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Profiling And Potential Use Of Bioactive Molecules From The Skin Mucus Of Two Cat Fishes From India

Surbhi Chaudhari[1*], Harshil Patel[2], Anjali Soni[3]

[1*]Department of Microbiology, R.K. Desai College, Vapi 396191, Gujarat, India.
[2]Thackeray Wildlife Foundation, Mumbai 400051, Maharashtra, India.
[3]Department of Biotechnology, Veer Narmad South Gujarat University, Surat 395007, Gujarat, India.
*Corresponding author: Surbhi Chaudhari
*Email: surbhichaudhari38@gmail.com

Abstract
Fish skin mucus layers are the main surface of exchange between fish and the environment, and they possess important biological and ecological functions. It comprises of diverse bioactive metabolites which plays an immense role in defense mechanisms and other important cellular activities for the fish. Present study aims to screen the unexplored skin mucus extract of two catfishes from India namely, Ompok pabda and Pangasianodon hypophthalmus. Profiling of the skin mucus of both the species was carried out by High Resolution-Liquid Chromatography Mass Spectrometry (HR-LCMS) to investigate the composition and properties of cutaneous secretions and their potential uses are also discussed.

Keywords: *Ompok pabda, Pangasianodon hypophthalmus, HR-LCMS, Bioactive molecules, Antimicrobial compounds.*

1. INTRODUCTION

Cutaneous secretion, also known as skin Mucus, is the primary surface of exchange among fish and their surroundings, thus plays an important role in intra- as well as interspecific chemical communication (Todd et al. 1967; Beklioglu et al. 2006; Reverter et al. 2018). The skin Mucus acts as a vital biochemical and physical barrier, performing various biological and ecological roles including osmoregulation (Shephard 1993, 1994), protection against abrasion (Oosten 1957), defence against environmental toxins and heavy metal poisoning (Coello & Khan 1996), parental care and parental feeding (Chong et al. 2006), defence against pathogens (Gomez et al. 2013), and chemical communication (Leonard et al. 2012). Skin Mucus of Teleosts is almost identical to mammalian mucus and is largely made of mucins (Shephard 1993). Fish skin Mucus also contains countless immune molecules, such as lysozymes, immunoglobulins, complements, lectins, and antimicrobial peptides (AMPs) (Salinas 2015), and other molecules like mycosporine-like amino acids (MAAs) (Zamzow 2007), toxins, and kairomones that mediate interspecific interactions by providing information that benefits individuals of another species and harms the emitter (Purcell & Anderson 1995; Sugiyama et al. 2005).

Composition of fish skin Mucus and rheological properties of the mucus are essential for the maintenance of various functions of mucus (Lai et al. 2009). Mucus surfaces are vital matrices whose composition differs among different taxa and with different endogenous (sex and developmental stage) and exogenous factors (stress, water temperature, pH and infections) (Esteban 2012). Stress (e.g., stress due to handling, confinement, food deprivation, vulnerability to toxic substances) can alter the mucus composition along with its rate of production (e.g., level of proteins and immune molecules), compromising health of the fish and increasing the fish vulnerability to pathogenic bacterias (Al-Zaidan et al. 2012; Terova et al. 2011; Easy & Rose 2010; Liu et al. 2013). Mucus viscoelasticity controls its potential to block various types of motile bacteria (Cone 2009), and many previous studies demonstrated that fish have tendency to escalate their mucus production and alter composition of mucus when exposed to harmful microbes (Gustafsson et al. 2013; Rajan et al. 2013; Van Der Marel et al. 2010), which may be a mode of protection against such pathogens. Additionally, infections of such pathogens (e.g., virus, bacteria) is capable to alter the mucosal microflora of fish, facilitating the increase of such pathogenic microbes (Llewellyn et al. 2017; Reid et al. 2017).

The order Siluriformes, of the class Actinopterygii (Ray-finned fishes), comprises of a diverse group of ray-finned fishes, having prominent barbels and are commonly known as catfishes. The aim of the present study is to investigate the composition and properties of cutaneous secretions of two catfishes namely, *Ompok pabda* and *Pangasianodon hypophthalmus*. *O. pabda*, commonly known as Pabdah catfish, is a native fresh water catfish of

south Asia, found in variety of habitats ranging from small steams and ponds to large water bodies like rivers and lakes (Menon 1999). *P. hypophthalmus*, commonly known as Striped catfish, a native catfish to Mekong river basin and some other rivers of adjoining regions in Vietnam and Thailand. However, it has gained popularity among aquarium hobbyists and as a food source in recent years, and now it is cultured in many parts of Asia, including India (Singh & Lakra 2012). Apart from some basic studies concerned with systematics, distribution, anatomy, growth and basic ecology; many aspects of these catfishes are yet to be investigated, including the composition and properties of cutaneous secretions.

