FTIR Spectroscopic Characterization of *Holothuria leucospilota* from Ratnagiri

MOHINI BAMANE[1], MADHURA MUKADAM[2]
[1, 2]*Member - Department of Zoology, Gogate Jogalekar College, Ratnagiri*

Abstract*— Sea cucumbers, belonging to the class Holothuroidea and phylum Echinodermata, are marine animals with great ecological significance and potential possible medical applications. The present work uses Fourier Transform Infrared (FTIR) spectroscopy to analyse the biochemical composition of Holothuria leucospilota procured from Ratnagiri, India. From the FTIR spectra, it has been revealed that several important functional groups such as proteins, lipids, carbohydrates, and sulphur-containing compounds were present in the Holothuria leucospilota. High levels of proteins and collagen found in the samples suggest the potential for wound healing and tissue engineering, while lipid peaks point to valuable omega-3 fatty acids which are known for their anti-inflammatory and cardioprotective properties. The presence of carbohydrates, which are probably glycosaminoglycans, renders anti-inflammatory and anticoagulant effectiveness. Sulphur-containing compounds present might have antioxidant and antiviral benefits. These findings justify the application of H. leucospilota for the development of drugs and functional foods, which requires more efforts to be directed towards the identification and isolation of active components.

Index Terms- bioactive compounds, functional groups, FTIR, Holothuria leucospilota.

I. INTRODUCTION

Sea cucumbers, belonging to the Holothuroidea class and Echinodermata phylum, are ancient sea creatures found worldwide (Zhao, 2015). They live on the ocean floor and play important ecological roles, mainly by recycling nutrients and improving sediment. Their feeding behaviours, including consuming marine sediments and straining seawater, create rich habitats for diverse marine microorganisms like bacteria and fungi to thrive (Xia *et al*., 2022). Globally, about 1,500 types of sea cucumbers have been identified (Bordbar *et al*., 2011). In Asia, particularly China, sea cucumbers are highly valued for their nutritional and medicinal benefits, with bioactive substances such as polysaccharides, saponins, lipids, and peptides being explored for drug development (Zhao *et al*., 2018).

Sea cucumbers are rich in essential nutrients like proteins, omega-3 and -6 fatty acids, vitamins, and minerals (Hossain *et al*., 2020; Janakiram *et al*., 2015). Compounds like chondroitin sulphate and sulphated polysaccharides exhibit antioxidant, antimicrobial, anti-inflammatory, anticancer, and antihypertensive effects (Pangestuti & Arifin, 2018, Dai *et al*., 2021). Despite their prevalence in coastal regions, species such as *Holothuria leucospilota* from Ratnagiri, have not been extensively studied for their potential medicinal benefits. While sea cucumbers have garnered attention for their nutritional properties, significant research gaps remain regarding species diversity and the analysis of their bioactive compounds.

In Southeast Asia, species like *Stichopus hermanni* have been utilized for medicinal purposes; however, scientific validation is limited. The full potential of sea cucumbers for drug discovery remains unexplored (Bordbar *et al.,* 2011). Advances in marine biochemistry indicate a need for further studies on new compounds such as alkaloids and functional peptides with therapeutic applications. Marine organisms represent a promising source of bioactive compounds; over 10,000 metabolites have been reported with notable pharmacological properties (Aydın *et al.,* 2011; Blunt *et al.,* 2010).

Despite its abundance along the Ratnagiri coast of Maharashtra, *Holothuria leucospilota* is often overlooked by local fishermen due to its appearance. This underutilization stems from limited awareness of its nutritional and medicinal benefits. Therefore, further research is essential.

Fourier Transform Infrared (FTIR) spectroscopy is a powerful analytical tool that identifies chemical

compounds based on their vibrational characteristics. Its application in marine biology remains largely unexplored. This study aims to utilize FTIR spectroscopy to examine *Holothuria leucospilota* from Ratnagiri to uncover essential molecular elements contributing to its biological functions.

