoic) acid C16:0]	7.574	2.519
Stearic (octadecanoic) acid [C18:0]	8.135	13.989
Oleic (*cis*-9-octadecenoic) acid [C18:1]	9.312	1.131
Linoleic (9,12-octadecadienoic) acid [C18:2]	11.018	1.283
Linolenic (9,12,15-octadecatrienoic) acid [C18:3]	11.874	1.065
Arachidic (eicosanoic) acid [C20:0]	12.781	0.498
Eicosapentaenoic acid [C20:5], (EPA)	13.484	1.113
Behenic (docosanoic) acid [C22:0]	13.626	1.879
Docosahexaenoic acid [C22:6], (DHA)	14.12	13.181
Lignoceric (tetracosanoic) acid [C24:0]	14.335	14.528

Table V.3. Fatty acid composition of *Garra gotyla*

Methyl esters of fatty acids	RT	Composition (%)
Capric (decanoic) acid [C10:0]	13.72	2.162
Lauric (dodecanoic) acid [C12:0]	14.12	23.346
Myristic (tetradecanoic) acid [C14:0]	15.93	4.178
Palmitic (hexadecanoic) acid C16:0]	16.26	0.684
Palmitoleic (*cis*-9-hexadecenoic) acid [C16:1]	16.92	0.317
Stearic (octadecanoic) acid [C18:0]	18.94	7.773
Oleic (*cis*-9-octadecenoic) acid [C18:1]	19.54	0.631
Linoleic (9,12-octadecadienoic) acid [C18:2]	20.31	0.605
Arachidic (eicosanoic) acid [C20:0]	21.08	2.395
Eicosapentaenoic acid (C20:5), C20;5n3 (EPA)	22.11	0.537
Docosahexaenoic acid (C22:6), C22:6n3 (DHA)	22.46	3.448
Lignoceric acid [C24:0]	22.46	32.594

Table V.4. Fatty acid composition of *Labeo pangusia*

Fatty acids	RT	Composition (%)
Capric (decanoic) acid [C10:0]	6.047	11.277
Lauric (dodecanoic) acid [C12:0]	8.143	11.126
Myristic (tetradecanoic) acid [C14:0]	14.12	3.807
Palmitic (hexadecanoic) acid C16:0]	15.931	0.615
Palmitoleic (*cis*-9-hexadecenoic) acid [C16:1]	16.712	0.938
Stearic (octadecanoic) acid [C18:0]	18.369	1.618
Oleic (*cis*-9-octadecenoic) acid [C18:1]	18.669	1.369
Linoleic (9,12-octadecadienoic) acid [C18:2]	18.928	5.043
Eicosapentaenoic acid (C20:5), (EPA)	20.308	4.448
Behenic (docosanoic) acid [C22:0]	21.091	14.649
Docosahexaenoic acid (C22:6), (DHA)	22.498	18.851
Lignoceric (tetracosanoic) acid [C24:0]	22.805	22.192

Table V.5. Fatty acid composition of *Neolissochilus hexagonolepis*

Methyl esters of fatty acids	RT	**Composition (%)**
Myristic (tetradecanoic) acid [C14:0]	14.883	3.674
Palmitic (hexadecanoic) acid C16:0]	15.184	25.821
Palmitoleic (*cis*-9-hexadecenoic) acid [C16:1]	16.803	0.541
Stearic (octadecanoic) acid [C18:0]	18.971	0.538
Oleic (*cis*-9-octadecenoic) acid [C18:1]	19.308	0.362
Linoleic (9,12-octadecadienoic) acid [C18:2]	19.805	27.207
Arachidic (eicosanoic) acid [C20:0]	21.921	5.624
Behenic (docosanoic) acid [C22:0]	23.581	14.801
Docosahexaenoic acid (C22:6), C22:6n3 (DHA)	24.688	1.702
Lignoceric (tetracosanoic) acid [C24:0]	25.906	8.276

