ISSN: 2347-5129
IJFAS 2015; 2(3): 87-92

www.fisheriesjournal.com
Received: 06-11-2014
Accepted: 07-12-2014

Md. Ashraful Hoque
Department of Applied & Environmental Chemistry, University of Chittagong, Chittagong 4331, Bangladesh.

Mohammad Helal Uddin
Department of Applied & Environmental Chemistry, University of Chittagong, Chittagong 4331, Bangladesh.

Md. Al-Amin
Applied Chemistry Research Laboratory, Department of Chemistry, University of Chittagong, Chittagong 4331, Bangladesh.

Md. Abul Hasan Roni
Department of Applied & Environmental Chemistry, University of Chittagong, Chittagong 4331, Bangladesh.

Correspondence
Mohammad Helal Uddin
Department of Applied & Environmental Chemistry, University of Chittagong, Chittagong 4331, Bangladesh.

Isolation, physico-chemical characterization and microbial studies of liver lipid of sting ray fish (*Dasyatis sephen*) of the Bay of Bengal

Md. Ashraful Hoque, Mohammad Helal Uddin, Md. Al-Amin and Md. Abul Hasan Roni

Abstract
Lipid was extracted from the liver of Sting Ray (*Dasyatis sephen*) by solvent extraction method and then purified by suitable conventional chromatographic method. Various physical and chemical characteristics of the lipid sample were determined by standard methods and compared with those of standard fats or oils. Fatty acid composition of the lipid sample was investigated by Thin Layer Chromatographic (TLC) examination. The liver lipid of Sting Ray was found to contain palmitic acid, stearic acid and oleic acid with some other unknown fatty acids. Antimicrobial activity (antibacterial activity against four bacteria and antifungal activity against four fungi) of the lipid was tested by standard methods. The lipid containing liver of Sting Ray was analyzed quantitatively for the determination of percentages of protein and mineral (N, P, K, Ca) contents by modified Kjeldahl method.

Keywords: Sting Ray fish, Liver, Antibacterial, Antifungal, TLC, PUFA.

1. Introduction
Bangladesh is a country with hundreds of rivers and ponds and is notable for being a fish-loving nation, acquiring the name "Machh-e Bhat-e Bangali" which means "Bengali by fish and rice". Most of the people in the developing countries like Bangladesh are dependent on fish as a source of animal protein and about 80% of the animal protein in our diet comes from fish alone [1]. Sting Ray constitutes the largest single species fishery in almost all the riverine, estuarine and marine water of Bangladesh commonly known as Gauru Leza Hauspata and locally known as Haus. It is valuable natural resource of Bangladesh quality protein and fat. The liver of *Dasyatis sephen* is the largest organ in the body and may comprise one-seventh to one-sixth of the weight of a Sting Ray [2]. Liver lipid of Sting Ray (*Dasyatis sephen)* is remarkably differs from vegetable oils in containing a great variety of fatty acids especially highly unsaturated fatty acids [3]. Many marine fishes are rich in lipid (oil) which provides mainly 16:0, 18:0 and 18:1 fatty acids but also a little bit pharmaceutically important and physiologically active ω-3 polyunsaturated fatty acids (ω-3 PUFA) [4]. The ω-3 fatty acid series are abundantly found in marine algae and phytoplankton and also in the marine fishes which eat these marine plants [5]. Nutritionists and food scientists need lipid and fatty acid composition data to aid them in dietary formulation, nutrient labeling, processing and product development [6]. Currently, the biochemical effects of fish oils in human health and nutrition [7, 8] have placed renewed emphasis on the apparent difference in the compositions of fish oils [9]. In the present investigation, the lipid and fatty acids of the liver of *Dasyatis sephen* were studied with a view to exploring the possibilities of commercial exploitation of the highly available species as a source of marine oils and polyunsaturated fatty acids of ω-3 series. The lipid sample was also evaluated with respect to biological activities (bacterial and fungal activities) for pharmacological aspects.

2. Materials and methods
2.1 Collection of the marine species
The Sting Ray fish was collected from the local fish market, Sadarghat, Chittagong Bangladesh and preserved in deep freezer for a few days. The liver was separated and

preserved until extraction and further chemical investigation.

