Content list available at http://epubs.icar.org.in, www.kiran.nic.in; ISSN: 0970-6429

Indian Journal of Hill Farming

December 2022, Volume 35, Issue 2, Page 47-52

# Antifungal activity of integumentary extract of selected freshwater fish species against selected microbes

N. P. Devi • S.K. Das • R.K. Sanjukta • S. G. Singh • N. U. Singh

ICAR-Research Complex for NEH Region, Umiam- 793103, Meghalaya

ARTICLE INFO

***Article history:***
Received: 22 September, 2022
Revision: 30 September, 2022
Accepted: 02 December, 2022

***Key words:*** *Antifungal activity, Freshwater fish species, Integumentary extracts, antifungal agent, Heteropneustes fossilis.*

**DOI:** 10.56678/iahf-2022.35.02.8

ABSTRACT

The present study reports on the antifungal activity of integumentary secretions of four selected freshwater fish species *viz.*, Catla (*Labeo catla*), Mrigal (*Cirrhinus mrigala*), and Magur (*Clarias batrachus*) *and* Singhi (*Heteropneustes fossilis*) against the four important fungal strains namely *Aspergillus fumigatus, Aspergillus flavus, Candida albicans and Fusarium solani*. The result showed that different fish species have exhibited a varying level of inhibition effect on the selected fungal strains growth. Comparatively, the integumentary extract of Singhi has produced the best-controlling effect on the growth of selected fungal strains where the highest antifungal activity was shown against *A. fumigatus* (16±0.71 mm). The integumentary extract of Catla, when compared to its equivalents, exhibits the lowest antifungal activity among the fish species. The integumentary extracts of various fish species were discovered to have an inhibitory impact on the fungal species of *A. fumigatus*, however this effect was found to be least effective on these species. Overall, our findings indicated that the fish integumentary extracts of Singhi and Magur had antifungal properties that were comparable to those of the positive control ketoconazole and may therefore be advised for the future synthesis of prospective antifungal medicines.

## 1. Introduction

The consumption and demand for fish production and its related valued added products have been increasing day by day since, most people considered fish as healthy food and a good source of protein (FAO 2012). By considering the importance of fish as the main food item and for its sustainable production at long run, the quality of the water ecosystem equally need to be a good and productive one. Disease is considered as one of the deciding factors for successful production of fish (Pethkar and Lokhande 2017). Moreover, the changing climate and its associated phenomenon along with the rising potential threat of water pollution would add to the impeding cause of fish production (Devi *et al.*, 2019). Specifically, fish live in the exposed environment where several microbes of diverse nature are also inhabited. Under such conditions, fish exhibited self-defense mechanism generally refers to innate immune system to escape the negative impact on their growth (Balasubramanian *et al.*, 2012). And it is noteworthy to mention that the issue of overdoing the application of antibiotics and its subsequent multi-resistant results has led to the more use of natural origin substances to fight against fish diseases (Benin and Dowell 2001).

It is generally believed that fish excretes protective substance (mucus) from their epidermal layer and act as a self-defensive mechanism. This secretive substance not only acts as disease resistance but also helps in carrying out several mechanisms of fish growth viz, respiration, communication, etc., (Ingram 1980). However, the quality and quantity of mucus secretion are highly influenced by the aquatic environmental condition. In this contention, it is highly needed to understand fish inherent characteristics and its interaction with their surroundings. Although studies related to the antibacterial properties of fish is being conducted across the country including the Northeastern part of India. However, there is little or meager information on the antifungal activity of different freshwater fish species, particularly in Northeastern state of India. In this context, the present studies were formulated with the aim to assess the antifungal property of some of the major freshwater fish

*Corresponding author: peetambarining@gmail.com

species inhabited in Northeastern region of Indian thereby helping in developing new agents based on natural origin to prevent fish diseases and thus improving the economic status of the region.

