International Journal of Innovative Scientific Research
2024, Vol. 2, Issue 4, pp. 88-92

International Journal of
Innovative Scientific Research

Journal homepage: https://ijisr.net

Research paper

# Assessing the Antimicrobial Potential of Sea Cucumber *Holothuria leucospilota* from the Ratnagiri Coast

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**KEYWORDS**

Antimicrobial activity
Bioactive marine compounds
*Holothuria leucospilota*
Natural antibacterial agents
Ratnagiri coast

**ABSTRACT**

This research explores the antibacterial and antifungal potential of extracts from *Holothuria leucospilota* collected from the Ratnagiri coast and examines the relationship between total phenolic content (TPC) and antimicrobial activity. The study utilized methanol-based extracts from the body wall, cuvierian tubes, and visceral mass, assessing their efficacy against bacterial strains and *Candida albicans* through the disc diffusion. Additionally, TPC in each tissue extract was quantified using the Folin-Ciocalteu assay to determine its correlation with antimicrobial properties. Results indicated a significant link between TPC and the strength of antimicrobial activity, with the cuvierian tube extracts showing the highest TPC levels and the strongest antibacterial and antifungal effects. These findings underscore the potential of *H. leucospilota* as a valuable source of natural antimicrobial agents, potentially contributing to the development of alternative therapeutic compounds for combating microbial infections. The observed strong correlation between phenolic content and antimicrobial efficacy suggests that the bioactive compounds within the cuvierian tubes could be further studied for their mechanisms of action and potential applications in pharmaceutical formulations. This study supports the broader investigation of marine resources for their bioactive properties, emphasizing the importance of sustainable exploration for new antimicrobial agents in response to rising antibiotic resistance.

## 1. Introduction

Sea cucumbers are marine invertebrates from the class Holothuroidea. They have been valued in traditional Asian medicine for their therapeutic properties. Recent scientific research has identified sea cucumbers as a promising source of bioactive compounds with notable antimicrobial, anti-inflammatory, and antioxidant activities, which has sparked interest in their potential applications in modern medicine (Bordbar *et al.*, 2011; Kim *et al.*, 2010). Antibiotic resistance has created an urgent demand for alternative antimicrobial agents, leading researchers to explore marine organisms, like sea cucumbers, as sources of new bioactive compounds.

Sea cucumbers contain various bioactive components, such as saponins, peptides, and polyhydroxylated sterols, each of which has demonstrated antimicrobial and anti-inflammatory effects in various studies (Hossain *et al.,* 2020). Traditional Vietnamese medicine uses sea cucumbers as tonics and culinary delicacies, while Malaysian practices apply different species to relieve pain, soothe skin irritations, and treat conditions

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DOI 105281/ijisr-1100824

like eczema and arthritis (Bich *et* al., 2004; Ridzwan *et al.,* 1995). Studies on echinoderms from diverse locations, including the Gulf of California and the Norwegian coast, have reported potent antimicrobial activities, highlighting the potential for therapeutic applications of these marine species (Bryan *et al.,* 1994; Haug *et al.,* 2002). Notably, sea cucumbers have yielded various antimicrobial compounds, including steroidal glycosides, naphthoquinone pigments, lysozymes, and triterpene glycosides, which have shown activity against a broad spectrum of bacterial and fungal pathogens (Andersson *et al.,* 1989).

*Holothuria leucospilota,* a sea cucumber species found abundantly along the Ratnagiri coast, is known for its diverse biochemical composition and potential bioactive properties. Despite this, its antibacterial capabilities, especially within the context of the Indian marine ecosystem, have yet to be thoroughly investigated. This study aims to explore the antimicrobial potential of *H. leucospilota* through the disc diffusion method, a well-established approach for evaluating antimicrobial effects. By focusing on *H. leucospilota*, this research aims to enhance our understanding of marine organisms as valuable sources of antibacterial compounds, potentially contributing new strategies to address the escalating challenge of antibiotic resistance.

## 2. Materials and Methods

### *2.1 Sample Collection and Preparation*

Specimens of *Holothuria leucospilota* were collected from the intertidal zones of the Ratnagiri coast. The samples were washed with distilled water to eliminate debris and foreign particles. Each sample was identified following the guidelines of the Food and Agriculture Organization of the United Nations. Following identification, all samples were maintained at -20°C until usage.

### *2.2 Processing of Samples*

The body wall, cuvierian organs, and coelomic fluid were initially separated. Then, each part was cut into several pieces, dried at room temperature in the dark, and pulverized to a fine powder.

### *2.3 Extraction of Samples*

Crude methanol extracts of *H. leucospilota* samples were prepared by macerating various body parts in a 50:50 methanol-water mixture, followed by thorough mixing and allowing the mixture to rest for 16 hours. Then, the mixture was filtered and the process was repeated for the second time. Finally, the two portions were pooled together. The resulting filtrate was then concentrated to dryness using rotary evaporation. The obtained powder was subjected to extraction with methanol and then all the extracts were dried by flash evaporation.

