British Journal of Pharmaceutical Research
7(5): 353-364, 2015, Article no.BJPR.2015.117
ISSN: 2231-2919

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Lipid Charecterization and Fatty Acid Profiles of *Clarius batrachus* and Its Defense Activity

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Authors' contributions

This work was carried out in collaboration between all authors. Author GB designed and conducted the study, wrote the protocol, and wrote the first draft of the manuscript. Authors CA and SV managed the literature searches, analyses of the study performed the spectroscopy analysis and authors KSM and NS managed the experimental process and author PS did the statistics and of the study. All authors read and approved the final manuscript.

Article Information

DOI:10.9734/BJPR/2015/17066
Editor(s):
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(2) Abdelwahab Omri, Department of Chemistry and Biochemistry and Departments of Biomolecular Sciences, Laurentian University, Canada.
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(4) Anonymous, Medical Univeristy of Lublin, Poland.
Complete Peer review History: http://sciencedomain.org/review-history/10010

Original Research Article

Received 25th February 2015
Accepted 4th May 2015
Published 2nd July 2015

ABSTRACT

Innate Defense Components were being useful to identify and understand their mode of action on microbial pathogens. The present study portrays the qualitative and quantitative determination of endogenous lipids from the skin, intestine, kidney and liver of *Clarius batrachus*. The skin lipids were identified by TLC, which constitutes phosphatydyl serine (Ptd Ser) (48%),

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glycerophospholipids (23%), phosphoglyceride (20%), and 7% of cholesterol (C). The GC-MS FAME profile of the Skin comprised, the saturated fatty acid (SFA) with n-16 and n-18 as major fatty acids, 25.04% of palmitic (PAM) and 10.23% of stearic acid (STA). The fatty acid profiles and lipid classes of other organs were also identified and quantitated. The results from the present study bestowed to the skin lipid from the C.batrachus can be developed as a novel drug for multidrug resistant bacterial pathogens.

Keywords: GC-MS FAME analysis; thin layer chromatography (TLC); lipid classes; multidrug resistant pathogens; Clarius batrachus.

1. INTRODUCTION

The epidermal mucosal surfaces of fishes provide first-line defense against microbial invasion through their complex secretions. The antimicrobial activities of peptides in these secretions have been well documented, but the contributions of lipids to mucosal defense have not yet been defined. The functions of innate host defense systems were to kill microbes through membrane permeabilization and disruption of membrane-bound multi-enzyme complexes [1] and to modulate the immune response through chemotactic activity and sequestration of pro-inflammatory microbial products [2]. The skin, intestine, kidney and liver were susceptible and constantly challenged with variety of pathogens through contact with environment like air borne, water borne, food borne and aspirated microbes and its integrity depends on continuous removal of microbes by mucociliary clearance. The mucosal secretions have been intensely studied, and important defense functions have been attributed to antimicrobial polypeptides such as lysozyme, defensins and magainin. The cutaneous mucosal delivery was originated from the epidermal layer of skin.

The secretion includes not only peptides but also conjugates with bound lipids. The lipids were diverse in its functions. Lipids are attributed to essential components of all biological membranes, but the membrane lipid composition differs greatly between eukaryotes and prokaryotes [2]. Lipids are also precursors for hormones, used for energy storage, and they have a prominent role as messengers and regulators of inflammation [3]. In fatty acids, the Poly-unsaturated fatty acids (PUFA) play a vital role in the immune response and overall health of salmon fish [4]. The possible mechanism of PUFA was executed through the influence of immune response. Initially the structural changes in the leukocyte membrane which influence cellular functions such as enzyme kinetics, ion transport and permeability, receptor expression and signal transmission, and secondly via., eicosanoids (chemical mediators of the immune response). Further the lipids in lung surface, predominantly phospholipids, are essential for reducing the surface tension in the alveoli, thus preventing their collapse during exhalation [5]. On the other hand the Fish oils with a high degree of unsaturation were widely used in the food and pharmaceutical industries, due to their high nutritional value. The nutritional value is due to the association of high levels of polyunsaturated fatty acids (PUFA) and the presence of vitamins of A and D groups [6]. However, oxidation of fish PUFA easily occurs during storage, which impairs their further applications [7]. Although, there are a few report on the lipid composition of leucocytes from the blood of Atlantic salmon (*Salmo salar L.*) [8], channel catfish and rainbow [9] trout were previously reported. But there are no reports on the fatty acid profiles and the lipid composition of the cells specifically involved in the immune response of *Clarius batrachus*, In this rationale, the present study identifies the lipid class and its fatty acid compositions of kidney, skin, liver and intestine of *C. batrachus* by Gas Chromatography and Mass Spectra and the defense activity was performed against bacterial pathogens by *In vitro* antimicrobial assay.