II. MATERIALS AND METHODS

1. Sample Collection

Specimens of Holothuria leucospilota were collected from the intertidal zone of Alava Beach, Ratnagiri, Maharashtra, in January 2022. This location was selected due to its rich biodiversity. Specimens were identified based on morphological features such as body shape, colour, and tentacle structure. Ethical guidelines for handling marine organisms were strictly followed to adhere to minimize ecosystem disruption.

2. Sample Preparation

After collection, specimens were thoroughly washed with seawater to remove surface debris, like sand and algae, and were then stored at -20°C to prevent degradation of biological compounds. For FTIR analysis, the protein-rich body wall tissue was excised, dried at 40°C for 48 hours, and finely ground to ensure optimal interaction with the infrared beam during FTIR analysis.

3. FTIR Spectroscopy

For FTIR analysis, 2 mg of freeze-dried powder was mixed with 200 mg of potassium bromide (KBr), ground, and compressed into a transparent pellet. FTIR spectra were recorded in the mid-infrared range (4000 to 400 cm^{-1}) using a Bruker Tensor 27 spectrometer. Data were collected with 32 scans at 4 cm^{-1} resolution. Background correction was performed using a pure KBr pellet. OPUS software was used to analyse the spectra and assign peaks to functional groups based on wavenumbers.

4. Data Analysis

Spectral data were processed using OMNIC software, which baseline-corrected and normalized the spectra. Key absorption bands corresponding to functional groups were identified and analyzed.

III. RESULTS

The FTIR spectra of *Holothuria leucospilota* displayed characteristic peaks corresponding to various molecular vibrations. The analysis revealed eight distinct absorption peaks associated with specific functional groups and biochemical components.

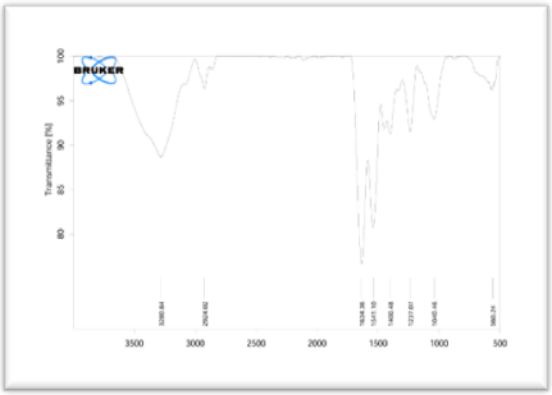

Fig. 1 FTIR Spectrum of *Holothuria leucospilota*

- Sample from Ratnagiri Coast

The FTIR analysis revealed eight distinct absorption peaks, each corresponding to specific functional groups and biochemical components, as detailed in the following table of absorption peaks and associated bioactive compounds.

Table No. I - FTIR Absorption Peaks and Associated Bioactive Compounds in *Holothuria leucospilota*

Sr No	Peak (cm^{-1})	Functional Group	Type of Vibration	Associated Bioactive Compound
1	3280.84 cm-1	hydroxyl groups	O-H stretching	Carbohydrates (Polysaccharides)
2	2924.02 cm-1 and 2854.36 cm-1	aliphatic groups	C-H stretching	Lipids (Fatty acids, omega-3)
3	1634.36 cm-1	carbonyl groups	C=O stretching	Proteins (Amide I band, collagen)

4	1541.10 cm-1	amide groups	N-H bending	Proteins (Peptides, collagen)
5	1400.48 cm-1	alkyl groups	C-H bending	Hydrocarbons, lipids
6	1237.07 cm-1	polysaccharides or glycosidic linkages	C-O-C stretching	Glycosaminoglycans (Chondroitin sulfate)
7	1040.46 cm-1	alcohols or Polysaccharides	C-O stretching	Glycosaminoglycans, carbohydrates
8	560.24 cm-1	sulfur-containing Compounds	C-S stretching	Sulfated polysaccharides

IV. DISCUSSION

The FTIR analysis revealed a range of bioactive compounds consistent with previous studies on other sea cucumber species. The presence of proteins, lipids, carbohydrates, and sulphur-containing compounds emphasizes its potential as a source of bioactive molecules for biomedical applications.

