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GC-MS Analysis of Biochemical Compounds Present in the Mucus of Zebra Fish (Danio Rerio)

R. Christy shaila[1], Dr. P. Kathireswari[2], Dr. K. Saminathan[3]
[1, 2, 3]*Department of Zoology, Kongunadu Arts and Science College, Coimbatore, India.*

***Abstract:** Zebra fish (Danio rerio) are small fresh water fish that are used as model organisms for biochemical research. The mucus layer of the fish covers the external body surface and to reduce body friction against water. The mucosa plays an important role in keeping the homeostasis of the fish and preventing entry of invading pathogens . In the present study the zebra fish mucus were analysed by Gas chromatography – mass spectrometry (GC-MS) to identify the various bio compounds present in the mucus of zebra fish. The result of the present study revealed that the presence of many important components (compounds) which plays an active role in the lifecycle that are widely used for antibiotic resistance.*
***Keywords:** Biochemical , mucus, zebra fish, pathogens, antibiotic.*

I. INTRODUCTION

In modern-life, one of the most significant issues in the world is the contact to man made chemicals that cause intervention of regular activities such as reproduction and development of different organisms in the environment. Some of them are dangerous and present likely or actual hazard to human health, wild life, aquatic organisms and or surroundings(Stone et al 1994). Fish is an important and good quality source of protein, vitamins and minerals but it deteriotes fastly when decarbolase enzyme is formed by bacterial growth causing production of bioactive amine(Asif Ali et al 2016) .Gas Chromatography- Mass spectrometry (GC-MS) is a combination analytical technique that couples the separation capabilities of GC with the discovery properties of MS to provide a higher effectiveness of sample analysis. While GC can separate the volatile compounds in a sample , MS helps fragment the components and identify them on the basis of their mass. GC-MS provides improved sample identification , higher sensitivity , an increased range of analyzable samples and faster results , which enable a new range of applications for GC-MS in several areas(Stone et al 1994). In medicinal chemistry , GC-MS is used in the production and characterization of compounds .The zebra fish is an increasingly accepted vertebrate model and it offers many advantages compared to rodent models. The reasons for its rising popularity include its genetic significance to humans , low cost of animal husbandry and possible for high through put studies.

II. MATERIALS AND METHODS

Danio rerio were purchased from an aquarium in Coimbatore, Tamil Nadu. The purchased zebra fishes were brought to the laboratory and accilimatized in tap water for one week. After one week the fishes were used for mucus collection.

A. Collection of Mucus from Fish

The mucus from the fish was carefully scraped from the dorsal body using a sterile spatula. Mucus was not collected in the ventral side to avoid internal and sperm contagion. The mucus samples were collected aseptically from the fish and carefully mixed with equal quantity of sterilized physiological saline.

B. Extraction

Hexane was chosen as the extraction solvent solvent and 350 ml of hexane was added to the sample tube and centrifuged under exact conditions to remove solid remnants.

C. Gcms Analysis

1.0 µl of the sample were inject splitlessly using Thermo GC- Trace Ultra Ver:5.0,GC-455 (30m x 0.25 mm i.d, 0.25 µm film thickness). The inlet temperature was set at 260□C at 6c/min. Helium was used as the carrier gas at a stable flow rate of 1.0 ml min – 1. The column temperature was primarily maintain at 70□C for 1 min and then increased at 260□C at a rate of 6□C min-1 and additional increased at 25□C min-1 to 300□C where it remain for 5 min . The column effluent was introduce into the ion source temperature at 230□C. The mass spectrometer was operate in electron impact (EI) mode (70 eV). Data acquisition was performed in full scan mode from m/z 50 -550 with a scan time of 0.5 s . GC-MS was analysed using electron impact ionization at 70 eV and

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data was evaluated using total ion count (TIC) for compound recognition and quantification . The spectrums of the components were compared with the database of spectrum of known components stored in the GC-MS library.

