Uttar Pradesh Journal of Zoology

Volume 45, Issue 18, Page 727-735, 2024; Article no.UPJOZ.4080
ISSN: 0256-971X (P)

Quantitative Estimation and Identification of Phospholipids in Gill, Liver, Intestine, Muscle, Brain Tissue of Fresh Water Fish Channa Punctatus (Bloch) in Thin Layer Chromatography (TLC) Sprayed with Dittmer –Lester Reagent

Venkateswara Rao Mandalapu [a++*] and Venkaiah Yanamala [b#]

[a] Department of Zoology, Government Degree College, Yellandu, Bhadradri Kothagudem Dist. Telangana State, India.
[b] Department of Zoology, Kakatiya University, Warangal, Telangana State, India.

Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.56557/upjoz/2024/v45i184490

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://prh.mbimph.com/review-history/4080

Original Research Article

Received: 14/07/2024
Accepted: 18/09/2024
Published: 25/09/2024

[++] Assistant Professor;
[#] Professor;
**Corresponding author: Email: venkatmandalapu18@gmail.com;*

Cite as: *Mandalapu, Venkateswara Rao, and Venkaiah Yanamala. 2024. "Quantitative Estimation and Identification of Phospholipids in Gill, Liver, Intestine, Muscle, Brain Tissue of Fresh Water Fish Channa Punctatus (Bloch) in Thin Layer Chromatography (TLC) Sprayed With Dittmer –Lester Reagent". UTTAR PRADESH JOURNAL OF ZOOLOGY 45 (18):727-35. https://doi.org/10.56557/upjoz/2024/v45i184490.*

ABSTRACT

Phosphoipids were quantified and observed in different tissues i.e. gill, liver, intestine, muscle and brain tissue of fresh water fish *Channa punctatus* through Thin Layer Chromatography (TLC). Gill, liver, intestine, muscle and brain tissues of *Channa punctatus punctatus* dissected and homogenated in chloroform and methanol mixture (2:1 ratio) and centrifuged. The supernatant homogenate was used for the experiment. The sample tissue is loaded in TLC plate and dipped in a beaker which consists of mixture of chloroform and methanol (2:1 ratio) that works as mobile phase. TLC is a sheet of aluminium foil which is coated with a thin layer of adsorbent that works as a stationary phase. The various lipids present in the sample tissue travels across the TLC plate. The distance travelled by the lipid substance is divided by the distance travelled by the mobile phase is called as Retardation factor (Rf Value). After the experiment, the TLC plate is drawn from the beaker and dried, sprayed with various reagent i.e. Dittmer-lester reagent. Spots with blue colour were appeared on the TLC plate. Rf values and individual spots were marked with pencil and calculated. Results reveals that brain tissue exhibited seven phopsholipids spots in blue colour with Rf value 80±0.5 and 90±0.5 were with high intensity with dark blue, 10±0.5, 30±0.5 and 70±0.5 were thick, Rf value 20±0.5, 60±0.5 were not clearly stained, followed by gill tissue showed six lipid spots with Rf value 50±0.5 and 90±0.5 were very dark, 10±0.5 and 20±0.5 spots were darkly stained, and 40±0.5 and 60±0.5 spots were unclear, Liver tissue exhibited five phospholipid spots with Rf value 50±0.5 is very darkly stained with blue colour, Rf value 30±0.5 spot was darkly stained, Rf value 10±0.5 and 40±0.5 spots were moderately stained, Rf value 20±0.5 spot was unclear stained. Intestine tissue showed four phosphiolipid spots with Rf value 10±0.5, 50±0.5, 70±0.5 and 90±0.5 were moderately stained. and muscle tissue exposed three phospholipid spots with Rf value 50±0.5 and 90±0.5 were moderately stained, Rf value 40±0.5 were unclear.The results indicates that brain tissue of Channa punctatus has more phospholipids. Similar Rf values were detected in different tissue indicates the similarity in lipid composition in the tissue.

