



Original Research Paper

***Hydraclathratus clathratus* was assessed for phytochemical fingerprinting and multifunctional bioactivity, including antioxidant, antibacterial, and carbohydrate hydrolysing enzyme inhibition.**

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Key Words

Abstract

DPPH, ABTS, antibacterial activity, α -amylase, α -glucosidase

Marine macroalgae represent a valuable source of natural bioactive compounds with potential applications in pharmaceutical, biomedical, and nutraceutical research. The present study investigated the preliminary phytochemical composition and antioxidant, antibacterial, and antidiabetic activities of the hydroalcoholic extract of *Hydraclathrus clathratus* (HCAE). Preliminary phytochemical screening revealed the presence of several major classes of secondary metabolites and other biologically relevant constituents, including saponins, glycosides, tannins, flavonoids, proteins, amino acids, phenolic compounds, lipids, and steroids. The antioxidant potential of HCAE was evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical-scavenging assays. The extract exhibited concentration-dependent radical-scavenging activity, with IC₅₀ values of 67.970 μ g/mL for DPPH and 58.718 μ g/mL for ABTS, indicating its capacity to neutralize free radicals under the experimental conditions. The antibacterial activity of HCAE was assessed against four bacterial pathogens, namely *Escherichia coli*, *Klebsiella pneumoniae*, *Vibrio cholerae*, and *Staphylococcus aureus*. The observed antibacterial effects suggest that the extract contains constituents with potential inhibitory activity against both Gram-negative and Gram-positive bacteria. Furthermore, the antidiabetic potential of HCAE was investigated by evaluating its inhibitory effects on α -amylase and α -glucosidase, two carbohydrate-hydrolysing enzymes involved in postprandial glucose regulation. The extract demonstrated enzyme-inhibitory activity, with IC₅₀ values of 56.562 μ g/mL for α -amylase and 59.006 μ g/mL for α -glucosidase. These findings indicate that HCAE possesses multiple biological activities that may be associated with its diverse phytochemical composition. In particular, its antioxidant activity, antibacterial effects, and inhibition of carbohydrate-digesting enzymes highlight the potential of *H. clathratus* as a candidate for further natural-product research. However, isolation and structural characterization of individual bioactive compounds, toxicity assessment, mechanistic investigations, and in vivo validation are necessary to establish its efficacy, safety, and potential therapeutic applications. Overall, this study provides a preliminary scientific basis for exploring *H. clathratus* as a marine-derived source of biologically active compounds for future pharmaceutical and functional-product development.

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1. Introduction:

Marine macroalgae are becoming attractive sources of structurally varied bioactive chemicals for pharmacological, nutraceutical, and functional applications [1–2]. Seaweeds produce phenolic compounds, phlorotannins, flavonoids, terpenoids, steroids, fatty acids, polysaccharides, pigments, proteins, and secondary metabolites [3]. Marine algae are intriguing candidates for natural bioactive agents since these chemicals exhibit antioxidant, antibacterial, anti-inflammatory, antidiabetic, anticancer, and other biological effects [4]. *Hydroclathrus clathratus*, a brown macroalga, may contain physiologically active metabolites. Phytochemical studies of *H. clathratus* found fatty acids, sugars, carboxylic acid derivatives, triterpenoids, steroids, and other chemicals, suggesting its bioactive potential [5]. Environmental factors and species-specific metabolic pathways affect brown algae's chemical composition, resulting in biological diversity [2,6].

Oxidative stress is caused by excessive reactive oxygen species formation and an imbalance between oxidant production and antioxidant defence. Phenolic compounds, phlorotannins, flavonoids, pigments, and other secondary metabolites from marine algae can scavenge free radicals [7]. Thus, antioxidant activity utilising complementary assays like DPPH and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) might give preliminary information about algal extracts' radical-scavenging capability [8]. Antimicrobial substances may come from marine algae. Phenolics, fatty acids, terpenoids, and other secondary algal metabolites inhibit several bacterial microorganisms [9]. Prior research on *H. clathratus* has shown antibacterial activity in algal-derived preparations, indicating its use as a source of antimicrobials [5]. Thus, testing the extract or algal-derived compounds against Gram-positive and Gram-negative bacteria can reveal their antibacterial properties. In addition