III.RESULTS

Gas chromatography mass spectroscopy study was carried out in crude extract of the fish sample using Hexane. The total ion chromatogram (TIC) of Hexane extract of Zebra fish show the GC-MS profile of the compounds identified is given in Fig 1 respectively. The peaks in the chromatogram were incorporated and were compared with the database spectrum of known components stored in the GC-MS library . The detailed tabulations of GC-MS analysis of the extracts are given in Table 1 respectively. GCMS analysis of various compounds from zebra fish mucus was performed using Thermo GC Trace ultra Ver 5.0 and typical ion chromatogram(TIC) of the given sample.

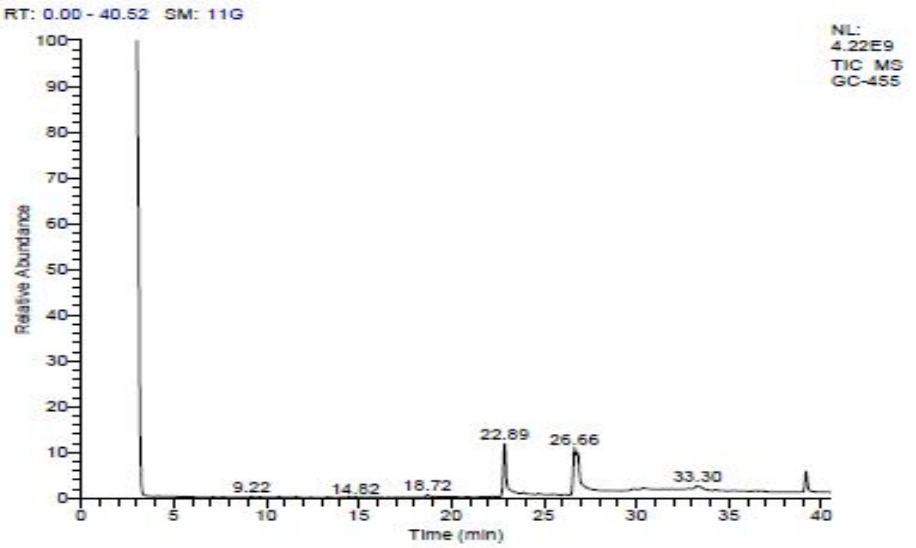
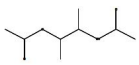
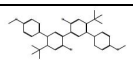
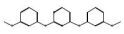
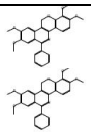
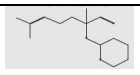
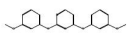
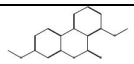
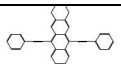
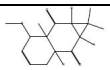
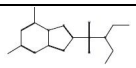
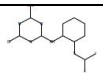


Figure 1. Total Ion Chromatogram (TIC) of Hexane extract of Zebra fish Mucus.

Table 1. Biochemical compounds identified in the Hexane Extract of the Zebra Fish Mucus.

S.No	RT	IUPAC Name	Molecular formula	Molecular weight	Chemical structure	Probability		Nature and uses
1	4.18	3-Acetamido-2-acetoxy butane	C8H15NO3	173		31.21		Clear liquid, may be toxic by inhalation or skin absorption and irritating to skin. used as a gasoline additive.
2	4.82	2,2- Dibromo-5,5-di (4-methoxyphenyl) 4,4-di – tert-butyl biphenyl.	C34H36Br202	634		65.57		Flavouring agent, Food additive and anti inflammatory.
3	11.61	2,6-bis (3- methoxy phenyl) sulfanyl pyrimidine	C18H16N202S2	356		40.52		It is used as food additives and flavouring agents.
4	14.51	12-phenyl-2,3,7,8-tetramethoxy-tetramethoxy-5H-(1)-benzopyrano(4,3-C) isoquinoline	C26H23No5	429		65.43		It is used in the manufacture of dyes, paints, insecticides and anti fungal.
5	19.08	Dimer of 2,3 dihydro-2,2,5 trimethyl-4-pyrone	C16H28O4	284		27.73		Used as flavour enhancer in the food industry.

