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ORIGINAL RESEARCH

Phytochemical Profiling, Elemental Constituents and Antimicrobial Efficacy of the Sponge *Haliclona oculata* Collected off the Bay of Bengal.

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Abstract

The Bay of Bengal holds unexplored marine riches. Due to inadequate funding and technological limitations, these resources are underutilized. This study investigated the phytochemical composition, and elemental constituents using an Atomic Absorption Spectrophotometer (AAS), and the antibacterial activity of *Haliclona oculata*. The sponge was collected from Saint Martin Island's southeast shore during low tide. The sponge extract was separated using acetone as solvent at room temperature. Phytochemical screening was performed to reveal the presence of various secondary metabolites and bioactive compounds in the sponge extract and study its diverse properties. Phytochemical screening revealed seven types of secondary metabolites and bioactive compounds, with high quantities of flavonoids, steroids, and terpenoids. Sodium and chloride were the main elements found in the samples (148.538 and 16.504 mg/dl respectively). Antibacterial tests were conducted using gram-positive (Staphylococcus aureus) and gram-negative (*Escherichia coli*) bacteria at concentrations of 10, 20, 30, and 40 µg/ml. The inhibition zone for *Escherichia coli* at 40% concentration was 21 mm, while for Staphylococcus aureus it was 16 mm. This indicates that *Escherichia coli* (gram-negative) has a higher potential inhibition zone than Staphylococcus aureus (gram-positive) at the same concentration of acetone extracts of *Haliclona oculata.*

1. INTRODUCTION

Approximately 80% of all biota on earth are marine species. Because of their isolation difficulties and complexity of accessibility, marine life has frequently received less research attention than upland lifeforms, even though they are frequently solitary sources of naturally occurring bioactive compounds with novel mechanisms of action. Marine sources have recently become the focus of more natural product studies due to their greater pharmacological and biological novelty over terrestrial sources. When compared to natural molecules from terrestrial habitats, those isolated from aquatic environments (basically marine) generally exhibit greater and more substantial bioactivity (Zineb et al., 2017). Until now, marine microbes have been underappreciated as a potential source for synthesizing new bioactive pharmacological chemicals (Fahmy & Abdel-Tawab, 2021). Organic chemists owe a great debt of gratitude to these natural molecules for sparking several innovations, including improvements to synthesis methods and the prospect of pharmacological and pharmaceutical agents with comparable effects. Secondary metabolites and natural products are often used simultaneously; both come from natural sources like plants, animals, or microbes (Zineb et al., 2017). A considerable percentage of the approximately 200 novel active chemicals found annually in marine environments are attributed to sponges (Blunt et al., 2009). The specific types of active chemicals found in sponges are diverse. To protect themselves from potential threats, marine sponges have evolved biologically active compounds due to the unique characteristics of marine environments compared to their terrestrial counterparts (Gogineni & Hamann, 2018).

It has proven possible to extract over 10,000 medical and bioactive substances from invertebrates found in the ocean. As the earliest multicellular creatures, sponges are boneless invertebrates that belong to the phylum Porifera. Since 1965, sponges have been the leading source of physiologically active chemicals among marine organisms, accounting for the largest secondary metabolites identified in 2003. Approximately 5000

metabolites have been retrieved from sponges globally to date, making up roughly thirty percent of the total compounds that have been extracted from marine ecosystems. Since the beginning of the last decade, there have been about 200 new bioactive compounds found that are generated from sponges which are disclosed annually (Kibungu et al., 2021). Sponge species range in number from 9,000 to 15,000 (BHADEKAR N. S., 2021). Since they make up around 30% of all naturally occurring compounds found to come from marine resources, sponges are acknowledged as one of the most abundant sources of multifunctional natural goods (Paul et al., 2021). Sponge production of several secondary metabolites is necessary for survival in the marine environment. Furthermore, such organisms typically encompass about 35% of their entire body weight in a wide variety of communities of microbial organisms (Martignago et al., 2023). Bioactive chemicals have been found in sponges more than other marine life forms. After a lengthy 700–800 million years evolution period, the sponges represent perhaps the most rudimentary of all multicellular creatures of all kinds (Bhimba & Vakati, 2013).

Researchers have recently taken keen interest in studying sponges analytically since these marine species have been discovered to accumulate large amounts of different elements, especially heavy metals, which could be caused by humans (Le Pennec et al., n.d.). Because of this, they can now be used as an indicator of environmental pollution. The materials' selectivity is shown by the composition and size of the grain in the environment, which is affected by the substrate to which the sponge is attached. The processes involved and the purpose of the material in such creatures are both uncertain (Pozzolini et al., 2010). The development and growth of all living things rely on trace elements. It is not well- known what elements contain sponges from the Bay of Bengal or how they deal with trace metals. This study compiles the components identified in sponges into an initial database. It provides the necessary groundwork for further research into the processes by which sponges regulate their metal consumption, accumulation, and preservation. A surprising lack of information regarding the trace metal content of the sponges found at Cox's Bazar remains a mystery, despite the fact that this area has long been favored for coral reef research. The main external source of trace elements, therefore, is atmospheric aerosols.

