CHROMATOGRAPHIC ANALYSIS OF POTENTIAL BIOACTIVE COMPOUNDS FROM JANIA SEAWEED SPECIES FROM THE BAY OF BENGAL, BANGLADESH

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ABSTRACT

Algae or seaweed is a known rich natural source of nutritional and medicinal values, associated with its availability along the coastal areas of the world. Meanwhile, marine sources are known to exhibit biologically active natural products, and a few reports have been published on the detection of bioactive compounds from Jania species of coralline red algae in the division Rhodophyta, such as those found on Bangladeshi St. Martin's Island. Preliminary phytochemical screening of the ethanolic extract showed the presence of glycosides, alkaloids, saponins, and steroids. Although this species exhibited little antioxidant and antimicrobial activity, the cytotoxicity bioassay showed significant findings, which might be considered an indication for further exploration. High-Performance Liquid Chromatography (HPLC), Ultraviolet and visible (UV-Vis) spectroscopy, and Nuclear Magnetic Resonance (NMR) analysis suggest that some active metabolites are present in plants. These results highlight the potential use of *Jania* species as a promising candidate for drug discovery and development; however, further studies are necessary to identify its bioactive principles and test its pharmacological applications.

KEYWORDS: Seaweed, *Jania*, bioactive compounds, Saint Martin's, Bangladesh.

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Introduction

Oceans cover 71% of Earth's surface, producing over 50% of global oxygen and absorbing CO2 at rates 50 times higher than the atmosphere, making marine ecosystems vital for ecological balance (1). Bangladesh's Bay of Bengal, a nutrient-rich deltaic region, supports diverse seafood resources traditionally used for food and medicine (1).

Advances in biotechnology frame this potential within the Blue Economy, promoting sustainable ocean prospecting for economic growth while conserving ecosystems (2, 3, 4). Such a strategy would focus on the prospecting of the oceans and their sustainable use to promote economic growth and conserve marine ecosystems. A growing interest of researchers is focusing on marine organisms for obtaining secondary metabolites and bioactive substances (5) that can be screened by employing high-throughput technologies. Seaweeds, key marine resources, provide nutrients like amino acids, lipids, vitamins, and bioactive metabolites (polysaccharides, phenols, carotenoids) with applications in food, feed, fertilizers, medicine, and phycocolloids (6, 7, 8, 9, 10).

Seaweeds exhibit antitumor effects (suppressing proliferation, metastasis, apoptosis), biofuel potential as sustainable biomass, and remediation of heavy metals, dyes, and effluents (11, 12, 13, 14, 15, 16, 17).

Jania (Rhodophyta, Corallinaceae), a pale pinkish-white coralline alga, shows antifouling, anti-ulcer, anthelmintic, antimicrobial, and cytotoxic activities in prior *J. rubens* studies (brominated diterpenes, oxysterols like 16β-hydroxy-5α-cholestane-3,6-dione) (18, 19, 20, 21, 22, 23, 24, 25).

Despite these reports, no analyses exist for Bay of Bengal *Jania* specimens, where unique salinity, temperature, and pollutants may drive distinct secondary metabolites via adaptive evolution. This study fills this gap by profiling phytochemicals and bioactivities using UV-Vis, HPLC, and NMR, advancing marine natural products for pharmaceuticals and Blue Economy goals.

Materials and Methods

Sample Collection

The shallow coastal water tended to be collected for seaweed samples at Cherra Dwip and Coral Island of St. Martin's Island, Cox's Bazar, Bangladesh. The samples were

transported in a well-preserved condition to the laboratory at Bangladesh Maritime University, Mirpur, Dhaka.

Study Area

This research was conducted on St. Martin's Island, a small coral island located approximately 10 km northwest of the Bangladesh mainland. The island is located between 20º34' – 20º39' N and 92º18' - 92°21'E geographical coordinates.

Experimental Workflow

The study followed a standardized procedure of sample preparation, extraction, analysis, and compound identification as illustrated in **Figure 1**.

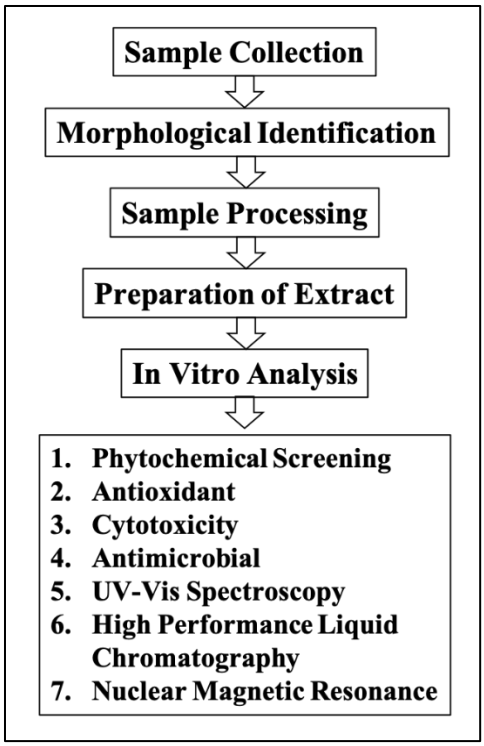

Figure 1. Experimental workflow of this study.