Proteins and Collagen: The amide I (1634 cm^{-1}, C=O stretching) and amide II (1541 cm^{-1}, N–H bending) peaks indicated a high content of proteins, particularly collagen, in *Holothuria leucospilota*. These findings aligned with earlier studies that established sea cucumbers as rich sources of collagen, known for their application in wound healing and tissue engineering due to their biocompatibility (Chen *et al*., 2011; Arumugam *et al.,* 2018).

Lipids and Fatty Acids: The peaks at 2924 cm^{-1} and 2854 cm^{-1} linked to C–H stretching confirmed the presence of lipids and fatty acids, particularly omega-3 fatty acids like EPA and DHA. These compounds were well-documented for their anti-inflammatory and cardioprotective effects (Zhang *et al.,* 2017; Kiew & Don, 2012).

Carbohydrates and Glycosaminoglycans: The bands at 1237 cm^{-1} (C–O–C stretching) and 1040 cm^{-1} (C–O stretching) indicated the presence of glycosaminoglycans, such as chondroitin sulphate. These compounds were known for their anti-inflammatory, anticoagulant, and anticancer properties (Liu *et al.,* 2019).

Sulphur-Containing Compounds: The C–S stretching peak at 560.24 cm^{-1} suggested the presence of sulphur-containing compounds, such as sulphated polysaccharides, known for their antioxidant and anti-inflammatory properties.

When compared with other sea cucumber species, such as *Holothuria scabra*, the FTIR spectrum of *H. leucospilota* showed a similar biochemical profile. However, variations in peak intensities could be attributed to environmental factors such as diet and habitat (Zhang *et al.,* 2017). The bioactive compounds identified in *Holothuria leucospilota* offered promising opportunities for applications in pharmaceuticals, nutraceuticals, and cosmetics. Collagen and glycosaminoglycans were already being investigated for their roles in anti-ageing, wound healing, and cancer treatment. Additionally, the presence of omega-3 fatty acids pointed to potential in developing heart health supplements, while sulphur-containing compounds were of interest for their antioxidant therapies. These findings were consistent with earlier research on the therapeutic potential of sea cucumbers (Dong *et al.,* 2011).

CONCLUSION

The FTIR analysis revealed a rich biochemical profile in *Holothuria leucospilota*, highlighting proteins, lipids, polysaccharides, and sulphur-containing compounds. These findings confirm the species' potential for applications in pharmaceuticals and nutraceuticals, particularly in wound healing, anti-inflammatory therapies, cardiovascular health, and antiviral treatments. The presence of collagen, glycosaminoglycans, omega-3 fatty acids, and sulphur-containing compounds suggests that *Holothuria leucospilota* could be a valuable resource for developing functional foods and drugs. The comparison with other sea cucumber species reinforces this potential, although further research is

necessary to fully characterize and isolate these compounds for commercial use.

Given its ecological abundance in Ratnagiri yet underutilization highlights the need for sustainable exploitation of this species, which could benefit both local economies and the pharmaceutical industry. Future research should focus on the detailed isolation and characterization of these bioactive compounds, with an emphasis on clinical trials to confirm their therapeutic efficacy.

ACKNOWLEDGEMENT

The authors would like to express their gratitude to the Principal of Gogate-Jogalekar College, Ratnagiri, for their support.

REFERENCES

[1] Arumugam, V. A., Ramesh, S., Sumantran, G. S., & Thirunavukkarasu, T. Sea cucumbers as a potential source of bioactive compounds for wound healing applications: A review. Journal of Tissue Engineering and Regenerative Medicine, 2018, 12(4), e1939-e1951. https://doi.org/10.1002/term.2589

[2] Aydın, M., Sevgili, H., Tufan, B., Emre, Y., & Köse, S. Proximate composition and fatty acid profile of three different fresh and dried commercial sea cucumbers from Turkey. International Journal of Food Science & Technology, 2011, 46(3), 500-508 https://doi.org/10.1111/j.1365-2621.2010.02511.x

[3] Blunt, J. W., Copp, B. R., Munro, M. H. G., Northcote, P. T., & Prinsep, M. R. Marine natural products. Natural Product Reports, 2010, 27(2), 165-237.