2. MATERIALS AND METHODS

2.1 Collection of Animals

The live healthy *Clarius batrachus* fishes of approximately 6 months old, weighing about 400 gm each were purchased from the lake located at navalpattu near, Mathoor, Tiruchirappalli, Tamilnadu, India.

2.2 Acclimatization

The procured fishes were brought and acclimated to laboratory condition in the tap water and maintained for 10 days. Then they were released into flow-through freshwater tanks

(28±2ºC) and fed ad libitum with commercial fish pellets to attain normalcy.

2.3 Collection of Various Organs from Fish

After a brief period of acclimatization, the fishes were anaesthetized with chloroform and carefully dissected the various organs like liver, intestine, kidney and skin. The organs were washed and buffered with phosphate buffered saline (PBS) then stored at 4ºC for further use.

2.4 Lipid Extraction

A lipid extract of each of the fish organs were obtained by the method described by [10]. 2 gms of fish organ such as liver, kidney, skin and intestine were homogenized separately in an Electronic homogenizer (500 mg/ml^{-1}) with chloroform–methanol (2:1, v/v) solvent system to isolate the lipids. After homogenization, centrifuged at 3000 rpm for 10 min. The crude lipid extracts were purified by "Folch wash". The Folch wash is 0.2 volume (4 ml for 20 ml) 0f 0.9% NaCl solution was added with lipids twice and centrifuge at 2000 rpm decant the upper phase to remove non-lipid contaminants.

2.5 Test Microorganisms

Five multidrug resistant pathogens were taken for the study. Among them four were Human pathogens and one was fish pathogen. The bacterial strains were *Escherichia coli* (BH-54), *Klebsiella pneumonia* (BH1991*), Staphylococcus aureus* (BH-87), *Pseudomonas putida* (BH-89) and *Aeromonas hydrophila* (BH-42). All the strains were procured from Department of Microbiology, Bharathidasan University, Tiruchirappalli.

2.6 Antimicrobial Assay

The antimicrobial activity was evaluated by Agar disc diffusion method. The nutrient agar medium (Hi-media) was inoculated with 100 µl of the inoculums (1×10^8 CFU) poured into the petri plate. The Drug loading disc of 8mm diameter was obtained from Hi Media, India. The 50 µl (50 µg) of lipid extract from various organs of *C. batrachus* was loaded on the solidified media containing test strains. For each bacterial strain controls were maintained. Extraction buffer (containing Methanol: Chloroform) were used as negative control and tetracycline (10 mg/ml) from which 50 µl was used as positive control. The plates were incubated overnight at 37ºC. Antimicrobial activity was determined by measuring the zone of inhibition.

2.7 Thin Layer Chromatography

The identification of the lipid classes were done by using thin layer chromatography [11].The standards such as glycerolipids (1-acylglycerol-3-phosphate) ,glycero phospho lipid (1, 2-Dihexadecanoyl- rac - glycero - 3- phospho- rac-(1-glycerol) ammonium salt), cholesterol(C), gangliosides (Mono Sialoganglioside) GM2, squalamine and phosphatidyl serine (Ptd Ser) (1,2-Dimyristoyl-sn-glycero-3-phospho-L-serine sodium salt) from Sigma, India were used to identify the lipids. The above standards were dissolved at 1 mg/ml of chloroform. The standards were developed in same chromatogram.

2.8 Estimation of Free Fatty Acids

A small quantity of free fatty acids is generally present in the fishes along with triglycerides. The quantity of free fatty acids was measured by acid number/acid value [12].

2.9 Preparation of Fatty Acid Methyl Esters

The lipid extract (100 mg) was dissolved in 4 mL of 5% hydrochloric acid in methanol and 0.5 mL benzene and then the mixture was refluxed in a silicone bath at 80–100ºC for 2 h. After cooling, the methyl esters were extracted with petroleum ether, simultaneously neutralized and dried over a sodium sulphate–sodium bicarbonate mixture. The solvent was evaporated to dryness under reduced pressure at 40ºC on a rotary evaporator (Heidolph, Laborota 4000). These fatty acid methyl esters (FAME) were then analyzed by GC–MS.

3. FAME ANALYSIS

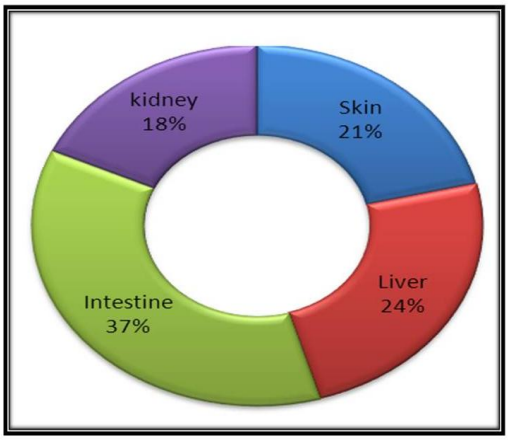
The fame analyses were performed on a Shimadzu QP-5000 GC–MS equipped with FID and a 25 m×0.25 mm, 0.25 µm film thickness WCOT column coated with 5% diphenyl siloxane. Helium was used as the carrier gas at a flow rate of 1.2 ml/min, at a column pressure of 42 KPa. The column temperature was programmed for fatty acid methyl esters (FAME) from 120–300ºC at a rate of 2ºC/min and held at 300ºC for 10