to antioxidant and antibacterial characteristics, sea algae may offer antidiabetic benefits. In vitro, inhibiting carbohydrate-hydrolysing enzymes such α -amylase and α -glucosidase can assess effects on carbohydrate digestion and postprandial glucose regulation [8–10]. Seaweed extracts and bioactive ingredients block these enzymes, with phenolic compounds and phlorotannins being studied for antidiabetic action [10,11]. Research indicates that marine algae extracts may have α -amylase inhibitory and antioxidant properties, suggesting a link between algal bioactive chemicals and metabolic health [12]. Thus, phytochemical characterisation is essential to understanding marine algal extract biology. Qualitative screening identifies the key phytoconstituent classes, while quantitative estimate of phenolics, flavonoids, saponins, tannins, steroids, and amino acids reveals their chemical makeup. This study examines the hydroalcoholic extract of *Hydroclathrus clathratus* for its phytochemical composition, antioxidant activity (DPPH and ABTS radical-scavenging assays), antimicrobial activity (selected bacterial strains), and α -amylase and α -glucosidase inhibition. These studies provide a preliminary assessment of *H. clathratus* hydroalcoholic extract's biological potential and potential as a source of bioactive chemicals.

2. Materials and Methods

2.1 Collection and Authentication of *Hydraclathrus clathratus*

Fresh thalli of *Hydraclathrus clathratus* were gathered from the designated coastal region and brought to the laboratory in sterile containers. The algal material was rinsed thoroughly with sterile distilled water to remove the sand, epiphytes and other foreign items attached to it. The cleaned material was certified by a qualified algal taxonomist using morphological and taxonomic characters. Voucher specimen was made and stored in the herbarium of the department for future reference.

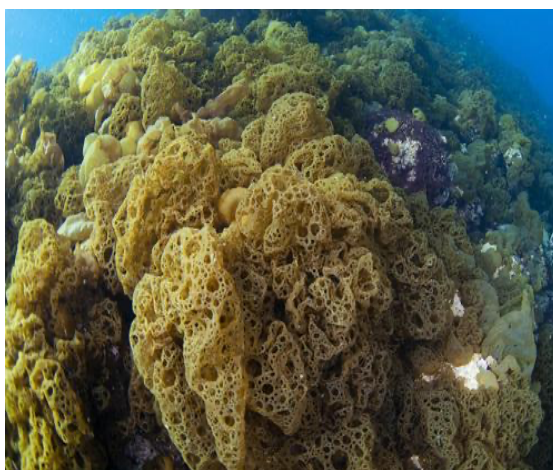


Figure 1. *Hydroclathrus clathratus* (C. Agardh)

2.2 Hydroalcoholic Extract Preparation

The algal thalli washed were dried under shade at room temperature till uniform weight was attained. The dry material was ground in a laboratory grade mechanical grinder and kept in an airtight container until extraction. About 100 g of powdered algal material was extracted by maceration with hydroalcoholic solvent [ethanol:distilled water, 70:30 (v/v)]. Extraction was done for 72 h with intermittent shaking. The extract was filtered through Whatman No. 1 filter paper and the filtrate was concentrated under decreased pressure using a rotary evaporator at a temperature not higher than 45 °C. The concentrate was further dried and stored in a sealed container at 4 °C for subsequent analysis. This hydroalcoholic extract was identified as HCHAE (*Hydraclathrus clathratus* hydroalcoholic extract).

2.3 Phytochemical Screening Qualitative:

Qualitative Phytochemical Screening of HCHAE The preliminary qualitative phytochemical screening for carbohydrates, reducing sugars, saponins, glycosides, tannins, fats and oils, flavonoids, proteins/amino acids, phenolic compounds, starch and steroids of HCHAE was carried out by using standard procedures described by Harborne with minor modifications [13]. Carbohydrates were identified by Molech's test, reducing sugars by Fehling's test, saponins by foam test, glycosides by Salkowski's reaction, tannins and phenolics by ferric chloride test, fats and oils by solubility test, flavonoids by alkaline reagent test, proteins and amino acids by ninhydrin test, starch by iodine test and steroids by Liebermann-Burhard reaction. The formation of characteristic colour

change, precipitate or persistent foam was regarded suggestive of the particular phytoconstituents. The intensity of the reactions was graded as absent (–), faintly positive (+), moderately positive (++), or very positive (+++).

