Comparative Study of Lipids in Brain Tissue of Fresh Water Fish Channa Punctatus in Thin Layer Chromatography

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Abstract: - Different types of lipids were quantified in brain tissue of *Channa punctatus* on Thin Layer Chromatography (TLC) by using different reagents i.e Dittmer-Lester reagent (to identify phospholipids), Dragondroff"s reagent (to identify choline lipids), Iodine vapors (to identify general lipids), Ninhydrine reagent(to identify aminogroup of lipids) and sulphuric acid reagent (to identify glycolipids). Brain tissue of *Channa 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, Dragondroff's reagent, Iodine vapors, ninhydrine reagent, Sulphuric acid reagent. Spots with different colours were appeared on the TLC plate. Rf values and individual spots were marked with pencil and calculated.Results reveals that brain tissue exhibited more phopsholipids at Rf value 10±0.5,20±0.5,60±0.5. High choline lipids were quantified at Rf value 30±0.5, 40±0.5. General lipids were high at Rf value 20±0.5; 60±0.5; 90±0.5. Amino group of lipids were high exhibited at Rf value 70±0.5; 80±0.5. And high concentration of glycolipids were quantified at Rf value 10±0.5, 60±0.5 and 90±0.5. Over all this tissue showed a high quantity of general lipids followed by phospholipids

Key Words:- Channa punctatus, brain, TLC, Reagents, Rf value

Introduction:- TLC has a strong potential as a surrogate chromatographic model for qualitative and quantitative analysis.Among chromatography methods, the thin-layer chromatography (**TLC**) must be considered for using in routine laboratories due to a number of advantages such as practicality, fast results and effectiveness, low cost, and simultaneous determination of analytes (Monterio et al., 2016). The conditionally essential very-long-chain polyunsaturated fatty acids (VLC-PUFAs), such as eicosapentaenoic acid (EPA, C20:5 n-3), play a vital role in human nutrition (Kolas Makay, 2024). Phospholipids are vital molecules for cellular homeostasis and are involved in various regulatory and structural functions, as well as being fundamental components of cell membranes. There are more than 1000 different phospholipids found in nature that vary in their chemical structures. Phospholipids consist of phosphorylated alcohol and at least one fatty acid (FA) connected to a glycerol or sphingosine backbone (Burri, L et al., 2012).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 (Ward OP *et al.*, 2005).Thin-layer chromatography (TLC) is widely used in laboratories throughout the world for food analysis and quality control. Numerous applications of TLC have been reported in the areas of food composition, intentional additives, adulterants, contaminants, and decomposition involving determinations of compound classes such as amino acids (protein quality), lipids and fatty acids (quality and adulteration of fat), sugars (beverage quality), biogenic amines (storage stability), vitamins (added as nutrients, colorants, and antioxidants), and organic acids (preservatives) (Panel Joseph Sherma, 2000). Lipids are important cellular components of biological systems and are involved in membrane formation of cells and cellular structures, signaling and energy storage (Fahy et al., 2011). At present, fishes, livestock animals, and grain resources are used on a massive scale as major dietary protein and fat sources; however, their use causes severe environmental problems and the feed-to-meat conversion efficiency for livestock animals is low (Sánchez-Muros et al., 2014). Therefore, the development of alternative and sustainable food materials is required.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 controlls (Sujatha K. *et al*., 2013). World fish lipid demand is continuously increasing, cholesterol in seasonal differences and significant losses may occur during processing and storage of foods (Spinelli J *et al*., 1982).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 (Kumaran R. *et al*., 2019).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 (Svetlana A.M *et al*., 2013).Adult population is recommended to consume fish as it consists of low cholesterol rather than other meat (Chabungbam *et al*., 2012). The physiological conditions of fish and habitat and nutritional value of fish is determined by the chemical composition of protein and lipids (Moghaddam HN *et al*., 2007; Aberoumad A *et al*., 2010; Sanatan Singh *et al.,* 2016). Fish lipids are excellent source of the essential polyunsaturated fatty acids (PUFAs), such as docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA) derive mainly from fish (Vignesh 2012).

Materials and methods:- Lipids were quantified and observed in brain tissue of *Channa punctatus* through Thin Layer Chromatography (TLC). Brain tissue of *Channa. Punctatus was* 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 reagents i.e. Dittmer-leister, Dragondroffs, iodide, ninhydrine and sulphuric acid reagents. Spots with different colours i.e. blue, orange, yellow, purple and black respectively were appeared on the TLC plate when sprayed with above reagents. And The Rf values were measured with a scale to quantify the different types of lipids in the brain tissue.