6	19.84	2,6-bis(3-methoxyphenyl)sulfanyl pyrimidine	C18H16N2O2S2	356		27.27		Food additives and flavouring agents.
7	20.79	Ethane peroxoic acid , cyano cyclohexyl phenyl(methyl ester (CAS)	C16H19NO3	273		25.24		Fatty acid ester, used to produce detergents and biodiesel
8	22.89	3,7-Dimethoxy-6 H – dibenzo (b,d) pyran- 6-one	C15Hi2O4	256		49.38		Used as solvent, manufacture of perfumes, resins, adhesives, paint strippers.
9	26.15	5-Benzyl oxymethyl – 1-methylene 3,3,5-trimethyl-2-vinylcyclopentane	C19H260	270		15.47		Colourless liquid with petroleum odour, intermediate propellents and blowing agents and personal care products.
10	28.37	5,12-Bis(phenyl ethynyl)-7,8,9,10-tetrahydro naphthacene	C34H24	432		89.77		Polycyclic aromatic hydrocarbon, used in organic field effect transistors
11	30.41	1 H – cyclopropa (b) naphthalene – 2,7-dione , 1,2 a-trimethyl	C15H2OO3	248		26.82		Intermediate in the manufacture of dyes, antioxidant in the rubber industry
12	31.08	5,7- Dimethyl-(1,2,4)triazolo(1,5-a)pyrimidine-2-sulfonic acid diethylamide	C11H17N5O2S	283		7.65		Clear white odourless material, used as recreational drug and for spiritual reasons.
13	31.88	4-chloro – 6 – (2-difluromethylthio phenyl amino)	C10H8CIF2N5S	303		4.20		Volatilearomatic amine, Manufacture of polyurethane and other industrial chemicals.
14	34.28	7-bromo-p-menthane 1,8 diol	C10H19BrO2	250		3.25		Colourless liquid . a hair straightening formula.
15	35.26	14-(4- Nitrophenyl)-15-H-benzo(h)benzo(6,7)indolo(3,2-b)quinoline	C29H17N3O2	439		2.77		Colourless hygroscopic liquid, used in manufacture of dyes , solvent for resins and trepenes.
16	36.40	1,2, Benzene Di carboxylic acid butyl 2-methyl propyl ester (CAS)	C16H22O4	278		3.78		Solvent, used as solvent for lacquer and nitro cellulose
17	36.77	5-Hydroxyl- 1-(4-methoxy phenyl)5-pheny-phenyl-pyrolidine – 2,3-dione	C17H15NO4	297		2.43		Antibacterial, anti fungal, food additives , flavouring agents
18	37.74	(5z,7E) – 5,7 dodecadienal	C12H2OO	180		8.28		Aromatic substance, used as in butter and chips.

IV. DISCUSSION

The GCMS analysis of the hexane extract resulted in many compounds which have diverse use. Compounds having anti-inflammatory , anti fungal, skin conditioning and flavoring agents, dyeing agents have been identified(Ashby et al 1997).Mucus of the zebra fish also contain fatty acids. The compound 5- hydroxyl 1-(4-methoxyl-phenyl) has antibacterial, antifungal properties.(Sanahuja et al 2015). The compound 2,2 Di bromo 5-5 di(4-methoxyphenyl) contains flavouring agents and have anti-inflammatory activities (Easy et al 2012).Ethano peroxic acid is a fatty acid ester and used to produce detergents and biodiesel(Bhogaonkar 2006). The compound 7 bromo –p-methane 1,8 diol is a colourless liquid and it is used as a very famous hair straightening formula in many countries(Elizabeth Thomas et al 2013). 1-H-Cyclopropa(6) naphthalene is an intermediate in the manufacture of dyes and used as anti-oxidants in the rubber industry(Tanakol et al 1999).3 acetamido-acetoxybutane is a clear liquid and may be toxic by inhalation as a skin absorbent and irritating to skin. It is also used as a gasoline additives.

V. CONCLUSION

The present study explores the goodness of the mucus of zebrafish which has a commendable sense of purpose like anti bacterial, anti fungal, anti inflammatory and it can be used in pharmaceutical purposes.