min, with a total run time of 100 min. The EI ionization voltage was 70 eV. Peak identification was performed by comparison of the obtained mass spectra with those available in the Wiley and NIST libraries (Shimadzu–Wiley Registry TM, 8th Edition Mass Spectral Library, Shimadzu and the NIST 08 Mass Spectral Library (NIST/EPA/NIH) – new 2008 version).

4. RESULTS

4.1 Total Lipid and Fatty Acid Composition of Various Organs in *C. batrachus*

The concentrations of total lipid and free fatty acid content in the endogenous lipid ligands associated with defense proteins in the first line innate defense system of various organs such as the Skin, Liver, Kidney and Intestine were estimated. Among the four organs fatty acid content, the Intestine (3.366±0.62 mg) and Liver (2.24±0.21 mg) were significantly higher than the Skin (1.96±0.81 mg) and Kidney (1.68±0.45 mg) as indicated (Fig. 1). The lipid content was observed highest in intestine 37% followed by Liver 24% followed by Skin and Kidney (21% and 18%) respectively.



Lipid Fig-1

Fig. 1. Estimation of total lipids in the skin, liver, kidney and intestine of *C. batrachus*

The total lipid content of the endogenous ligands bound with peptide of mucillary lineage of various organs.100 mg of samples were taken and assayed. The lipid content of the each organ was expressed in the percentage

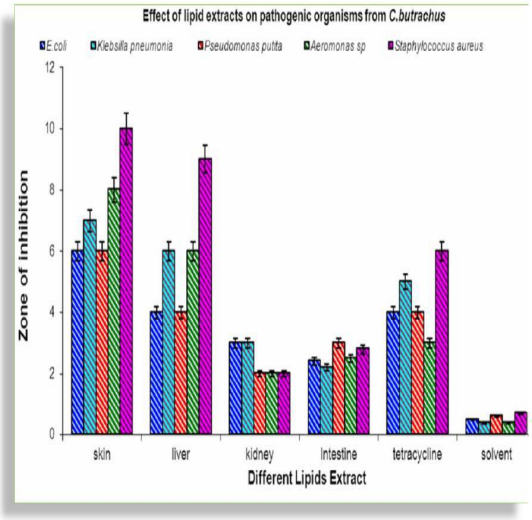
4.2 Defense (anti-infective) Property of Lipid Extracts from Various Organs of the Catfish *Clarius batrachus*

To ascertain the lipid extract of various organs contribute to inherent (anti-infective activity) defense activity the Skin, Liver, Kidney and Intestine were evaluated. The lipid extract from all the organs exhibited broad spectrum antimicrobial activity (Fig. 2). Amid the organs Skin lipid extract possess utmost antibacterial activity against *E. coli*, *K. pneumoniae, S. aureus*, *A. hydrophila* and *P. putida* than other organs extract. The kidney and the intestine indicate less antimicrobial against bacterial pathogens. The inhibitory effect of Skin (9.6±0.68 mm) and liver (8.6±0.82 mm) was significantly strong against *S. aureus* when compared to Positive control the drug Tetracycline (6.2±0.37 mm). Whereas the skin lipids extract exposure cause highest zone of inhibition for fish pathogens *A. hydrophila* (8.3±0.26 mm) and *P. putida* (6.9±1.03 mm). Whereas the nosocominal pathogen *K. pneumoniae* imparted (7.0±0.16 mm) and *E. coli* (5.8± 0.39 mm) activity. The other lipid extracts of kidney and intestine displays less than 4.0±2.09 mm for all the bacterial pathogens. The Positive control Tetracycline drug was used as a yardstick to compare the antimicrobial activity of different organs lipid extract. The positive control exhibit a high zone of inhibition in the *S. aureus* (6.3±0.18 mm), *K. pneumoniae* (5.07±0.42 mm), *P. putida* (4.1±0.51 mm) and *A. hydrophila* (3.41±0.28 mm). The negative control (the homogenizing buffer) was exposed to ensure the antimicrobial activity which showed 0.2 mm against all the Bacterial pathogens. This is due to the presence of organic solvents Methanol and Chloroform. All the values of zone of inhibition were treated statistically (Six different observations ± S.D).