There have been reports of a wide range of activities for the bioactive chemicals found in sponges, including anti-inflammatory, cancer-preventing, immune suppressive, curative, antiseptic, and anti-fouling properties (Krishnan & Keerthi, 2016). Sponge phytochemicals have been shown to offer protection against aging, cancer, coronary artery disease, Alzheimer's disease, blindness, retinal degeneration, and other conditions linked to elevated oxidative stress (Chairman et al., 2012). One of the world's greatest areas for marine biodiversity is the Bay of Bengal. Saint Martin, an exceptionally isolated island in Bangladesh, is a habitat of coral and a multitude of other local wildlife. Organic material derived from species associated with the coral ecosystem has been employed to build the island. The island is located 9 km off the tip of Bangladesh's Cox's Bazar peninsula and 8 km west of Myanmar's north coast. There are abundant aquatic and terrestrial resources on St. Martin Island that are noteworthy for biodiversity (Hossain et al., n.d.). The distinct marine organisms are highly worthwhile from a scientific standpoint for study and research. The abundance of sponges around the shoreline of Saint Martin was validated by reviews of relevant literature and unofficial database analysis. However, our understanding of the species found in Saint Martin's sponge is unfortunately lacking in published research. Prior research on isolating the secondary metabolite and assessing the antibacterial effectiveness has not been conducted on this species of marine sponge. It is the first thorough paper that we are aware of that evaluates the antibacterial activity, elemental analysis, and phytochemical profiling of the sponge species *Haliclona Oculata*, which is collected from the shores of Saint Martin island.

2. MATERIALS AND METHODS

2.1 Sponge Collection and Identification

In February 2023, we gathered sample sponge specimens from several locations around Saint Martin Island's southeast shore during low tide in the Bay of Bengal. The samples were primarily cleaned with seawater to get free of any attached sediment, dirt, debris, mud, and organisms. Immediately after being cleaned, the sponge was placed in a sealed icebox to be frozen for an overnight period and brought to the lab the next day. The collected marine sponge specimen had been identified at the Marine Natural Products Research Laboratory, University of Chittagong, Bangladesh. Utilizing spicules sorted by digestion of nitric acid and employing established recognition keys, the species were taxonomically identified (Hooper, 2002; Thomas, 1986).

2.2 Extract Preparation

Following multiple times of washing and rinsing with tap water and distilled water to remove any remaining impurities, the specimens of sponges were chopped into very small pieces and allowed to air dry before the material was crushed into a fine powder. At room temperature, powdered sponge and acetone (2.5%) were macerated for 72 hours. The suspension was shaken many times a day and the slurry was filtered through a sieve. Following that, the suspension was filtered using Whatman's filter paper No. 1. To obtain the crude extract, the filtrate was moved to a volumetric flask and dried using a rotary evaporator (H50-500, LabTech) with a maximum temperature setting of 40 °C. Following the extraction of this mixture in the presence of acetone, the organic layer was separated and dried on a rotary evaporator to yield the crude extract. The extracts were stored in a freezer at 4°C till more study was conducted.