(Dorsal View)

(Ventral view)

Fig 1: Sting Ray (*Dasyatis sephen*)

2.2 Extraction of lipid

Oil extraction from liver was carried out by solvent extraction method using acetone and ethyl acetate as solvent. Combined extract was recovered with a rotary evaporator at 45 ˚C to obtain lipid and dried under flushing with a slow stream of nitrogen gas for the removal of residual solvent. Extracted lipid was used for physico-chemical characterization, microbial studies and for analysis of fatty acids pattern by TLC. Analytical grade chemicals were used and solutions were prepared according to the standard procedure [10, 11, 12].

2.3 Physical characterization

The amount of total lipid was determined gravimetrically. Crude fat, crude fibre and ash contents of the liver of Sting Ray were determined by standard methods [13]. A weight portion of the liver of Sting Ray was first dried in an oven at about 100-105 ˚C for 4-5 hours to remove moisture and then burnt into ash in a muffle furnace at about 600 ˚C for 4 hours [14]. The refractive index of the lipid sample was determined by Abbe refractometer (Model; DTM-1 Atago Co. Ltd). The specific gravity of the lipid sample was determined by Specific Gravity bottle. The viscosity of the lipid sample was determined by Ostwald's viscometer at 30 ˚C.

2.4 Chemical characterization

Various chemical properties of the lipid sample were determined under the specific condition of the standard methods. Saponification value, saponification equivalent value, acid value and percentage of free fatty acid, iodine value, acetyl value, peroxide value, thiocyanogen value, Richert-Meissl value and Polenske value, Henher value, Kirschner value, Titre value, Marine oil test, Elaiden test, cholesterol content and quantity of unsaponifiable matter of the lipid were determined by standard methods **[15, 16, 17, 18]**.

2.5 Antimicrobial screening

The antimicrobial activity of liver lipid of Sting Ray was studied against four bacteria and four fungi [19]. For the detection of antibacterial activities the disc diffusion method was followed. The antifungal activity was assessed by food poison technique. Nutrient agar (NA) and potato dextrose agar (PDA) were used as basal medium for the test of bacteria and fungi respectively. Chloroform was used as a solvent to prepare desired solution (5% and 10%) of the lipid sample. Proper control was maintained with chloroform.

2.6 Estimation of minerals

By applying the standard methods, percentages of minerals (N, P, K and Ca) of lipid containing liver were determined.

2.7 Chromatographic examinations

The liver lipid of Sting Ray was subjected to TLC examination and its fatty acid composition was identified by comparing the R_f values of different spots of chromatograms with those of standard fatty acids reported earlier in different solvent systems **[20, 21]**.

3. Results and Discussion

3.1 Physical characteristics [12]

The amount of total lipid content of Sting Ray was found to be 30.93 mg/g. From this data it is evident that the lipid contents of Sting Ray per gram of liver were much more in comparison to other fish sources. This may claim valuable demands of Sting Ray for edible purpose due to its higher lipid level. The refractive index of the liver lipid of Sting Ray was found to be 1.4699 (Table 1) at 24 ˚C. The present result indicates that the lipid from the specimen contained moderate amount of unsaturated fatty acids. This was also supported by its iodine value.

The specific gravity of the liver lipid solution was found to be 0.9621 at 30 ˚C (Table 1). This sample was found in semisolid condition. From the result of this experiment, we got an idea about the specific gravity of the original lipid.

The viscosity of the liver lipid solution was found to be 303.665 milipoise at 30 ˚C (Table 1). From the result of viscosity we got an idea about the inter-molecular hydrogen bonding in the lipid sample. The present result suggested that there are a few hydroxyl groups and few free acid molecules present in the lipid sample. This observation is supported by low acetyl value and low acid value of the lipid sample.

Table 1: Physical constants of the liver lipid of Sting Ray

Name of the sample	Refractive index	Specific gravity	Viscosity (mp)
Linseed oil	1.4790-1.4800	0.931-0.938	296.084
Sunflower oil	1.4655-1.4721	0.924-0.926	331.125
Brain lipid of Kerani Chingri	1.4748	0.9180	287.060
Brain lipid of Baghda Chingri	1.4736	0.941	303.260
Liver Lipid of Blue Spotted Fantail Ray	1.4760	0.9575	325.325
Liver Lipid of Sting Ray	**1.4699**	**0.9621**	**303.665**

Moisture content of the lipid sample was found to be 1.21%. Crude fat, crude fibre and ash content of the lipid containing liver of Sting Ray were determined as shown in Table 2.