Preparation of Extract

In the laboratory, all seaweed samples were rinsed free of debris and air-dried at ambient temperature. Dried samples were ground into powders, which were steeped in conical flasks containing 50% ethanol (1:10 w/v). The extract was incubated at 27°C with mild shaking at 150 rpm for extraction. Extracts were filtered under reduced pressure through 11 cm double rings of Whatman filter paper, collected, and kept for further analysis.

Phytochemical Screening

Phytochemical screening was done to determine different bioactive compounds in the seaweed extract (6). Qualitative tests to detect the presence of glycosides, alkaloids, tannins, phenols, saponins, steroids, flavonoids, and terpenoids were carried out as described elsewhere.

Antioxidant Activity Assay

DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay method described by Blois (25) was used to determine the antioxidant activity. This technique is based on the power of antioxidants to donate hydrogen, reduce DPPH, and change color measurably (26).

Cytotoxicity Test

Cytotoxicity of the extract was determined by the Brine Shrimp lethality test using *Artemia salina* larvae death as an indicator for bioactivity, following standard procedures (27, 28, 29).

Antimicrobial Activity Assay

The infectious resistance was examined by the disc diffusion method to evaluate the inhibitory ability of seaweed extract on certain microbial strains (30).

Characterization of Bioactive Compounds

2.9.1 UV-Vis Spectroscopy

The absorption spectra of the extracts were recorded on a dual-beam UV-spectrophotometer (Shimadzu UV-1900) in the range of 190–1100 nm, using 50% ethanol as the blank.

High-Performance Liquid Chromatography (HPLC)

The analysis was performed on a YL9100 HPLC system (YOUNG IN Chromass, South Korea) with 100% water as the mobile phase at a flow rate of 0.5 mL/min. A detection wavelength range of 190–600 nm, a 36°C temperature and a pressure of 4000 psi were used. All samples were finally prepared with a concentration of 30 ppm.

NMR Spectroscopy (Nuclear magnetic resonance)

The NMR spectra were recorded using a BRUKER instrument at the Bangladesh Council of Scientific and Industrial Research. DMSO-dissolved extracts (2 mg/mL) were measured at 23°C. Structure determination was made through comparison of chemical shifts and coupling constants with those of reference compounds.

This broad-based methodology allowed the efficient culling, characterization, and identification of bioactive compounds from *Jania* species collected from St. Martin's Island.

Results

Phytochemical Screening

The phytochemical screening of the 50% ethanolic extract of *Jania* species confirmed the presence of glycosides, alkaloids, saponins, and steroids. But, Tannins, Phenols, flavonoids, and terpenoids were not found (**Table 1**). These results suggest the presence of bioactive secondary metabolites in these species.

Table 1. Summary of the phytochemical screening results.

Serial	Test Name	Results
1	Salkowski's Test	+++
2	Alkaloidal Screening	+++
3	Tannins Screening	Negative
4	Phenol Screening	Negative
5	Saponins Screening	++
6	Steroids Screening	+
7	Flavonoids Screening	Negative
8	Terpenoids Screening	Negative

Antioxidant Activity

The free radical scavenging activity of *Jania* extract was analyzed using DPPH, with ascorbic acid as a positive control. The experiment used five concentrations of the *Jania* extract, measured in dark conditions at 30 and 60 minutes post-incubation (**Figures 2 and 3**).

Statistical analysis for both the extract and positive control showed P values higher than 0.05 at most concentrations/period incubation periods, indicating no statistical difference (**Table 2**). No differences (P 100 mg/mL), when IC_{50} values are higher than 50–100 mg/mL, as moderate (IC_{50} of 50-100 mg/mL) and strong scavengers (values between 10–50 mg/mL). As presented with the scaled assay, this *Jania* extract did not show a moderate or strong scavenging activity as its inhibition (%) plotted remained low and did not close to IC_{50} in the tested concentrations. Remarkably, the scavenging activity increased with concentration (R^2 = 0.9962), whereas at higher concentrations, it decreased and became negative as incubation time increased to 60 minutes (R^2 = 0.4855), suggesting assay limitations or pro-oxidant effects during extended incubation periods.