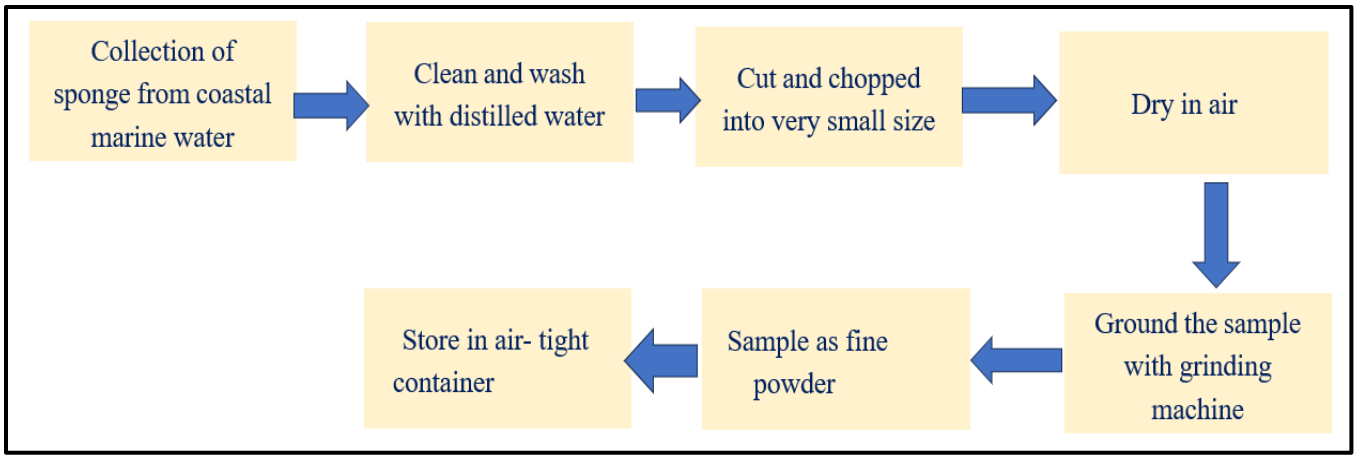

Figure 1: Flow chart of the preparation of sponge powder

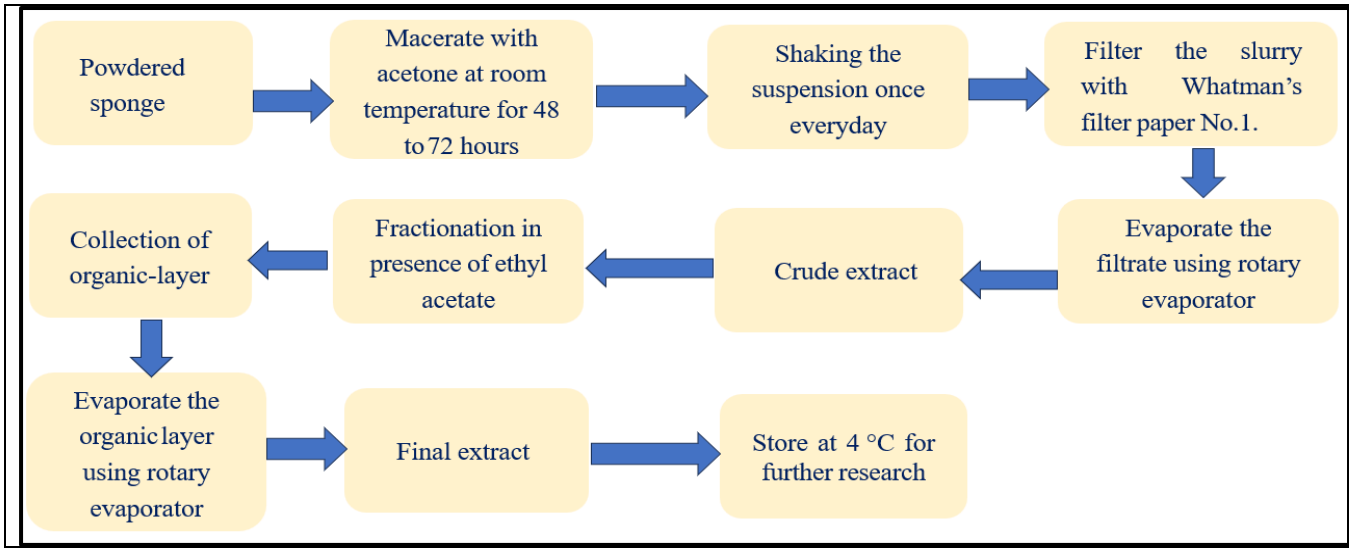

Figure 2: Flow chart of the preparation of sponge extracts

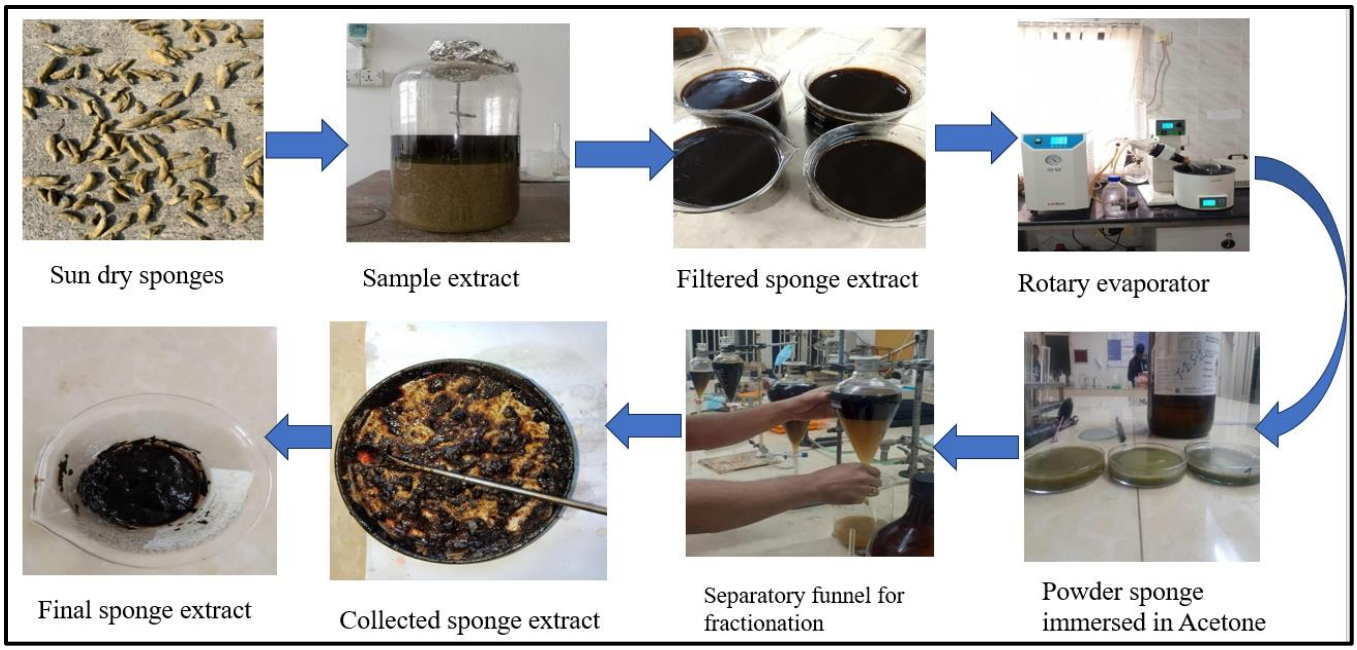

Figure 3: Preparation of the extract of marine sponge sample.

2.3 Phytochemical Screening

To determine the secondary metabolites employing standard techniques, the extract was put through a series of phytochemical experiments (Prashant Tiwari* & Bimlesh Kumar, 2011).

The following tests were performed for the phytochemical screening of the marine sponge samples.

1. **Test for Alkaloids**: Diluted hydrochloric acid was used to dissolve the extract, which was then filtered.
   - i) **Wagner's Test**: Wagner's reagent, which is iodine in potassium iodide, was applied to the filtrate. When a brown or reddish precipitate forms, an alkaloid is present.
   - ii) **Dragendorff's Test:** Dragendorff's reagent (potassium bismuth iodide solution) was applied to the filtrate. The existence of alkaloids is shown by the appearance of red precipitate.
   - iii) **Mayer's Test:** Mayer's reagent (potassium mercuric iodide) was added to the filtrate. The occurrence of alkaloids can be detected by the formation of a yellow-colored precipitate.
   - iv) **Hager's Test**: The filtrate underwent treatment using Hager's reagent, which is a solution of saturated picric acid. The abundance of alkaloids is demonstrated by the precipitate's yellow coloration.

2. **Test for Tannins**:
   Gelatin Test: One percent gelatin solution with sodium chloride was added to the extract. Tannin agents are indicated by the formation of a white precipitate.