## 2. Materials and Methods

### *Collection of fish*

To evaluate the antifungal property of selected freshwater fish species *viz*., Catla (*Labeo catla*) and Mrigal (*Cirrhinus mrigala*) were procured from the experimental farm of the ICAR-Research Complex for North-eastern Region, Umiam, Meghalaya, and Magur (*Clarias batrachus*) *and* Singhi (*Heteropneustes fossilis*) were sampled from the wild. The collected fishes were brought to the laboratory and fed with an artificial feed for five days to acclimatize the fishes under laboratory conditions.

### *Preparation of integumentary extract*

The fish integumentary extraction was performed in accordance with method described by Balasubramanian *et al.* (2012). Extraction was done in aseptic condition and the final supernatant was made after processing with a centrifuge at 1500 rpm for fifteen minutes and stored at 4°C.

### *Selection of fungal strains*

The antifungal activity of different freshwater fish species was assessed against the four selected fungal species *viz., Aspergillus fumigatus, Aspergillus flavus, Candida albicans and Fusarium solani*. The selected fungal strains were procured from HiMedia Laboratories.

### *Antifungal activity assessment*

Assessment of antifungal activity of different freshwater fish species was carried out by following the Brumfitt *et al.* (1990). For this, Potato Dextrose Agar media was used for inoculation purpose by keeping 2-3 days at 25°C.−Then the paper disc was infused aseptically on the media. Following this, the integumentary extracts of different fish species are loaded on the respective plates of selected microbes' triplicate. The growths of fungal infection were recorded by observing zone of inhibition (mm) after incubating the plates for 2 days at 25°C. Lastly, the recorded results were compared with performance of the positive control antibiotic Ketoconazole.

### *Statistical Analysis*

In order to elucidate the comparative performance of different species, one-way ANOVA, Duncan's Multiple Range Test (DMRT) at $p < 0.05$ was carried out using SPSS 17.0 (SPSS Inc., Chicago, USA).

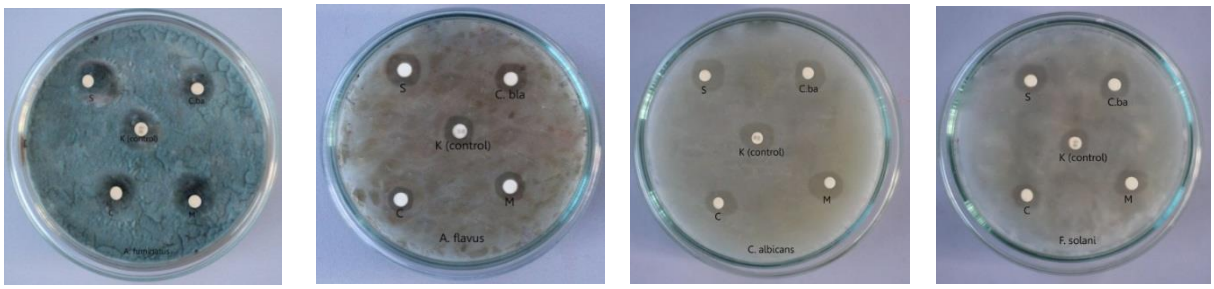
## 3. Results

The antifungal activity of integumentary extract of *Labeo catla, Cirrhinus mrigala, Clarias batrachus and Heteropneustes fossilis* against the four selected fungal species *viz., Aspergillus fumigatus, Aspergillus flavus, Candida albicans and Fusarium solani* showed significant ($p<0.05$) variation (Table 1). Among the fish species, maximum zone of inhibition was observed against *Aspergillus fumigatus* (16±0.71 mm in diameter) by the integumentary extract of *Heteropneutes fossilis followed by C. mrigala against A. fumigatus* (15±0.32 mm), *C. batrachus* against *A. fumigatus* (15±0.55 mm) as compared to other results. Specifically, other than the *L. catla* all the remaining fish species have shown similar inhibitory effects against *Aspergillus fumigatus, Candida albicans, and Fusarium solani* except *Aspergillus flavus* where *Heteropneutes fossilis* integumentary extract (15±0.55 mm) outweigh the other counterparts (Figure 1). Irrespective of different fish species, all the studied extracts were significantly effective against the fungal strain *Aspergillus fumigatus* as compared to other counterparts. Among the fish species, *L. catla* has exhibited the least antifungal activity compared to rest of the species. Interestingly, all the studied fish extracts also produced higher antifungal activity than the performance of positive control (Ketoconazole) except the extract of *L. catla*.