In comparison, ascorbic acid (the positive control) exhibited stable scavenging activity across all considered concentrations, with a percentage inhibition increasing at 30 and 60 min (R^2 = 0.8233 and 0.9874, respectively). The calculated IC_{50} values for ascorbic acid were in good agreement with those of powerful antioxidants, also verifying the assay suitability. The further investigation of the standard deviation analysis revealed a more stable result for ascorbic acid than *Jania* extract, which demonstrated increasing deviations with higher concentrations and longer incubation times.

The IC_{50} could not be determined for the *Jania* extract due to low inhibition at 30 minutes and negative values at 60 minutes, which emphasizes the weak antioxidant capacity observed. By comparison, IC_{50} values for ascorbic acid were stable and repeatable across conditions indicating it to be highly efficient in scavenging under these experimental settings (**Table 3**).

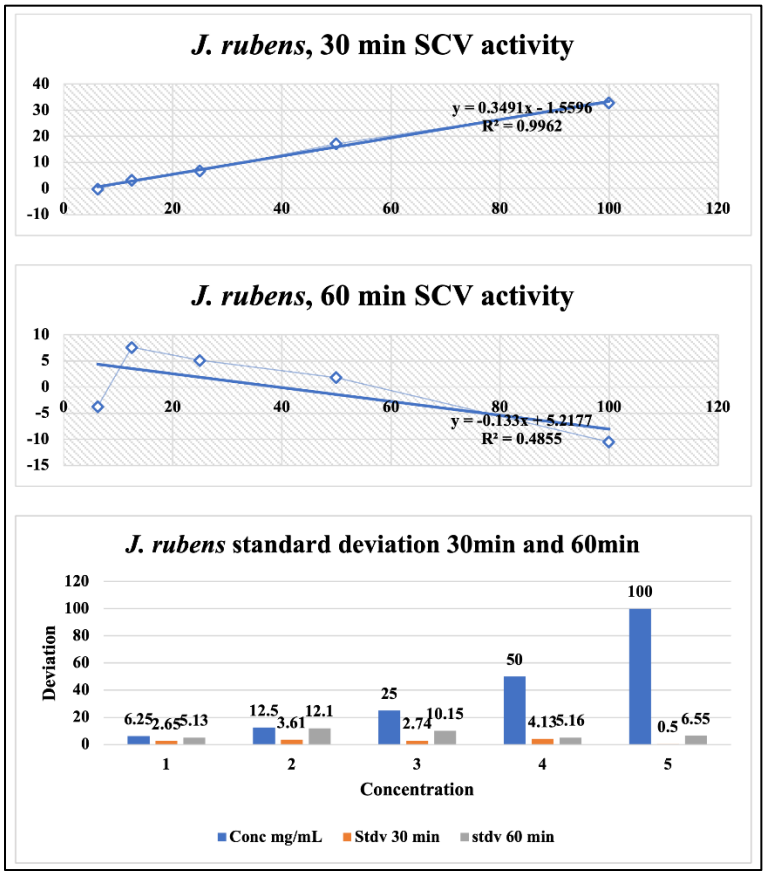

Figure 2. DPPH Scavenging Activity of the extract after 30 and 60 Minutes and Standard Deviation.