Keywords: Phopsholipis; Channa punctatus; TLC; dittmer-lester reagent; Rf value; chloroform-methanol mixture.

1. INTRODUCTION

Fish diet has a major impact on the chemical composition of fish tissues and especially on the fatty acid (FA) composition of the fish lipids [1–4]. Fish is highly nutritious, easily digestible and much sought after food. Nutritional value of fish depends on their biochemical composition which is affected by water pollution [5] Fishes are the major component of aquatic fauna, and chief source of protein, carbohydrate and fats for humans and domestic animals [6,7].

Fishes are the excellent models for monitoring environmental contamination in aquatic system [8,9]. Phospholipids constitute a major component of cell membranes, where they play essential role in vital cellular processes [10]. These lipids are the highly important in the ecosystem and biochemical monitoring and testing of aquatic species. The role of lipids cellular metabolism is very significant although three main functions were identified: energetic; structural and bioeffector roles [11]. Long chain omega -3 fatty acids, PUFA, particularly EPA and DHA are available in fish lipid, consumption of these PUFA s have been perceived to be important in human food, health and disease controls [12]. World fish lipid demand is continuously increasing, cholesterol in seasonal differences and significant losses may occur during processing and storage of foods [13]. The fish liver oils contain high level of cholesterol but the consumable part of fish i.e. Muscle and brain contain less quantity of lipid [12]. Cholesterol works as a defensive action and transports fat to liver in the form of cholesterol ester for oxidation. It helps in formation of bile acids and bile salts, 7-dehydrocholesterol and vitamin D3, corticosteroid hormone, androgens and Progesterone. Cholesterol helps in granulation of cell division and acts as antagonist to phospholipids [12]. Lipids and fatty acids play an important role in membrane and directly control the membrane mediated process i.e.

osmoregulation, nutrient assimilation and transport. The nature quantity of the lipids in fish differ in different organisms and habit [14]. Consumption of fish has been linked to health benefits as the long chain PUFA has gained attention because of the benefits of the prevention of human coronary artery disease [15]. Adult population is recommended to consume fish as it consists of low cholesterol rather than other meat [16]. The physiological conditions of fish and habitat and nutritional value of fish is determined by the chemical composition of protein and lipids [17]. (Moghaddam HN *et al*., 2007; [18,19]. Fish lipids are excellent source of the essential polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) derive mainly from fish [20]. n3 polyunsaturated FA (n-3 PUFA) in the diet has been recognized to have important beneficial properties for the prevention of human coronary artery disease [21]. Fish are quite different from the other animal food sources, because they provide low energy and have high-level proteins, which contain all essential amino acids. So they are beneficial nutrition sources [22] “Fish are not only beneficial protein source but also contain considerable amount of unsaturated fatty acids, and thus the studies on lipid biochemistry have been considered so important recently [23-28]. The lipids are the most important biochemical compounds of fish” [29]. Fish store the lipids in various organs; particularly in muscles and liver. On the contrary, the mammals store in adipose tissue. A great amount of these lipids are transferred to the different parts of the body to be used for various physiological actions” [30].

2. MATERIALS AND METHODS

Different tissues I.e gill, liver, intestine, muscle and brain were collected from the fresh water fish *Channa punctatus* by Folch [31] method. And Tissues were weighed to nearest milligram and homogenized with chloroform methanol mixture (v/v) (2:1) to a final dilution of 10fold the weight of tissue i.e., the homogenate 1 gm of tissue was diluted to a volume of 10 ml of choloform methanol mixture solution. The mixture was homogenized and centrifuged at 2000 rpm for 5 mins. The organic layer was collected and evaporated in vaccum at 350C and the residue was dissolved in 0.5ml of solvent (Chloform: Methanol. 2:1). The TLC was run on a ready made silica gel plates (E-merck) in a solvent system of Chloroform, methanol, water (65:25: 10) system. The plates were dried at room temperature. The plates were observed under UV lamp and the spots were marked with pencil. For routine analysis of plates were sprayed with Dittmer-Lester reagent. Rf values of all the spots were determined immediately.

