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IJCPA, 2015; 2(3): 188-192

International Journal of
CHEMICAL AND PHARMACEUTICAL
ANALYSIS

eISSN: 2348-0726 ; pISSN : 2395-2466

Research Article

IDENTIFICATION AND CHARACTERIZATION OF LIPIDS BY TLC IN TISSUE EXTRACT OF *ARION ATER* (BLACK SULG)

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Received: 3 March 2015 / Revised: 14 April 2015 / Accepted: 27 April 2015/ Online publication: 1 July 2015

ABSTRACT

The molecular diversity of chemical compounds found in aquatic animals offers a good chance for the discovery of novel bioactive compounds of unique structures and diverse biological activities. Slugs are not protected by a shell and produce chemicals for various ecological uses, including defense against predators, have attracted great interest for their lipid composition. Lipid analysis of slugs revealed dominant phospholipids, sterols and monoalkyldi-acylglycerols. Among polar lipids, 1-alkenyl-2-acyl glycerophospholipids (plasmalogens) and ceramide-aminoethyl phosphonates were found in the mollusks. The fatty acid compositions of the gastropods differed greatly from those of other mollusk's and exhibited a wide diversity: very long chain fatty acids known as demospongic acids, a series of non-methylene-interrupted fatty acids and an abundance of various odd and branched fatty acids typical of bacteria. Symbiotic bacteria revealed in some species of slugs participate presumably in the production of some compounds serving as a chemical defense for the mollusks. The unique fatty acid composition of the gastropods are determined by food supply, inherent biosynthetic activities and intracellular symbiotic microorganisms. The potential of gastropods as a source of biologically active lipids and fatty acids. The present investigation was carried out to study the lipids qualitatively in the tissue extract of *Arion ater* (Black slug) for the presence of various types of lipids by thin layer chromatography (TLC) using chloroform. Methanol and water (65:25:4) as solvent system. The chromatograms revealed many lipid spots with different R_f values indicating the presence of different types of lipids such as sterols, Glycolipids, phospholipids in tissue extract of *Aron ater*.

Keywords: TLC, *Arion ater*, R_f value, Chromatogram

1. INTRODUCTION

Mollusks are widely distributed throughout the world and have many representatives such as slugs, whelks, clams, mussels, oysters, scallops, squids and octopods in the marine and estuarine, fresh water ecosystem. Many classes of bioactive compounds exhibiting anti-tumor, anti-leukemic, antibacterial and antiviral activities have been reported worldwide. Among the mollusk's some have pronounced pharmacological activities or other properties which are useful in the biomedical arena. It is surprising to find that some of the pharmacological activities are attributed to the presence of polysaccharides particularly sulphated mucopolysaccharide and lipids. Moreover, they are synthesized without dedicated cells or tissues and they can rapidly diffuse to the point of infection. The potential of gastropod as a source of biologically active products is largely explored in India.

The molecular diversity of chemical compounds found in aquatic animals is the result of the evolution of the organisms and their unique physiological and biochemical adaptations and offers a good chance for the discovery of novel bioactive compounds with a variety of unique structures and diverse biological activities. Mollusks have become the focus of many chemical studies aimed at isolating and identifying novel natural products, which often are very colorful, are not protected by a shell and are named slugs, have attracted strong interest for their secondary metabolites, which are active in chemical defenses against predators (Cimino *et al.,* 2006)[1]. These compounds exhibit a large variety of chemical structures and have been shown to possess ichthyotoxic, feeding-deterrent and cytotoxic properties, to have antibacterial activity, to act as sexual pheromones and are responsible for various bioactivities, such as antitumor, anti-inflammatory and antioxidant activities. Clearly, dietary sources contribute significantly to the chemical diversity of metabolites found in some mollusks. However, their *de novo* biosynthesis has been reported for several mollusk species. The secondary metabolites isolated from mollusks fall into a wide range of structural classes, with some compounds predominating in certain taxa. In the gastropod, terpenes dominate, whereas fatty acid derivatives are relatively uncommon (Benkendorff *et al*., 2010)[2].