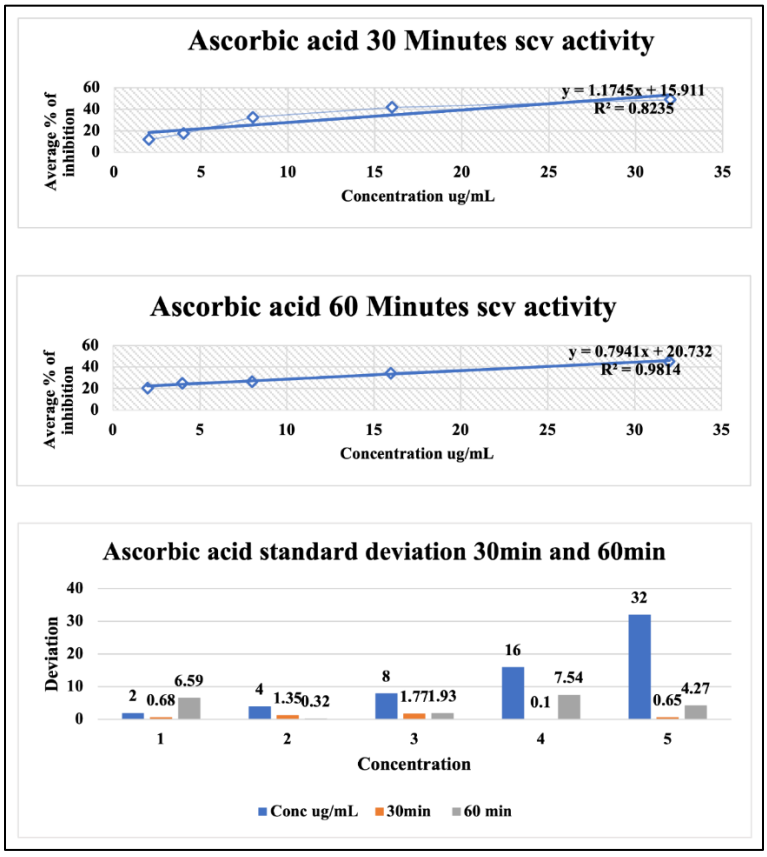

Figure 3. DPPH Scavenging Activity of Positive Control after 30 and 60 Minutes and Standard Deviation.

Table 1. P-value (T-test) of different concentrations of the sample extract and positive control.

Extract Conc.	**P-value (T-test)**	**Positive control Con.**	**P-value (T-test)**
6.25 mg/mL	0.26	2 ug/mL	0.08
12.5 mg/mL	0.47	4 ug/mL	0.01
25 mg/mL	0.3	8 ug/mL	0.06
50 mg/mL	0.79	16 ug/mL	0.17
100 mg/mL	0.18	32 ug/mL	0.21

Table 2. Determination of IC_{50} (Inhibitory Concentration 50%) Value.

IC_{50} Value		
Extract	30 minutes	1158.45
	60 minutes	-336.71
Positive control	30 minutes	29.02
	60 minutes	36.86

Cytotoxicity Assay

The brine shrimp lethality assay also demonstrated a strong cytotoxic response to the *Jania* extract. Mortality of the *Nauplii* was monitored at 45, 90, 180, 360, 540, and 1440 min after exposure to different concentrations of the sample extract and the positive control. Negative (ethanol) control treatment results were subtracted to correct for observed mortality rates.

Statistical analysis revealed a clear trend of increase in the number of dead larvae with increased incubation time and extract concentration, resembling a dose and time-dependent cytotoxicity. In the first observation (45 min), the LC_{50} (lethal concentration for 50% of test organisms) of the *Jania* extract body was found to be 168.42, while the positive control presented an LC_{50} of 338.70. During the experiment, the LC_{50} for both treatments decreased, with values of 138.33 for the extract treatment and 68.75 for the positive control after 1440 minutes.

This decreasing tendency of LC_{50} over time indicates that the cytotoxic potency, both for *Jania* extract and positive control, was enhanced after longer exposure (31, 32). The sequential fall/worsening decrease in LC_{50} values proposed that something is more toxic to brine shrimp as the duration of exposure increases (**Table 4, Figure 4**). These results emphasize the potential bioactivity of components in the *Jania* extract and encourage further study on their mode of action as well as applications.

