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Research paper

Phytochemical Analysis of *Holothuria leucospilota,* a Sea Cucumber from the Ratnagiri Coast

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ABSTRACT

This study presents a qualitative biochemical analysis of extracts from *Holothuria leucospilota*, a sea cucumber species widely recognized for its potential medicinal and nutraceutical properties. Using various solvents, the extracts were screened for the presence of key secondary metabolites, including alkaloids, phenolic compounds, saponins, glycosides, reducing sugars, tannins, flavonoids, and steroids. The analysis revealed that *H. leucospilota* contains several bioactive compounds with promising therapeutic potential. These findings contribute to a better understanding of the species' medicinal properties and offer avenues for future research in marine-derived bioactive substances.

1. Introduction

The marine environment is an exceptional source of secondary metabolites, featuring structures that are typically not found in the natural products of terrestrial plants. Over the past decade, a wide variety of new structures isolated from marine habitats have revealed that marine organisms produce numerous novel bioactive compounds (Fenical and Jensen, 2006 and Sawadogo et al., 2015). In comparison to microorganisms, marine plants, terrestrial plants and animals, marine animals are a particularly rich source of potent secondary metabolites with pharmaceutical properties (Munro et al., 1999). As a result, marine invertebrates are an ideal source of cytotoxic compounds with high potential for therapeutic use (Faulkner, 1984).

Holothuria leucospilota, commonly known as the black sea cucumber, is a marine invertebrate found along India's coasts, particularly in the Ratnagiri region. Sea cucumbers are valued for their nutritional and therapeutic potential and have been used in traditional medicine across various cultures. Extracts of *H. leucospilota* are known to contain multiple

bioactive compounds with diverse biological activities, including anti-inflammatory, anticancer, antioxidant, and antimicrobial properties. These therapeutic effects are largely attributed to the presence of secondary metabolites. While some studies have investigated the biological activities of *H. leucospilota* extracts, comprehensive analyses of its chemical profile are still limited. Most research on the chemical composition of *H. leucospilota* has been conducted on samples from the South China Sea (Van Dyck et al., 2010 and Han et al., 2010).

This study aims to provide a detailed phytochemical analysis of *Holothuria leucospilota* from the Ratnagiri coast, with a focus on identifying the unique bioactive compounds present in this regional species. Specifically, it seeks to examine the phytochemical constituents, including secondary metabolites such as saponins, alkaloids, flavonoids, terpenoids, and phenolic compounds, which are commonly associated with diverse biological activities.

2. Materials and Methods

2.1 Sample Collection and Preparation

Specimens of *Holothuria leucospilota* were collected from coastal waters. The samples were thoroughly washed to remove debris, cut into small pieces, and air-dried. The dried samples were ground into a fine powder and subjected to extraction using different solvents, including ethanol, methanol, water, petroleum ether, and chloroform.

2.2 Extraction Procedure

The ground sample was soaked in each solvent for 72 hours with occasional shaking. Afterwards, the mixtures were filtered, and the filtrates were concentrated using a rotary evaporator. These crude extracts were subsequently utilized for biochemical analysis.

2.3 Qualitative Biochemical Tests

The phytochemical analysis of extracts for the identification of bioactive chemical constituents was done using standard procedures by Trease (1989) Harborne (1973) and Sofowara (1983).

2.3.1 Tannins

About 0.5 g of the sample was put in a test tube, 20 ml of distilled water was added, and the mixture was heated to boiling. The mixture was subsequently filtered, 0.1% $FeCl_3$ was added to the resulting filtrate and observations were recorded. A brownish-green or blue-black colouration indicated the presence of tannins.

2.3.2 Saponins

The crude solvent extract was combined with 5 ml of water and shaken vigorously. The appearance of a stable foam indicated the presence of saponins.

2.3.3 Flavonoids

Approximately 1 g of the extract was mixed with a few small pieces of magnesium ribbon (0.5 g), followed by the addition of a few drops of concentrated hydrochloric acid. The development of a pink or magenta-red colour after 3 minutes signified the presence of flavonoids.

2.3.4 Terpenoids

The solvent extract of the sea cucumber was taken in a clean test tube, 2 ml of chloroform was added and vigorously shaken, then evaporated to dryness. Two milliliters of concentrated sulfuric acid were added to the mixture and heated for approximately 2 minutes. The appearance of a greyish colour indicated the presence of terpenoids.

2.3.5 Glycosides

a. Salkowski's test: The solvent extract was mixed with 2 ml of chloroform and 2 ml of concentrated sulphuric acid was carefully added and shaken gently, then the observations were made. A red-brown colour indicated the presence of a steroidal ring (glycone portion of glycoside)

b. Liebermanns test: The solvent extract was mixed with 2 ml of chloroform and 2 ml of acetic acid. The mixture was cooled in ice and observations were made. A colour change from violet to blue to green indicated the presence of a steroidal nucleus (the glycone portion of glycosides).

c. Keller-Kilani test: The solvent extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of a 2% $FeCl_3$ solution. This mixture was then carefully poured into a test tube containing 2 ml of concentrated sulfuric acid. The formation of a brown ring at the interface of the two solutions indicated the presence of cardiac glycosides.