2.1 TLC Plates

Ready made marketed (E. Merck AG, Darmstadt, Germany) silica gel-G coated on aluminium plates with thickness of the layer 0.2mm were used, the solution of total lipids prepared in (Chloroform: Methanol, 2:1) was applied on the plates at a distance of 1 cm from lower edge of the plate. The loaded plates were developed in the solvent system mentioned above.

2.2 Reagent Preparation

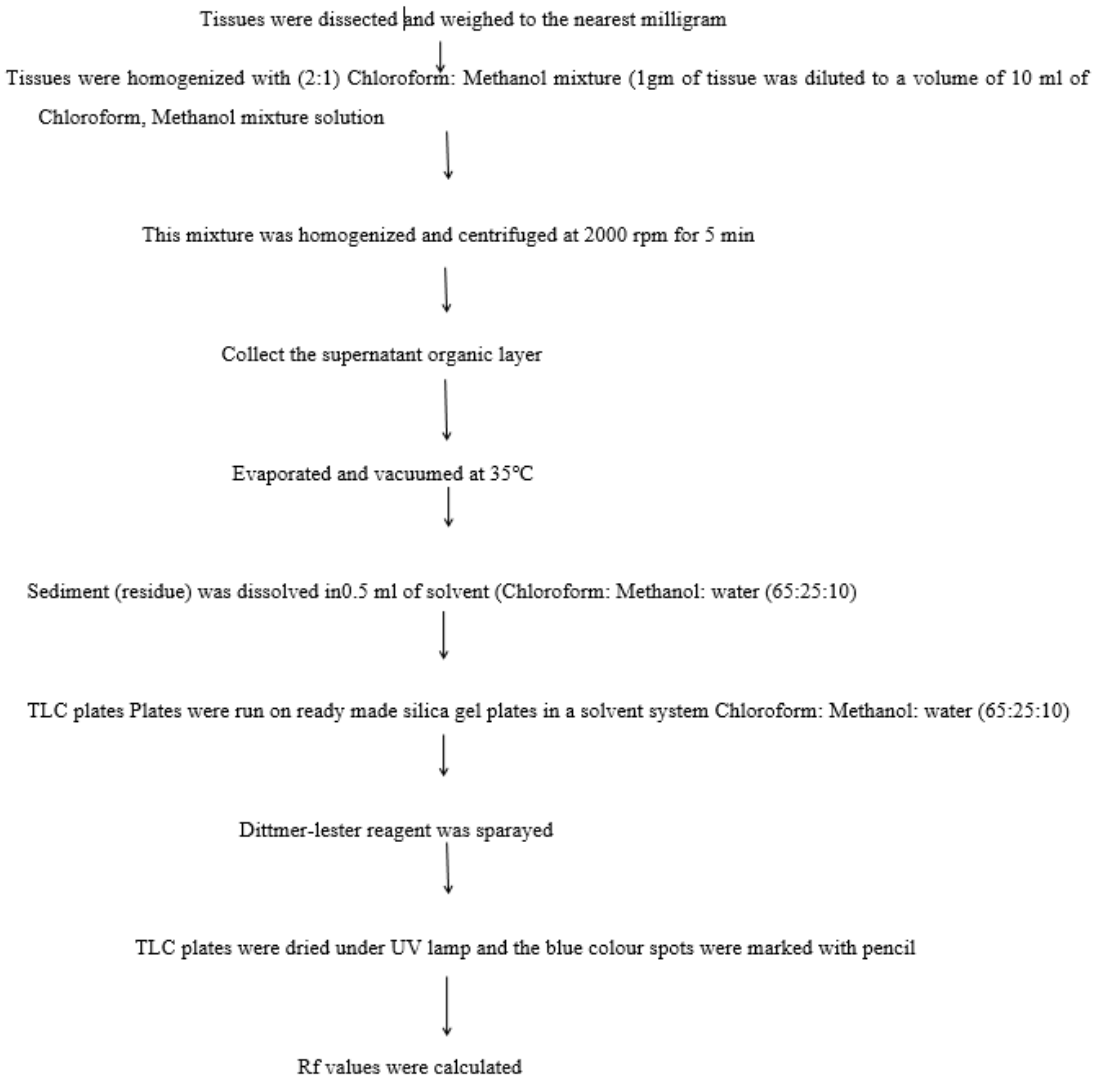
Following reagent systems were employed in identifying the lipid fractions in accordance with the procedures given by Kates (1986).