4.3 Identification of FAME (Fatty Acid Methyl Esters) of Total Lipid and Its Proximity of Skin, Liver, Kidney and Intestine from *C. batrachus*

The saturated and unsaturated fatty acid composition and the amount in various organs were identified (Fig. 3 & Table 1). In the skin, the saturated fatty acid (SFA) with n-16 and n-18 as major fatty acids contribute 25.04% and 10.23% respectively were identified as palmitic (PAM) and steric acid (STA). The chemical systemic naming was hexadecanoic and octadecanoic family. The Unsaturated fatty acids n-18:1, 18:2

were found and identified as oleic (OLA) and linoleic acid (LNA). Long chain fatty acid n-20:4 were detected in the GC-MS as eicosanoids especially recognized as arachidonic acid (ARA) as 20.69%. The liver possesses hexadecanoic acid as the saturated fatty acid (SFA) undecylic acid and unsaturated fatty acid as lauric acid 0.76%. The kidney exhibits very long chain fatty acids n-20:4, W6, 12&15c belonging to the eicosinoic groups, which forms 60.98% of arachidonic acid. Also the unidentified new fatty acids 14:0 OH/16:1ISO-1 occurs 39.02%. Intestine exhibits more types of saturated fatty acids (SFA) such as 11:0, 13:0, 14:0, 15:0, 16:0, 17:0, 19:0 and 20:0 their percentages were 0.07%, 0.08%, 2.96%, 1.31%, 26.59%, 1.12%, 0.23% and 0.21% respectively. Among the saturated fatty acids (SFA), the myristic acid possess highest percentage of 26.59% which systemically viz., -cis-9, 10-ethylenehexadecanoic acid. Small amounts of unsaturated fatty acids such as n-9:0, 13:0, 14:0, 15:0, 16:0, 17:0, 19:0 and 20:0ANTEISO were found in the percentage of 0.10, 0.29, 0.32, 0.71, 0.48, 0.99, 0.25, 0.82, 1.14, 0.26 and 0.34 respectively. The ω-fatty acid occurred as 23.72%, highest level in the intestine. The branched chain fatty acid such as n-12:0, 13:0, 14:1, 14:0, 15:0, 16:0,17:1,18:1,19:1 and 20:0 ISO were found in the percentage of 0.49, 0.17, 0.05, 0.38, 0.71, 0.78, 0.97,0.50,0.12 and 1.13 respectively.



Lipid Fig. 2

Fig. 2. Antibacterial efficacy of peptide associated endogenous lipids from various organ of *C. batrachus*

The zone of Inhibition of Skin, Liver, Kidney and Intestine were attained against Bacterial pathogens at 100µl concentration using Disc diffusion assay. Tetracyline as Positive control and Extraction solvent (Methanol: Chloroform) As Negative control