**Table 1.** Antifungal activity of the integumentary extract of fish species against selected four fungal species

| Fungal strains | *Labeo catla* | *Cirrhinus mrigala* | *Clarias batrachus* | *Heteropneustes fossilis* | Positive control (Ketoconazole) |
|---|---|---|---|---|---|
| *Aspergillus fumigatus* | $13\pm0.55^{a}$ | $15\pm0.32^{b}$ | $15\pm0.55^{b}$ | $16\pm0.71^{b}$ | $15\pm0.71^{b}$ |
| *Aspergillus flavus* | $10\pm0.45^{a}$ | $13\pm0.55^{b}$ | $14\pm0.71^{bc}$ | $15\pm0.55^{c}$ | $13\pm0.63^{b}$ |
| *Candida albicans* | $10\pm0.55^{a}$ | $14\pm0.84^{b}$ | $13\pm0.71^{b}$ | $14\pm0.45^{b}$ | $13\pm0.45^{b}$ |
| *Fusarium solani* | $11\pm0.32^{a}$ | $13\pm0.45^{b}$ | $13\pm0.84^{b}$ | $13\pm0.71^{b}$ | $12\pm0.55^{ab}$ |

Values followed by different alphabets in parenthesis are significantly different at $p < 0.05$ based on Duncan's multiple range test (DMRT).



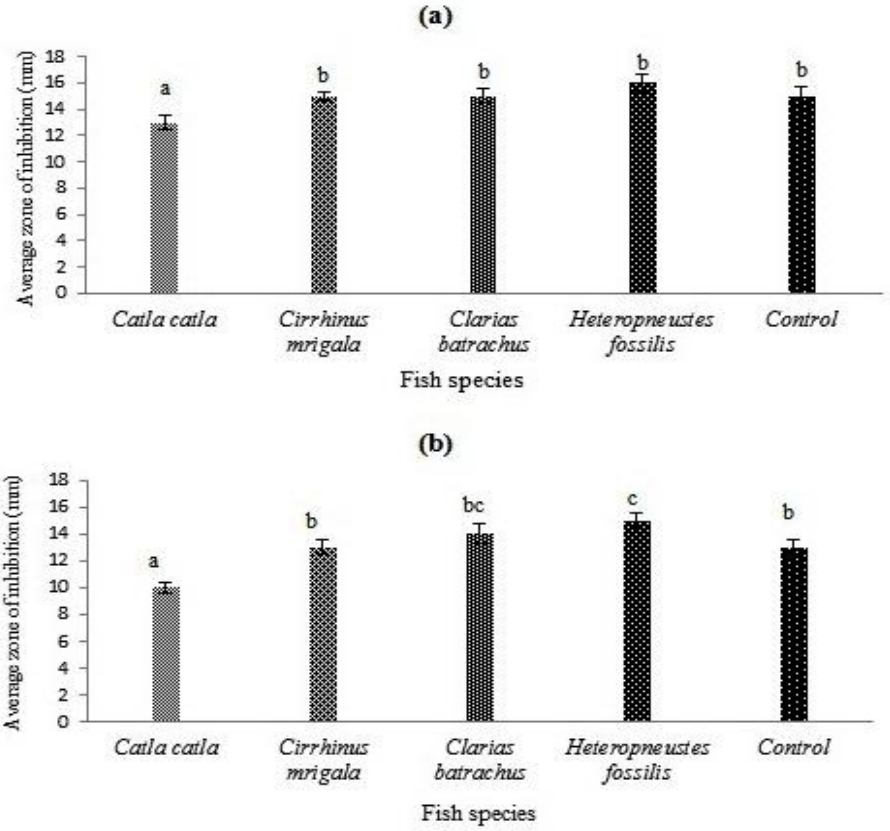

A B C D

**Figure 1.** Antifungal activity of different freshwater fish species integumentary extract against selected microbes

A: *Aspergillus fumigatus*; B: *Aspergillus flavus*; C: *Candida albicans*; D: *Fusarium solani*