3. **Test for flavonoids**:
   - i) **Alkaline Reagent Test:** Some drops of sodium hydroxide solution were added to the extract. When diluted acid is added, the strong yellow color that formed before turning colorless signifies the existence of flavonoids.
   - ii) **Lead acetate Test**: Several drops of lead acetate solution were added to the extract. Precipitate that has a yellow hue suggests that it has flavonoids.
   - iii) **Shinoda Test:** 5 ml of methanol was applied to the sample (1 g) in a test tube of 20 ml. In addition, three magnesium chip pieces and a few drops of strong HCl were added to the sample. There's a purple hue that suggests flavonoids are present.
   - iv) **Sulfuric Acid Test**: When a small amount of the extract is dissolved in one milliliter of strong sulfuric acid, a color shift that suggests the existence of flavonoids is seen.

4. **Test for saponins:**
   - i) **Froth Test**: After diluting the extract with 20 ml of distilled water, it was agitated for 15 minutes in a graduated cylinder. The occurrence of saponins is shown by the appearance of a one-centimeter-thick layer of foam.
   - ii) **Foam Test**: Two milliliters of water were mixed with half a gram of extract. The possibility of saponins is obvious if the foam formed lasts for 10 minutes

5. **Test for Glycosides**: After hydrolyzing the extract with dilute hydrochloric acid, the glycosides were tested.
   i) **Modified Borntrager's Test**: After treating the extract with a solution of ferric chloride, it was submerged in warm water for approximately five minutes. Equal parts of benzene were added to the mixture once it had cooled. After being separated, the benzene layer was given an ammonia solution treatment. Anthranol glycosides are present when the ammoniacal layer begins to take on a rose-pink hue.
   ii) **Legal Test:** Sodium nitroprusside was applied to the extract along with sodium hydroxide and pyridine. Cardiac glycosides are present when a pink to blood-red tint forms.
   iii) **Ferric chloride Test:** After adding 5 milliliters of strong H_2SO_4 to a tiny amount of the extract, it is heated for 15 minutes. After cooling, 20% KOH is added to neutralize this. There are two parts to the solution. Phenolic glycone is produced when a ferric chloride solution (3 drops) is added to one of the sections. This is because the hydrolysis of the glycoside produces a green-to-black precipitate.
   iv) **Kella-Killani Test:** A test tube (20 ml) holding 0.5 g of the sample was filled with 5 ml of glacial acetic acid, which included residues of ferric chloride. Holding the test tube at a 45-degree angle, 1 milliliter of pure sulfuric acid was carefully added down the side. There is a purple ring-colored area at the junction, which suggests the existence of cardiac glycoside.

6. **Test for Steroid:**
   Salkowski Test: Two phases are formed by adding strong sulfuric acid (1 ml) and 1 ml of chloroform to a 0.5 g sample in a test tube of 20 ml. The emergence of a yellow color is considered to be an indicator of the presence of sterols.

7. **Test for Terpenoids**:
   Liebermann-Buchard Test: Strong sulfuric acid was applied after acetic anhydride (few drops) and one milliliter of chloroform was added to 0.5 g of sample in a test tube of 20 ml. After carefully mixing the combination, the resultant solution had a blue color that shifted over time, suggesting the presence of terpenes.

8. **Test for Phenolic compound**:
   Ferric Chloride Test: Three to four drops of ferric chloride solution were added to the extract. Phenols are present when a blue-black color begins to form.

9. **Test for Carbohydrate**: Extract was dissolved in 5 ml distilled water and filtered. The filtrate was used to test for the presence of carbohydrates.
   i) **Molisch's Test:** A test tube containing two drops of an alcoholic α-naphthol solution was used to treat the filtrate. The abundance of carbohydrates is shown by the violet ring forming along the junction.
   ii) **Benedict's Test:** Benedict's reagent was applied to the filtrate, and it was then gradually heated. Reducing sugars are detected by the appearance of an orange-red precipitate.
   iii) **Fehling's Test:** The filtrate was heated using Fehling's A and B solutions, neutralized with alkaline substances, and hydrolyzed using dilute hydrochloric acid. Red precipitate denotes the presence of reducing carbohydrates.

10. **Test for Phytosterol:**
   i) **Liebermann Burchard's Test**: Chloroform was applied to the extract before it was filtered. After adding some drops of acetic anhydride to the filtrate, it was heated and allowed to cool. Concentrated sulfuric acid was introduced. The development of a brown ring at the intersection denotes an abundance of phytosterols.
   ii) **Salkowski's Test**: Chloroform was applied to the extract before it was filtered. A few drops of concentrated sulfuric acid were added to the filtrate, which was then agitated and left to stand. Triterpenes are identified by their golden-yellow appearance.

11. **Test for Proteins and Amino Acids:**
   i) **Xanthoproteic Test:** A few drops of strong nitric acid were added to the extract. The appearance of yellow color signifies the existence of proteins.
   ii) **Ninhydrin Test:** A 0.25% w/v ninhydrin reagent was added to the extract and allowed to boil for a short while. Amino acid content is indicated by the development of a blue hue.

2.4 Elemental Analysis

A crucible was stuffed with six grams of the sample dried in an oven. After that the crucible was heated to 500 °C for 4 hours in a muffle furnace. After the furnace had receded to approximately 120 °C, the crucible was taken out and left to cool in a desiccator for an hour before being weighed. The entire procedure continued until a consistent weight was achieved. . Each digesting tube was filled with the 0.5 g weighted ash-like samples. HNO_3 (5 ml), distilled water (5 ml), and $HClO_4$ (5 ml) were poured into the tube. The digesting tubes were stirred continuously for a uniform mixture of the components in it. The digester's temperature was set to 160 °C, and the digestion continued for 80 minutes within the digestion block, which was kept within a fume cabinet. After that, the temperature was raised to 250 °C, and the well was digested for a further 30 minutes (the stage known as "white fuming"). After lowering the temperature of the

digester to 140 °C once more, HCl (1ml) was introduced to the tubes. The temperature of the digest was lowered and reached room temperature to avoid the emergence of an insoluble residue ($KClO_4$). The tube's contents were combined and filtered after additional water had been introduced to bring it up to par. An atomic absorption spectrophotometer was used to analyze the elements in the resultant solution.