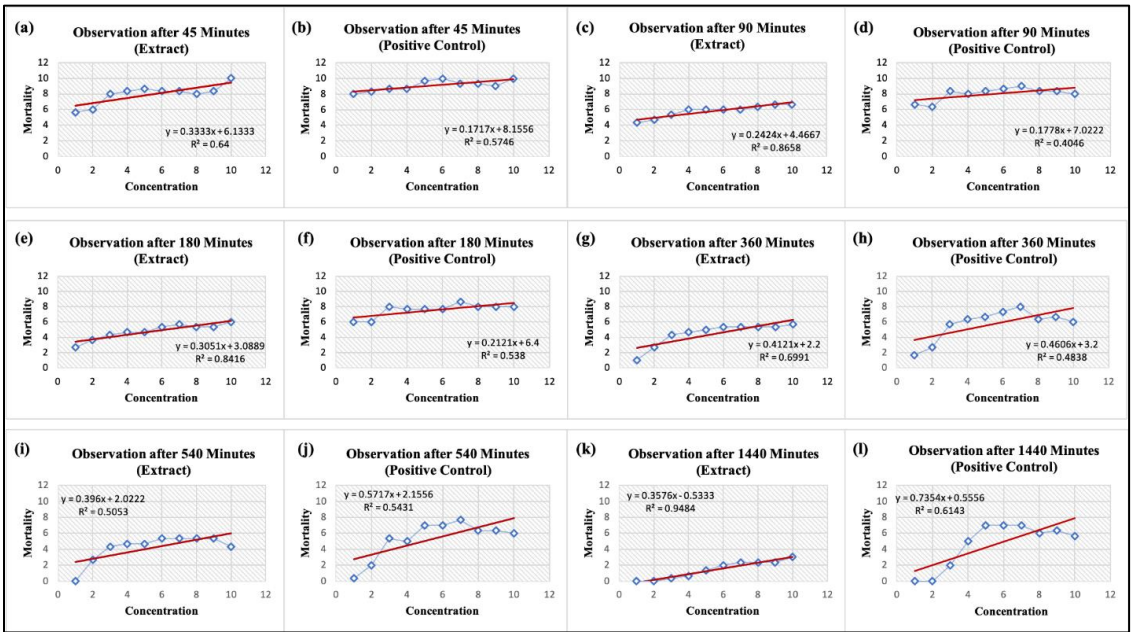

Figure 4. Brine Shrimp Lethality Bioassay. First observation after 45 minutes **(a, b),** second observation after 90 minutes (**c, d**), third observation after 180 minutes (**e, f**), fourth observation after 360 minutes (**g, h**), fifth observation after 540 minutes (**i, j**), sixth observation after 1440 minutes (**k, l**) of inoculation of *Nauplii* both sample extract and positive control.

Table 3. LC_{50} (Lethal Concentration 50%) Value result of different observations of 50% ethanolic extract and positive control after different time intervals.

Observation	**Sample extract or Positive control**	**LC_{50}**
First Observation	Ethanolic Extract	168.42
	Positive Control	338.70
Second Observation	Ethanolic Extract	224.7
	Positive Control	320.71
Third Observation	Ethanolic Extract	174
	Positive Control	265.91
Fourth Observation	Ethanolic Extract	129.09
	Positive Control	115.50
Fifth Observation	Ethanolic Extract	131.37
	Positive Control	91.23
Sixth Observation	Ethanolic Extract	138.33
	Positive Control	68.75

Antimicrobial Activity

Jania extract showed no antimicrobial activity toward the tested pathogens (*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Staphylococcus haemolyticus*) with the disc diffusion method. Discs containing 25 µL of extract (125, 250, and 500 µg/mL) exhibited no zones of inhibition after incubation at 37°C for up to 12 or 24 h. Bacterial growth patterns in the presence of the extract were similar to those seen with a negative control, i.e., half-strength ethanol.

Characterization of Bioactive Compounds

UV-Vis Spectroscopy

The absorption peaks at 255.50 nm, 684.50 nm, 881 nm, and 974.50nm were observed in UV-Vis spectrometry of *Jania* extract (**Figure 5**). The maximum of the strongest absorption was at 255.50 nm with a relative intensity of 3.954. Correlating this value to typical literature peaks, such as this, can be indicative of benzene-related structures since clear absorption for benzene at 255 nm is visible. This result is the first indication of existence for aromatic components in the extract.