S: *Heteropneustes fossilis*; Cba: *Clarias batrachus*; C: *Catla catla*; M: *Cirrhinus mrigala*;

K: Ketoconazole



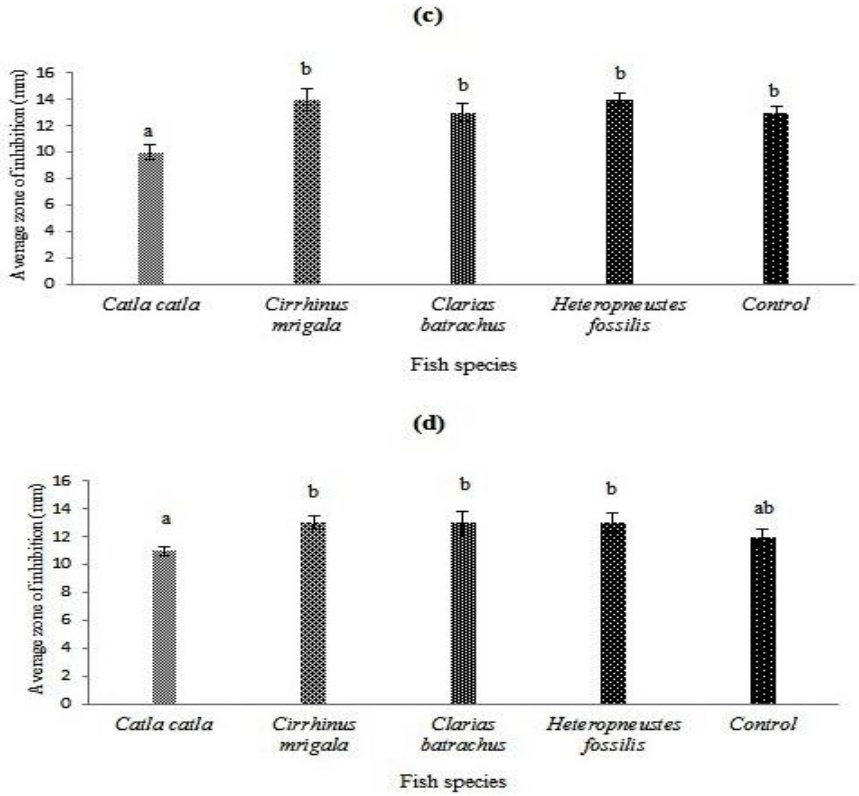



**Figure 2.** Antifungal activity of integumentary extract and control Ketoconazole against a) *A. fumigatus*; b) *A. flavus*; c) *C. albicans* and d) *F. solani*

