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Iodine Vapor Spray For the Detection of General Lipids on Thin Layer Chromatography in Fresh Water Fish Channa Punctatus

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ABSTRACT:
Thin Layer Chromatography (TLC) was used to detect and quantify general lipids in the gill, liver, gut, muscle, and brain tissues of the freshwater fish Channa punctatus. Dissected and homogenized in a chloroform and methanol combination (2:1 ratio), the tissues of the brain, gills, liver, intestines, and muscles of the Channa punctatus punctatus were then 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 retardation factor (Rf Value) is the product of the mobile phase's travel distance and the lipid substance's travel distance. After the experiment, the TLC plate is drawn from the beaker and dried, sprayed with Iodine vapour reagent. Spots with yellow colour were appeared on the TLC plate. Rf values and individual spots were marked with pencil and calculated. And the results reveals that brain tissue quantified more lipids followed by liver, muscle and intestine while gill tissue exposed less quantity of lipids. Brain tissue exhibited six yellow colour lipid spots in which lipid spots of Rf value 10±0.5; 40±0.5; 80±0.5 and 90±0.5 were very darkly stained whereas Rf value 30±0.5 and 60±0.5 were moderately stained. Liver tissue has shown five yellow colour spots with Rf value 50±0.5 and 60±0.5 were highly stained with yellow colour, Rf value 10±0.5; 20±0.5; 80 ±0.5 were moderately Stained.Muscle tissue has shown four yellow colour spots with Rf value 90±0.5 were highly Stained, Rf value 30±0.5 and 50±0.5 were moderately stained, Rf value 70 ±0.5 were unclear. Intestine tissue has shown five yellow colour spots with Rf value 10±0.5 and 90±0.5 were moderately stained, Rf value 30±0.5; 40±0.5 and 50 ±0.5 were unclear. Whereas gill tissue has shown three yellow colour spots with Rf value 90 ±0.5were moderately Stained, Rf value, 40±0.5; 50±0.5 were unclear. Similar Rf values were detected in different tissue indicates the similarity in lipid

composition in the tissue.

Keywords:
General lipids, Gill, liver, intestine, muscle, brain, *Channa punctatus*; TLC; Iodine vapour reagent, Rf value; chloroform,methanol mixture.

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1. INTRODUCTION

Studies have demonstrated that the chemical makeup of fish tissues, particularly the fatty acid (FA) composition of fish lipids, is significantly affected by the food that fish consume. (Caballero MJ, 2002; Menoyo D López-Bote CJ, 2007; Jobling M, 2008; Turchini GM, 2009). In addition to being a popular and healthy meal option, fish is also easy to digest. Water contamination alters the biochemical makeup of fish, which in turn affects their nutritional value (Gehan H Fahmy, 2012). The majority of aquatic species are fish, and fish are the principal food source for people and domestic animals in terms of protein, carbohydrates, and lipids. (Tripathi PK, 2003; Prado R, 2009). According to Anonymous (2008), India is second in inland fish output and third overall in global fish production.The nutritional benefit of fish, which includes vital fatty acids, is well recognized, and the intake of fish is on the rise. Calder (2004) evaluated the research on the use of fish oils for secondary prevention after myocardial infarctions and in preventing cardiovascular illnesses by modifying risk factors such as hyperlipidemia and hypertension.Researchers looked studied the lipid profiles of the roe, muscle, and viscera of mullet (Liza carinata) fish (Joe et al., 1988). For neutral lipids, a hexane to ethyl acetate volume ratio of 9:1 was utilized. For glycolipids, the chloroform to methanol volume ratio was 170:24:25:4, and for phospholipids, the chloroform to methanol volume ratio was 65:25:4. Scientists used spraying reagents such α-naphthol for glycolipids and ammonium molybdate-perchloric acid for phospholipids to identify which portions had eluted (Mangold, 1961). The Dragendorff reagent was utilized for phosphatidylcholine (PC) and lysophosphatidylcholine (LPH) (Vashkovsky and Kostetsky, 1968), whereas ninhydrin was employed for phosphatidylethanolamine (PE) (Mangold 1961; Vashkovsky and Kostetsky 1968).Essential dietary components known as polyunsaturated fatty acids (PUFAs) have been proposed as treatments for several diseases (Roy et al., 2020; Yadav et al., 2018). According to Scott GR (2004) and Shinde SCS (2007), fish are great examples of organisms that can track contamination in water systems.The demand for fish lipids across the world is on the rise, and there are seasonal variations in cholesterol levels as well as the possibility of substantial food loss due to processing and storage. (Spinelli J, 1982). 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 (Sujatha KA, 2013).Adult population is recommended to consume fish as it consists of low cholesterol rather than other meat (Chabungbam, 2012).The chemical makeup of protein and lipids determines the physiological circumstances, habitat, and nutritional value of fish (Moghaddam HN et al., 2007; Aberoumad A, 2010; Sanatan Singh, 2016). Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are two examples of polyunsaturated fatty acids (PUFAs) that are abundant in fish lipids (Vignesh SR, 2012). Integrating gas chromatography with thin layer chromatography of silver nitrate enhances the identification of trans fatty acids. (W M Nimal Ratnayake, 2019).