2. MATERIAL AND METHODS

live individuals of *Ompok pabda* and *Pangasionodon hypophthalmus* were collected from the native water bodies (*O. pabda*) and aquaculture farms (*P. hypophthalmus*) and transferred to the laboratory (Surat, India). A total of 10 fish specimens per species were maintained in a 1000 L of fish tank at a water temperature 27 ± 2°C and pH of 7 ± 2. The total length of the fish for *O. pabda* ranged from 12.3 to 21.7 cm and for *P. hypophthalmus* ranged from 18.2 to 28.6 cm. Half of the water in tank was changed on alternate days to retain hygiene conditions. They were daily monitored for their health, as only healthy fish were sampled for mucus collection and care was taken to remove any fish with lesions or laceration. They were fed every day with the prepared feed of wheat flour, rice bran, groundnut oil cake, and mixture of minerals at 4% of their body weight during the acclimation period.

After seven days of acclimation in laboratory conditions, fishes were starved for a day and washed with 2% of potassium permanganate before collection of mucus. Mucus sample was collected with the help of a sterile spatula by softly scraping from dorsal side in anterior to posterior direction, from head to tail, at regular intervals in a day. No anesthesia or chemical was used. Mucus samples were filtered before running them in the HR-LCMS.

Biochemical metabolites present in the mucus extracts were carried out using Ultra High-Performance Liquid Chromatography with Photodiode Array (UHPLC-PDA)-Detector Mass Spectrophotometer (HR-LCMS 1290 Infinity UHPLC System), Agilent Technologies®, Santa Clara, California, USA. The liquid chromatographic system consisted of a HiP sampler, binary gradient solvent pump, column compartment and quadrupole time of flight mass spectrometer (MS Q-TOF) with dual Agilent Jet Stream Electrospray (AJS ES) ion source. First, 10 µL of sample was injected into the system, followed by separation in SB-C18 column (2.1 × 50 mm, 1.8 µm particle size). Then, 1% formic acid in deionized water (solvent A) and acetonitrile (solvent B) were used as solvents. Flow rate of 0.350 mL/min was used, while, MS detection was performed in MS Q-TOF. Metabolites were identified via their mass spectra and their unique mass fragmentation patterns.

3. RESULTS

The mucus extract of both the cat fishes were analysed with the help of HR-LCMS for the identification of bioactive metabolites present in the fish skin mucus of the respective species. They were putatively recognized with their thorough mass spectra data, absorbance spectra, and retention times compared with human metabolome database. A large number of metabolites were identified from the skin mucus by using both positive (+ESI) and negative electrospray (-ESI) ionization (Figures 1 and 2). Skin mucus of both the species is composed of different types of bioactive metabolites including fatty acids, lipids, amino sugars, amino alcohols, small peptides, etc. (Table 1 and 2). A total of 84 compounds were identified in the cutaneous mucus of both the cat fish species. However, both species had different qualitative and quantitative distribution of the compounds. The maximum number of total identifiable compounds detected in a species was no more than 50, found in *O. pabda*; whereas 45 compounds were detected in *P. hypophthalmus*. 11 compounds were present in both the species (Figure 3), viz., Sultopride, Solanocapsine, Colneleic acid, C16 Sphinganine, Porson, Tridecyl phloretate, 7alpha,12alpha-Dihydroxy-5beta-chol-3-en-24-oicAcid, N-Pentadecylcyclohexanecarboxamide, PA(18:3(6Z,9Z,12Z)/20:1(11Z)), Arcaine, AL 34662.

In *O. pabda*, 35 compounds were found while using positive (+ESI) electrospray and remaining 15 compounds were detected while using negative (-ESI) electrospray. Similarly, in *P. hypophthalmus*, 31 compounds were found while using positive (+ESI) electrospray and remaining 14 compounds were detected while using negative (-ESI) electrospray.