Table 2: Crude fat, crude fibre content and ash content of the liver of Sting Ray

Name of the sample	Crude fat (%)	Fibre content (%)	Ash content (%)
Liver of Sting Ray	5.18	2.18	2.013

3.2 Chemical characteristics [12]

The saponification value of the liver lipid of Sting Ray was found to be 283.58 (Table 3). The saponification equivalent value of the liver lipid of Sting Ray was found to be 197.83. The saponification value is inversely proportional to the average molecular weight or chain length of the fatty acid present in the fat or oil. The saponification equivalent value is directly proportional to the average chain length of fatty acid present. This result clearly indicate that the lipid sample contain higher proportion of high molecular weight fatty acids.

The acid value of the liver lipid of Sting Ray was found to be 1.43 (Table 3). The percentage of free fatty acid (F.F.A.), as oleic, was calculated from acid value and was found to be 0.71 (Table 3) for the lipid sample. Acid value indicates the proportions of free fatty acid in the oil or fat. Low acid value is an indication of freshness of the oil or fat and suitability of the lipid for edible purpose. The ester value of the liver lipid of Sting Ray was found to be 282.15 (Table 3). This result indicates that ester may present in the lipid sample.

The iodine value of the liver lipid of Sting Ray was found to be 118.87 (Table 3). Iodine value gives an estimation of the degree of unsaturated fatty acids in the triglyceride molecules of the fat or oil. The value indicates that the lipid sample contain moderate proportion of unsaturated fatty acid and is of semidrying type which is supported by Elaiden test.

The peroxide value is an indication of unsaturation present in fats or oils. The more unsaturated fats or oils absorb more oxygen, form greater amounts of hydroperoxides and show higher peroxide value. The peroxide value of the liver lipid of Sting Ray was found to be 112.72 (Table 3). It can be concluded from the result that the liver lipid under investigation contained good amount of unsaturated fatty acids.

The acetyl value is a measure of hydroxylated fatty acids in a fixed oil or fat. The acetyl value of the liver lipid of Sting Ray was found to be 13.71 (Table 3). The result indicates low content of free hydroxyl groups present in the sample.

The Thiocyanogen value of the liver lipid of Sting Ray was found to be 63.87 (Table 3).

This observation is in conformity with the findings that the lipid sample has moderate iodine value and peroxide value.

The titre vatue of the liver lipid of Sting Ray was found to be 26.8 (Table 3). The result indicates that the lipid sample is of fat type which support their semisolid condition at room temperature.

The Henher value of the liver lipid of Sting Ray was found to be 78.86 (Table 3). The result indicates the higher percentage of water insoluble nonvolatile fatty acids present in the lipid sample.

The unsaponifiable matter of the liver lipid of Sting Ray was found to be 1.64 (Table 3). Unsaponifiable matter is defined as those substances which are not saponified by alkali and which are soluble in ether or petroleum ether. In general, if a fixed oil or fat contains unsaponifiable matter in excess of about 2% there is reason to support adulteration.

The result indicates that the lipid sample may contain a small amount of unsaponifiable matter such as sterols, vitamins A & D, hydrocarbons etc.

The Polenske value of the liver lipid of Sting Ray was found to be 0.66 (Table 3). The Polenske value represents a measure of volatile water insoluble but alcohol soluble fatty acids. The Polenske value as obtained is a support of the small amount volatile water insoluble but alcohol soluble fatty acids in the lipid sample.

The Richert-Meissl value of the liver lipid of Sting Ray was found to be 0.97 (Table 3). Since the Reichert-Meissl value is a measure of the volatile water soluble lower fatty acids present in the fat or oil, so the lower R-M value of the lipid sample is an indication of low content of volatile water soluble fatty acids.

The Kirschner value of the liver lipid of Sting Ray was found to be 0.371 (Table 3). The result indicates the presence of trace amount of fatty acids in the Reichert-Meissl distillate which form soluble silver salt.

The liver lipid of Sting Ray was found to form cloudy solution with bromine and a precipitate appeared due to the insoluble bromide during the experiment. Hence, the lipid is marine oil (fish oil).

The liver lipid of Sting Ray was found to form treacle-like consistency with mercuric nitrate solution after 24 hours during the experiment. Hence, the lipid is of semi-drying type (Elaiden test). Semidrying oils absorb oxygen from air slowly and thicken after keeping exposed to air for some time but do not dry up and the iodine value varies between 95 and 140.