2. MATERIALS AND METHODS

The freshwater fish Channa punctatus was harvested using the Folch technique for the purpose of obtaining several tissues, including gills, liver, intestines, muscles, and brain. The tissues were measured to the closest milligram and then mixed with a chloroform-methanol

solution in a 1:1 ratio until the final concentration was 10 times the tissue weight; for example, 1 gram of homogenate was diluted to 10 milliliters of the chloroform-methanol solution. The mixture was well mixed before centrifugation at 2000 rpm for five minutes. Once the organic layer was gathered, it was vacuum-evaporated at 350 degrees Celsius. After that, the residue was dissolved in 0.5 milliliters of a 2:1 solvent combination of methanol and chloroform. The TLC was performed on pre-made silica gel plates (E-merck) using a solvent combination of chloroform, methanol, and water (65:25:10) instead of water. 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

The plates were coated with aluminum and prepared silica gel-G from Darmstadt, Germany's E. Merck AG. The plates were covered with a full lipid solution (1 cm from the bottom edge) in a 2:1 chloroform:methanol ratio. The solvent solution that was stated prior to was used to generate the loaded plates.

2.2 Reagent Preparation

Since iodine is soluble in both saturated and unsaturated lipids, its vapors may be utilized as a universal lipid detector. The blotting sheets were filled with iodine crystals and then wetted with chloroform. Afterwards, the TLC plates were placed in a Petri dish and let to dry at room temperature. Iodine fumes caused lipid patches to become yellow. The spots were quickly scanned and their Rf values were recorded after being exposed to iodine fumes.

3. RESULTS

Gill tissue has shown three yellow colour spots with Rf value 90 ±0.5were moderately Stained, Rf value, 40±0.5; 50±0.5 were unclear.

Liver tissue has shown five yellow colour spots with Rf value 50±0.5 and 60±0.5 were highly stained with yellow colour, Rf value 10±0.5; 20±0.5; 80 ±0.5 were moderately Stained.

Intestine tissue has shown five yellow colour spots with Rf value 10±0.5 and 90±0.5 were moderately stained, Rf value 30±0.5; 40±0.5 and 50 ±0.5 were unclear.

Muscle tissue has shown four yellow colour spots with Rf value 90±0.5 were highly Stained, Rf value 30±0.5 and 50±0.5 were moderately stained, Rf value 70 ±0.5 were unclear.

Brain tissue has shown six yellow colour spots with Rf value 10±0.5; 40±0.5; 80±0.5 and 90±0.5 were very darkly stained, Rf value 30±0.5 and 60±0.5 were moderately stained.

Hence the staining pattern and intensity of spots staining it is observed that general lipids were highest present in brain tissue, followed by liver, muscle, Intestine and gill respectively.

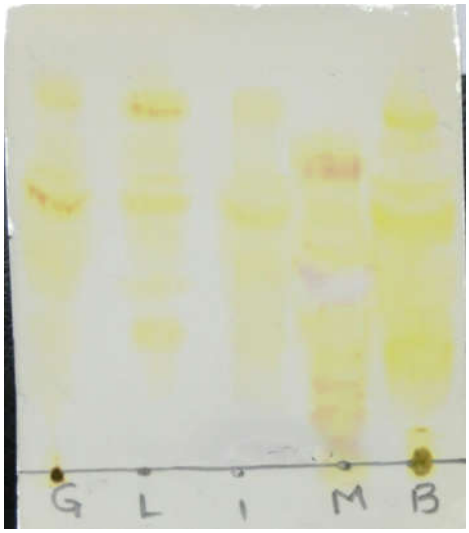

Figure 1: TLC of Gill, Liver, Intestine, Muscle, Brain tissue of *Channa punctaus* (Bloch) stained with Iodine vapours regent

Table 1: Rf Values of different tissues of Channa punctatus sprayed with Iodine vapours reagent

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

LANE	TISSUE	Rf value 10±0.5	Rf value 20±0.5	Rf value 30±0.5	Rf value 40±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-2	LIVER	++	++	-	-	++++	++++	-	++	-
LANE-3	INTESTINE	++	-	+	+	-	-	-	-	++
LANE-4	MUSCLE	-		++	-	++	-	+	-	+++
LANE-5	BRAIN	++++	-	++	++++	-	++	-	++++	++++