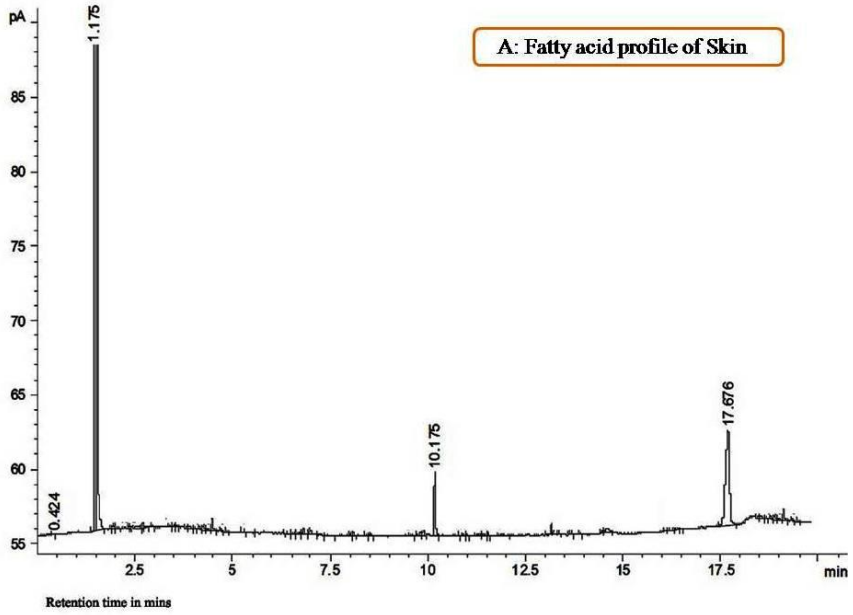


Fig. 3A. Skin GCMS

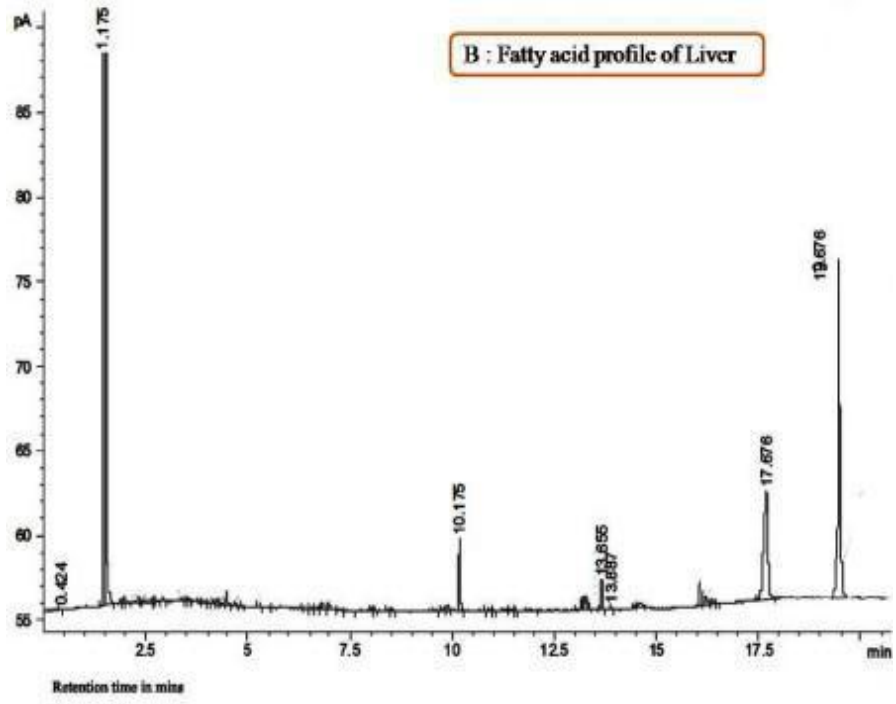


Fig. 3B. liver

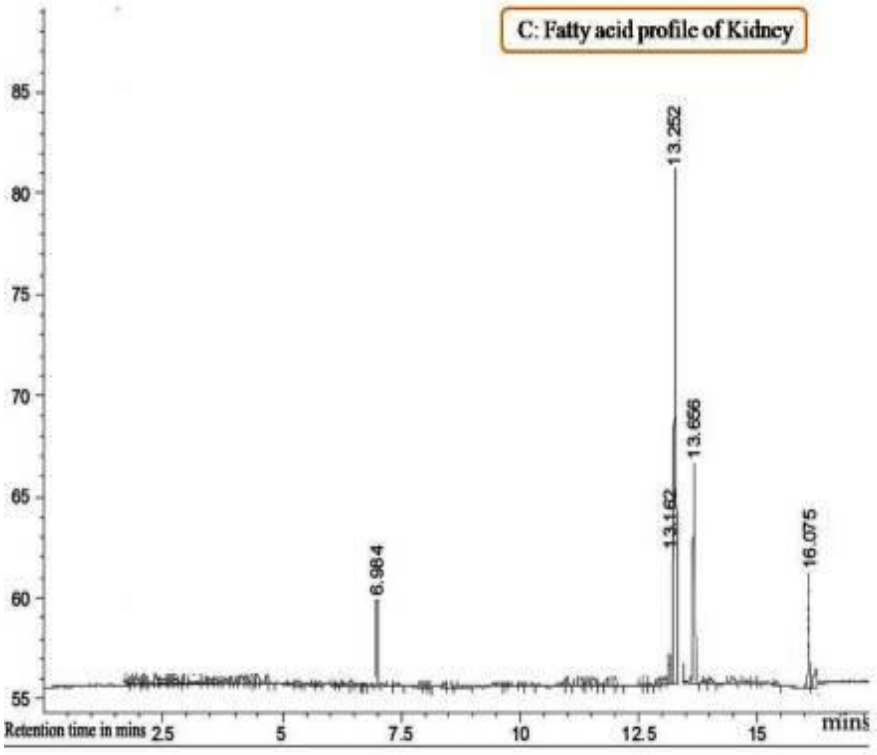


Fig. 3C. Kidney GCMS

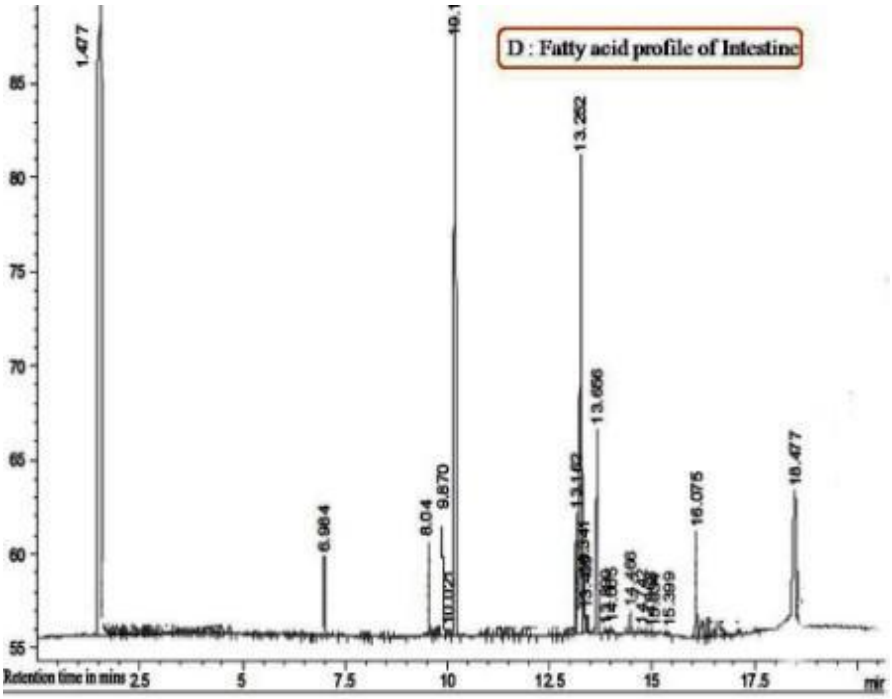


Fig. 3D. Intestine GCMS

Fig. 3. GC-MS analysis of fatty acid methyl esters (FAME) in skin, liver, kidney and intestine - total lipids from *C. batrachus*

4.4 Detection of Lipid Classes in the Skin, Liver, Kidney and Intestine of *C. batrachus* by Thin Layer Chromatography (TLC)

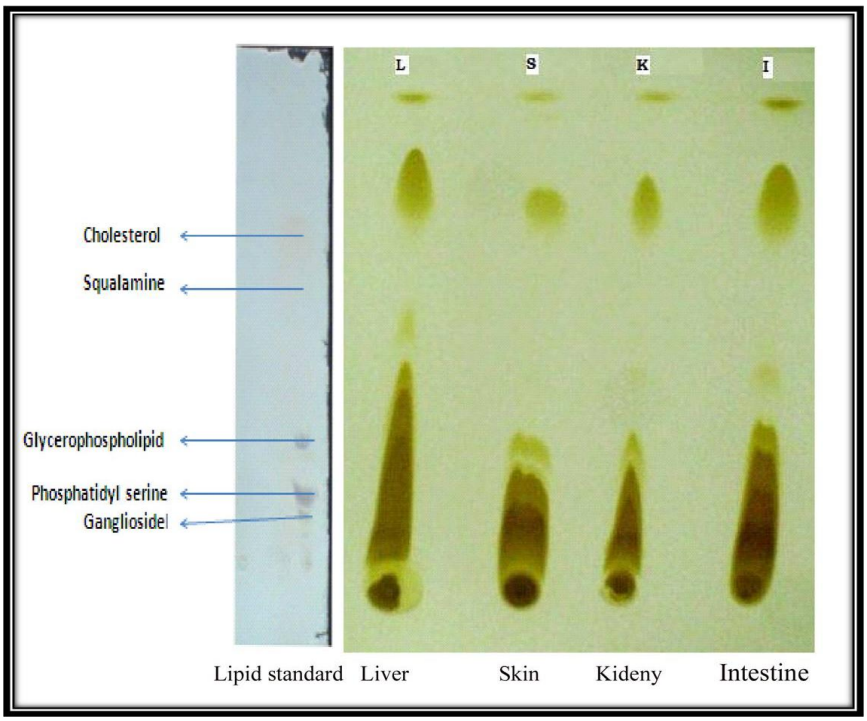
The lipid samples from the skin, liver, kidney, intestine and standards were eluted in chromatogram (Fig. 4). The relative front from the each samples and the band area were analyzed and it was calculated by comparing with the Standards. Skin illustrates the presence of phosphoglyceride (20%), phosphatydyl serine (Ptd Ser) (48%), glycerophospholipids (23%) and 7% of cholesterol (C). The liver displays the presence of 25% of phosphoglycerides, phosphatidyl serine (Ptd Ser) (24%), glyero-phospholipid (21%), 12% of cholesterol (C) and interestingly squalamine was appeared only in liver with 14%. kidney exposes the presence of phosphorglyceride (10%), phosphatidyl serine (Ptd Ser) (27%), glycerophospholipids (11%), cholesterol (C) (6%) and ganglioside (20%). Intestine depicts 12% of glycerophopholipids, phosphatidyl Serine (15%), glycero phospho lipids 20%, cholesterol(C) 17% and ganglioside 04%. In the above composition of lipids detected in the various organs, the skin reveals the highest percentage of phosphatidyl serine (Ptd Ser) (48%) and (23%) glycerophospholipids. Notably the ganglioside was present mainly in the intestine (9%) and kidney (20%) (Table 2).