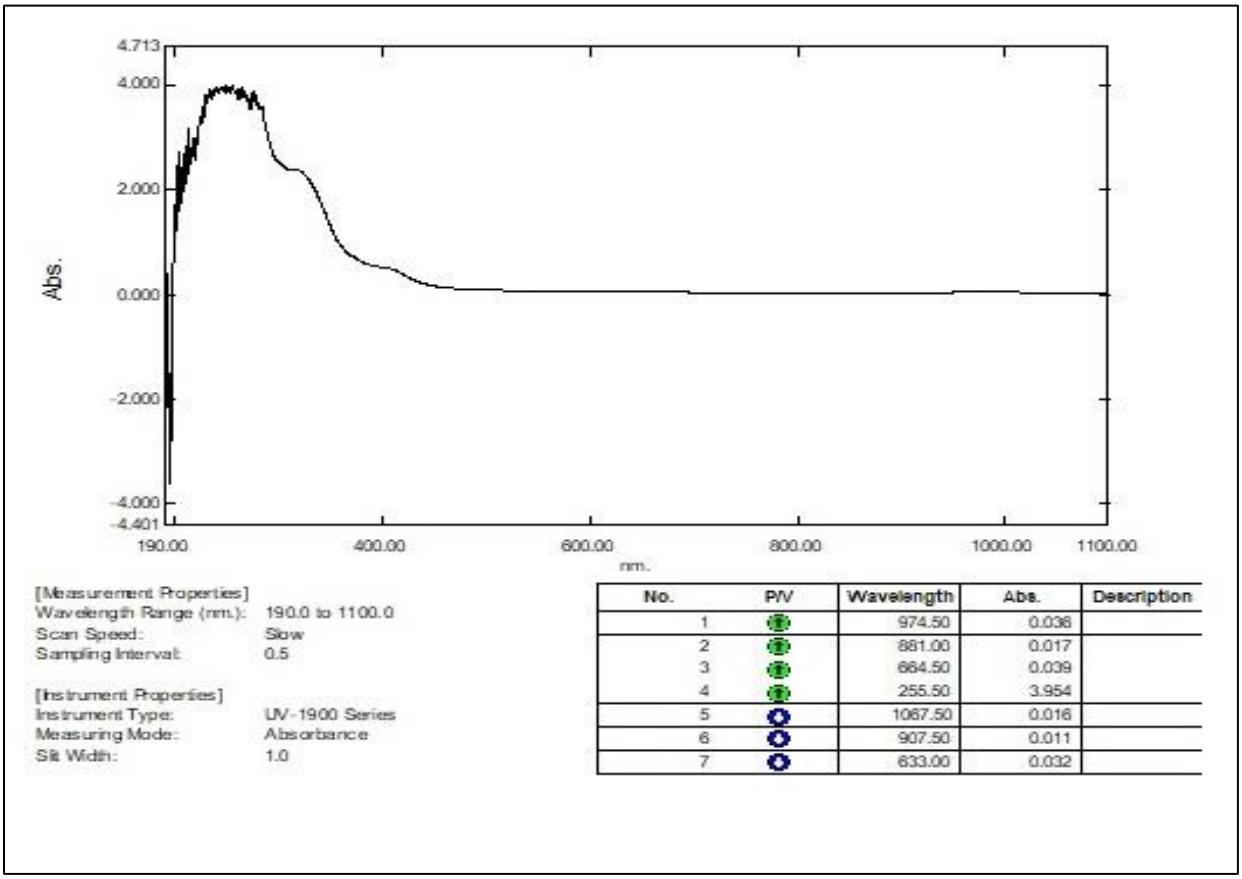

Figure 5. UV-Spectrophotometer absorption peak of the sample extract.

High-Performance Liquid Chromatography (HPLC)

HPLC detection (water/acetonitrile) also showed two major peaks with retention times 5.712 and 5.598 min, respectively (**Figure 6**). The visible of these large peaks in this area suggest the existence of rather polar to polar bioactives. These retention times are in agreement with the possible attendance of alkaloids or glycosides, as known elution profile.