++++: Highly darkly stained with yellow colour
+++: Darkly stained with yellow colour
++: Moderately stained with yellow colour
+: Unclearly stained with yellow colour
-: Rf value spot is absent

4. DISCUSSION

Results obtained from the current research study reveals that Brain tissue of *Channa punctatus* exhibited highest quantity of general lipids. Brain tissue exposed a total six yellow colour lipid spots when this tissue was exposed to Iodine vapour reagent. Out of six lipid spots three lipid spots with Rf value 10±0.5, 40±0.5, 80±0.5, 90±0.5 were highly darkly stained which quantifies more general lipids in the tissue. Whereas liver tissue exhibited two highly darkly stained lipid spots at Rf value 50±0.5 and 60±0.5 The remaining lipid spots with Rf value 10±0.5, 20±0.5 and 80±0.5 were darkly stained. Muscle tissue exhibited four yellow colour lipid spots, Rf value 90±0.5 was darkly stained remaining are moderate. Intestine tissue showed five lipid spots Rf value 10±0.5 and 90±0.5 were moderate. Less lipids were estimated in gill tissue which expressed three lipid spots in which Rf value 90±0.5 was moderate and remaining are unclear. The results reveals brain tissue contains more lipids. Thin-layer chromatography (TLC) has been used for PL detection and separation ever since chromatography was first developed, and it is still commonly used today. According to Steele and Banks (1994), there are several kinds of silica gel, which is commonly employed as a stationary phase in normal phase chromatography for PL separation.

The polar lipid content of fatty fish oils and its changes throughout an accelerated oxidation test were studied by Jukka K. Kaitaranta and Paul J. Ke (1981) using quantitative thin layer chromatography-flame ionization detection (TLC-FID). Two common freshwater fish, the murrel (Channa striatus) and the rohu (Labeo rohita), have had their fatty acid content and lipid profiles published using TLC (P.G. Prabhakara Rao et al., 2010). Two popular freshwater fish species, mrigal and Catla catla, have had their lipid profiles and fatty acid concentrations shown in studies using thin-layer chromatography (TLC). (P.G. Prabhakara Rao et al., 2013). Classification and subclassification of freshwater fish body lipids was performed. A total of seven subclasses were identified within the nonpolar lipid class. Triacylglycerols, sterol esters, and free fatty acids' fatty acid compositions were ascertained (Mita Ghosh et al., 2007).A number of studies, including those by Mukhopadhyay and Ghosh (2007), Prabhakara Rao et al. (2010), Mukhopadhyay et al. (2004), and Mukhopadhyay and Ghosh (2003), found that the phospholipid portion of roe possessed substantial quantities of EPA and DHA. Mirja Kaizer Ahmed et al. (2023) reported on the characteristics, extraction, measurement, and use of lysophosphatidylcholine obtained from marine fish to brain health. An HPTLC method was devised by Marion Papin et al. to measure alkyl and alkenyl glycerolipids in chimera and shark oils and tissues. Katarzyna Dyńska-Kukulska and Witold Ciesielski recorded techniques for thin-layer chromatography and biomaterial phospholipid extraction.A mixture of chloroform, methanol, and 2 n ammonia in a volume ratio of 65:24:4 was found by Vecchini et al. (1995) to be the mobile phase that enabled the separation of PC, PE, PI, and PS completely on a single chromatoplate, as well as their quantitative detection. In order to estimate total lipids, Iverson et al. (2001) compared the Bligh, Folch, and Dyer techniques in great detail. Folch and Bligh-Dyer's methods are commonly used to examine biological fluid and tissue samples (Schiller et al., 2004). Avalli and Contarini (2005) and Donato et al. (2011) utilized the Folch approach to extract PLs from samples of various dairy products, including cow and donkey milk. A detailed comparison of the Bligh, Dyer, and Folch methods for determining total lipids was carried out by Iverson et al. (2001). Folch and Bligh-Dyer's methods are commonly used to examine biological fluid and tissue samples (Schiller et al., 2004). The Folch technique was used to extract PLs from samples of cow and donkey milk and other dairy products by Avalli and Contarini (2005) and Donato et al. (2011). Separation of various PL classes may be accomplished rapidly and affordably using TLC. In this study, we provide spray reagents that may be used as an alternative to more costly methods like HPLC, GC, or MS for quantitative PL measurement. It was found by Dittmer, JC, and Lester, R.L. that thin-layer chromatograms may be used to identify phospholipids with simple technique. Our results are in consonance

with (Bheem Rao et al., 2024 and Venkateswara Rao et al., 2024).

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Competing Interests
Authors have declared that no competing interests exist.

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