4. DISCUSSION

During the past few decades, antimicrobial properties and potential of various natural elements have been the central pillar for researchers working for therapeutic innovations. Such natural elements are considered safer, as obtained from natural resources (Adnan et al. 2017a, 2017b, 2018; Patel et al. 2020), hence not affecting the surfaces and surroundings while acting upon them. A popular example is Hagfish, evolutionarily among the most primitive vertebrate species that lacks vital and required adaptive defence apparatus that includes antibody-based immunity and thymus, which are generally found in teleost fish (Raison & Dos Remedios 1998; Rolff 2007). They are known scavengers of the ocean's muddy bottom and live in adverse situations (Spitzer & Koch 1998). To flourish in such conditions without any defence components, they have a mechanism to secrete huge amount of skin mucus composed of efficient antimicrobial compounds, that may include bioactive peptides/proteins, lysozyme, and proteases (Subramanian et al. 2007). Therefore, in the quest of finding natural antimicrobial and bioactive compounds, we selected mucus extract of the two previously understudied cat fishes, *O.pabda* and *P. hypophthalmus*. The mucus extract of such potentially medicinally important fishes exhibited broad spectrum of bioactive compounds.

A large number of pathogenic and non-pathogenic microbes inhabit the aquatic ecosystems and fishes are always in connection with such surrounding. The epidermal mucus secretion and the epidermis itself acts as a natural barrier between the potential pathogenic microbes of its environment and fish (Shephard 1993). Fish mucus is multifaceted, as it plays a crucial role in various activities, such as communication, respiration, feeding, reproduction, excretion, ionic and osmotic regulation, nest building, and resistance to diseases (Button & Boucher 2008). Various studies have showed that fish mucus is as a potent source of novel antimicrobial compounds. It also serves as a first line of defence against pathogenic microbes (Austin & McIntosh 1988; Fouz et al. 1990; Hjelmeland et al. 1983; Grinde et al. 1988; Nagashima et al. 2001; Sarmaşik 2002).

Bioactive metabolites with antimicrobial potential and different classes of bioactive metabolites including fatty acids, lipids, amino sugars, amino alcohols, small peptides, etc., were found in the skin mucus of both *O.pabda* and *P. hypophthalmus*, via HR-LCMS analysis (Table 1 and 2). Metabolites from the skin mucus of *O.pabda* such as Amantadine, N-Acetylisatin, Solanocapsine, Colneleic acid, Porson, Bactobolin, Carbenicillin and N-Feruloyltyramine could play a pivotal role in antibacterial, antiviral, antifungal and antibiofilm potential of *O.pabda* mucus extract. Similarly, metabolites from the skin mucus of *P. hypophthalmus* such as Eudistomin C, D-Phenylalanine, Solanocapsine, Colneleic acid, Porson, (1R,6S)-gamma-himachalene, Methyl Arachidonyl Fluorophosphonate, Gentianine, Xestoaminol C, 4-O-Methylmelleolide and Methyl orsellinate could play an important role in antibacterial, antiviral, antifungal and antibiofilm potential of *P. hypophthalmus* mucus extract. It has been reported that free fatty acids constitute major part of fish mucus and contribute in protection against a variety of fungal and bacterial diseases similar to human sebum (Lewis 1970).

In addition to antimicrobial properties, Xestoaminol C and Guanine have shown to be anti-parasitic (Avila et al. 1987; Prommaban et al. 2020). Two other noteworthy metabolites, Bactobolin and Eudistomin C, reported in the present study have reported anti-tumour properties (Kondo et al. 1979; Gul & Hamann 2005). Collectively, the present study revealed that skin mucus of both the catfishes is rich with a diverse class of bioactive metabolites that may have an unexpected antimicrobial potential among other uses. Identified bioactive compounds and peptides should be tested individually for efficacy and potency, that will provide a far more realistic prediction of every compound and their peptide activity. Further studies to investigate the broad action of fish skin mucus should be conducted, and efforts should be made to investigate its therapeutic and pharmaceutical applications.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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FIGURE CAPTIONS:

Figure 1: Chromatogram of skin mucus of *Ompok pabda* produced by HR-LCMS. (A) positive mode, (B) negative mode.

Figure 2: Chromatogram of skin mucus of *Pangasianodon hypophthalmus* produced by HR-LCMS. (A) positive mode, (B) negative mode.

Figure 3: Chemical structures of compounds identified by HR-LCMS, that are present in skin mucus of both the species. (A) Sultopride, (B) Solanocapsine, (C) Colneleic acid, (D) C16 Sphinganine, (E) Porson, (F) Tridecyl phloretate, (G) 7alpha,12alpha-Dihydroxy-5beta-chol-3-en-24-oic Acid, (H) N-Pentadecylcyclohexanecarboxamide, (I) PA(18:3(6Z,9Z,12Z)/20:1(11Z)), (J) Arcaine, (K) AL 34662.