Table V.6. Fatty acid composition of *Raiamas bola*

Methyl esters of fatty acids	**RT**	**Composition (%)**
Capric (decanoic) acid [C10:0]	6.155	26.505
Lauric (dodecanoic) acid [C12:0]	8.184	9.388
Myristic (tetradecanoic) acid [C14:0]	11.071	0.943
Palmitic (hexadecanoic) acid C16:0]	14.401	11.081
Palmitoleic (*cis*-9-hexadecenoic) acid [C16:1]	14.4	1.141
Stearic (octadecanoic) acid [C18:0]	15.997	1.611
Linoleic (9,12-octadecadienoic) acid [C18:2]	16.763	3.469
Arachidic (eicosanoic) acid [C20:0]	18.198	3.035
Eicosapentaenoic acid (C20:5), C20;5n3 (EPA)	18.443	5.385
Behenic (docosanoic) acid [C22:0]	18.746	7.932
Lignoceric (tetracosanoic) acid [C24:0]	19.001	8.855

Table V.7. Fatty acid composition of *Tor putitora*

Methyl esters of fatty acids	RT	Composition (%)
Myristic (tetradecanoic) acid [C14:0]	14.883	2.151
Palmitic (hexadecanoic) acid [C16:0]	15.184	15.113
Stearic (octadecanoic) acid [C18:0]	16.803	0.316
Oleic (*cis*-9-octadecenoic) acid [C18:1]	17.532	0.268
Linoleic (9,12-octadecadienoic) acid [C18:2]	18.971	0.315
Linolenic (9,12,15-octadecatrienoic) acid [C18:3]	21.921	3.292
Arachidic (eicosanoic) acid [C20:0]	24.006	27.301
Eicosapentaenoic acid (C20:5), C20;5n3 (EPA)	24.934	22.304
Docosahexaenoic acid (C22:6), C22:6n3 (DHA)	25.906	4.844
Lignoceric (tetracosanoic) acid [C24:0]	27.439	19.604

Table V.8. Fatty acid composition of *Cyprinion semiplotum*

Methyl esters of fatty acids	RT	Composition (%)
Lauric (dodecanoic) acid [C12:0]	13.777	5.195
Myristic (tetradecanoic) acid [C14:0]	14.275	8.179
Palmitic (hexadecanoic) acid C16:0]	14.405	2.468
Palmitoleic (*cis*-9-hexadecenoic) acid [C16:1]	14.771	0.560
Stearic (octadecanoic) acid [C18:0] ester	16.771	0.212
Linoleic (9,12-octadecadienoic) acid [C18:2]	17.959	1.065
Linolenic (9,12,15-octadecatrienoic) acid [C18:3]	17.955	1.267
Arachidic (eicosanoic) acid [C20:0]	18.435	20.756
Behenic acid [C22:0]	19.012	26.989
Docosahexaenoic acid (C22:6), C22:6n3 (DHA)	22.662	1.258
Lignoceric acid [C24:0]	22.787	25.675

Table V.9. Fatty acid composition of *Barilius barna*

Methyl esters of fatty acids	RT	Composition (%)
Capric (decanoic) acid [C10:0]	5.859	4.641
Lauric (dodecanoic) acid [C12:0]	5.971	10.949
Myristic (tetradecanoic) acid [C14:0]	8.147	10.451
Palmitic (hexadecanoic) acid C16:0]	13.62	2.165
Stearic (octadecanoic) acid [C18:0]	14.11	2.972
Oleic (*cis*-9-octadecenoic) acid [C18:1]	14.32	9.927
Linolenic (9,12,15-octadecatrienoic) acid [C18:3]	15.01	0.298
Arachidic (eicosanoic) acid [C20:0]	15.93	2.733
Gadoleic (cis-9-eicosenoic) a*cid [*C20:1]	16.7	6.949
Behenic (docosanoic) acid [C22:0]	18.37	6.052
Docosahexaenoic acid (C22:6), (DHA)	18.68	1.450
Lignoceric (tetracosanoic) acid [C24:0]	18.93	11.24