2.5 Antimicrobial Activity

The ability of the test agents to suppress the development of microbes around the discs, which results in a distinct zone of inhibition, serves as a gauge of their antimicrobial potency. For this purpose, Petri dishes and other glassware were sterilized in an autoclave for about 1 hour at 121 °C and 15 lbs/sq inch of pressure. To prepare the test plate (simply plating), the nutrient agar medium was maintained at 55 °C in a water bath to prevent precipitation. Plating was conducted in a Biological Safety Cabinet (BSC) with UV modes, lights, and fans on to assist solidification and disinfection. The BSC was covered with thick black cloths for UV protection. Nutrient agar was evenly distributed on sterilized plates and left in the BSC for 50 minutes to solidify. During this time, the lights and fans were off, and the UV lid was closed. Streaking (preparation of subculture) was performed in an aseptic laminar air cabinet. Test organisms from fresh cultures were transferred to slanted agar and incubated at 37 °C for 24 hours to generate pure cultures. This study used gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria stored at -70 °C to -80 °C. Streaking was done in the BSC with UV mode on, lights, and fans off. Bacteria were transferred using a sterilized loop, which was flamed after each use to prevent contamination. This process was repeated several times to ensure purity.

4. RESULTS

Table 1: Qualitative phytochemical screening of sponge sample

Constituents	Test	Reagent used	Observation	Sponge extract
Alkaloid	Wagner's Test	Potassium iodide	A brown or reddish precipitate	-
	Dragendroff's Test	Potassium bismuth iodide solution	Red precipitate	
	Mayer's Test	Potassium mercuric iodide	Yellow precipitate	
	Hager's Test	Saturated picric acid solution	Yellow precipitate	
Tannins	Gelatin Test	Gelatin solution	White precipitate	-
Flavonoids	Alkaline Reagent Test	Sodium hydroxide solution	Strong yellow color formation	+++
	Lead acetate Test	Lead acetate solution	Yellow precipitate	
	Shinoda Test	Methanol	Purple color formation	
	Sulfuric Acid Test	Strong sulfuric acid	Color shifts	
Saponins	Froth Test	Distilled water	Thick layer of foam	+
	Foam Test	Distilled water	Foam formation	
	Modified Borntrager's Test	Ammonia solution	A rose-pink hue.	

Glycosides	Legal's Test	Sodium nitroprusside, sodium hydroxide	Pink to blood red tint forms	+
	Ferric chloride Test	Ferric chloride solution	Green to black precipitate	
	Kella-Killani Test	Glacial acetic acid	Purple ring-color	
Steroid:	Salkowski Test	Sulfuric acid and chloroform	Emergence of yellow color	++
Terpenoids	Liebermann-Buchard Test	H_2SO_4 and acetic anhydride	Blue color solution	++
Phenolic compound	Ferric Chloride Test	Ferric chloride solution	Blue-black color solution	+
Carbohydrate	Molisch's Test	Alcoholic α-naphthol solution	Violet ring formation	-
	Benedict's Test	Benedict's reagent	Orange-red precipitate	
	Fehling's Test	Fehling's A and B solutions	Red precipitate	
Phytosterol	Libermann Burchard's test	Acetic anhydride and conc. H_2SO_4	Brown ring development	-
	Salkowski's Test	Chloroform and conc. H_2SO_4	Golden yellow appearance	
Proteins and amino acids	Xanthoproteic Test (Protein)	Strong nitric acid	Yellow color appearance	+
	Ninhydrin Test (Amino acid)	Ninhydrin reagent	Development of blue hue	

[+: present moderately; ++: present highly; +++: present very strongly; -: indicates absence]