Fig.1

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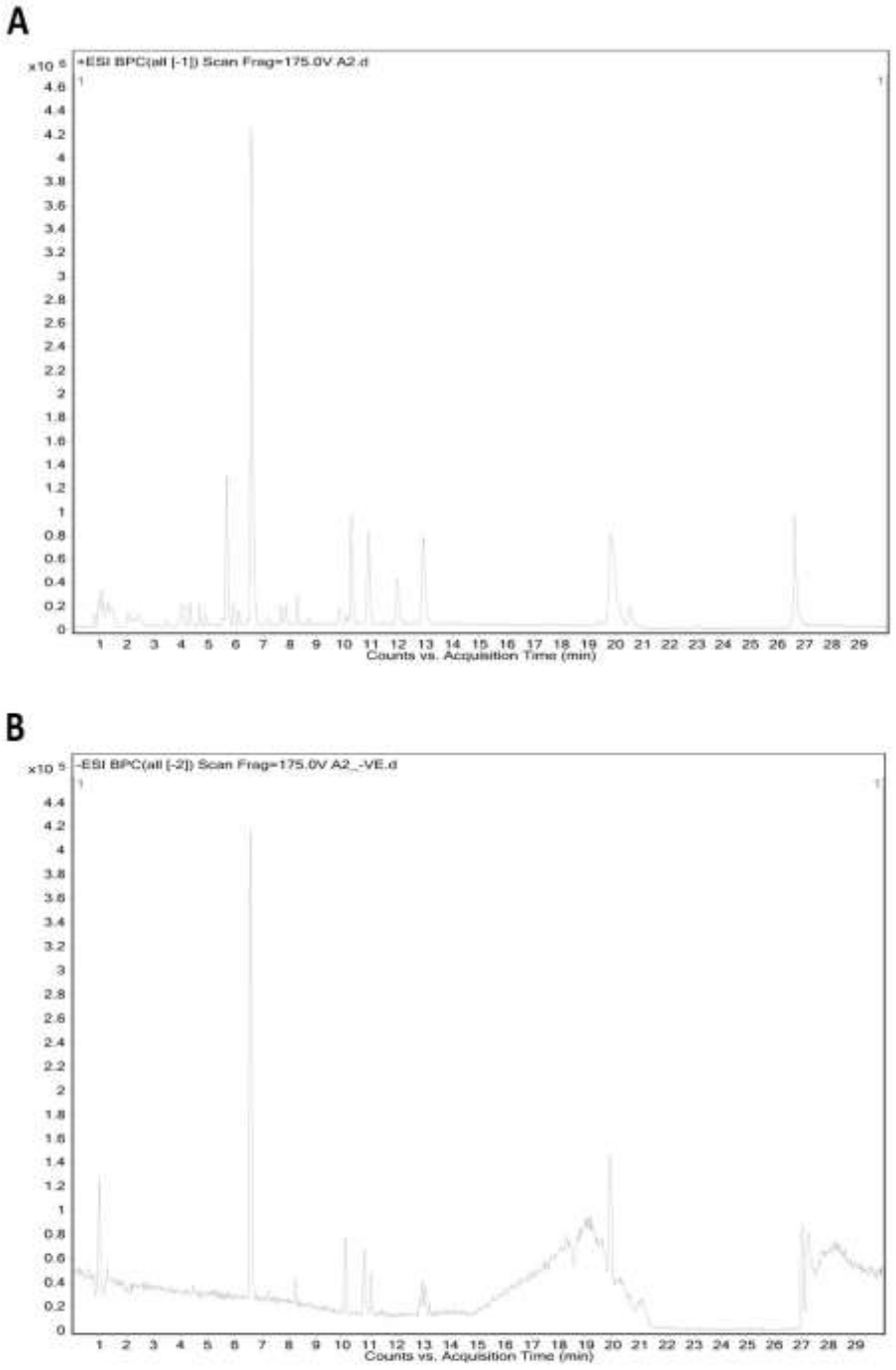