Mollusks, as well as the invertebrates, in general, constitute a source of lipid bioactive compounds offering a variety of nutraceutical and pharmaceutical applications. Among them, the omega-3 polyunsaturated fatty acids (PUFA), such as eicosapentaenoic acid, 20:5*n*-3, and docosahexaenoic acid, 22:6*n*-3, are known for their beneficial effects on human health. These PUFA *n*-3 fatty acids are widely known for their capacities for cardioprotection; they reduce triacylglycerol and cholesterol levels and have anti-inflammatory and anticancer effects (Wendel et al, 2009)[3]. Numerous experiments on animals confirmed the cancer preventive properties of PUFA *n*-3 fatty acids from aquatic sources. Some other aquatic lipids also show many potential bioactive properties. Monogalactosyl di acylglycerols and digalactosyl di acylglycerols from the aquatic microalga, *Nannochloropsis granulata*, have been reported to have a nitric oxide inhibitory activity. The betaine lipid from microalgae *N. granulata*, diacylglyceryltrimethylhomoserine, shows a nitric oxide inhibitory activity, indicating a possible value as an anti-inflammatory agent. The glycolipid, sulfoquinovosyl diacylglycerol, from red alga *Osmundaria obtusiloba* and from brown alga *Sargassum vulgare* (Plouguerne *et al* 2013)[4] exhibits a potent antiviral activity against herpes simplex virus type 1 and 2. This glycolipid from a brown alga, *Lobophora variegata*, possess a pronounced antiprotozoal activity (Cantillo-Ciau *et al*., 2013)[5]. Studies on glycosphingolipids from marine sponge *Axinyssa djiferi* proved their good antiplasmodial activity (Farokhi, *et al* 2013)[6].

Although interest in the fatty acid composition of mollusks has not been abated, it has become increasingly obvious that phyla of aquatic invertebrates may be a source of unusual aquatic lipids, such as plasmalogens, phospholipids, glycolipids and diverse fatty acids.

Lipids are essential to living systems which are a diverse group of compounds with a variety of functions like structural components in cellular membranes; they are most potent energy storage source or cell signaling molecules. Lipids are hydrophobic or amphiphilic small molecules whose biosynthesis originates from two building blocks, a ketoacyl group or isoprene group. Lipid classes are divided into natural lipids such as triglycerides, polar lipids such as Phospholipids and cholesterol. TLC can be used as a separation technique for analysis of lipids. TLC can be used as a separation technique for analysis of lipids. Ridzwan Hashim *et al* (2014)[7], reported that the steroids are synthesized by many species of *Arion ater* slugs of the genus *Arion*. A mixture of steroidal compounds was separated by TLC in the tissue extract of *Arion ater* and *In-vitro* and animal models have suggested this species being rich pharmacological activities, so it could be used in prevention and improve the treatment of cardiovascular diseases.

2. MATERIALS AND METHODS

2.1 Protocol for Identification of Lipids

The protocol followed for identification of lipids is depicted in Fig.1.

2.2 Collection and identification of *Arion ater*

The Slugs were collected from fields of Komatipally village, Warangal, Telangana, India. The collected animals were identified by using standard manuals.

2.3 Extraction and Collection of Samples

The tissue extract was collected by the method of Folch *et al.,* (1957)[8]. The *Arion ater* was collected and transferred to the laboratory and washed with distilled water and taken the tissue extraction.

Collected the slugs and crushed the whole body

↓

The tissue was weighed to the nearest milligram

↓

Homogenized the tissue extract with (2:1) Chloroform: Methanol mixture (1gm of secretion was diluted to a volume of 10ml of Chloroform Methanol mixture solution)

↓

The mixture was homogenized and centrifuged at 2000rpm for 5 minutes

↓

Collect the organic layer (upper layer)

↓

And kept it and evaporated and vacuumed at 35^{0}C

↓

Sediment (residue) was dissolved in 0.5ml of solvent (Chloroform: Methanol, 2:1)

↓

The TLC plates were run on readymade silica gel plates in a solvent system,
Chloroform, methanol, water (65: 25: 10)

↓

The plates were dried at room temperature

↓

The plates were dried under UV lamp and the spots were marked with pencil.

Fig. 1: Protocol for identification of lipid

2.4 Experimental procedure for preparation of TLC

The tissue extract was weighed to the nearest milligram and homogenized in chloroform, methanol mixture 2:1 ratio. The mixture was centrifuged at 2000 rpm for 5 minutes and filtered. The supernatant containing organic layer was collected and evaporated. The particulate matter (dry matter) of the tissue extracts were determined by heating at 110^{o}C for 48 to 72 hours until a constant weight of the dry material was obtained. The residue was dissolved in 0.5 ml of chloroform, methanol (2:1) solvent. The solutions were spotted at a distance of 1 cm on to readymade TLC silica gel plates (Emerc, Germany) and run in a solvent system of chloroform, methanol and water (65:25:4). The plates were dried at room temperature.