## 4. Discussion

The ever-increasing demand for fish production and intensive aquaculture programs would require minimizing the economic loss due to mortality. To tackle the problem of disease outbreaks, the uses of chemical antibiotics have become the most common method. However, the negative impact of excessive use of such chemical antibiotics has been realized, and stressed on the use of natural origin drugs has become the most viable alternative option. Fishes are exposed to a wide range of environments where several microbes of varying natures are also inhabited. Fish have produced their own mechanism to survive under challenging threats of several microbes by showing their innate immune system (Bragadeeswaran *et al.*, 2011). Since, the mechanism related to innate immune system is largely responsible to produce several enzymes and antimicrobial proteins from the fish mucus layer (Dalmo *et al.*, 1997, Hellio *et al.*, 2002). On this contention, Jung *et al.* (2012) also stated that secretion of mucus or other protective substances from fishes is largely attributed by nature of fish species and their environmental condition. Antimicrobial properties of several fish species including both freshwater and marine species against medically important pathogens have been reported by several workers during the last few decades. For instances, Lirio *et al.* (2019) have studied the antimicrobial activities of freshwater fish species *viz.*, *Oreochromis niloticus*, *Clarias batrachus*, *Channa striata* against several medically important microbes in Philippines and found that all the three fish species possessed antimicrobial potent where *Clarias batrachus* exhibited the most inhibitory effect. Similarly, Fuochi *et al.* (2017) while studying the antimicrobial activity of marine fish *Dasyatis pastinaca* (Linnaeus, 1758) found the skin mucus of *D. Pastinaca* possesses antimicrobial activity against several microbes namely *Klebsiella pneumoniae*, *Candida glabrata* etc.

Concisely all the selected freshwater fish species *viz,* catla, mrigal, magur and singhi has been playing a major role with respect to overall fish production of the region. The comparative performance of the antifungal activity of different fish species has shown a different level of inhibition against the growth of selected microbes in the present study. Among the fish species, the integumentary extract of *Heteropneutes fossilis* has produced a better inhibitory effect in all the four selected microbes. On this contention, Subramanian *et al.* (2007) had suggested that different fish species have possessed varying level of mucus enzymes and antimicrobial proteins in their bodies. Nevertheless, Ellis (2001) have stressed that the quality and quantity of mucus production are also controlled by the prevailing aquatic ecosystem and their interactive behavior with other components of the ecosystem involving the living microbes. Moreover, the variation in mucus or integumentary secretion

from fishes due to difference in medium of extraction and their associated enzymatic activities cannot be ruled out.

Overall, in the present study, the integumentary extract of *H. fossilis* and *L. catla* exerted the maximum and minimum inhibitory effect respectively against the growth of all selected microbes. Similar results have been reported by Kumari and Yadav (2020) in acidic mucus extraction of *C. catla*, *C. carpio* and *H. fossilis.* However, our result is not consistent with the finding of Balasubramanian *et al.* (2012) where *C. catla* has produced a higher inhibitory effect against *A. flavus* (17 mm) as compared to the finding of the present study (10±0.45 mm). Specifically, among the fungal species, *A. flavus* have produced the maximum resistance against the effect of the fish integumentary extract on fungal growth. A study conducted by Kumari and Yadav (2020) have reported that comparatively *A. flavus* is more inhibitory resistance than *C. albicans* against the organic mucus extraction of *C. catla* and *C. carpio* while reverse trend was observed in acidic mucus extraction of the same fish species. On this contention, Subramanian *et al.* (2012) have suggested that the different peptide substances have a significant role in inhibiting the growth of microbes on the fish mucus layer via disintegrating the cell membrane of the microbes.

## 5. Conclusion

The present study demonstrated the importance of fish mucus or integumentary extract in controlling the growth of fungal microbes and thus helps in minimizing the economic losses due to fish mortality. In this present study, all the selected fish species have produced antifungal activity against the selected microbes. Overall, our study has revealed that *Heteropneustes fossilis* have shown similar effects as compared to the chemical antibiotic Ketoconazole. Therefore, our results will eventually help to promote the use of drugs based on natural origin fish mucus or integumentary extract to control the fungal diseases of fish. Moreover, it not only helps in reducing the negative impact of multi-resistant of antibiotics but also ensures a safer aquatic ecosystem.

## 6. Acknowledgement

The authors are grateful to the Director, ICAR Research Complex for NEH Region, Umiam, Meghalaya for facilitating the required services to undertake the present study under the project "Antimicrobial activity of integumentary secretion from selected cultivable fishes" PIMS Project Code: IXX13390.