Dittmer – Lester Reagent (Modified by vaskovasky and kostetsky) This reagent was used to detect phospholipids for which it is specific, and can detect as little as 10µg of lipid. The phospholipids appeared as blue spots on a white background with few minutes without heating.

2.3 Reagent Preparation

Solution A: 16 gm of Ammonium molybdate in 120 ml distilled water.

Solution B: 40 ml of Con, HCL, 10ml of mercury and 80 ml of sol. A were added, shaken for 30 minutes and filtered. To reminder of the solution (A) 200 ml of Con. H2SO4 and all the solution (B) were added, cooled and stored. This was bright green coloured solution and was stabled at room temperature. Just before use this solution was diluted with distilled water (1:3 v/v). the final spray reagent was Amber colored.



3. RESULTS

The spot pattern of phospholipids observed through TLC in different tissues under examination is presented in Fig. 1 and Rf values of individual spots and the staining patterns with Dittmer-lester reagent is shown in Table 1.

Gill tissue of *Channa punctatus* showed six blue colour spots with Rf value 50±0.5 and 90±0.5 were very dark, Rf value 10±0.5 and 20±0.5 spots were darkly stained, Rf value 40±0.5 and 60±0.5 spots were unclear.

Liver tissue of *Channa punctatus* shown five blue colour spots with Rf value 50±0.5 is very darkly stained with blue colour, Rf value 30±0.5 spot was darkly stained, Rf value 10±0.5 and 40±0.5 spots were moderately stained, Rf value 20±0.5 spot was unclear stained.

Intestine tissue of *Channa punctatus* shown four blue colour spots Rf value 10±0.5, 50±0.5, 70±0.5 and 90±0.5 were moderately stained.

Muscle tissue of *Channa punctatus* shown three blue colour spots with Rf value 50±0.5 and 90±0.5 were moderately stained, Rf value 40±0.5 were unclear.

Brain tissue of *Channa punctatus* shown seven blue colour spots with Rf value 80±0.5 and 90±0.5 were with high intensity with dark blue, Rf value 10±0.5, 30±0.5 and 70±0.5 were thick, Rf value 20±0.5 and 60±0.5 were unclear.

Hence it is identified that high quantity of phospholipids were observed in brain tissue followed by gill, liver, intestine and least in muscle tissue of *Channa punctatus* when these tissues were sprayed with Dittmer – lester reagent.

Table 1. Rf Values of different tissues of Channa punctatus sprayed with Dittmer-lester reagent

Lane	Tissue	Rf value 10±0.5	Rf value 20±0.5	Rf value 30±0.5	Rf value40±0.5	Rf value 50±0.5	Rf value 60±0.5	Rf value 70±0.5	Rf value 80±0.5	Rf value 90±0.5
Lane-1	Gill	+++	+++	-	+	++++	+	-	-	++++
Lane-1	Liver	++	+	+++	++	++++	-	-	-	-
Lane-3	Intestine	++	-	-	-	++	-	++	-	++
Lane-4	Muscle	-	-	-	+	++	-	-	-	++
Lane-5	Brain	+++	+	+++	-	-	+	+++	++++	++++

++++: Highly darkly stained with blue colour
+++: Darkly stained with blue colour
++: Moderately stained with blue colour
+: Unclerly stained with blue colour
-: Rf value spot is absent

Fig. 1. TLC of Gill, Liver, Intestine, Muscle, Brain tissue of *Channa punctaus* (Bloch) stained with Dittmer-lester reagent