V.2. Results and Discussion

The fatty acid compositions of the nine fish species detected by GC-MS technique (**Fig. V.1–9**) are shown in T**ables V.1–9**. In the present study, fatty acid composition in variable amounts was seen among the different fish species (T**able V.10**), and this may be because of the different fish species, the location and age of the species. It is seen that the total saturated fatty acids (SFAs) were found to be the highest (89.47 %) in *C. semiplotum* and the lowest (51.20 %) being in *B. barna*. The three SFAs such as palmitic acid, myristic acid and stearic acid could be detected in all nine fishes. Myristic acid ranged from 0.89 % in *B. bendelisis* to 37.75 % in *C. chagunio*, palmitic acid ranged from 0.62 % (*L. pangusia*) to 25.82 % (*N. hexagonolepis*), and the stearic acid varied from 0.21 % (*C. semiplotum*) to 13.98 % (*C. chagunio*). Similarly, Jabeen et al. (2011) investigated the profiles of fatty acid of the three fish species. They reported that the myristic acid content varied from 0.34 to 3.78 %, palmitic acid varied from 1.89 to 47.71 %, and stearic acid varied from 0.44 to 11.99 %. Tenyang et al. (2016) also studied the profiles of fatty acids of the four freshwater fishes and reported myristic acid concentration in the range of 2.47–3.37 %, palmitic acid in the range of 20.07–

23.99 % and stearic acid in the range of 6.37–9.97 %. Ghassem et al. (2009) investigated the fatty acid profile of four freshwater fish species. They reported the myristic acid concentration in range of 0.93 to 3.11 %, palmitic acid in the range of 20.80 to 24.34 % and stearic acid in the range of 4.68 to 6.06 %. Dhaneesh et al. (2012) also studied the fatty acid compositions of the ten fish species and reported that palmitic acid, myristic acid, and stearic acid contents were in the range of 13.97–16.96 %, 7.11–10.66 %, and 0.33–0.68 %, respectively. This study could detect capric acid only in four fish species and these are *L. pangusia* (11.28 %), *G. gotyla* (2.16 %), *B. barna* (4.64 %), and *R. bola* (26.51 %). Lauric acid was detected in seven fish species except in *N. hexagonolepis* and *T. putitora*. Arachidic acid was found to be present in eight fish species that ranged from 0.49 to 27.30 %, but it was not detected in *L. pangusia.* Behenic acid could be found in seven fish species, except in the two fish species *viz. T. putitora* and *G. gotyla.* Behenic acid was found to be the highest in *B. bendelisis* (45.12 %) species and the lowest being in *C. chagunio* (1.88 %). Lignoceric acid which was found in the range of 8.28–32.59 % was identified in the eight fishes except in *B. bendelisis.*

The total quantity of the unsaturated fatty acids (UFAs) in the nine fishes ranged from 4.15 % in *C. semiplotum* to 42.69 % in *B. bendelisis* (T**able V.10**). The total amount of monounsaturated fatty acids (MUFAs) was found to be the highest in the *B. bendelisis* (19.68 %) and the lowest was in *T. putitora* (0.27 %). Palmitoleic acid was not found in *B. barna, C. chagunio* and *T. putitora*, but it was detected in other six fish species that varied from 0.25 to 1.14 %. Oleic acid was the most abundant MUFA found in the seven fishes that ranged from 0.27 % (*T. putitora*) to 17.79 % (*B. bendelisis*). Jabeen and Chaudhry (2011) also investigated and reported that oleic acid (2.19–25.64 %) was the most abundant MUFAs found in their study, which are higher compared to the present investigation. Tenyang et al. (2016) reported the oleic acid as the most abundant MUFAs in their study and mentioned the concentration of oleic acid in 21.10–32.96 % range. Ghassem et al. (2009) also mentioned oleic acid as the most abundant MUFAs in their study and reported oleic acid content in the range of 28.45–39.26 %. However, Dhaneesh et al. (2012) reported the oleic acid contents of some fishes of Lakshadweep, which were found in the range of 5.15–6.88 %. Gadoleic acid was found only in one species *viz. B. barna*, and gondoic acid was detected only in *B. bendelisis.*

Table V.10. Fatty acid (methyl ester) composition of nine fish species in the % of total fatty acids by the peak area