2.4 Determination of Total Phenolic Content

Total phenolic content of HCHAE was evaluated by Folin–Ciocalteu colorimetric technique with some adjustments [14,15]. Briefly, the extract in a suitable concentration was combined with the Folin–Ciocalteu reagent. After addition of sodium carbonate solution, the reaction mixture was incubated for a brief period at room temperature in the dark. The absorbance was read spectrophotometrically at 760–765 nm against a reagent blank. Gallic acid was utilised as the standard reference and calibration curve was created from varying amounts of gallic acid. Total phenolic content was reported in mg gallic acid equivalents (GAE)/g extract.

2.5. Total Flavonoid Content Determination

The total flavonoid concentration was determined by the aluminium chloride colorimetric technique [14,15]. To summarise, a known volume of HCHAE was combined with methanol, aluminium chloride solution and potassium acetate and then incubated at room temperature. Absorbance was measured at 415 nm with an appropriate reagent blank. Experimental standardisation chosen for the present study was based on catechol as the reference standard. The results were represented as mg catechol equivalents (CE)/g of extract.

2.6 Determination of Total Saponin Content

The total saponin content was estimated by the spectrophotometric technique using diosgenin as a reference standard. The selected chromogenic reagent was allowed to react with suitable quantities of HCHAE under controlled conditions and the absorbance was recorded spectrophotometrically against the reagent blank. Diosgenin was used to generate a calibration curve. Total saponin content was reported in mg diosgenin equivalents (DSG)/g extract. The assay was carried out three times [10].

2.7 Total Tannin Content Determination

Total tannin content was determined by spectrophotometric technique using tannic acid as standard. Appropriate quantities of HCHAE were reacted with the corresponding colour development reagent and the absorbance was measured against the reagent blank. A calibration curve was produced with tannic acid. The tannin concentration was reported as mg of tannic acid equivalents (TAE)/g extract [10].

2.8 Estimation of steroid content

The total steroid content was determined by a colorimetric method using an appropriate steroid standard. The selected chromogenic reagent was reacted with a known concentration of HCHAE under standardised circumstances and the absorbance was determined spectrophotometrically. The amount of steroid was determined using a standard calibration curve and given as mg of standard equivalents/g of extract [10].

2.9 Determination of the Total Amino Acid Content

The total amino acid content was measured by a colorimetric ninhydrin test. Different quantities of HCHAE were reacted with ninhydrin reagent and heated under regulated conditions for the development of the distinctive chromophore. The absorbance was then measured spectrophotometrically at the relevant wavelength after cooling. Glycine was utilised as the reference standard and the results were represented as mg glycine equivalents (GLY)/g extract [10].

2.10 DPPH Radical-Scavenging Activity

The free-radical-scavenging activity of HCHAE was evaluated by the 2,2-diphenyl-1-picrylhydrazyl

(DPPH) test according to the method reported by Brand-Williams et al. with slight modifications [16,14]. The HCHAE concentrations of 100-500 µg/mL were synthesised in the suitable solvent. An aliquot of each concentration was added to newly produced DPPH solution. The reaction mixtures were incubated in the dark at room temperature for about 30 min. The decrease in absorbance was measured at 515–517 nm as compared to the control. Ascorbic acid (AA) was employed as a positive control.

DPPH radicals inhibition % was computed as follows:

$$\text{DPPH inhibition (\%)} = [(A_0 - A_1)/A_0] \times 100$$

where A_0 is the absorbance at zero concentration and A_1 is the absorbance of the sample or standard. The concentration inhibiting 50% of DPPH radical activity (IC_{50}) was derived from the concentration-response curve. The experiment was repeated three times. The DPPH technique is based on the reduction of DPPH radical with a concomitant drop in its characteristic absorbance .