Lane-1: Gill tissue; Lane-2: Liver tissue; Lane-3: Intestine tissue; Lane-4: Muscle tissue; Lane-5: Brain tissue

4. DISCUSSION

The results obtained from the current research study reveals that Phopsholipid concentration is observed more in the brain tissue of *Channa punctatus.* Brain tissue has exhibited 07 phopholipid spots. Spots with 80±0.5 and 90±0.5 were highly stained (++++) and Rf value 10±0.5, 30±0.5 and 70±0.5 were darkly stained (+++). Next to the brain tissue gill tissue exposed 06 phospholipid spots in which highly stained spots (++++) were 02 with Rf value 10±0.5 and 20±0.5. Liver tissue exhibited 05 phospholipid spots. Spots with Rf value 50±0.5 was highly stained (++++). Spots with Rf value 30±0.5 were darkly stained (+++). In intestine and muscle tissue the highly stained lipids spots were not exposed. Intestine tissue has exhibited 03 moderately stained spots (++) with Rf value 10±0.5, 50±0.5, 90±0.5. And muscle tissue exposed only 02 moderately stained spots(++) with Rf value 50±0.5 ,90±0. Determination of the fatty acids in fish tissue and feed comparison of different methods and statistical evaluation was reported [32]. Bligh [33] Discovered the rapid method of total lipid extraction and purification. Phospholipid changes in muscle from stored frozen lake Michigan coho Salman reported [34]. Folch [35] reported a simple method of isolation and purification of lipids from animal tissues. Fraser [36] investigated a method is described whereby a complete analysis of individual neutral lipid and phospholipid classes in marine animal total lipid can be achieved using an latroscan TLC- FID analyser. [37] studied qualitative and quantitative analysis of lipid classes in fish oils by Thin-Layer Chromatography (TLC). [38] studied the metabolism and functions of fatty acids in Teleost fish. Indrasena [39] reported the Thin layer chromatography determination of phospholipids in biological samples.Previous studies revealed an interestingly tight correlation between the low cardiovascular disease occurrence rates in Greenlandic Inuit people and Japanese and their high intake of seafood fat [40,41]. Fatty acids are essential for life due to their vital roles as a source of membrane constituents, energy, and metabolic and signaling mediators [42]. Our results are in consonance with Bheem Rao [43].

5. CONCLUSION

Fish is the cheapest source of good quality animal protein readily available to the masses throughout the world and its demand is always

on increase. Lipids rich in PUFA are potential sources of anti-aging, anti-thrombotic, anti-inflammatory, anti-cholesterelemic and anti-cancer drugs to immunostimulant and immunosuppressant therapeutics. Freshwater fish meat is an important source of n–3 long-chain polyunsaturated fatty acids (PUFA), principally eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which have an indisputable effect on human health and prevent the genesis of human coronary disease, arrhythmia prevention. Vascular relaxation improvement, anti-inflammatory responses, platelet aggregation inhibition, enhancement of plaque stability and antiatherosclerotic effects. Due to its low cost, high nutritious value and low cholesterol and high protein the fish is consumed by many people.The current research study emphasized on the quantification of lipids in TLC. Due to heavy anthropocentric activities mainly the pesticide pollution caused the deterioration of aquatic life. Over usage of pesticides has altered the fish health, indirectly the human. Any change in the water quality, feed quality reflects on the fish health. So present research study definitely proves that fish can be used as a model. Which help in identification of water toxicity. When both quantity and quality of fish declines that could definitely effect the human health too. So protection of aquatic life is the global need and responsibility. fish Our findings will definitely give an insight.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

ACKNOWLEDGEMENTS

The authors would like to thank the chair of the Zoology Department at Kakatiya University for providing the necessary laboratory space for this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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ISSN 0256-971X.
DOI: 10.56557/upjoz/2024/v45i73989

Peer-review history:
The peer review history for this paper can be accessed here:
https://prh.mbimph.com/review-history/4080