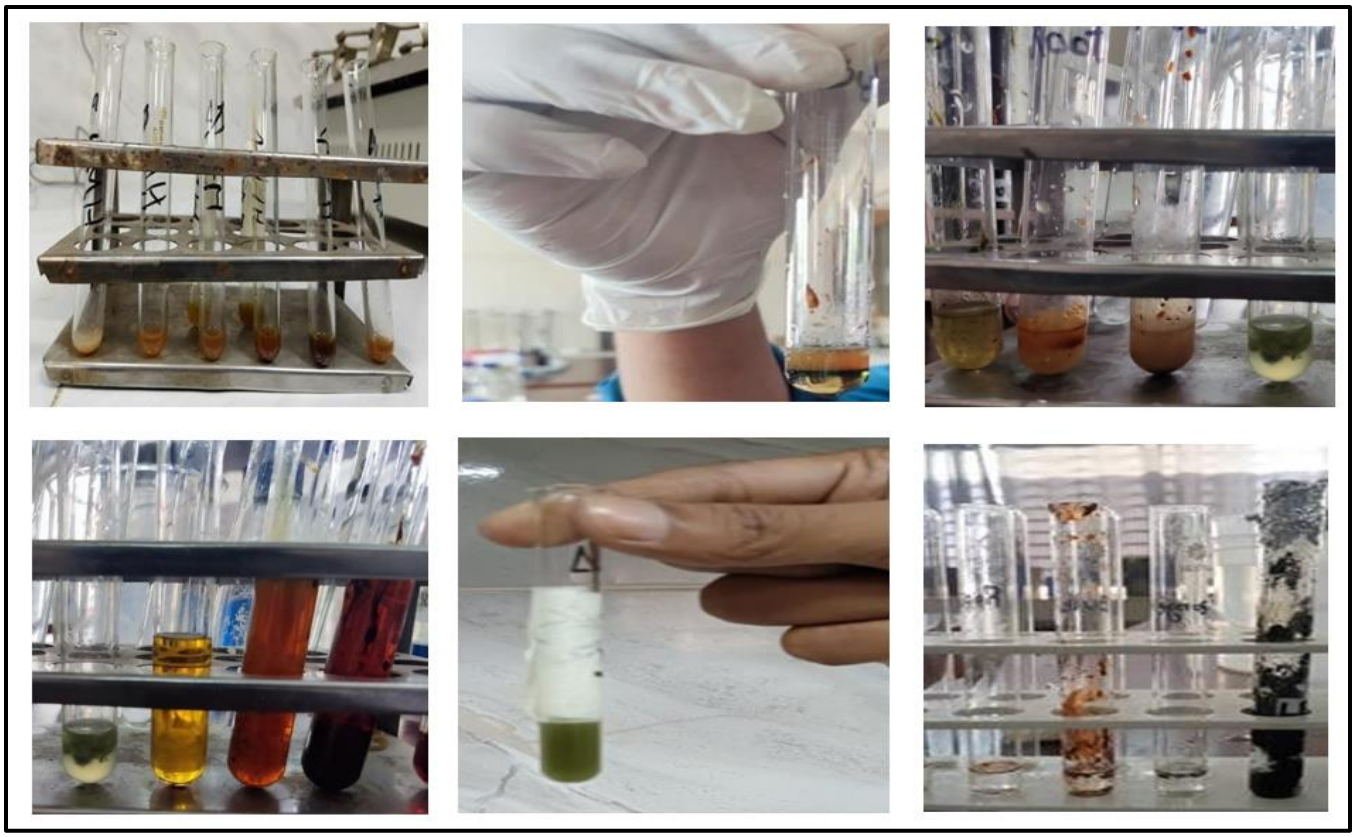

Figure 4: Qualitative test for phytochemical metabolites

Table 2: Different applications of the phytochemicals derived from the acetone extract of *Haliclona Oculata.*

Phyto chemicals	Field of Applications	Different Applications
Flavonoid	Health benefits	Possess the capacity to offer protection against a range of ailments, including diabetes, cancer, metabolic disorders, and cognitive and cardiovascular issues (Soobrattee et al., 2005)
	Food industry	In addition to animal feed, the prevention of microbiological deterioration and lipid oxidation in meats protects vitamins and enzymes (Huvaere & Skibsted, 2015).
	Skin-carebenefit	Flavonoids found in dermatological products may maintain the overall health and beauty of the skin (Huvaere & Skibsted, 2015)
Saponins	Biological application	Saponins are used biologically as immune-stimulating agents (Guclu-Ustundag & Mazza, 2007)
	Use in cosmetics	By eliminating dirt and oil from the skin's surface, saponins promote high skin surface activity, which serves as a cleaning agent (Touriño et al., 2008).
	Medicinal use	Saponins prevent platelet aggregation, reduce lipid levels in the blood, blood glucose response, risks of cancer, and cholesterol (Tiwari et al., 2009).
Glycoside	Physiological effects	In addition to being advantageous as nutritional supplements, these nonmetabolizable food additives do not cause tooth decay (Tiwari et al., 2009).
	Medicinal use	Glycosides are medications used to treat heart arrhythmias and heart failure.
	Stability in food matrices	Under the recommended conditions, glycosides, as food additives, stay stable in the food matrix throughout the whole manufacturing and storage process (Yang et al., n.d.)
Steroids	Effect on body	People who utilize anabolic steroids usually experience very quick increases in muscle strength and lean muscle mass
	Medicinal use	A class of anti-inflammatory medications called corticosteroid tablets, or steroids, is used for the treatment of asthma, allergies, Addison's disease, inflammatory bowel disease, and osteoarthritis.
	Poultry growth	Due to the high cost of steroids and hormones, the chicken industry chooses not to use them out of extreme avarice rather than consideration for the wellbeing of its animals.
Terpenoid	Medicinal value	Terpenoids have antiseptic, antiviral, anti-inflammatory, antifungal, and anti-parasitic properties in addition to their ability to prevent cancer through chemotherapy (Newman et al., 2000)
	Therapeutic use	These terpenoids exhibit a broad spectrum of biological activity against several infectious illnesses, cancer, inflammation, and malaria (Panche et al., 2016)
	Use in the food industry	Among the flavor compounds produced by microbial fermentation are terpenoids, which have potent floral, fruit, rose, and tobacco odors, particularly sesquiterpenes.
Proteins and amino acids	Fertilizer	The ability of amino acids to chelate is often used in fertilizers to accelerate the uptake of minerals by plants, hence treating mineral shortages such as iron chlorosis (H. DeWayne Ashmead, 1986)
	Pharmaceutical	A variety of amino acid compounds are used in the pharmaceutical business. A few examples are eflornithine, 5-HTP (5-hydroxytryptophan), which is being tested as a treatment for depression (Sanda & Endo, 1999), L-DOPA (L-dihydroxyphenylalanine) for Parkinson's disease (Auddy et al., n.d.)
	Animal feed	Animal feed is supplemented with amino acids because several of its ingredients are deficient in certain important amino acids, especially tryptophan, lysine, and methionine (Schwardmann et al., 2023)

Table 3: Elemental (Mineral) content of marine sponge *Haliclona oculata* extracts.

Components	Obtained Minerals (mg/dl)
Potassium	2.47
Phosphorus	2.73
Chloride	16.504
Magnesium	0.703
Sodium	148.538
Copper	0.181
Calcium	1.806

Table 4: Antibacterial activity of acetone extract of *Haliclona oculata* against two strains of bacteria.