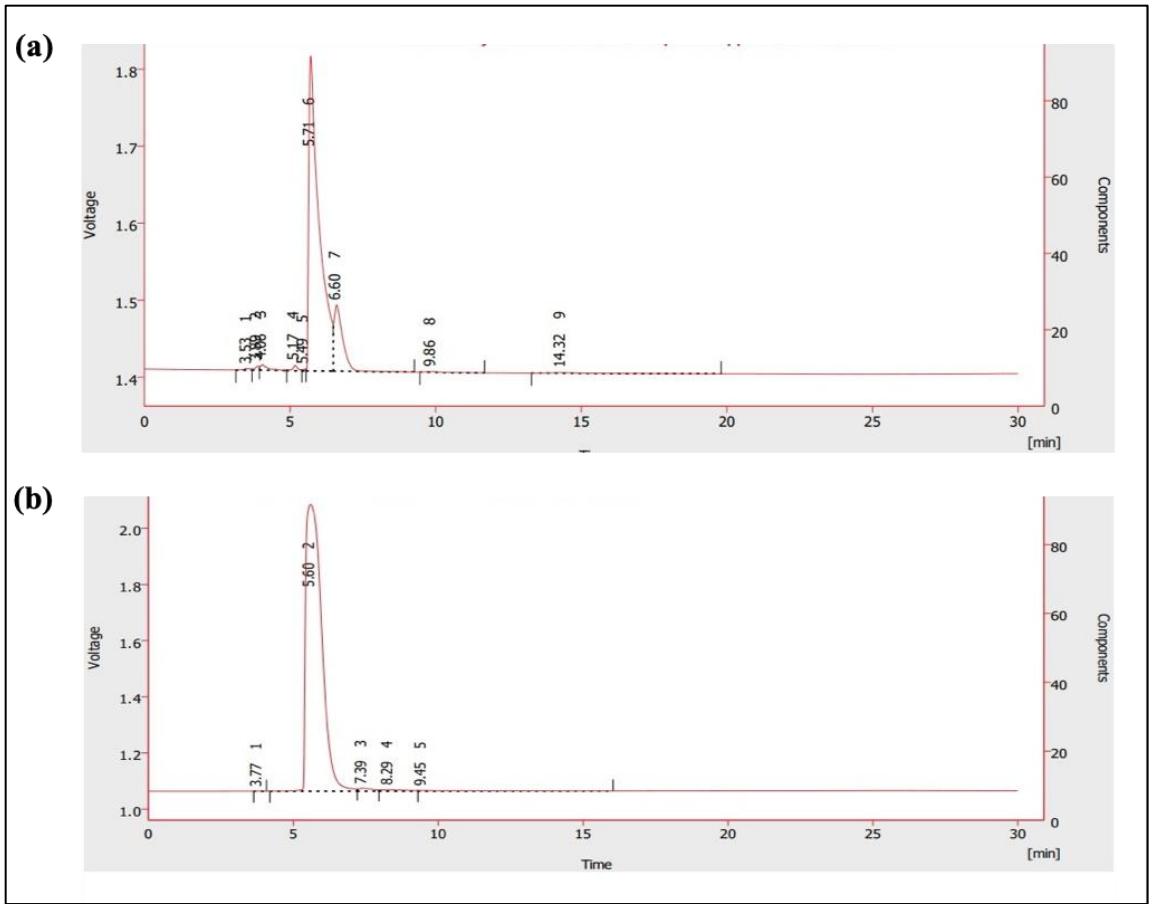

Figure 6. HPLC analysis of ethanolic extract of *Jania* species dissolved in methanol using water (a) and acetonitrile (b) as a mobile phase.

Nuclear Magnetic Resonance (NMR)

The proton NMR spectrum (**Figure 7**) of the extract displayed signals for aliphatic protons at δ 1.2–1.8 ppm and aromatic protons at δ 6.8–7.5 ppm. Other resonances in the δ 3.5–4.0 ppm region were also observed, which are typically attributed to an oxygenated methylene group, corroborating the glycosidic or steroidal structures of the sample.

Taken together, these spectroscopic and chromatographic findings also reveal the presence of various biofunctional compounds in the *Jania* extract. These structural attributes (aromatic and glycosidic/steroidal) seen in the chemical composition further explain the cytotoxicity of extracts, whereas its antioxidant and antimicrobial activities were less significant.