VI. ACKNOWLEDGEMENT

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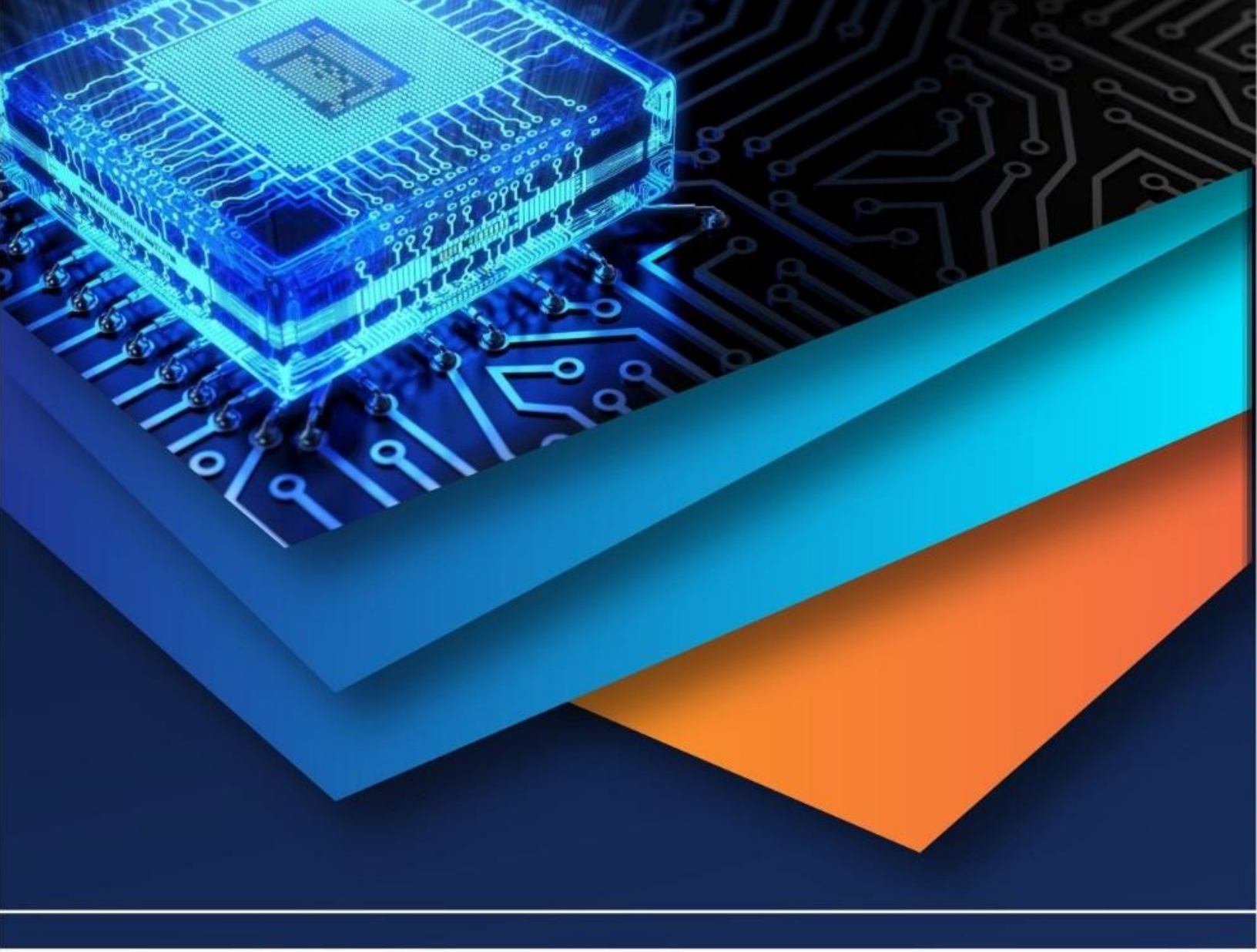

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