Sample ID	Concentration (µg/ml)	Zone of inhibition at various concentrations (Diameter in mm)	
		Escherichia coli	***Staphylococcus aureus***
S1	10	3	Nil
S2	20	8	5
S3	30	15	11
S4	40	21	16

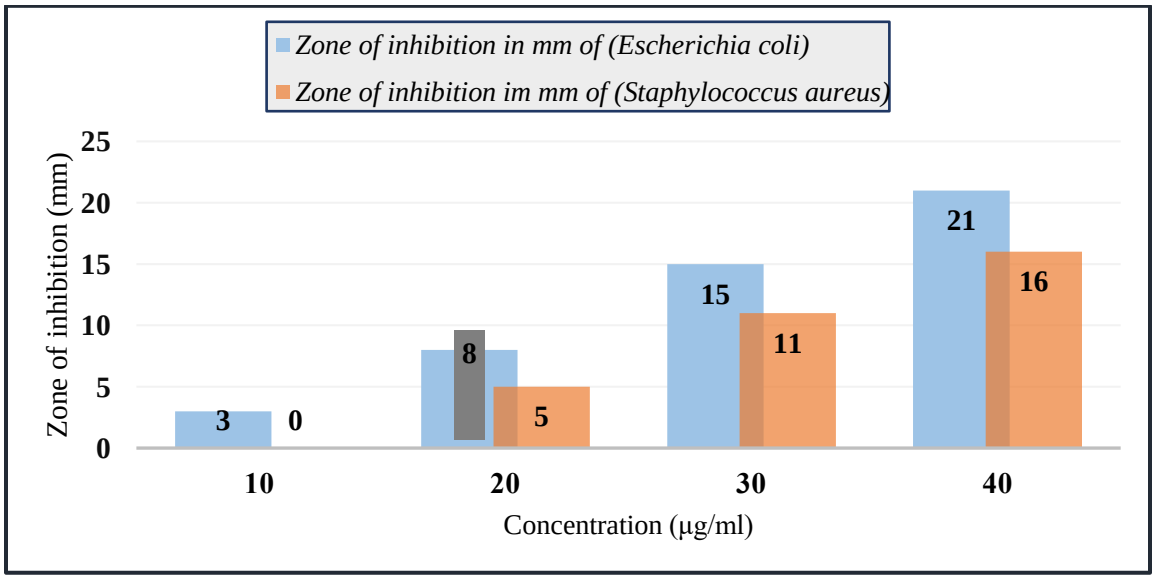

Figure 5: The zone of inhibition (in mm) of the crude extract of *Haliclona oculata* against various concentrations.

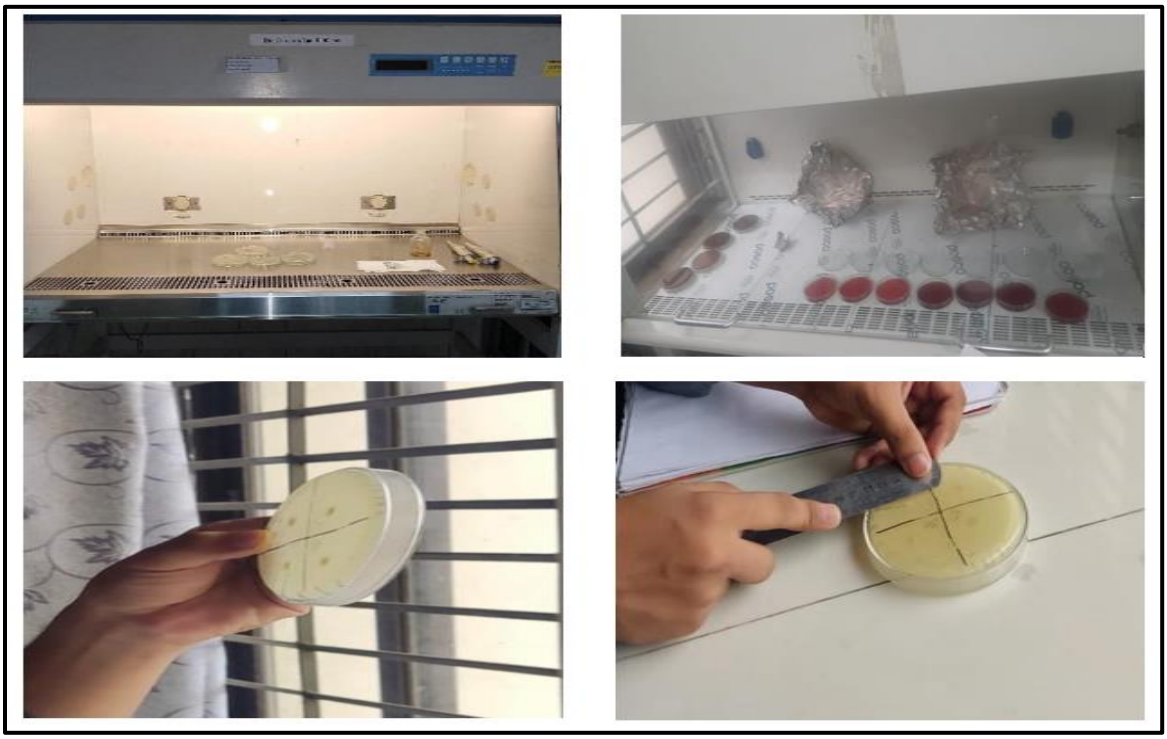

Figure 6: Antibacterial test of the crude sample of *Haliclona oculata*.

