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Investigation of functional groups in epidermal mucus of endemic bagridae fishes of Western Ghats

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Abstract
The epidermal mucus secreted by the epidermal cells of Bagridae fishes help to reduce the body friction against the water, play a vital role in maintaining the homeostasis and innate immune system to prevent the entry of invading pathogens. A preliminary investigation was carried out to isolate the epidermal mucus from two endemic and endangered Catfish species of Western Ghats *viz., Hemibagrus punctatus* and *Sperata aorides*. The isolated epidermal mucus of two catfishes were subjected to FT-IR analysis to find out the functional groups present in the mucus. The result of the present study revealed that the functional groups present in the mucus samples of two species are slightly varied. The alcohol as OH stretch at peak value 3900 – 3300cm^{-1},primary amide is present as C═O stretching of proteins at 1643cm^{-1} and secondary amide is present as N–H bending and C–N stretching of proteins at 1550 cm^{-1},alkane as C=C bending at 680-670 cm^{-1},halo compounds and iodo compounds as C-Br / C-Cl and C-I stretching at 670 – 400 cm^{-1}are present in the epidermal mucous of the catfishes.

Keywords: Catfishes, epidermal mucus, FT-IR, functional groups

Introduction
India has a rich diversity of fish fauna with nearly 11% of the total fish species of the world [1]. Fishes have a significant role in food. In fishes, generally the body surface is covered by a mucus coat, secreted by skin gland cells. The mucus coat significantly varies in kinds of nature like thickness and viscosity in different fish species and this mucus plays a significant role in mechanical and physiological protection. Certain fishes and their by-items add valuable to ayurvedic and Unani drugs for the treatment of duodenal ulcers, skin sicknesses, night visual deficiency, general shortcoming, loss of hunger, colds, hacks, bronchitis, asthma, tuberculosis, and so forth.The fishes have the number of accessory structures interspersed among their skin connective tissues that greatly extend the apparent simple function of a body covering. Among the most evident of these structures are the mucus cells which produce the slimy lubricating secretion so evident when handling fish. Epidermal mucus is a significant part of the inborn invulnerable system in fish and gives a first physical and substance hindrance against microorganisms. Few quantitative chemicals are available on the chemistry of fish epidermal secretions and the secretion of fishes and mammals have many similarities [2]. A major product of epidermal mucus cells is mucin, a mixture of glycoproteins joined by disulphide bonds, a protein core with rich amino acids threonine, serine and proline, 70 to 80% dry weight carbohydrate, and considerable structural variability due to the presence of various functional groups [3]. The highly hydrated complex proteoglycan molecules are responsible for the viscous properties of mucus and account for the slippery, coating properties of fish skin secretions. The structure of the epidermal mucus is an entire factor among species and inside species, sex, formative stages, and natural conditions.
Catfishes generally have thick skin and is covered by gel-like epidermal mucus. The mucus is mostly proteinous and possesses antimicrobial peptides for its defence system [4]. The family Bagridae, commonly found throughout fresh and brackish water bodies in Asia and Africa, consists of 244 species belongs to the 19 genera. In the last decade 1 new genus, 23 new species was documented from this family. Bagridae, one of the largest catfish family recognised by [5]. Among the 19 genera, the genus of *Sperata* and *Hemibagrus* are also important economic values group but less studies of taxonomy status, distribution and biology, still need to explore these groups. Present study was carried to find out the functional groups present in the mucus composition by using FTIR analysis of *Sperata aorides* and *Hemibagrus*

punctatus which are endemic to Cauvery River, Western Ghats.

Materials and methodology

1. Study area

Fishes were collected from morning time while fishing by the native fishermen by using a gill net. The selected fishes are *Hemibagrus punctatus* (figure.1) which is an endemic and endangered catfish to the Western Ghats captured at Nellithurai at Coimbatore district in Bhavani River and *Sperata aorides* (figure.2) which is endemic catfish of river Cauvery that was captured at Polampatti in Erode district. Both the catfishes are commercial catfishes available in local markets in that area.

2. Collection of samples

Mucous of two different species was collected in different sterilized 5 ml sample tube with a sterilized spatula by gentle scraping as soon as after we captured the fish to prevent the mixing of mucus of other fishes. After that spatula is wiped and cleaned by ethanol and dried up before being used for the sample collection. The samples were named F1 and F2 for each sample for future analysis. The collected samples were kept in the deep freezer at -40 ºC [6].