5. DISCUSSION

Lipid extracts from animal and plant tissues were usually used as the materials to study the antimicrobial activity of lipid origin. Antimicrobial effect of many fatty acids and their esters were studied in detail [13]. Several studies suggest that antimicrobicidal lipids play a role in innate non-specific immunity of humans and animals, which is their first line of defense against invading pathogens. Lipid secretion may with held the resident micro biota on mucosal surface possessing the antimicrobial peptides [14]. Hence the present results of skin, liver, kidney and intestine evidenced that endogenous lipids associated with antimicrobial peptides in mucoid lineage of innate immunity contribute to inherent defense activity against bacterial pathogens. Similar innate host defense activity (Antibacterial activity) was established in respect to antimicrobial lipids from the nasal secretions [15]. Among the lipid extracts of various organs,

the skin lipid extracts expressed very strong antibacterial activity against all the 5 multidrug resistant bacterial pathogens (*E. coli, K. pneumoniae, A. hydrophila, S. aureus* and *P. putida*). This might be due to the lipophilic nature of molecules act to concentrate the compound existing inside lipid-rich sebaceous follicle in the Skin as was reported by [16] in the Human sebaceous follicle, which possesses Bacteriostatic and Bactericidal activity. Moreover the, skin was found to destroy many species of microorganisms and this property was due to the presence of long chain fatty acids in the skin lipids [17]. Lipids of sebaceous gland origin are known to have multiple functions in maintaining skin homeostasis, for example to be responsible for the three-dimensional organization of the skin surface lipids and the integrity of the skin barrier [18] in addition to exhibit strong antimicrobial activity.

The lipid classes from all the organ extract of *C. batrachus* possess defense activity. In addition the lipid class of skin and liver possess significant anti-infective activity. The other two organs kidney and intestine possess low antibacterial activity. The lipid content in the fish showed explicit activity and it varies from species to species, Sex, maturity, Age, geography and organ to organ within the host. This implies significant difference in the composition to total lipids [19]. The present study also supports the above findings that the compositions of lipids in different organs vary considerably as evidenced in the GC-MS FAME and TLC analysis of *C. batrachus*. Similarly quantification and identification of lipids employing the above techniques have already done in nasal fluids. Trailed by this study, presently the lipid class such as phosphoglycerides, phosphatidyl serine (Ptd Ser), glycolipids, squalene, cholesterol (C) and ganglioside were identified and quantified by TLC. The cholesterol (C) was found lesser concentration in Skin and kidney of *C. batrachus* than liver and intestine because they were the food processing organs.



Lipid Figure-4

Fig. 4. Thin layer chromatogram of skin, liver, intestine and kidney of *C. batrachus*

The thin layer chromatogram of various organs of C. batrachus contains L=Liver, S=Skin, I=Intestine and K=Kidney, subsequent spots were developed and the Relative front of Each spot as identified and quantified with the support of Standards (Phosphoglycerides, Phosphatidyl serine(Ptd Ser), Squalamine & Cholesterol (C)) and Compound library

Table 1. GC–MS analysis of FAME of total lipid of skin, liver, kidney and intestine from *C. batrachus*

Organs	Systematic name	Trivial name	Saturated fatty acids	(%)	Systematic name	Trivial name	Unsaturated fatty acids	(%)	Branched fatty acids	(%)
Skin	Hexadecanoic	Palmitic	16:0	25.04	Octadecanoic	Stearic	18:1 w9c	10.23	19:1 ISO	14.30
	Octadecanoic	Stearic	18:0	10.23	Octadecanoic	Stearic	18:2 w6,9c	6.54		
					Eicosanoic	Arachidic	20:4 w6,9,12,15c	20.69		
Liver	Hexadecanoic Acid	Undecylic	11:0		Dodecanoic Acid	Lauric	12:0 ISO		11.0 ISO	0.76
Intestine	Hexa decanoic	Undecanoic	11.0	0.07	Nonanoic	Pelargonic	9.0	0.10	12:0 ISO	0.49
	acid	Myristic acid	13:0	0.08	-*cis*-9,10-		13:0 Anteiso	0.29	13:0 ISO	0.17
			14:0	2.96	ethylenehexadecanoic		14:0 Anteiso	0.32	14:1 ISO E	0.05
	Tetra		15:0	1.31	acid		15:0 Anteiso	0.71	14:0 ISO	0.38
	decanoic Acid		16:0	26.59			16:0 Anteiso	0.48	15:0 ISO	0.71
			17:0	1.12			16:1 w9c	0.99	16:0 ISO	0.78
			19.0	0.23			17:1 w9cISO	0.25	17:1 ISO	0.97
			20:0	0.21			17:0 Anteiso	0.82	18:0 ISO	0.50
							17:1 w8c	1.14	18:1 ISO H	0.12
							17 : 0 cyclo	0.26	19:1 ISO I	1.13
							18:3 w6c (6,9,12)	0.34	19:0 ISO	0.32
							18:1 w9c	23.72	20:0 ISO	0.54
							18:1 w7c	2.49		
							18:1 w6c	0.60		
							20:1 w9c	0.32		
Kidney							Unidentified	39.02	14:0 OH/16:1 ISO I	
					Eicosanoic	Arachidonic	20:4 w6,9,12,15c	60.98		