## 7. References

Balasubramanian S, Prakash M, Senthilraja P and Gunasekaran G (2012) Antimicrobial properties of skin mucus from four freshwater cultivable fishes (*Catla catla, Hypophthalmichthys molitrix, Labeo rohita* and *Ctenopharyngodon idella*). African Journal of Microbiology Research 6(24):5110-20

Bragadeeswaran S and Thangaraj S (2011) Hemolytic and antibacterial studies on skin mucus of eel fish, *Anguilla anguilla* Linnaues, 1758. Asian Journal of Biological Science 4 (3):272-276

Brumfitt W, Hamilton-Miller JM and Franklin I (1990) Antibiotic activity of natural products: 1. Propolis. Microbios 62(250):19-22

Dalmo RA, Ingebrigtsen K and Bøgwald J (1997) Non-specific defence mechanisms in fish, with particular reference to the reticuloendothelial system (RES). Journal of fish diseases (4):241-73

Devi NP, Das SK, Sanjukta RK and Singh SG (2019) A comparative study on antibacterial activity of integumentary extract of selected freshwater fish Species and Neem extracts against gram-positive and Gram-Negative bacteria. Journal of Entomology and Zoology Studies 7(2): 1352-1355

Ellis AE (2001) The immunology of teleosts, In: RJ Roberts (ed) Fish Pathology 3rd edition, Elsevier, New York, pp.133-150

FAO (2012) The State of World Fisheries and Aquaculture, FAO Fisheries and Aquaculture Department, Rome, 209 pp

Fuochi V, Li Volti G, Camiolo G, Tiralongo F, Giallongo C, Distefano A, Petronio Petronio G, Barbagallo I, Viola M, Furneri PM and Di Rosa M (2017) Antimicrobial and anti- proliferative effects of skin mucus derived from *Dasyatis pastinaca* (Linnaeus, 1758). Marine drugs15(11):342

Hellio C, Pons AM, Beaupoil C, Bourgougnon N and Legal Y (2002) Antibacterial, antifungal and cytotoxic activities of extract from fish epidermis and epidermal mucus. International Journal of Antimicrobial Agents 20 (3):214-219

Ingram GA (1980) Substances involved in the natural resistance of fish to infection-a review. Journal of Fish Biology 16: 23-60

Jung TS, Del Castillo CS, Javaregowda PK, Dalvi RS, Nho SW, Park SB, Jang HB, Cha IS, Sung HW, Hikima JI and Aoki T (2012) Seasonal variation and comparative analysis of non-specific humoral immune substances in the skin mucus of olive flounder (*Paralichthys olivaceus*). Developmental & Comparative Immunology 38(2):295-301

Kumari S and Yadav S (2020) Study of Antifungal Activity of Epidermal Mucus of Three Fresh Water Fishes. Annals of Biology 36(1):75-80

Lirio AGC, De Leon JAA, and Villafuerte AG (2019) Antimicrobial activity of epidermal mucus from top aquaculture fish species against medically-important pathogens. Walailak Journal of Science and Technology (WJST)16(5):329-40

Mainous III AG and Pomeroy C (2001) Management of antimicrobials in infectious diseases. Humana Press

Pethkar MR and Lokhande MV (1995) Antifungal activity of skin mucus of three cultivable fish species (*Catla-catla, Cirrhinus mrigala* and *Anguilla-anguilla*). International Journal of Zoology Studies 13: 61-92

Sheeshka J and Murkin, E (2002) Nutritional aspects of fish compared with other protein sources. Comments on Toxicology 8(4-6): 375-397

Subramanian S, Mackinnon SL and Ross NW (2007) A comparative study on innate immune parameters in the epidermal mucus of various fish species. Comparative Biochemistry and Physiology - Part B: Biochemistry & Molecular Biology 148:256-263

Subramanian S, Ross NW and Mackinnon SL (2008) Comparison of antimicrobial activity in the epidermal mucus extracts of fish. Comparative Biochemistry & Physiology 150(B):85-92