3. Analysis of sample

I. Preparation of sample

After being taken out from the freezer the samples were thawed at normal room temperature. 0.5 ml of skin mucous was taken out for the analysis.A small drop of the sample is placed on one of the KBr plates. The second plate is placed on top and makes a quarter turn to obtain a nice even film. The plates are placed into the sample holder and run a spectrum. After this procedure, the plate was thoroughly cleaned to prevent contamination of future samples. The windows are wiped with a tissue, then wash several times with methylene chloride then ethanol.

II. FTIR analysis

The samples were analyzed by using Shimadzu FTIR instrument. The emitted radiation from an IR source passes through an interferometer composed of a beam splitter, a fixed mirror, and a moving mirror. The interferometer measures the wavelength of emitted light via interference patterns that help to increase accuracy [7]. IR spectra are obtained by applying IR radiation to a sample and measuring the intensity of the passing radiation at a specific wavenumber. The number of scans can be adjusted based on the quality requirement for the sample analysis; currently, the most common number of scans used is 45 with a resolution of 16cm^{-1}.

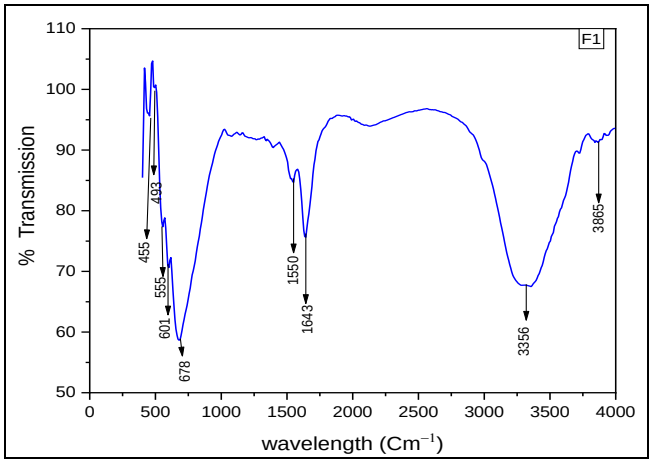
III. Analysis of results

IR radiation of certain molecular groups can be detected at specific wave numbers. The x-axis of the spectrum represents the wave number while the y axis represents absorbance or % transmittance.The graph was drawn by using origin 2018 software then the values are interpreted by using IR-pal software to know about the functional groups present in the given samples.

Result

The FTIR analysis was carried with skin mucous of two different species of catfishes. The peak values are incorporated in the graph was entered on the IR- pal software to know about the functional groups present in the samples. The values of the peak are shown in graph 1 and 2.In general, the IR spectrum can be split into four regions for interpretation, 4000-2500 cm^{-1} means absorption of single bonds formed by hydrogen and other elements, 2500-2000 cm^{-1} means absorption of triple bonds, 2000-1500 cm^{-1} means absorption of double bond 1500-400 cm^{-1} means region consist of many different complicated bands, called fingerprint region, it is rarely used for identification of the functional group. Table 1 and 2 shows similarity among elements present between the regions 3800- 3000 cm^{-1} has water and alcohol as OH stretch, 1700- 1500 cm^{-1} has amide, here there is the presence of two types of amides primary amide and secondary amide. At 1643 cm^{-1} primary amide is present as C═O stretching of proteins, at 1550 cm^{-1} secondary amide is present as N–H bending and C–N stretching of proteins. The fingerprint region on the graph of the two fishes has the same element in the region of 680-670 cm^{-1} alkane as C=C bending is present, from 670 – 400 cm^{-1} halo compounds and iodo compounds as C-Br/C-Cl and C-I stretching are present. In skin mucous of *Sperata aorides*, at the region 1300-1000 cm^{-1} ester group is present.