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Fig. 2

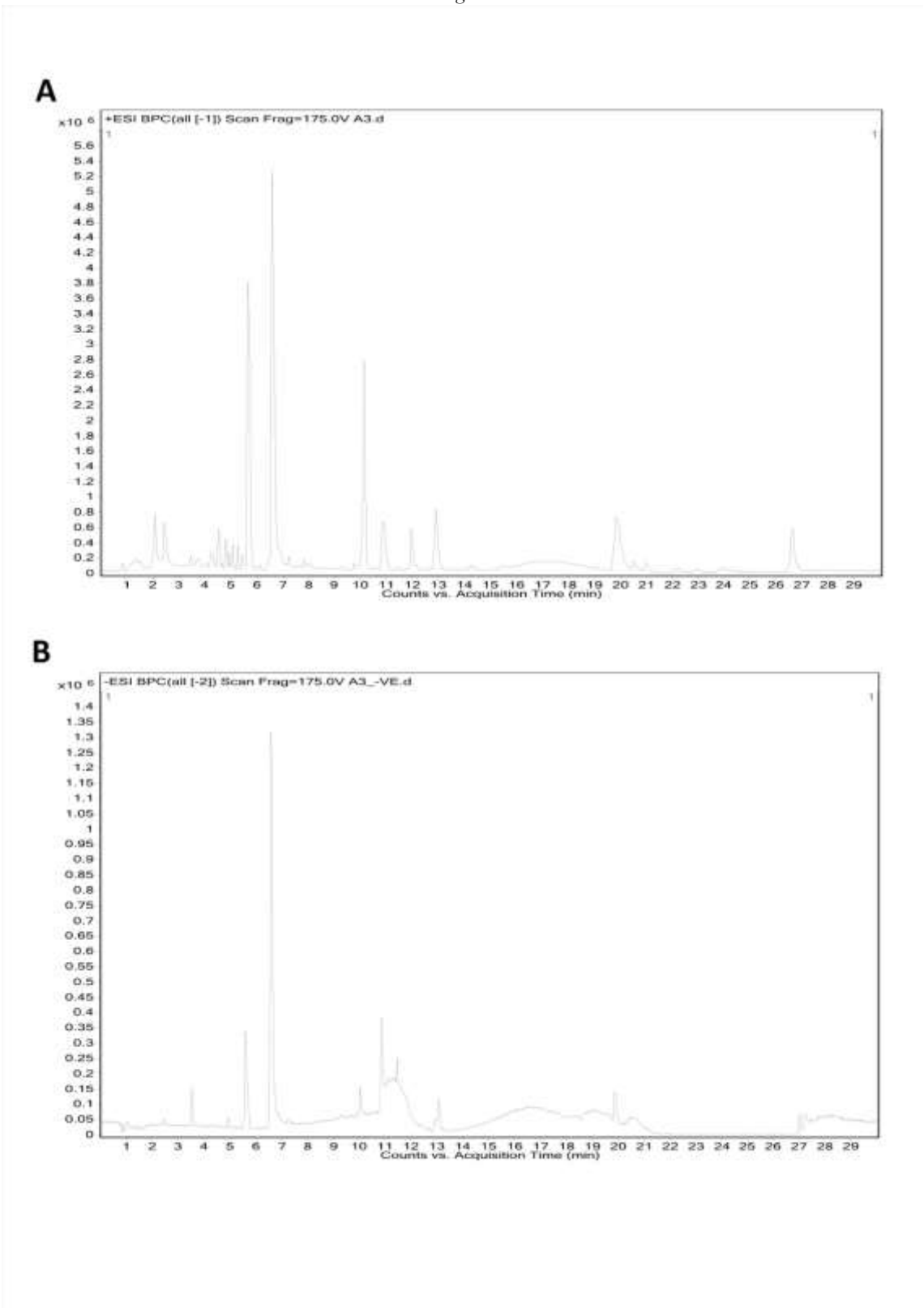

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Fig. 3

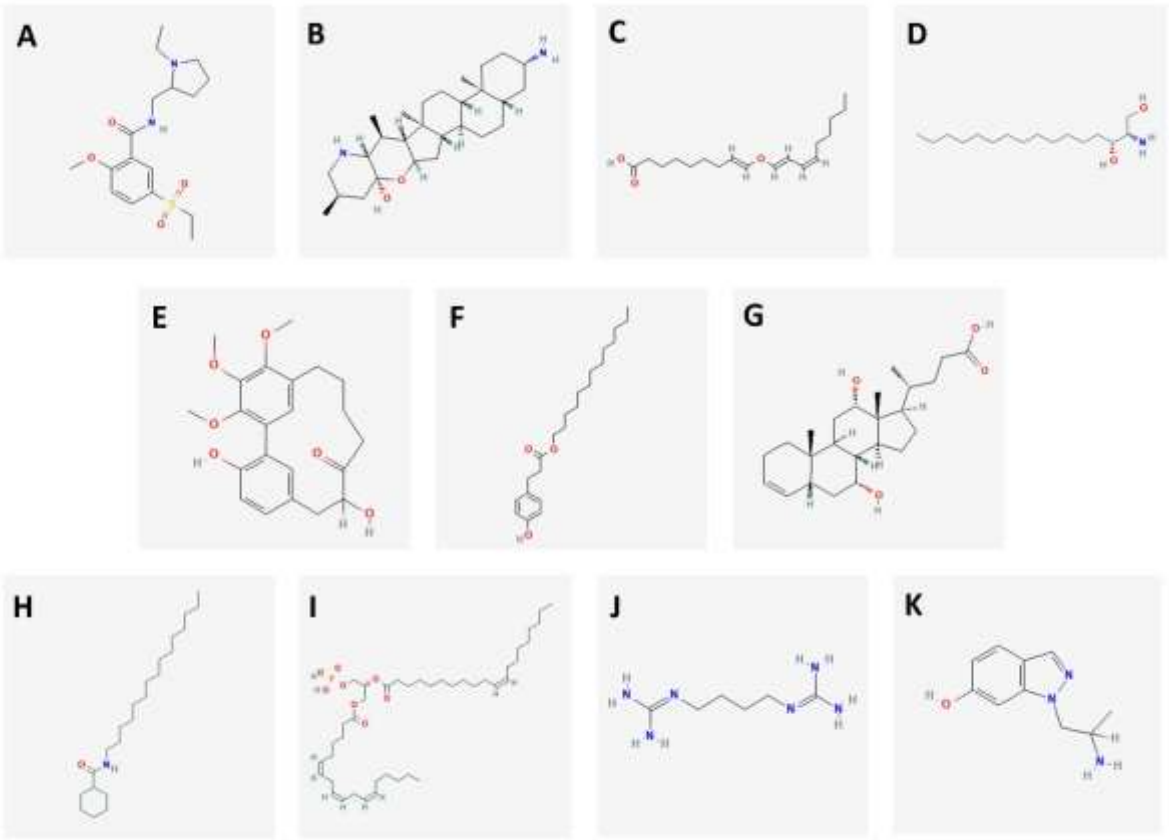

Table 1: Identified major bioactive metabolites by HR-LCMS from *Ompok pabda* mucus extract with their bioactivity.

No	Compound Name	RT	Mass	Formula	Mode of Analysis	Reported biological activity	Reference
1	3-(3,4,5-Trimethoxyphenyl)propanoic acid	0.964	240.102	C12 H16 O5	Positive		
2	2-Butyl-5-methyl-4-propyloxazole	1.187	181.148	C11 H19 N O	Positive		
3	Amantadine	1.198	151.137	C10 H17 N	Positive	antiviral and dopaminergic activity	Rejdak et al. 2022
4	Nitrazepam	1.263	281.08	C15 H11 N3 O3	Positive	anti-anxiety agent and anticovasulant	Podhorna & Krsiak 2000
5	Leu Val Glu	1.503	359.21	C16 H29 N3 O6	Positive		
6	3,5-Dihydroxy-phenylglycine	1.555	183.055	C8 H9 N O4	Positive	Excitatory Amino Acid Agonists	Haure-Mirande

							et al. 2019
7	p-Hydroxynorpseudoephedrine	1.571	167.097	C9 H13 N O2	Positive		
8	DL-Norephedrine	2.052	151.102	C9 H13 N O	Positive		