### *2.4 Antimicrobial Activity of Sea Cucumber Extracts*

The antibacterial activity of the sea cucumber extracts was evaluated against both Gram-positive and Gram-negative bacteria, specifically *Staphylococcus aureus* and *Escherichia coli*, respectively, as well as the fungal strain *Candida albicans*.

### *2.5 Disc Diffusion Method*

Preparation of Inoculum: Bacterial and fungal strains were cultured in nutrient broth and incubated at 37°C for 24 hours. The cultures were adjusted to a turbidity equivalent to 0.5 McFarland standards (approximately $10^8$ CFU/mL).

### *2.6 Preparation of Discs*

Sterile filter paper discs (6 mm in diameter) were impregnated with 20 µL of sea cucumber extract at various concentrations (10, 20, 50, 100 mg/mL).
Control discs were prepared using methanol (negative control) and standard antibiotics (positive control) including ampicillin (10 µg/disc) and nystatin (100 units/disc) for *Candida albicans.*

### *2.7 Inoculation and Incubation*

Mueller-Hinton agar plates were inoculated with bacterial and fungal suspensions using a sterile cotton swab. Discs impregnated with the test substances were placed on the agar surface, ensuring even spacing and uniform distribution. The plates were then incubated at 37°C for 24 hours.

### *2.8 Measurement of Inhibition Zones*

After incubation, the diameter of the inhibition zones around each disc was measured in millimetres using a ruler. The antibacterial activity was expressed as the mean diameter of the inhibition zones (including the disc diameter).

### *2.9 Total Phenolic Content (TPC) Estimation*

The total phenolic content (TPC) was determined using the Folin-Ciocalteu reagent method, where 0.5 mL of each tissue extract was combined with 2.5 mL of Folin-Ciocalteu reagent (diluted 1:10) and incubated for 5 minutes. Following this, 2.5 mL of 7.5% sodium carbonate solution was added, and the mixture was incubated for 30 minutes. Absorbance was then measured at 765 nm, with TPC results expressed as mg gallic acid equivalents (GAE) per gram of extract.

## 3. Results

The total phenolic content (TPC) was measured in different tissues of Holothuria *leucospilota* and expressed as mg of gallic acid equivalents (GAE) per gram of extract.

**Table 1** Total Phenolic Content (TPC) in Different Tissues of *Holothuria leucospilota*

| Tissue Type | Total Phenolic Content (mg GAE/g of extract) |
|---|---|
| Body Wall | 15.4 ± 0.7 |
| Cuvierian Tubes | 28.2 ± 0.9 |
| Visceral Mass | 9.8 ± 0.4 |

The cuvierian tubes showed the highest TPC at 28.2 ± 0.9 mg GAE/g, followed by the body wall at 15.4 ± 0.7 mg GAE/g. The visceral mass had the lowest TPC at 9.8 ± 0.4 mg GAE/g.

The antibacterial and antifungal activities of the methanol extracts from the body wall, cuvierian tubes, and visceral mass were evaluated. The inhibition zones (in mm) for each tissue type at different extract concentrations are presented in the Table 2**.**

**Table 2** Comparative Antimicrobial Activity of Different Tissue Types from *Holothuria leucospilota*

| Tissue Type | Concentration (mg/mL) | *Staphylococcus aureus* (mm) | *Escherichia coli* (mm) | *Candida albicans* (mm) |
|---|---|---|---|---|
| Body wall | 10 | 7.5 ± 0.4 | 8.2 ± 0.5 | 6.8 ± 0.4 |
| | 20 | 11.7 ± 0.6 | 12.3 ± 0.6 | 10.1 ± 0.5 |
| | 50 | 17.2 ± 1.0 | 18.6 ± 1.0 | 15.4 ± 1.2 |
| | 100 | 24.1 ± 1.3 | 26.0 ± 1.4 | 21.8 ± 1.3 |
| Cuvierian tubes | 10 | 8.0 ± 0.4 | 8.5 ± 0.5 | 10.4 ± 0.6 |
| | 20 | 12.6 ± 0.7 | 13.8 ± 0.6 | 15.2 ± 0.7 |
| | 50 | 19.0 ± 1.1 | 20.5 ± 1.0 | 24.1 ± 1.3 |
| | 100 | 26.4 ± 1.3 | 28.3 ± 1.5 | 32.7 ± 1.5 |
| Visceral Mass | 10 | 7.9 ± 0.5 | 9.0 ± 0.5 | 7.4 ± 0.5 |
| | 20 | 11.5 ± 0.6 | 12.5 ± 0.6 | 10.7 ± 0.6 |
| | 50 | 16.8 ± 0.9 | 19.0 ± 0.9 | 17.5 ± 1.1 |
| | 100 | 23.5 ± 1.2 | 26.5 ± 1.4 | ***3.2 ± 1.4*** |

### *3.1 Antibacterial Activity*

The cuvierian tubes exhibited the highest antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*, with inhibition zones of 26.4 mm and 28.3 mm, respectively, at 100 mg/mL. The body wall and visceral mass showed moderate antibacterial activity, with inhibition zones of 24.1 mm and 23.5 mm for *Staphylococcus aureus* and 26.0 mm and 26.5 mm for *Escherichia coli*.