Tissues were dissected and weighed to the nearest milligram

↓

Tissues were homogenized with (2:1) Chloroform: Methanol mixture(1gm of tissue was diluted to a volume of 10 ml of Chloroform, Methanol mixture solution

↓

This mixture was homogenized and centrifuged at 2000 rpm for 5 min

↓

Collect the supernatant organic layer

↓

Evaporated and vacuumed at 35℃

↓

Sediment (residue) was dissolved in0.5 ml of solvent (Chloroform: Methanol:water(65:25:10)

↓

TLC plates Plates were run on ready made silica gel plates in a solvent system Chloroform: Methanol: water (65:25:10)

↓

Reagents were sparayed

↓

TLC plates were dried under UV lamp and the blue colour spots were marked with pencil

↓

Rf values were calculated

Results Brain:- tissue of *Channa punctatus* shown seven blue colour spots of phospholipids with Dittmer -Lester reagent with Rf value 10±0.5,20±0.5,60±0.5 were with high intensity with dark blue (+++), Rf value 90±0.5 was thick(++), Rf value 30±0.5 was slightly thick(+) and 50±0.5, 70±0.5 were unclear (±). While nine orange colour spots of choline lipids were quantified with Dragondroffs reagent , Rf value 30±0.5 and 40±0.5 were more darkly stained with more intensity (+++), Rf value 10±0.5; 60±0.5; 80±0.5, 90±0.5 were highly stained (++), Rf value 70±0.5 is slightly thick (+) and 20±0.5 , 50±0.5 were unclear (±). The tissue has shown nine yellow colour spots of general lipids with Iodine vapors . Rf value 20±0.5; 60±0.5; 90±0.5 were very darkly stained (+++), Rf value 30±0.5 and 70±0.5 were moderately stained (++), Rf value 40±0.5 and 50±0.5 were slightly spoted (+), while Rf value 10±0.5 and 80±0.5 were unclear. When sprayed with ninhydrine reagent this tissue has exhibited six yellow colour spots of amino group of lipids. Rf value 70±0.5; 80±0.5 were very darkly stained (+++), Rf value 10±0.5 and 90±0.5 were moderately stained (++). Rf value 40±0.5,50±0.5 were faintly stained (+). And six black colour spots of glycolipids were exhibited when sprayed with Sulfuric acid reagent. Rf value 10±0.5, 60±0.5 and 90±0.5 were more darkly stained (+++), Rf value 30±0.5 was moderately stained (++); Rf value 20±0.5 and 50±0.5 were faintly stained (+). (Fig 1, Table1).

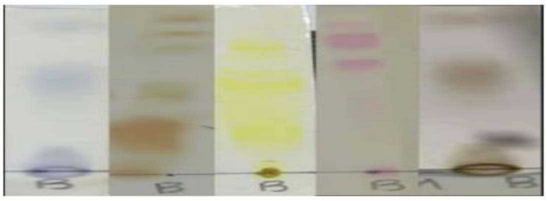

Fig1: Thin Layer Chromatography of Brain tissue of Channa punctatus treated with different reagents

S.No	Reagent	Brain tissue of Channa punctatus treated with different reagents for lipid identification Rf values X100								
		10± 0.5	20± 0.5	30± 0.5	40± 0.5	50± 0.5	60± 0.5	70± 0.5	80± 0.5	90± 0.5
L1	Dittmer lister(phospho lipids)	+++	+++	+	_	±	+++	±	-	++
L2	Dragen droff's(choline lipids)	++	±	+++	+++	±	++	+	++	++
L3	Iodine (all types of lipids)	±	+++	++	+	+	+++	++	±	+++
L4	Ninhydrine(FA A)	++	-	_	+	+	_	+++	+++	++
L5	H2So4 (glycolipids;st erols)	+++	+	++	_	+	+++	_	-	+++

Table1 : Rf values of lipids on Thin Layer Chromatography of brain tissue treated with different reagents

Discussion:- The current research study emphasized on quantitative estimation of lipids in brain tissue of *Channa punctatus* on TLC when sprayed with Dittmer Lester reagent, Dragondroff''s reagent, Iodine vapors, ninhydrine reagent and H2SO4 reagent. The concentration of phospholipids is very high at Rf value 10±0.5, 20±0.5,60±0.5. It was discovered that high concentration of choline lipids at Rf value 30±0.5, 40±0.5. General lipids were most at Rf value 20±0.5, 60±0.5, 90±0.5. Amino group of lipids were high in concentration at Rf value 60±0.5,80±0.5.

The high concentration of glycolipids and sterols was discovered at Rf value 10±0.5, 60±0.5, 90±0.5. These lipid sopts on TLC were more darkly stained. One-dimensional high-performance thin-layer chromatography (HPTLC)-based method was developed (Kolos Makay, 2024) that allows the separation and quantification of the main microalgal glycerolipid classes. Rapid quantification of neutral lipids and triglycerides during zebrafish embryogenesis was discovered (Prusothman et al., 2017). Our results are in consonance with Venakateswara Rao et al., (2024), Venkateswara Rao et al., (2024). 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). In order to estimate total lipids, Iverson et al. (2001) compared the Bligh, Dyer and Folch methods. Bheem Rao et al., (2024) discovered the variations in lipids in different tissue of of Heteropneustes fossilis on TLC.

Conclusion:- Fish is the most edible animal in world wide as well as in India. Due to its low cost, high nutritious value and low cholesterol and high protein the fish is consumed by many people. Fish Lipid is most important in human diet. It contain low cholesterol. Lipids play a vital role in the cellular metabolism.The current research study emphasized on the quantification of lipids in TLC. Lipids are also important nutrients. Fish contain the beneficial lipids. Fish meat contains low cholesterol and high protein value. The assessment of lipids in the fish is most important now a days. Due to heavy anthropocentric activities mainly the pesticide pollution caused the deterioration of aquatic life.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. Our findings will definitely give an insight.

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