Table 2. Detection of different types of lipids in skin, liver, intestine and kidney of *C. batrachus*

Lipids	Skin	Liver	Intestine	Kidney
Phosphoglycerides	20%	25%	12%	10%
Phosphatidyl Serine	48%	24%	15%	27%
Glycerophospholipids	26%	21%	20%	11%
Squalamine	--	14%	--	--
Cholesterol	07%	12%	17%	06%
Ganglioside	--	--	09%	20%

The kidney of *C. batrachus* exhibits the presence of ganglioside (20%) with other glycolipids and Phospholipids. Usually the presence of ganglioside (Monosialoyl) was reported in the kidney of teleost fish [20] particularly in the neuronal tissues and it was suggested to help maintain the membrane fluidity at low temperatures. The squalene was detected in the liver of *C. batrachus* as 14%, where as squalene was reported at trace level in human liver [21]. The intestine exhibits the presence of phospholipids, glycolipids, cholesterol (C) and gangliosides. Amid the lipid composition of various organs, the skin lipid comprises phosphatidyl serine (Ptd Ser) (48%), glycerol-phospholipid (26%), phosphoglycerides (20%) and cholesterol (C) (7%). This may be the reason for possessing the robust antimicrobial activity by the skin, which was evidenced by the highest zone of inhibition against all bacterial pathogens than other organ's lipids. Earlier study also opined that the presence of above antimicrobial lipids on the skin is not incidental but they are truly a part of the innate immune system and play an active role in the defense against invading pathogens [22]. Generally the fresh water fish have higher unsaturated fatty acid content (17-53%) than the saturated fatty acid (SFA) (12-38%). Similarly *C. batrachus* also showed higher unsaturated fatty acids in the Kidney and skin. However, interestingly the intestine displays the low unsaturated fatty acid content. The gram positive bacteria were more susceptible to antibacterial property of lipids than gram negative bacteria. However in the present study all the gram negative bacterial pathogens were highly sensitive to skin lipids of *C. batrachus*. This could supports the view that "there are many expectations, besides the reports" [23]. The gram negative bacteria *E. coli* and *Salmonella* sp. being extremely resistant to antibacterial lipids at neutral pH, but are killed effectively at acidic pH. *E. coli* is also susceptible to the combined effect of lipid at high temperature. In the present GC-MS study, Skin lipids posses highest antibacterial activity because it contains predominantly combination of the saturated fatty acids (SFA) such as myristic acid, stearic acid (STA), palmitic acid (PAM), and unsaturated Fatty acids oleic acid (OLA), arachidonic acid (ARA), palmitoleic and linoleic acids (LNA). They were most active against gram-positive bacteria and gram negative bacteria thus, these fatty acids could be the cause of antimicrobial property of *C. batrachus*. Also, the above fatty acids were well reported to possess antibacterial and antifungal activity [24]. The FAME analysis of kidney showed unidentified saturated fatty acid (SFA) C14:0 OH, 16:1 ISO (39%) and unsaturated fatty acid (60.98%) arachidonic acid, thus the kidney imparted only moderate antibacterial activity than skin. The Liver possesses good defense activity against pathogens because of the presence of squalene. Whereas the intestine imparted very trace level of antibacterial activity evidences by the spectral data of GC-MS displayed only ~1.0% of fatty acid contains lauric acid, myristic acid and majority 25% of (saturated fatty acid (SFA) palmitic acid (PAM). Lauric acid was reported as antimicrobial agent [25]. Nearly 15 types of unsaturated fatty acids with trace level were observed in the intestine, this may be due to the presence of dietary fatty acids in the feed. [26] opined that the lauric acid is the most effective fatty acid, because it is very active against pathogens even at very low concentration. Further the host derived antimicrobial lipids may exert their activity in conjunction with antimicrobial polypeptides, which have a well documented membrane-perturbing activity [27]. It is plausible that antimicrobial lipids damage microbial membranes by transferring their hydrophobic acyl or side chains, and this activity created lesions.

6. CONCLUSION

In conclusion, lipids extracted from different organs of *C. batrachus* possess different level of antibacterial activity, the selected fatty acids found in the lipids associated with the antimicrobial peptide may be the cause for the bioactivity. It ensures that the natural antibiotic substances present in the mucoid membrane acts as innate barrier against pathogens. Moreover the *in vivo* activity (Clinical Trials) will provide the efficiency of these identified lipids. And also the action mechanism of these lipids and their fatty acids should provide a solution to develop drug for many multi-drug resistant pathogens.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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