Methyl esters of fatty acids	Fish species								
	FS-1	**FS-2**	**FS-3**	**FS-4**	**FS-5**	**FS-6**	**FS-7**	**FS-8**	**FS-9**
Capric acid [C10:0]	–	–	2.16	11.28	–	26.50	–	–	4.64
Lauric acid [C12:0]	0.86	8.16	23.35	11.13	–	9.39	–	5.19	10.95
Myristic acid [C14:0]	0.89	37.75	4.18	3.81	3.67	0.94	2.15	8.18	10.45
Palmitic acid [C16:0]	1.14	2.52	0.68	0.62	25.82	11.08	15.11	2.47	2.17
Palmitoleic acid [C16:1]	0.74	–	0.32	0.94	0.54	1.14	–	0.56	–
Stearic acid [C18:0]	0.54	13.99	7.77	1.62	0.54	1.61	0.32	0.21	2.97
Oleic acid [C18:1]	17.79	1.13	0.63	1.37	0.36	–	0.27	–	9.93
Linoleic acid [C18:2]	14.13	1.28	0.60	5.04	27.21	3.47	0.32	1.07	–
Linolenic acid [C18:3]	–	1.07	–	–	–	–	3.29	1.27	0.29
Arachidic acid [C20:0]	4.31	0.49	2.39	–	5.62	3.04	27.30	20.76	2.73
Gondoic acid [C20:1]	1.15	–	–	–	–	–	–	–	–
Gadoleic acid [C20:1]	–	–	–	–	–	–	–	–	6.949
EPA [C20:5]	8.89	1.11	0.54	4.45	–	5.39	22.30	–	–
Behenic acid [C22:0]	45.15	1.88	–	14.65	14.80	7.93	–	26.99	6.05
DHA [C22:6]	–	13.18	3.45	18.85	1.70	–	4.84	1.26	1.45
Lignoceric acid [C24:0]	–	14.53	32.59	22.19	8.28	8.86	19.60	25.68	11.24
Total SFA	52.86	79.32	73.13	65.28	58.73	69.35	64.48	89.47	51.20
Total MUFA	19.68	1.13	0.95	2.31	0.90	1.14	0.27	0.56	16.87
Total PUFA	23.02	16.64	4.59	28.34	28.91	8.85	30.75	3.59	1.75
Total UFA	42.69	17.77	5.54	30.65	29.81	9.99	31.02	4.15	18.62
PUFA/SFA	0.44	0.21	0.06	0.43	0.49	0.13	0.48	0.04	0.03
Total FA identified	95.56	97.10	78.67	95.93	88.55	79.35	95.51	93.62	69.83

FS = Fish species; FS-1 = *Barilius bendelisis*; FS-2 = *Chagunius chagunio*; FS-3 = *Garra gotyla*; FS-4 = *Labeo pangusia*; FS-5 = *Neolissochilus hexagonolepis*; FS-6 = *Raiamas bola*; FS-7 = *Tor putitora*; FS-8 = *Cyprinion semiplotum*; FS-9 = *Barilius barna*; FA, Fatty acids; UFA, Unsaturated fatty acid; SFA, Saturated fatty acid; MUFA, Monounsaturated fatty acid; PUFA, Polyunsaturated fatty acid; EPA, Eicosapentaenoic acid; DHA, Docosahexaenoic acid.

Polyunsaturated fatty acids (PUFAs) are considered as the essential biochemical components for the human diet that can afford several important health benefits. In this study, the total amount of PUFAs was found to be the lowest in *B. barna* (1.75 %) and the highest in *T. putitora* (30.76 %). Along with the *T. putitora,* a high concentration of PUFAs was also