2.11 ABTS Radical Cation Decolorisation Activity

The ABTS radical cation scavenging activity was evaluated according to the method of Re et al. with slight changes [5]. Potassium persulfate was reacted with ABTS to produce the ABTS radical cation and left to stand in the dark to achieve a stable radical solution. The resultant solution was diluted with appropriate solvent to produce the necessary absorbance at 734 nm. The ABTS + solution was treated with various amounts of HCHAE (100–500 µg/mL) and incubated for a fixed period at room temperature. Absorbance was measured at 734 nm. Ascorbic acid was employed as positive control.

Percentage inhibition was computed as: ABTS inhibition (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of control and A_1 is the absorbance in the presence of HCHAE or AA. IC_{50} values were determined using concentration-response curves. All experiments were carried out in triplicates. The ABTS assay is based on the reduction of the preformed ABTS radical cation by hydrogen donating antioxidant chemicals [14, 16].

2.12 Antibacterials activities

The agar well/diffusion method was used to investigate the antibacterial effect of HCHAE on *Escherichia coli*, *Klebsiella pneumoniae*, *Vibrio cholerae* and *Staphylococcus aureus*. The bacterial cultures were grown on a suitable nutritional medium and adjusted to a turbidity comparable to roughly 0.5 McFarland standard before inoculation. Uniformly inoculated sterile Mueller Hinton agar plates with appropriate bacterial suspensions.

Different quantities (25, 50 and 75 μL) of HCHAE were added to the created sterile wells in the surface of the agar. Standard anti-bacterial precautions and appropriate solvent were used. The plates were incubated under adequate conditions for the test organisms and the diameter of the inhibition zones around each well was determined in mm. Each experiment was performed in triplicate and findings were expressed as mean $\pm$ standard deviation (SD). The overall agar diffusion process was performed using the standardised antimicrobial susceptibility-testing principles specified by CLSI M02 [8].

2.13 Inhibition of α -Amylase Activity

The α -amylase inhibitory activity of HCHAE (20–100 $\mu\text{g/mL}$) was measured by the DNS technique [1]. The extract was incubated with α -amylase and starch substrate at 37 °C and the reaction was stopped using DNS reagent. Absorbance was read at 540 nm. Acarbose was used as normal. The percentage of inhibition was computed and the IC₅₀ value was obtained from the curve concentration-response. All assays were done in triplicate.

2.14 Inhibitory activity of α -glucosidase

The α -glucosidase inhibitory effect of HCHAE (20–100 $\mu\text{g/mL}$) was assessed by the p-NPG substrate technique [1]. The extract and enzyme were pre-incubated at 37 °C and p-NPG was added. The formation of p-nitrophenol was estimated at 405 nm. Acarbose was employed as standard and inhibition percentage and IC₅₀ values were calculated. All experiments were performed in duplicates.

2.15 Statistical analyses

All tests were repeated at least three times and the findings were presented as mean $\pm$ standard deviation (SD). Statistical analysis was done with the appropriate statistical software. Statistical differences between experimental groups were determined by one-way analysis of variance (ANOVA) followed by the appropriate post-hoc multiple-comparison test where applicable. Values of $P < 0.05$ were considered statistically significant. For concentration-dependent antioxidant and enzyme-inhibition experiments, concentration–response curves were fitted and IC₅₀ values were determined using regression analysis.

3. Results and Discussion

3.1 Phytochemical Screening (Qualitative)

Preliminary phytochemical screening of Hydroalcoholic extract of *Hydracanthus clathratus* (HCHAE) demonstrated the presence of various bioactive phytochemical classes (Table 1). Saponins, glycosides, tannins, lipids and oils, flavonoids, proteins/amino acids and phenolic compounds were present while carbohydrates, reducing sugars and starch were absent. Positive for steroids. The discovered ingredients gave the strongest qualitative response (+++) for phenolic compounds and moderate reactions (++) for the others, i.e. saponins, tannins, lipids and oils, flavonoids and proteins/amino acids. The presence of phenolics and flavonoids is especially noteworthy as these chemicals are usually associated with electron- and hydrogen-donor characteristics and could contribute to the antioxidant activity of marine algae [17,10]. Moreover, saponins and glycosides might be responsible for biological activity related to the membrane. Tannins and other polyphenolic components have been described as having antioxidant and antibacterial activity [18,12]. Marine macroalgae are recognised as key sources of structurally varied phenolic compounds, terpenoids, sterols, polysaccharides and other secondary metabolites exhibiting potential therapeutic effects [10]. Thus, the qualitative phytochemical profile of HCHAE shows the existence of various classes of chemicals that may work singly or synergistically in the biological activities identified in the present investigation.