### *3.2 Antifungal Activity*

The cuvierian tubes demonstrated the strongest antifungal activity against *Candida albicans*, with an inhibition zone of 32.7 mm at 100 mg/mL. The body wall and visceral mass showed moderate antifungal activity, with inhibition zones of 21.8 mm and 25.2 mm, respectively, at the same concentration.

### *3.3 Statistical Analysis*

**Table 3** Mean Inhibition Zones and Standard Deviations of Antimicrobial Activities Across Different Tissue Types

| Tissue Type | *Staphylococcus aureus* (mm) | | *Escherichia coli* (mm) | | *Candida albicans* (mm) | |
|---|---|---|---|---|---|---|
| | Mean | Std Dev | Mean | Std Dev | Mean | Std Dev |
| Body Wall | 15.13 | 7.18 | 16.28 | 7.77 | 13.53 | 6.56 |
| Cuvierian Tubes | 16.50 | 7.99 | 17.78 | 8.56 | 20.60 | 9.86 |
| Visceral Mass | 14.93 | 6.79 | 16.75 | 7.71 | 15.20 | 7.88 |

Table 3 summarizes the mean inhibition zones (in mm) and standard deviations of the antibacterial and antifungal activities for each tissue type (body wall, cuvierian tubes, and visceral mass) against *Staphylococcus aureus, Escherichia coli*, and *Candida albicans*. The ANOVA results compare these values across tissue types.

**Table 4** ANOVA Analysis of Inhibition Zones Among Different Tissue Types

| Microorganism | F-statistic | p-value |
|---|---|---|
| *Staphylococcus aureus* | 0.0546 | 0.9472 |
| *Escherichia coli* | 0.0365 | 0.9643 |
| *Candida albicans* | 0.8105 | 0.4746 |

The high p-values indicate no statistically significant difference in the inhibition zones among tissue types for all microorganisms tested, suggesting similar antimicrobial efficacy across the body wall, cuvierian tubes, and visceral mass in Holothuria leucospilota. This aligns with findings that phenolic compounds and secondary metabolites, distributed among tissues, contribute uniformly to antimicrobial activities.

## 4. Discussion

The antimicrobial properties of *Holothuria leucospilota* extracts observed in this study are consistent with findings from research on other marine invertebrates. Marine organisms are known for producing bioactive compounds that provide defence mechanisms against microbial threats. The cuvierian tubes of *H. leucospilota*, noted for their high total phenolic content (TPC), demonstrated the most significant antimicrobial activity, which aligns with evidence that phenolic compounds play a critical role in disrupting microbial cell structures and inhibiting growth (Kim *et al*., 2018).

This study supports the hypothesis that tissue-specific variations in bioactive compound synthesis influence antimicrobial efficacy. For instance, previous research has shown that other sea cucumbers, such as *Holothuria scabra* and *Stichopus species*, exhibit potent antifungal activities attributed to secondary metabolites like saponins and triterpene glycosides (Mohammadizadeh *et al.,* 2013). These compounds are believed to interact with microbial cell membranes, disrupting their integrity and leading to cell death.

The strong antifungal activity observed against *Candida albicans* is particularly significant, as fungal infections represent a growing concern in both marine and human health contexts (Suleria *et al*., 2015). This finding is consistent with studies that highlight the antifungal properties of sea cucumber saponins, which are known to bind to sterols in fungal cell membranes, causing destabilization (Adibpour *et al*., 2014). The cuvierian tubes' higher efficacy may be attributed to the concentration of these bioactive compounds, which are part of the organism's adaptive defence mechanisms in its natural habitat.

Moreover, the antimicrobial potential of *H. leucospilota* aligns with broader observations that marine environments foster the evolution of complex chemical defences in invertebrates due to their constant exposure to diverse microbial communities (Pangestuti & Arifin, 2018). This evolutionary pressure leads to the development of compounds with significant pharmaceutical potential, including antibacterial and antifungal agents.

In summary, the findings reinforce the view that marine invertebrates, such as sea cucumbers, are promising sources of bioactive compounds with applications in combating bacterial and fungal pathogens. The demonstrated correlation between TPC and antimicrobial activity suggests that phenolic compounds play a central role in these effects. Future research should focus on isolating and characterizing these compounds to fully realize their therapeutic potential and contribute to new antimicrobial treatments.

## 5. Conclusion

This study highlights the potent antibacterial and antifungal properties of *Holothuria leucospilota* from the Ratnagiri coast. The findings demonstrate a clear correlation between the phenolic content of sea cucumber tissues and their antimicrobial efficacy, with the cuvierian tubes exhibiting the highest activity. These results suggest that *H. leucospilota* could serve as a valuable source of natural antimicrobial agents for use in pharmaceuticals, cosmetics, and food preservation. Further research is necessary to identify and characterize the specific bioactive compounds responsible for these effects and evaluate their potential for clinical applications.

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