2.3.6 Alkaloids

The crude extract was mixed with 1% HCl in a test tube. The test tube was then gently heated and the mixture was filtered. A few drops of Mayer's and Wagner's reagents were then added to the side of the test tube containing the filtrate. The formation of a precipitate confirmed the presence of alkaloids.

2.3.7 Phenols

The solvent extract was put in a test tube and treated with a few drops of 2% of FeCl3. Blue green or black colouration indicated the presence of phenols.

2.3.8 Steroids

1. Salkowski's Test: About 2 g of the solvent extract was taken in a test tube and mixed with 2 ml of chloroform. After thorough mixing, 2 ml of concentrated sulfuric acid was carefully added down the side of the test tube. A red colour at the interface indicated the presence of steroids.

2. Liebermann-Burchard's Test: Approximately 2 g of the extract was dissolved in 10 ml of chloroform and filtered. To 2 ml of this filtrate, 2 ml of acetic anhydride was added, followed by careful addition of concentrated sulfuric acid. A colour change from pink to blue or green indicated the presence of steroids.

2.3.9 Reducing Sugars Detection

Benedict's Test: About 2 g of the extract was mixed with 2 ml of Benedict's reagent in a test tube. The mixture was heated in a boiling water bath for 3–5 minutes. A colour change from blue to green, yellow, orange, or red indicated the presence of reducing sugars, with the colour intensity corresponding to the concentration of reducing sugars present.

3. Results

The qualitative biochemical analysis of Holothuria leucospilota extracts was carried out using a variety of solvents, including ethanol, methanol, water, petroleum ether, and chloroform. The analysis aimed to identify the presence of secondary metabolites such as tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids, phenolic compounds, reducing sugars, and steroids. The results of this analysis are summarized in Table 1, which shows the presence (+) or absence (-) of these metabolites in the different solvent extracts.

Table 1: Qualitative Biochemical Analysis of Holothuria leucospilota Extracts for Secondary Metabolites

Secondary Metabolite	Test	Ethanol Extract	Methanol Extract	Water Extract	Petroleum Ether Extract	Chloroform Extract
Tannins	Ferric Chloride	+	+	+	-	-
Saponins	Honeycomb	+	+	-	-	-
Flavonoids	Magnesium Ribbon	+	+	-	-	-
Terpenoids	Sulfuric Acid	+	+	-	-	-
Glycosides	Salkowski	+	+	-	-	-
	Liebermann	+	+	-	-	-
	Keller-Kiliani	+	+	-	-	-
Alkaloids	Mayer	+	+	-	-	+
	Wagner	-	-	-	-	+
	Dragendorff's	+	+	-	-	+
Phenolic Compounds	Ferric Chloride	+	+	+	-	-
	Ferric Sulfate	+	+	+	-	-
Reducing Sugars	Benedict's	+	+	+	-	-
Steroids	Salkowski	+	+	-	-	+
	Liebermann Burchard's	+	+	-	-	+

4. Discussion

The qualitative biochemical analysis of *Holothuria leucospilota* extracts from various solvents (ethanol, methanol, water, petroleum ether, and chloroform) revealed the presence of several bioactive secondary metabolites, including tannins, saponins, flavonoids, terpenoids, glycosides, alkaloids, phenolic compounds, reducing sugars, and steroids. These metabolites are known for their therapeutic potential and contribute to the bioactivity of marine organisms like sea cucumbers.

The qualitative analysis of *Holothuria leucospilota* extracts revealed the presence of several bioactive secondary metabolites. Tannins were detected in ethanol, methanol, and water extracts, aligning with other marine organisms' findings (Akinmoladun et al., 2007). Saponins were present in ethanol and methanol extracts, supporting their solubility in polar solvents (Rai et al., 2023). Flavonoids were found in ethanol and methanol extracts, consistent with previous reports of their antioxidant properties (Ghanimi et al., 2022).

Terpenoids were detected in ethanol and methanol extracts, demonstrating solubility in polar solvents (Bakkali et al., 2008). Glycosides were present in both ethanol and methanol extracts, reflecting their bioactive potential (Bhaskar et al., 2006). Alkaloids appeared in ethanol, methanol, and

chloroform extracts, indicating solubility in both polar and non-polar solvents (Kuppuswamy et al., 2025).

Phenolic compounds were found in ethanol, methanol, and water extracts, known for their antioxidant properties (Rios et al., 2005). Reducing sugars were detected in ethanol, methanol, and water extracts, which are commonly present in marine species (Murugaiyah et al., 2015). Steroids were identified in ethanol, methanol, and chloroform extracts, confirming their solubility in both polar and non-polar solvents (Kumarasamy et al., 2020).

Overall, *Holothuria leucospilota* contains a variety of bioactive compounds, especially in ethanol and methanol extracts, highlighting its pharmacological potential.

5. Conclusion

The ethanol and methanol extracts of *H. leucospilota* were the most bioactive, exhibiting a wide range of secondary metabolites including alkaloids, phenolic compounds, saponins, glycosides, reducing sugars, tannins, and steroids. These compounds, especially in ethanol and methanol extracts, demonstrate the significant pharmacological potential of this marine species. The findings highlight *H. leucospilota* as a promising source of natural bioactive substances, supporting its potential use in medicinal and therapeutic applications. Further studies on the isolation and characterization of these bioactive compounds are recommended to explore their specific pharmacological activities.

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