Table 1. Qualitative Phytochemical Screening of *Hydracanthus clathratus* hydro alcoholic extract

S. No	Phytochemical	Test Used	Result
1	Carbohydrates	Molisch's Test	-
2	Reducing Sugar	Fehling's Test	-
3	Saponins	Foam Test	++
4	Glycosides	Salkowski's Test	+
5	Tannins	Ferric Chloride Test	++
6	Fats and Oils	Solubility Test	++
7	Flavonoids	Alkaline Reagent Test	++
8	Proteins & Amino Acids	Ninhydrin Test	++
9	Phenolic Compounds	Ferric Chloride Test	+++
10	Starch	Iodine Test	-
11	Steroids	Liebermann–Burchard Test	+

3.2 Quantitative Phytochemical Constituents

The quantitative study showed that HCHAE contains detectable levels of phenolics, flavonoids, tannins, steroids, saponins and amino acids (Table 2). The total phenolic compounds were evaluated at the greatest concentration (5.43 ± 0.51 mg GAE/g extract) followed by the flavonoids (3.46 ± 0.80 mg CE/g extract) and tannins (2.60 ± 0.51 mg TAE/g extract). The extract included steroids, amino acids and saponins at levels of 1.53 ± 0.41 , 1.36 ± 0.56 and 0.60 ± 0.52 mg/g respectively. The observed radical scavenging properties in the following antioxidant assays may be partly due to the greater relative phenolic content. Phenolic hydroxyl groups can give hydrogen atoms or electrons to reactive radicals, thus stabilising free-radical intermediates [12, 19]. Radical scavenging is also performed by flavonoids via mechanisms of hydrogen donation and metal chelation [6].

The presence of tannins with 2.60 ± 0.51 mg TAE/g is also of interest since hydrolysable and condensed tannins have several hydroxyl groups that can interact with reactive oxygen species and proteins. Previous research have shown that the biological capabilities of seaweeds are substantially impacted by their phenolic and flavonoid composition, although the amounts can vary considerably depending on species, geographical location, season, climatic conditions and extraction solvent [20,7]. The hydroalcoholic solvent utilised in the present investigation might have contributed to the extraction of moderately polar phenolic and flavonoid chemicals. Thus, the quantitative phytochemical profile offers a reasonable chemical explanation for the antioxidant, antibacterial and enzyme inhibitory effects demonstrated by HCHAE.

Table 2. Total phenolic, flavonoid, saponin, tannins, steroid and amino acids contents *Hydracanthus clathrus* hydro alcoholic extract

Phytoconstituents	HCHAE
Phenol (mg GAE/g extract)	5.43 ± 0.51
Flavonoid (mg CE/g extract)	3.46 ± 0.80
Saponin (mg DSG /g extract)	0.60 ± 0.52
Tannins (mg TAE/g extract)	2.60 ± 0.51
Steroid	1.53 ± 0.41

mg TAE/g extract	
Amino acid (mg GLY/g extract)	1.36 ±0.56

*Note: GAE: Gallic acid equivalents; CE: Catecholequivalents; GLY: Glycine; HCHAE: *Hydraclathratus clathrus hydro alcoholic extract*; DSG: diosgenin; TAE: Tannic Acid Equivalent.