[4] Bordbar, S., Anwar, F., & Saari, N. High-value components and bio-actives from sea cucumbers for functional foods—A review. Marine Drugs, 2011, 9(10), 1761-1805. https://doi.org/10.3390/md9101761

[5] Chen, J., Li, S., Yang, X., & Hao, J. Collagen from sea cucumber and its applications in biomedicine. Marine Drugs, 2011, 9(6), 1452-1466. https://doi.org/10.3390/md9061452

[6] Dai, L., Zhou, Y., Liu, C., Wang, H., & Sun, L. Chemical composition, bioactive substances, and pharmaceutical and food applications of sea cucumbers (Holothuroidea). Marine Drugs, 2021, 19(3), 181. https://doi.org/10.3390/md19030181

[7] Dong, Y., Liao, M., Zhu, Z., & Li, X. Isolation and characterization of collagen from sea cucumber Acaudina molpadioides and its potential application in drug delivery. Journal of Biomaterials Science, Polymer Edition, 2011, 22(17), 2197-2214. https://doi.org/10.1163/092050611X591764

[8] Hossain, M. B., Patra, J. K., & Rangaswami, K. R. Nutritional and bioactive importance of sea cucumbers: A review. Food Bioscience, 2020, 34, 100527. https://doi.org/10.1016/j.fbio.2020.100527

[9] Janakiram, N. B., Mohammed, A., & Rao, C. V. Sea cucumber metabolites as potent chemopreventive agents. Marine Drugs, 2015, 13(5), 2909–2923. https://doi.org/10.3390/md13052909

[10] Kiew, P. L., & Don, M. M. Jewel of the seabed: Sea cucumbers as nutritional and therapeutic resources. Journal of Complementary and Integrative Medicine, 2012, 9(1), 1-24. https://doi.org/10.1515/1553-3840.1397

[11] Liu, X., Zhang, Y., Li, P., Kang, Y., & Huang, J. Marine collagen peptides from sea cucumber promote cell proliferation and cell cycle in human skin fibroblasts. Food Bioscience, 2019, 30, 197-204. https://doi.org/10.1016/j.fbio.2019.01.007

[12] Pangestuti, R., & Arifin, Z. Medicinal and health benefit effects of functional sea cucumbers. Journal of Traditional and Complementary Medicine, 8(3), 2018, 341-351. https://doi.org/10.1016/j.jtcme.2017.09.003

[13] Smith, B. C. Infrared Spectral Interpretation: A Systematic Approach. CRC Press, 2011.

[14] Xia, S., Yao, Y., Zhang, M., & Li, G. Sea cucumber aquaculture and its ecological impact on marine ecosystems. Marine Ecology Progress Series, 2022, 691, 123-134. https://doi.org/10.3354/meps14072

[15] Zhao, Y. Sea cucumber aquaculture: Farming Sea cucumbers for food and conservation. Reviews in Aquaculture, 2015, 7(4), 243-251. https://doi.org/10.1111/raq.12068

[16] Zhang, Y., Liu, W., Li, G., Shi, B., & Miao, Y. Collagen from marine biological resources: A review of its extraction, properties, and applications. Marine Drugs, 2017, 15(4), 102. https://doi.org/10.3390/md15040102

[17] Zhao, Y., Yang, H., Zhou, Y., Wang, X., Liu, X., Fan, S., & Chang, Y. Bioactive compounds from sea cucumbers (Holothuroidea): A review. Journal of Natural Medicines, 2018, 72(1), 18-42. https://doi.org/10.1007/s11418-017-1140-2