The amount of cholesterol in the liver lipid of Sting Ray was found to be 37.93 mg/100 g. A comparatively higher amount of cholesterol was observed in the liver lipid of Sting ray. It can be suggested that the liver of Sting Ray is less useful for edible purpose due to the cholesterol level.

The effect of storage time on the lipid sample showed a significant variation in different properties. Acid value, Peroxide value increased with increasing time of storage and Richert-Meissl value, Thiocyanogen, titre value, iodine value decreased with increasing time of storage. That means, the quality of the lipid deteriorated with increasing time of storage.

3.3 Antimicrobial activities [20]

In the present study, the lipid sample was selected and screened for antibacterial activities against four pathogenic bacteria and antifungal activity against four phyto-pathogenic fungi.

It is evident from Table 4 that, the lipid sample was found to be active against *Salmonella typhi, Staphylococcus aureus* and *Escherichia coli.*

Paper discs while soaked in lipid solutions (10% and 5%) were used, *Salmonella typhi* showed highest zone of inhibition for Sting Ray (20 mm). Salmonella typhi, Staphylococcus aureus and Escherichia coli were found to be sensitive towards the lipid sample at 10% and 5% but has no inhibitory activity against Bacillus cereus.

It is evident from the Table 5 that the lipid of Sting Ray did not show any inhibition on mycelial growth of *Aspergillus fumigatus*. Except this the mycelial growth of almost all test fungi was inhibited by the lipid sample. *Curvularia lunata* showed highest zone of inhibition for Sting Ray (20.1120 mm)

Table 3: Chemical constants of the liver lipid of Sting Ray and some related fats and oils

Name of the Sample	S.V.	S.E.V.	A.V.	F.F.A. (%)	E.V.	I.V.	P.O.V.	Acetyl Value (%)	T.V.	Titre value (°C)	H.V.	U.S.M.	P.V.	R.M.V.	K.V.
Olive oil	190-195	287-295	0.6-1.5	0.25-0.60	--	80-88	--	10.4	75-83	17.26	0.6	0.5-1.2	0.5	0.6-1.5	--
Sunflower oil	190-194	287-295	0.6-2.4	0.15-0.45	--	125-140	--	--	78.4-81.3	17	--	0.3-0.9	--	0.5	--
Cotton seed oil	192-198	283-292	1.0-5.0	0.4-0.9	--	103-111	--	0.7-12.2	61-69	30.37	94.2	0.8-1.8	--	0.95	--
Linseed oil	189-195	287-296	4.0	0.5-0.75	--	175-200	--	--	--	--	94.8	1.0-1.5	--	--	--
Soyabean oil	190-195	287-295	1.2-1.5	0.35-0.85	--	129-137	--	--	77-85	22.27	--	0.7-1.6	0.2.-1	0.5-2.55	--
Coconut oil	255-260	210-250	2.5-10.0	--	--	8.2-9.6	--	--	6.1-7.0	20-24	82	0.15-0.7	15-17	7.8-8.0	--
Palm kernel oil	248	220-250	220-250	--	--	15-18	--	--	--	--	94.2	--	--	28	--
Sardine oil	189.8-193.8	---	2.2-21.7	--	--	138-177	--	--	--	--	--	--	--	--	--
Whale oil	184-200	---	0.3-51	--	--	126.9	--	--	--	--	--	--	--	--	--
Muscle Lipid of Hilsa	203.25	276.01	3.108	1.56	--	92.55	55.05	10.255	52.54	--	93.27	0.74	0.761	0.965	--
Brain lipid of Baghda Chingri	229.255	244.71	1.11	0.56	28.14	95.83	194.95	10.58	43.63	27.2	95.32	0.566	0.796	1.04	--
Brain lipid of Kerani Chingri	214.11	262.06	1.04	0.52	13.07	100.38	192.26	10.82	45.29	26.7	92.19	0.641	0.694	0.95	--
Liver Lipid of Sting Ray	**283.58**	**197.83**	**1.43**	**0.71**	**282.15**	**118.87**	**112.72**	**13.71**	**63.87**	**26.8**	**78.86**	**1.64**	**0.66**	**0.97**	**0.371**

Abbreviations: S.V.= Saponification Value; S.E.V.= Saponification Equivalent Value; A.V.= Acid Value; F.F.A.= Free fatty acid ; E.V.= Ester value; I.V.= Iodine Value; P.O.V.= Peroxide Value; T.V.= Thiocyanogen Value; H.V.= Henher value; U.S.M.= Unsaponifiable Matter; P.V.= Polenske Value; R.M.V.= Reichert-Meissl Value; K.V.= Kirschner value.
"**--**"= Data not available.