3.3 DPPH Radical scavenging activity

HCHAE exhibited DPPH radical scavenging action in a concentration dependent manner (Table 3). HCHAE showed inhibition of 16.95 ± 0.34 , 27.39 ± 1.07 , 38.94 ± 0.62 , 59.20 ± 0.33 and $73.94 \pm 0.92\%$ at doses of 100, 200, 300, 400 and 500 $\mu\text{g/mL}$ respectively. Conversely, ascorbic acid generated 33.13 ± 0.43 , 43.65 ± 0.70 , 57.06 ± 0.39 , 68.62 ± 0.06 and $81.88 \pm 0.34\%$ inhibition at the respective doses. The computed IC₅₀ values for HCHAE and ascorbic acid were 67.970 and 48.800 $\mu\text{g/mL}$, respectively. The gradual increase in inhibition with increase in extract concentration is indicative of a concentration-dependent free-radical-scavenging effect. The DPPH assay assesses the ability of antioxidant compounds to donate hydrogen or electron to the stable DPPH radical, causing a decrease in absorbance [8]. The lower activity of HCHAE relative to ascorbic acid in the conditions examined is consistent with the relatively high total phenolics and flavonoids concentrations seen in Table 2. DPPH can be quenched by hydrogen donation from phenolic compounds, and flavonoids can stabilise the resultant radical intermediates by resonance delocalisation [7, 21]. Marine algae are rich sources of a variety of antioxidant elements such as phenolic compounds, flavonoids, pigments, terpenoids and other secondary metabolites [7,10]. Thus, the activity reported may not be due to a single chemical but due to additive or synergistic interactions between numerous ingredients. The IC₅₀ value of 67.970 $\mu\text{g/mL}$ reflects significant

antioxidant activity. However, caution is required when comparing with other algal extracts as extraction methods, test methodologies and phytochemical composition could significantly impact IC₅₀ values.

3.4 ABTS Radical Scavenging Activity

The antioxidant capacity of HCHAE was further validated by the ABTS experiment (Table 3). HCHAE showed 27.96 ± 0.33 , 37.02 ± 0.92 , 47.04 ± 0.05 , 64.61 ± 0.28 and $77.40 \pm 0.80\%$ inhibition at 100-500 $\mu\text{g/mL}$, respectively. Ascorbic acid showed maximum inhibition at all the doses (42.00 ± 0.37 to $84.28 \pm 0.30\%$). IC₅₀ values were 58.718 $\mu\text{g/mL}$ and 36.297 $\mu\text{g/mL}$ for HCHAE and ascorbic acid, respectively. The increase in ABTS radical inhibition is concentration dependant and is in agreement with the DPPH results. The ABTS assay is based on the ability of antioxidant molecules to quench the generated ABTS radical cation by electron- or hydrogen-transfer processes [22,23]. The difference in the chemical characteristics and reaction processes of the two radicals may be the reason for the lower IC₅₀ values of HCHAE in the ABTS test than in the DPPH assay. DPPH activity can be affected by steric accessibility and solvent compatibility [10,24], while ABTS can react with a wider variety of hydrophilic and lipophilic antioxidant chemicals. The antioxidant activity found in both experiments and the amounts of phenolic and flavonoid compounds indicate that HCHAE includes compounds able to scavenge reactive radicals. Similar antioxidant potential has been observed for various marine macroalgae with phenolic contents suggested to be key contributors to antioxidant activity [10, 24].

Table 3 Comparative analysis of radical scavenging activity by *Hydraclathratus clathrus hydro* alcoholic extract and Ascorbic Acid (AA) in a) DPPH assay, b) ABTS assay.

Concentration (Ig/ml)	Inhibition %			
	DPPH		ABTS	
	AA	HCHAE	AA	HCHAE

100	33.13 ± 0.43	16.95 ± 0.34	42.00 ± 0.37	27.96 ± 0.33
200	43.65 ± 0.70	27.39 ± 1.07	51.13 ± 0.61	37.02 ± 0.92
300	57.06 ± 0.39	38.94 ± 0.62	62.75 ± 0.33	47.04 ± 0.05
400	68.62 ± 0.06	59.20 ± 0.33	72.78 ± 0.54	64.61 ± 0.28
500	81.88 ± 0.34	73.94 ± 0.92	84.28 ± 0.30	77.40 ± 0.80
IC50 Value	48.800	67.970	36.297	58.718

AA: Ascorbic acid **DPPH:** 2,2-diphenyl-1-picrylhydrazyl; **ABTS:** 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)