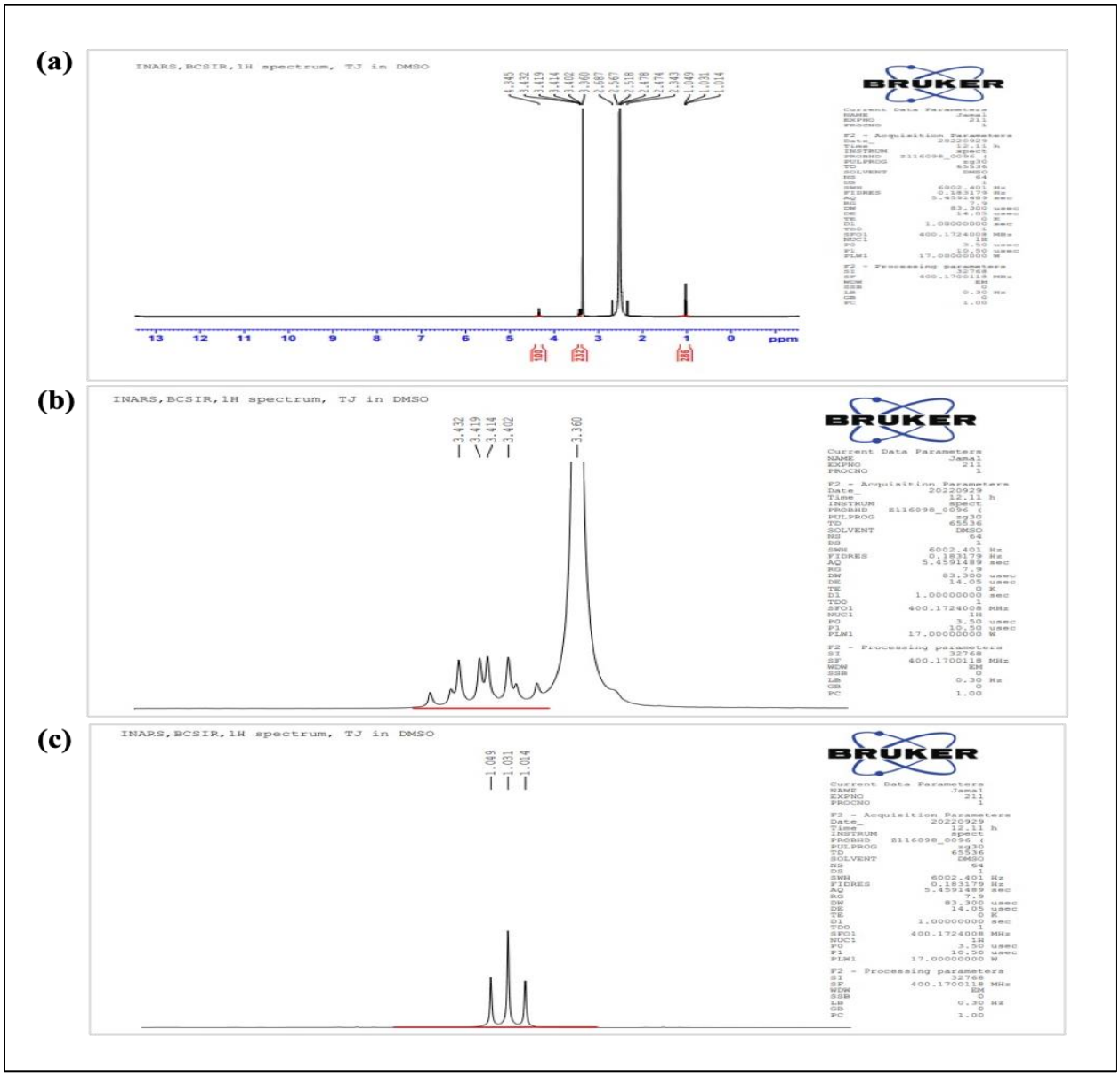

Figure 7. The representation of a ^{1}H atom of a principal compound present in the experimental extract (a, b, and c).

Discussion

In this work, the bioactivity and chemical diversity of Bay of Bengal *Jania* species were investigated, extending the current understanding of secondary metabolite composition and associated biological properties for this marine alga (33, 34, 35, 36). The systematic sampling and accurate treatment of samples contributed to the reliability and reproducibility of the following assays, and conventional methods contributed to a robust characterization (37, 38).

Chemical Composition

Phytochemical screening confirmed glycosides, alkaloids, saponins, and steroids—consistent with *Jania rubens* but lacking flavonoids/phenols typical of other seaweeds, suggesting unique Bay of Bengal biosynthesis possibly driven by pollution stress (6, 38, 39, 40, 41). UV-Vis (255.50 nm peak) indicates aromatic benzene derivatives (42, 43); HPLC peaks (5.598-5.712 min) suggest polar glycosides/alkaloids (44); NMR shows aliphatic (1.2-1.8 ppm), aromatic (6.8-7.5 ppm), and oxygenated methylene (3.5-4.0 ppm) protons characteristic of glycosidic/steroidal skeletons. These structural classes in red algae frequently disrupt cell membranes, explaining observed cytotoxicity while weaker antioxidants align with absent phenolics.

Biological Activities

DPPH assay revealed weak antioxidant activity (IC_{50} >1158 µg/mL at 30 min, negative at 60 min vs. ascorbic acid 29-37 µg/mL) with no statistical differences ($P>0.05$). No antimicrobial zones formed against *E. coli, K. pneumoniae, P. aeruginosa, S. haemolyticus* at 125-500 µg/disk. However, brine shrimp lethality showed strong dose/time-dependent cytotoxicity (LC_{50} 168.42 µg/mL at 45 min decreasing to 138.33 µg/mL at 1440 min vs. positive control 338.70→68.75 µg/mL), with highest mortality at >100 µg/mL/long exposures (31, 32).

Interpretations and Limitations

The LC_{50} decrease reflects time-dependent toxicity enhancement, but crude ethanolic extracts have limitations: solvent interference, absent positive cytotoxicity controls, matrix effects (pro-oxidant shift at 60 min DPPH), and lack of fractionation. Brine shrimp lethality predicts general cytotoxicity but correlates poorly with mammalian anticancer efficacy serving as preliminary screen requiring tumor cell line validation, not therapeutic proof (32, 33, 42, 45, 46, 47).

Compared to *J. rubens* (cytotoxic oxysterols LC_{50} ~10-50 µg/mL, bromoditerpenes), Bay of Bengal specimens show similar steroid/glycoside profiles but stronger brine shrimp LC_{50} (138-168 µg/mL), possibly from regionally unique sterols adapted to deltaic stressors (21, 24, 46, 47).

Implications

This first Bay of Bengal *Jania* analysis reveals novel low LC_{50} values and regional chemical profiles absent from prior Bangladesh studies, advancing marine pharmacognosy and Blue Economy by identifying cytotoxic leads for fractionation/isolation (34, 35, 36, 37, 38, 39).

Future Directions

Fractionate actives via activity-guided isolation, apply metabolomics/DNA barcoding for species confirmation, test against human cancer cell lines, and validate in vivo. These steps will identify therapeutic compounds while supporting sustainable seaweed bioprospecting.

Conclusions

In summary, though *Jania* species showed some prospect of being a source of bioactive compounds, including alkaloids and glycosides, it is unimpressive with respect to both antioxidant and antimicrobial actions. Further efforts should be directed toward species identification through DNA barcoding, characterization of the main compounds and evaluating their efficacy against several diseases.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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