2.5 Staining for identification and characterization of lipid Spots

For the identification of separated lipid classes. The TLC plates were treated with specific reagents for identification and characterization specific lipid types. Viz., 1). Iodine vapors, 2). Sulphuric acid (H_2SO_4), 3). Ninhydrine, 4). Dittmer-leister reagent and 5) Dragendorff's reagent according to the procedure of Kates Rf values of all the spots were determined immediately**.**

3. RESULTS AND DISCUSSION

The results obtained on the characterization and identification of lipids by the TLC in tissue extract of *Arion ater* are presented in Fig.2 and Table 1. The Retardation Factor (R_f) values of lipid spots were determined according to the procedure Folch *et al.,* (1957)[8] and presented in Tables 1. The results obtained indicate that the tissue extract stained with Dittemer leister reagent showed eight blue colour spots (Lane1) of phospholipids at R_f values 70, 60, 58, 54.1, 41.7, 32, 28.1, 12.7. Among the spots of R_f 70, 60, 58 and 28 were stained with more intensity.

The Tissue extract stained with Dragendorff's staining reagent showed five spots of choline lipids (Lane 2) at R_f 70, 60, 58, 54.1, 43 and 32 among the spots at R_f 70 and 60 were more intensity. The spot at R_f 32 was not clearly visible in the tissue extract of *Arion ater.*

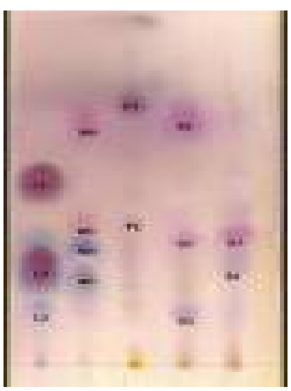
The sulphuric acid reagent for lipids showed only four black glycolipid spots in tissue extract (Lane3) spots at R_f 70, 60 , 58 and 54.1 among the R_f 70 was more intensity and remaining three spots were stained with moderate intensity.

The iodine is a general stain for lipids. Staining of TLC plates under iodine vapours resulted in the appearance of seven yellow spots in tissue extract of *Arion ater* (Lane4) spots at R_f 70, 60, 58, 54.1, 43, 32 and 25.8 among the R_f 70 and 60 were moderate intensity remaining five moderate intensity and R_f 12.2 was not visible in tissue extract of *Arion ater*.

On staining for lipids with Ninhydrine, lipids containing amino group appeared as four purple spots in tissue extract of *Arion ater* (Lane5) spots at R_f 70, 60, 58 and 54 among the R_f 70 and 60 were high intensity and R_f 58 was moderate intensity, R_f 25.8 was not clearly visible. R_f 12.2 was completely absent in the tissue extract of *Arion ater.*

Table 1: Tissue extraction staining of lipid spots with

S. No	Reagent	70	60	58	54.1	43	32	25.8	12.2
L1	Dittmer-leister reagent	++	++	++	++	++	++	+	+
L2	Dragendorff's reagent	++	++	+	+	+	±	-	-
L3	Sulphuric acid reagent	+++	+	++	++	-	-	-	-
L4	Iodine reagent	++	++	+	+	+	+	+	-
L5	Ninhydrine reagent,	+++	+++	++	+	-	-	±	-



L1 L2 L3 L4 L5

Fig.2: Tissue extraction of Arion ater (Black slug) TLC patterns of lipid lanes

Qualitative studies on characterization and identification of lipids by thin layer chromatography in tissue extract of *Arion ater* showed a considerable variation, between the presence lipids, the highest number of spots present in the Dittmer leister reagent (L1) that is indicates the more phospholipids are present in the tissue extract of *Arion ater* and lowest number of spots are appeared in the Ninhydrin reagent (L5) that is indicates lipid contain amino groups are very less and remaining lipids such as Glycolipids, choline lipids, glycerophospolipids are present average in the tissue extract of *Arion ater*

Wendel *et al.,* reported that the biochemical profile of lipids, number and types of biochemical constituents of gastropods and cephalopods species and these are differed significantly and forms different geographic locations. The biochemical components of lipids relative and absolute concentrations were varied based on the different solvent extraction methods.

4. CONCLUSION

Mollusks, as well as the invertebrates in general, constitute a source of lipid bioactive compounds offering a variety of activities. This study has demonstrated for the first time that gastropods exhibit a wide diversity of lipids that differed greatly from that of other mollusks. Lipids of slugs were composed mainly of phospholipids rich in plasmalogen PE and plasmalogen PS. The slugs exhibited some unique features in their fatty acid composition. The current study has shown that these mollusks may be an important resource of a wide range of bioactive compounds

In view of above results it can be concluded that the tissue extraction of *Arion ater* consists of different lipid components with different staining reagents. The selection of solvent system is also very important for the extraction of targeted lipid components and several bioactive compounds, which are species specific. This needs further investigations in various solvent and extraction methods in *Arion ater*.

5. ACKNOWLEDGEMENTS

The authors would like to acknowledge Professor Vijay Kumar, Department of Zoology, for providing TLC plates and laboratory of Eco-Physiology and Toxicology, Dept. of Zoology and Kakatiya University, Warangal, for providing laboratory facilities.

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