3.5 Antimicrobial Activity

HCHAE displayed antibacterial efficacy against all the four tested bacterial strains but the level of inhibition differed across the species (Table 4). The inhibition zones against *Escherichia coli* were 1.46 ± 0.54 , 2.80 ± 0.51 and 3.26 ± 0.41 mm at 25, 50 and 75 μ L respectively. *Klebsiella pneumoniae* displayed zones of inhibition of 1.63 ± 0.44 , 2.96 ± 0.57 and 3.63 ± 0.39 mm and *Vibrio cholerae* 0.80 ± 0.52 , 2.43 ± 0.15 and 3.20 ± 0.63 mm. The highest inhibition was obtained against *Staphylococcus aureus* with zones of 1.80 ± 0.66 , 3.32 ± 0.42 and 4.96 ± 0.54 mm correspondingly. The rise in inhibition with increasing volume of extract suggests that the antibacterial effects are concentration dependent. The highest inhibition zone of the examined organisms was *S. aureus* at 75 μ L, whereas

the lowest response was in *V. cholerae* at the lowest concentration. The susceptibility differences may correlate with differences in bacterial cell-envelope structure, membrane composition and permeability. The antibacterial activity of HCHAE may be attributed to phenolics, flavonoids, tannins and saponins that act by different mechanisms including microbial membrane rupture, modification of membrane permeability, protein interaction and interference with cellular metabolic processes [25,26]. Extensively studied sources of antibacterial metabolites from marine macroalgae include phenolics, terpenoids, fatty acids, halogenated chemicals and other secondary metabolites [12,25]. The present results show that HCHAE has a detectable antibacterial activity, however the relatively modest inhibition zones suggest that the extract should not be considered as a substitute for a conventional antibiotic. Further investigations employing MIC and MBC determination would be needed to quantify its antibacterial potency.

Table.4 Antimicrobial activity of *Hydracanthus clathrus hydro alcoholic extract* –mediated against selected bacterial strains, expressed as zone of inhibition (mm).

S.No	Strains	Sample	Zone of inhibition (mm)		
			25 μ L	50 μ L	75 μ L
1	<i>E. coli</i>	HCHAE	1.46 ± 0.54	2.80 ± 0.51	3.26 ± 0.41
2	<i>Klebsiella pneumoniae</i>		1.63 ± 0.44	2.96 ± 0.57	3.63 ± 0.39
3	<i>Vibrio cholerae</i>		0.80 ± 0.52	2.43 ± 0.15	3.20 ± 0.63
4	<i>Staphylococcus aureus</i>		1.8 ± 0.66	3.32 ± 0.42	4.96 ± 0.54

3.6 α -Amylase Inhibitory Activity

Concentration dependent inhibition of the α -amylase activity was observed by HCHAE (Table 5).

The extract at doses from 20 to 100 µg/mL produced 14.45 ± 0.44 , 32.39 ± 0.44 , 46.40 ± 0.25 , 65.73 ± 0.06 and $82.04 \pm 0.02\%$ inhibition, respectively. The positive control Acarbose demonstrated more inhibition at each concentration range from 25.90 ± 0.08 to $89.91 \pm 0.35\%$. The IC_{50} values were $56.562 \mu\text{g/mL}$ for HCHAE and $47.501 \mu\text{g/mL}$ for acarbose. The increasing inhibition suggests that HCHAE might inhibit the α -amylase-mediated hydrolysis of starch in vitro. α -Amylase is a major digestive enzyme which hydrolyses dietary starch into smaller carbohydrate products and inhibition of this enzyme can decrease the pace of carbohydrate digestion and the subsequent availability of glucose [1,27]. The observed activity could be attributed to

phenolics, flavonoids, tannins and other substances capable of interacting with the enzyme catalytic or substrate binding sites.

Polyphenolic substances can block carbohydrate-hydrolyzing enzymes by hydrogen bonding, hydrophobic interactions and interactions with amino acid residues near enzyme active sites [28]. Hence, the α -amylase inhibitory reaction could be explained by the relatively high phenolic content of HCHAE. However, as the present investigation was an in vitro enzyme assay, the results suggest the possibility of enzyme inhibition rather than the definite antidiabetic activity.

Table 5. Antidiabetic activity of the hydroalcoholic extract of *Hydracanthus clathrus hydro* alcoholic extract determined by the α -amylase inhibition assay.