Fig 1: *Hemibagrus punctatus*



Graph 1: Shows the result for epidermal mucus of *Hemibagrus punctatus*

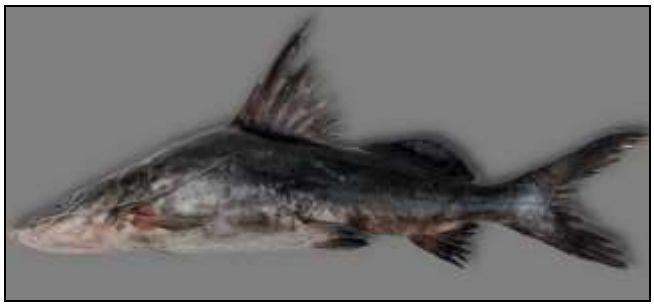
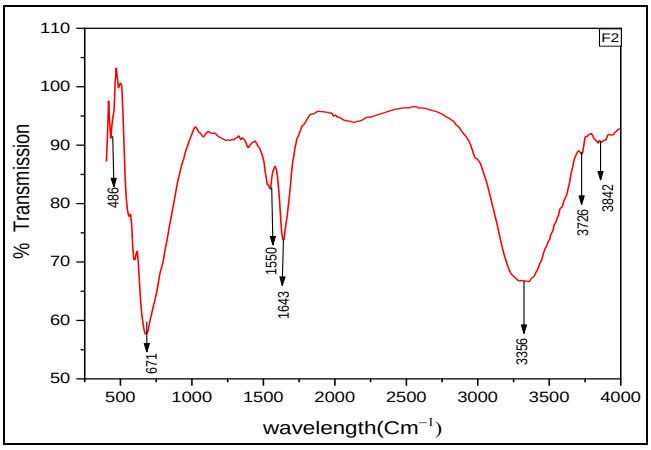


Fig 2: *Sperata aorides*



Graph 2: Shows the result for epidermal mucus of *Sperata aorides*

Table 1: Table for graph 1- FTIR result for epidermal mucus of *Hemibagrus punctatus*

Wavenumber (cm^{-1})	Definition of spectral arrangement
3865	OH stretch
3356	RCO-OH dimer OH - carboxylic acid
1643	Amide I: mainly C═O stretching of proteins
1550	Amide II: N–H bending and C–N stretching of proteins
678	Alkane, C=C bending
601	RC#CH #C-H bend, halo compounds
555	C-Br stretching, halo compounds
493	C-I stretching
455	C-I stretching

Table 2: Table for graph 2- FTIR result for epidermal mucus for *Sperata aroides*

Wavenumber (cm^{-1})	Definition of spectral arrangement
3842	OH stretch
3726	OH stretch
3356	RCO-OH dimer OH – carboxylic acid
1643	Amide I: mainly C═O stretching of proteins
1550	Amide II: N–H bending and C–N stretching of proteins
1396	RCO-O-, C-O stretch
1080	ESTERS, RCOOR C-O stretch
671	Alkane, C=C bending
594	C-Br stretching, halo compounds
563	C-Br stretching, halo compounds
486	C-I stretching, aliphatic iodo compounds
432	C-I stretching, aliphatic iodo compounds

Discussion

Secretions from epidermal mucus cells meet many different needs. They are carefully controlled and can be selectively stimulated by a variety of stimuli. Catfishes have moderate to high levels of epidermal mucus secretions also have proteinaceous club cells distributed widely throughout the epidermis. The skin mucus secreted by the catfishes have the properties such as an anti-predator defensive mechanism, analogues to repellent, or venom or secreted alarm substance. It can repair the injury [5]. The biochemical compositions of the skin mucous of catfishes are mostly protein and it consists of a trace amount of carbohydrate and lipids [7]. The skin mucus of some catfish has the property of healing non-diabetic foot ulcers, chronic back pain and some neurological disorders [8]. Antimicrobial compounds have been found associated with the epithelial mucus secreting cells of fishes. Inhibition effect may be due to the pore-forming properties against several bacterial strains and these suggested that fish secrete antibacterial proteins able to permeable the membrane of the target cell and thus act as a defence barrier [9]. This work is the basic study about the fish mucus by using the FT-infrared spectrum. IR spectroscopy is an analytical tool able to detect the characteristic vibrational modes of individual chemical groups and bonds. It has been used to examine and provide important data on a wide variety of biological molecules [10]. In this FTIR result, the functional group region 4000-2000 cm^{-1} are similar in two species such as water and alcohol as OH stretch. Here there is also the presence of amide groups it refers to the proteins, as the primary amide and secondary amides. In the fingerprint region ester group is present in the *Sperata sp.* and alkanes, halo compounds and iodo compounds are present in the mucus of both the species as common. The present study shows the functional groups present in the skin mucous of the cat fishes *Hemibagus punctatus* and *Sperata aorides*. By using the epidermal mucus, it can be commendable for its anti-microbial, anti-inflammatory and anti-coagulant properties.

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