5. DISCUSSION

5.1 Bioactive Substances

Marine sponges are known for their ability to synthesize a diverse array of secondary metabolites that exhibit a variety of biological activities. An initial qualitative phytochemical analysis was carried out with the use of a sample of sponge extract. For qualitative evaluation, the following phytochemicals were taken into consideration as well: alkaloid, flavonoid, glycoside, tannin, saponin, phenolic compound, carbohydrate, reducing sugar, protein and amino acid, acidic compound, phytosterol, and terpenoid phytochemicals. The presence of relative phytochemicals in the extract of the test sample was indicated by using the plus sign (+). To indicate that the secondary metabolites were not present, the symbol (-) was utilized. According to the phytochemical data, the extracts' constituents have distinctive chemical compositional patterns. **Table 1** displays the findings from the screening of phytochemicals.

In our current analysis, the most prevalent substances identified were flavonoids, saponins, glycosides, proteins, amino acids, steroids, and terpenoids. Notably, our sponge sample lacked alkaloids, tannins, phenolic compounds, carbohydrates, reducing sugar, acidic compounds, or phytosterol **(Table 1)**. This aligns with findings by (Mahmood Belal Haider et al., 2022), highlighting the pharmacological potential of marine sponges, including cytotoxicity, antibacterial, thrombolytic, antioxidant, antiviral, and antifungal properties. Marine sponges have biological functions that include cytotoxicity, antimicrobial, thrombolytic, antioxidant, antiviral, and antifungal. All of these functions are related to its antioxidant capability. To protect cells from free radical damage, antioxidants are vital to human health. Certain bioactive compounds, also known as secondary metabolites, are referred to as phytochemicals within the scientific community. Phytochemically active components have been shown to improve how we maintain our internal systems by improving metabolism or regulating hormones. They assist in protecting us against contaminants from the environment. It has been demonstrated that many bioactive chemicals possess unique biological properties and may have potential medicinal applications. (Mushtaq et al., 2018)

5.2 Mineral Content Analysis

Sodium and chloride were the primary constituents in our sample species, with concentrations of 148.538 mg/dl and 16.504 mg/dl, respectively. Phosphorus and potassium were also present in significant amounts (2.73 mg/dl and 2.47 mg/dl, respectively), along with trace levels of calcium, magnesium, and copper **(Table 3)**. Atomic Absorption Spectroscopy (AAS) was used to analyze the mineral content of our marine sponge specimen. Energy Dispersive X-ray Spectrometry (EDXA/EDS) has also enabled the detection of various elements in recent research (Pallela et al., 2011).

An analysis of a sponge sample reveals that different components may concentrate within the sponge tissues along with the food particles that have been consumed, depending on the structural differences between them. This results in unequal deposition on the surface of the sponges. Marine sponges can be used as a bio-indicator for heavy metal detection in maritime coastal areas by undergoing ICP-AES analysis, which aids in the identification of Cu, Zn, Pb, Cd, Fe, As, and other heavy metals (Kumar & Shah, 2014).

5.3 Antibacterial Activity

Our study proved that acetone extracts of *Haliclona oculate* inhibit the growth of bacteria. The extract was tested against *Escherichia coli* and *Staphylococcus aureus* at concentrations of 10, 20, 30, and 40 µg/ml **(Table 4)**. *Escherichia coli* showed the highest inhibition zone of 21 mm at 40 µg/ml concentration, with the lowest inhibition zone of 3 mm at 10 µg/ml. For *Staphylococcus aureus*, the highest inhibition zone was 16 mm at 40 µg/ml, with no inhibition observed at 10 µg/ml. These results suggest that the gram-negative strain of *Escherichia coli* has a higher potential inhibition zone than *Staphylococcus aureus*.

Bioactive substances in marine sponges are crucial for the treatment of many diseases. Phenolic compounds, known for their antiseptic properties, contribute significantly to the antibacterial effects observed in marine sponges. These phenolic substances can denature proteins and interact with cell membrane phospholipids, altering their permeability and providing strong antibacterial effects. Flavonoids and phenols in marine sponges support their therapeutic value (M. J. Pelczar, 1988). The differences observed in the antimicrobial efficacy against various bacterial strains underscore the impact of these environmental conditions on the production of bioactive compounds in marine sponges. The fluctuations in antimicrobial activity may be ascribed to environmental factors surrounding the sponges, which may encourage the development of particular defense mechanisms. These results suggest that marine sponges and other aquatic habitats may present a distinct challenge for symbionts, necessitating the evolution of novel survival mechanisms, such as antimicrobial chemicals.

6. CONCLUSION

In this study, a comprehensive analysis of a marine sponge sample from Saint Martin Island's coastal shore was conducted, focusing on its qualitative phytochemical profile, mineral content, and antibacterial activity. The results have revealed a high flavonoid content, along with the presence of secondary metabolites such as terpenoids, saponins, glycosides, proteins, amino acids, and steroids. The remarkable antibacterial capabilities of these compounds have played a crucial role in the creation of novel medicines and topical antibacterial treatments. The rich array of essential nutrients, including vitamins, minerals, and amino acids, also highlights the potential of these sponges for inclusion in dietary supplements and various industries such as food, cosmetics, and health. The findings underscore the critical role of marine sponges, particularly *Haliclona oculata*, in bioprospecting for novel bioactive compounds. This

species shows great promise for contributing to the production of innovative medications and therapeutic agents. The antibacterial properties identified suggest substantial implications for the development of new antimicrobials, which is crucial in the fight against antibiotic resistance. This research also paves the way for breakthroughs in the food, pharmaceutical, cosmetic, and health sectors, supporting the sustainable use of marine resources.

Further investigations into the proximate composition and diverse biological activities, such as anticancer, anti-inflammatory, immunomodulatory, and antioxidant properties, could open up new avenues for medical and environmental sustainability. Novel antimicrobial agents should be the primary focus of future research, with a focus on characterization, detailed cell toxicity assessments, and screening of additional microbial strains.

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Declaration of Interests

We, the authors of this research manuscript, declare that we have no financial interest. We have provided written comment to publish the paper in this journal.

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