9	Guanine	2.304	151.051	C5 H5 N5 O	Positive	antiprotozoal	Avila et al. 1987
10	N-Acetylisatin	4.106	189.045	C10 H7 N O3	Positive	antibacterial activity against different gram-positive and gram-negative bacteria	Olomola et al. 2013
11	Methyldopexamine	4.313	370.262	C23 H34 N2 O2	Positive		
12	Sultopride	4.745	354.161	C17 H26 N2 O4 S	Positive		
13	Lys Pro Lys	4.916	371.246	C17 H33 N5 O4	Positive		
14	17alpha-Estradiol-3-glucosiduronic-acid	5.621	448.21	C24 H32 O8	Positive		
15	Solanocapsine	5.704	430.36	C27 H46 N2 O2	Positive	antibacterial activity	Boll et al. 1956
16	5beta-Cholestane-3alpha,6beta,7beta,25,26-pentol	5.772	452.342	C27 H48 O5	Positive		
17	Vanillyl alcohol	6.495	154.065	C8 H10 O3	Positive		
18	DG(20:2(11Z,14Z)/20:5(5Z,8Z,11Z,14Z,17Z)/0:0)	6.619	656.529	C42 H72 O5	Positive		
19	1-Ipomeanol	7.642	168.081	C9 H12 O3	Positive		
20	Ro 31-7549	8.25	398.175	C24 H22 N4 O2	Positive	Protein Kinaze inhibitor	Kim et al. 2016
21	Istamycin B1	9.797	417.262	C18 H35 N5 O6	Positive		
22	Pro Glu	9.827	244.103	C10 H16 N2 O5	Positive		