Table 4: Antibacterial activity of the liver lipid of Sting Ray

Name of bacteria	Type of Sample	Zone of inhibition (diameter in mm) after 48 hours		
		Treatment	Control	Differences
Salmonella typhi	10% 5%	20 10	0 0	20 10
Staphylococcus aureus	10% 5%	15 7	0 0	15 7
Escherichia coli	10% 5%	15 7	0 0	15 7
Bacillus cereus	10% 5%	0 0	0 0	0 0

Table 5: Percent growth inhibition of test fungi by the liver lipid of Sting Ray

Name of the Fungi	Type of Sample	Zone of inhibition(diameter in nm) after 5 days
		lipid of Sting ray
Fusarium equiseti	10%	13.1423
Aspergillus fumigatus	10%	-12.1012
Alternaria alternata	10%	8.1801
Curvularia lunata	10%	20.1120

(-) means no inhibition.

From these result it can be said that this work may provide valuable information about the prospect of derivation of pesticides and pharmaceuticals from the liver lipid of Sting ray fish.

3.4 Estimation of minerals [17]

Most of the people of our country have been suffering to a great extent from protein malnutrition. From Table 6, it is evident that Sting Ray contains a good amount (3.976%) of nitrogen as well as protein (proteinaceous nitrogen) which is well balance in respect of essential amino acids.

The percentage of phosphorus (1.176) indicates that phospholipid may present in the lipid sample. The percentage of potassium (1.125) in the lipid sample may be helpful to increase blood pressure for those people having low blood pressure. The percentage of calcium (0.610) may help formation of rigid bone structure of the community children in their growing age who eat these marine species. Above all, the maximum catch and use of Sting Ray should be encouraged for ready supply to different remote regions under careful processing to avoid putrefaction.

Table 6: Percent of N, P, K and Ca in liver of Sting Ray with other samples

Name of the sample	N	P	K	Ca)
Brain of Kerani chingri	3.090	0.5506	1.061	0.798
Brain of Baghda Chingri	3.540	0.7262	1.123	0.914
Liver of Blue Spotted Fantail Ray	4.099	2.7500	1.180	0.641
Liver of Sting Ray	**3.976**	**1.176**	**1.125**	**0.610**

3.5 Chromatographic analysis [22]

The liver lipid of Sting Ray was subjected to TLC examination and their fatty acid composition were identified by comparing the R_f values of different spots of chromatograms with those standard fatty acids as reported (Table 7) in different solvent systems. It was found from the chromatograms that the lipid sample produced about 3-5 spots. Among the spots, three spots were identified as palmitic acid, stearic acid and oleic acid in the liver lipid of Sting Ray.

Table 7: The R_f values of thin layer chromatographic examination of the liver lipid of Sting Ray

Solvent systems	R_f values of standard fatty acids			R_f values obtained from the spots of lipid sample			
	OA	PA	SA				
P:E (60:40)	0.921	0.947	0.283	0.288	0.949	0.402	0.508
P:E:A (70:30:1)	0.942	0.961	0.417	0.945	0.963	0.418	0.254
P:H (80:20)	0.813	0.832	0.316	0.811	0.327	0.852	0.459
H:E (80:20)	0.815	0.820	0.201	0.814	0.828	0.207	0.192

PA- Palmitic Acid, SA- Stearic Acid, OA-Oleic Acid

4. Conclusion

Physico-chemical characterization and microbial studies of liver lipid of Sting Ray fish (*Dasyatis sephen*) have been performed in the present study. The presence of moderate amount of unsaturated fatty acids in the lipid sample was confirmed by R.I., S.V., I.V. and T.V. Low content of volatile water-soluble and volatile water-insoluble fatty acids were established by R.M.V. and P.V. Low free hydroxyl group content was confirmed by the acetyl value of the lipid sample. Percentage of F.F.A. validated suitability of the oil for edible purpose. Semidrying nature of the lipid was pointed out by I.V. and confirmed by Elaiden test. Chromatographic examinations substantiated the presence of some important fatty acids in the lipid sample. However, no decisive conclusion can be drawn from antimicrobial screening results with the lipid sample.