detected in *L. pangusia* (28.34 %), *N. hexagonolepis* (28.91 %) and *B. bendelisis* (23.02 %). Dhaneesh et al. (2012) described higher concentrations of PUFAs in some fish species of Lakshadweep Archipelago which were found in the range of 30.32–35.11 %. Tenyang et al. (2016) reported the level of PUFAs in four freshwater fish species that ranged from 11.62 to 32.41 %. Ghassem et al. (2009) also reported PUFAs in some Malaysian freshwater fish species that ranged from 23.19 to 33.79 %. Similarly, Ljubojevic et al. (2013) reported the levels of PUFAs in some fish species of Inland waters that ranged from 17.07 % to 28.15 %. The ω-6 fatty acid *viz.* linoleic acid detected in the eight fishes except in *B. barna* was the most abundant PUFAs found in this study that ranged from 0.32 % in *T. putitora* to 27.21 % in *N. hexagonolepis*. Low concentration of linolenic acid (ω-3 fatty acid) was detected only in four fish species, and they are *B. barna* (0.29 %), *C. chagunio* (1.07 %), *C. semiplotum* (1.27 %) and *T. putitora* (3.29 %). Eicosapentaenoic acid (EPA) is a *ω*-3 fatty acid and it was observed in six fish species, which was found in the range of 0.54–22.30 %. High levels of EPA were observed in *T. putitora* (22.30 %) and *B. bendelisis* (8.89 %). Docosahexaenoic acid (DHA) is also a *ω*-3 fatty acid and it was detected in seven fishes varying from 1.26 % (*C. semiplotum*, lowest) to 18.85 % (*L. pangusia*, highest). Mohanty et al. (2016) also investigated the fatty acid compositions of important freshwater fish species of India. They reported that the EPA contents varied from 0.9 to 6.8 % and the levels of DHA were found in the range of 0.4 to 8.9 %. In this investigation, it is found that the results of this study are higher than comparable to that of the results reported by Mohanty et al. (2016). Dhaneesh et al. (2012) also reported the EPA content of some fish species of Lakshadweep that ranged from 3.3 to 5.17 %, and the DHA content which was found ranging from 2.0 to 4.81 %. In our study, the concentrations of DHA and EPA of some fishes are in good agreement with the data reported by Dhaneesh et al. (2012). In contrast to this, some fishes of our study showed higher concentrations of EPA and DHA (**Table V.10**). Hence, the fish species of this study have the potentials to be served as natural dietary supplements for ω-3 fatty acids such EPA and DHA. The ω-3 fatty acids (PUFAs) such as DHA and EPA are the essential biological molecules which on being consumed could improve the biological processes of life and can reduce the risk of the untimely death of human beings. EPA is influential on the behavior and mood, whereas DHA is essential for brain growth and infant developments and it is needed for normal functioning of the brain also in adults (Karuppasamy et al., 2013; Mohanty et al., 2016). DHA and EPA can support in reducing the risk of coronary heart disease, atherosclerosis, hypertension, cancer, lung diseases, rheumatoid arthritis, old age diseases such as Alzheimer's disease, dementia and macular degeneration (Muhamad and Mohamad,

2012; Mahanty et al., 2014; Mohanty et al., 2016). Deficiency of DHA and EPA can cause neurobehavioral disorder and most commonly occur in adolescence and childhood that can be prohibited by consumption of ω-3 PUFAs (Feldman and Reiff, 2014; Mahanty et al., 2014). As per the US Dietary Guidelines 2010, for a person with both higher and average cardiovascular disease, consumption of DHA + EPA at least 250 mg in a day in average and 1,750 mg in a week is recommended (Washington, 2010). The quality of fats is indicated by the ratio of PUFA/SFA, and According to Wood et al. (2008), if the ratio is above 0.4, the fish species would be considered as the most favorable one. In the present study, PUFA/SFA value was found in the range of 0.03 to 0.49 and the four fishes whose values were above the mentioned limit are considered as the most favorable one. They are *B. bendelisis* (0.44), *N. hexagonolepis* (0.49), *L. pangusia* (0.43) and *T. putitora* (0.48). The total FAs identified were seen to be the lowest in the *B. barna* (69.83 %) and found to be the highest in *C. chagunio* (97.09 %).

Conclusion

In this investigation, the fatty acid compositions ranged from 51.20–89.47 % of SFAs, 0.27–19.68 % of MUFAs and 1.75–30.75 % of PUFAs. EPA and DHA varied respectively from 0.54–22.30 % and 1.26–18.85 %. The fish species are found to be rich in ω-3 fatty acids such as EPA and DHA. Hence, the fish species of this study have the potentials to be served as natural dietary supplements for ω-3 fatty acids and its consumption can fulfil malnutrition problems and can reduce the risk of various health diseases.