23	Colneleic acid	9.838	294.223	C18 H30 O3	Positive	antifungal activity	Croft et al. 1993
24	Val Ile Leu	10.09	343.24	C17 H33 N3 O4	Positive		
25	C16 Sphinganine	10.15	273.27	C16 H35 N O2	Positive		
26	Porson	10.91	386.177	C22 H26 O6	Positive	anti-tubercular (antibacterial) activity	Ting et al. 2014
27	beta-Butoxyethyl nicotinate	11.15	223.124	C12 H17 N O3	Positive		
28	Armillarin	11.92	414.209	C24 H30 O6	Positive		
29	Tridecyl phloretate	13.26	348.27	C22 H36 O3	Positive		
30	7alpha,12alpha-Dihydroxy-5beta-chol-3-en-24-oic Acid	19.62	390.281	C24 H38 O4	Positive		
31	Boviquinone 4	19.69	412.264	C26 H36 O4	Positive		
32	N-Pentadecylcyclohexanecarboxamide	20.5	337.339	C22 H43 N O	Positive		
33	10-Hydroxydesipramine	26.56	282.171	C18 H22 N2 O	Positive		
34	Bactobolin	26.59	382.068	C14 H20 Cl2 N2 O6	Positive	antibacterial activity, anti-tumour activity	Kondo et al. 1979
35	S-Farnesyl Thioacetic Acid	26.61	296.181	C17 H28 O2 S	Positive		
36	2,4-Dichlorotoluene	0.924	159.985	C7 H6 Cl2	Negative		
37	Carbenicillin	1.084	378.09	C17 H18 N2 O6 S	Negative	antibacterial activity	Butler et al. 1970
38	5'-Butyrylphosphouridine	1.09	394.083	C13 H19 N2 O10 P	Negative		
39	2-Hydroxyethanesulfonate	1.092	125.999	C2 H6 O4 S	Negative		
40	Thiosulfic acid	1.133	113.944	H2 O3 S2	Negative		
41	Isocitrate	1.279	192.025	C6 H8 O7	Negative		

42	PA(18:3(6Z,9Z,12Z)/20:1(11Z))	6.582	724.504	C41 H73 O8 P	Negative		
43	N-Feruloyltyramine	7.226	313.131	C18 H19 N O4	Negative	antibacterial activity	Samita et al. 2016
44	Arcaine	10.07	172.144	C6 H16 N6	Negative		
45	Tanacetol A	11.02	294.181	C17 H26 O4	Negative		
46	AL 34662	11.06	191.105	C10 H13 N3 O	Negative		
47	10-Oxo-13-hydroxy-11-octadecenoic acid	12.9	312.228	C18 H32 O4	Negative		
48	3alpha,15-Dihydroxymarasmene	17.87	266.152	C15 H22 O4	Negative		
49	2,4-Dimethyl-tetradecanoic acid	18.54	256.238	C16 H32 O2	Negative		
50	Oleic acid(d2)	19.85	284.269	C18 H32 D2 O2	Negative		

Table 2: Identified major bioactive metabolites by HR-LCMS from *Pangasianodon hypophthalmus* mucus extract with their bioactivity.

No	Compound Name	RT	Mass	Formula	Mode of Aalysis	Reported biological activity	Reference
1	Ethyl 3-iodo-2E-acrylate	0.87	225.9465	C5 H7 I O2	Positive		
2	Eudistomin C	1.864	369.0143	C14 H16 Br N3 O2 S	Positive	antiviral and anti-tumor activity	Gul & Hamann 2005
3	D-Phenylalanine	2.081	165.0808	C9 H11 N O2	Positive	antibacterial activity	Kawai & Nagai 1978
4	Eudistomin C	2.25	369.0145	C14 H16 Br N3 O2 S	Positive	antiviral and anti-tumor activity	Gul & Hamann 2005
5	trans-Zeatin	2.443	219.1126	C10 H13 N5 O	Positive		
6	Glycerol tripropanoate	3.245	260.1263	C12 H20 O6	Positive		
7	10-Hydroxynortryptyline	3.567	279.1639	C19 H21 N O	Positive		
8	(1R,6S)-gamma-himachalene	3.788	204.1885	C15 H24	Positive	Antimicrobial and antioxidant	Choudhury et al. 2008
9	Methyl Arachidonyl Fluorophosphonate	4.433	370.2427	C21 H36 F O2 P	Positive	Antimicrobial and anti-tubercular	Yang et al. 2020
10	Lys Gln Ile	4.636	387.2507	C17 H33 N5 O5	Positive		