5. Acknowledgement

I am highly indebted to Professor Dr. Hossain Jamal and Professor Dr. Abdul Kader, Institute of Marine Sciences and Fisheries, University of Chittagong for identifying the marine species and their thoughtful suggestions during this work. I am also highly delighted to express my indebtedness to Mr. Sreevash Bhattacharjee, Senior Scientific Officer, B.C.S.I.R. Laboratories Chittagong for providing necessary facilities to complete some experiments.

6. References

1. Begum M, Ahmed ATA, Das M, Parveen S. A comparative microbiological assessment of five types of selected fishes collected from two different market. J Advan Biol Res 2010; 4:259-265.
2. Debasish P, Dipankar B, Tarun KP, Amarendra P, Amitabha G. Liver lipids and fatty acids of the sting ray *Dasyatis bleekeri* (Blyth) Journal of the American Oil Chemists Society 1998; 75(10):1373-1378.
3. Stansby ME. Fish oils, their Chemistry, Technology, Stability, Nutritional properties and Uses, Avi Publishing Company. Inc 1968, 3-9.
4. Lehninger AL, Biochemistry, Edn 2, Worth Publishers.

Inc., NY, 1978, 279-301.
5. Ackman RG. Fish in Marine Biogenic Lipids, Fats and Oils. CRC Press, Inc., Boca Raton 1989; 2:145-178.
6. Ackman RG. Nutritional Composition of Fats in Seafoods. Prog Food Nutr Sci 1989; 13:161–241.
7. Ackman RG. Perspectives on Eicosapentaenoic Acid (EPA). n-3 News, Massachusetts General Hospital, Boston 1986; 1(4):1-4.
8. Lands WEM. Fish and Human Health. Academic Press, London, 1986, 1–186.
9. Ackman RG. Fatty Acid Composition of Fish Oils, Nutritional Evaluation of Long Chain Fatty Acids in Fish Oil. Edited by SM. Barlow and ME. Stansby, Academic Press, London, 1982, 25-88.
10. Uddin MH, Majid MA. Physico-chemical characterization and study of microbial activities of the brain lipid and chemical analysis of the brain of Kerani Chingri (*Metapenaeus affinis*) of the Bay of Bengal. Chittagong Univ J Science 2000; 24:83-89.
11. Vogel AI. A Text Book of Practical Organic Chemistry. Addission Wesley Longman Ltd, London, 1975; 163.
12. Uddin MH, Majid MA, Mistry AC, Manchur MA. Isolation, Characterization and Study of the Microbial Activities of the Brain Lipid and Chemical Analysis of the Brain of Baghda Chingri (*Penaeus monodon*) of the Bay of Bengal. Pak J Sc Ind Res 2004; 41:121-125.
13. Ranganna S. Handbook of Analysis and Quality for Fruit and Vegetable Products, Edn 2, India, 1991, 3-226.
14. Marshall AJ, Williams WD. Text book of Zoology Invertebrate, Edn 7, 1995, 709-732.
15. Griffin RC. Technical Method of Analysis, Edn 2, 1972, 309-342.
16. Morris JB. The Chemical Analysis of Foods and Food Products, 1965, 375-382.
17. Al-Amin M, Uddin MH, Afrin A, Nath KB, Barua S. Extraction, Physico-Chemical Characterization and Antimicrobial Screening of the Muscle Lipid of Cuttle Fish (*Sepia esculenta*) of the Bay of Bengal. Int Lett Chem Phy Astr 2014; 17(1):87-97.
18. Willams KA, Oils, Fats and Fatty Foods, Edn 4, 1966, 124-391.
19. Ahmed S, Rahaman MS, Chowdhury JU, Begum J, Anwar MN. Antimicrobial Activities of Leaf Extracts and Crude Alkaloids of *Glycosmis pentaphylla*. Chittagong Univ J Sci 1998; 22:77-81.
20. Rahman MM, Uddin MH, Islam MW, Majid MA. Analytical Characterization and Antimimicrobial Screening of the Hilsha (*Tenualosa ilisha*) Fish Lipid from the Bay of Bengal. Hamdard Medicus 2009; 52(3):23-28.
21. Randerath K. Thin-layer Chromatography. Rein-Hold Publishing Corp., Chapmann and Hall Ltd. London 1988, 47, 10-137.
22. Ahmed HAU, Uddin MH, Mannan MA, Barua S, Hoque MA. Studies on the Isolation, Physico-chemical Characterization and Microbial Activites of Melon (*Cucumis melo*) Seed Oil. Int J Innov Sci Res 2014; 11(1):105-111.