11	Sultopride	4.685	354.1605	C17 H26 N2 O4 S	Positive		
12	3-Hydroxy-10'-apo-b,y-carotenal	4.843	392.2678	C27 H36 O2	Positive		
13	1-(O-alpha-D-glucopyranosyl)- 3-keto-(1,25R)-hexacosanediol	5.531	574.4491	C32 H62 O8	Positive		
14	Solanocapsine	5.682	430.3583	C27 H46 N2 O2	Positive	antibacterial activity	Boll et al. 1956
15	Malyngamide J	5.688	607.3829	C33 H53 N O9	Positive		
16	5beta-Cholestane-3alpha,6beta,7beta,25,26- pentol	5.772	452.3408	C27 H48 O5	Positive		
17	PE(15:0/18:2(9Z,12Z))	6.705	700.4932	C38 H72 N O8 P	Positive		
18	DG(19:0/20:5(5Z,8Z,11Z,14Z, 17Z)/0:0)[iso2]	6.768	656.5284	C42 H72 O5	Positive		
19	Gentianine	6.913	175.0649	C10 H9 N O2	Positive	antibacterial activity against different gram-positive and gram-negative bacteria	Mansoor et al. 1999
20	17-[[3-(1-Pyrrolidinyl)propyl]imino]androst-5-en-3beta-ol acetate Pyrrolidinyl)propyl]imino]andr ost-5-en-3beta-ol acetate	7.202	440.3404	C28 H44 N2 O2	Positive		
21	Colneleic acid	9.836	294.2227	C18 H30 O3	Positive	antifungal activity	Croft et al. 1993
22	C16 Sphinganine	10.141	273.2707	C16 H35 N O2	Positive		
23	Xestoaminol C	10.21	229.2434	C14 H31 N O	Positive	antimicrobial, antiparasitic, anti-tubercular and antifungal activities	Prommaban et al. 2020
24	Porson	10.869	386.177	C22 H26 O6	Positive	anti-tubercular (antibacterial) activity	Ting et al. 2014
25	4-O-Methylmelleolide	11.951	414.2088	C24 H30 O6	Positive	antimicrobial activity	yoon et al. 1994
26	5-epi-5-J2-IsoP	12.941	334.2158	C20 H30 O4	Positive		
27	D-erythro-Sphingosine C-15	14.286	257.2385	C15 H31 N O2	Positive		
28	Tridecyl phloretate	15.495	348.2699	C22 H36 O3	Positive		
29	PE(18:1(11Z)/14:0)	16.646	689.5065	C37 H72 N O8 P	Positive		
30	7alpha,12alpha-Dihydroxy-5beta-chol-3-en-24-oic Acid	19.763	390.2809	C24 H38 O4	Positive		

31	N-Pentadecylcyclohexanecarboxamide	20.495	337.338	C22 H43 N O	Positive		
32	Mitotane	0.87	317.9531	C14 H10 Cl4	Negative		
33	Methyl orsellinate	3.567	182.0564	C9 H10 O4	Negative	antifungal activity	Thadani et al. 2012
34	Phenylacetaldehyde	5.613	120.0564	C8 H8 O	Negative		
35	Mandelic acid, methyl ester	5.668	166.0615	C9 H10 O3	Negative		
36	2-(9R-(5Z,9Z-tetracosadienoyloxy)-3-methyl-2Z-decenoyloxy)-ethanesulfonic acid	6.625	654.4537	C37 H66 O7 S	Negative		
37	PA(18:3(6Z,9Z,12Z)/20:1(11Z))	6.663	724.5042	C41 H73 O8 P	Negative		
38	Arcaine	10.074	172.1437	C6 H16 N6	Negative		
39	C.I. 14700	10.782	436.0398	C18 H16 N2 O7 S2	Negative		
40	9-Hydroxydecanoic acid	10.897	188.1388	C10 H20 O3	Negative		
41	AL 34662	11.068	191.1046	C10 H13 N3 O	Negative		
42	9R,10S-dihydroxy-stearic acid	13.137	316.2585	C18 H36 O4	Negative		
43	Doisynoestrol	17.146	298.1579	C19 H22 O3	Negative		
44	Centrolobine	19.882	312.1729	C20 H24 O3	Negative		
45	Methyl (9Z)-10'-oxo-6,10'-diapo-6-carotenoate	20.321	312.1731	C20 H24 O3	Negative		