Concentration (µg/mL)	% Inhibition of α -amylase by acarbose (Positive control)	% inhibition of α -amylase α -amylase
20	25.90 ± 0.08	14.45 ± 0.44
40	48.68 ± 0.60	32.39 ± 0.44
60	59.43 ± 0.39	46.40 ± 0.25
80	73.94 ± 0.74	65.73 ± 0.06
100	89.91 ± 0.35	82.04 ± 0.02
IC_{50}	47.501	56.562

3.7 α -Glucosidase Inhibition Activity

A similar concentration-dependent pattern of inhibition was seen in the α -glucosidase assay (Table 6). HCHAE exhibited inhibition of 28.91 ± 0.48 , 37.15 ± 0.50 , 48.32 ± 0.31 , 60.84 ± 0.11 and 77.77 ± 0.13 at 20, 40, 60, 80 and 100 µg/mL respectively. Acarbose produced 35.67 ± 0.48 , 48.09 ± 0.35 , 63.60 ± 0.43 , 73.19 ± 0.48 and $87.35 \pm 1.74\%$ inhibition at identical doses. The IC_{50} values calculated were $59.006 \mu\text{g/mL}$ for HCHAE and $41.968 \mu\text{g/mL}$ for acarbose. The inhibitory impact of HCHAE on α -glucosidase is of particular interest because this enzyme catalyses the last step of carbohydrate digestion to produce absorbable monosaccharides. Inhibition of α -glucosidase might delay the quick appearance of glucose after carbohydrate intake, which can slow down the digestion of carbohydrates [1, 28,]. The observed inhibition may be attributed to the phenolic and flavonoid contents that have been documented to interact with digestive enzymes and decrease their catalytic activity [10,29]. The

somewhat greater IC_{50} value of HCHAE in comparison with acarbose indicated that the crude extract was less powerful than the reference inhibitor under the experimental conditions. However, the detectable inhibitory action of HCHAE against both α -amylase and α -glucosidase indicates the extract contains compounds with potential carbohydrate hydrolysing enzyme inhibitory capabilities. Bioassay-guided fractionation and chemical characterisation are needed for the identification of the active molecules. The results as a whole indicate that HCHAE comprises different types of phytoconstituents and possesses antioxidant, antibacterial, α -amylase and α -glucosidase inhibitory actions. The relatively high phenolic and flavonoid contents were in agreement with the concentration-dependent antioxidant activity in both DPPH and ABTS experiments. The same groups of chemicals may also be responsible for the antibacterial and carbohydrate hydrolysing enzyme inhibitory actions. Marine macroalgae are

recognised as a storehouse of chemically varied metabolites with antioxidant, antibacterial and enzyme regulating characteristics [1,31-37]. The combined results indicated that *H. clathratus* is a prospective source of physiologically active ingredients. However, the present results were obtained using a crude hydroalcoholic extract and in vitro testing. Thus, the activities cannot be readily

construed as therapeutic benefits. Further work with chromatographic fractionation, LC–MS/MS or GC–MS profiling, identification of individual active compounds, MIC/MBC determination, enzyme kinetic studies and appropriate cellular or *in vivo* models would be required to establish mechanisms and biological relevance of the observed activities.

Table 6. In vitro α -glucosidase inhibitory activity of the *Hydraclathratus clathrus* hydro alcoholic extract compared with acarbose.

Concentration ($\mu\text{g/mL}$)	% Inhibition of α -glucosidase by acarbose (Positive control)	% inhibition of α -glucosidase
20	35.67 ± 0.48	28.91 ± 0.48
40	48.09 ± 0.35	37.15 ± 0.50
60	63.60 ± 0.43	48.32 ± 0.31
80	73.19 ± 0.48	60.84 ± 0.11
100	87.35 ± 1.74	77.77 ± 0.13
IC ₅₀	41.968	59.006

Conclusions:

The present investigation revealed the hydroalcoholic extract of *Hydraclathratus clathratus* consists of different phytochemical ingredients such phenolics, flavonoids, tannins, saponins, steroids and amino acids. The high levels of phenolic and flavonoid compounds correlated with significant and dose-dependent antioxidant activity in DPPH and ABTS experiments. HCHAE also showed antibacterial action against both Gram positive and Gram negative bacterial strains. The highest inhibition was obtained against *Staphylococcus aureus* at the largest extract volume tested. The extract also suppressed the activities of α -amylase and α -glucosidase in a concentration dependent manner, indicating a possible interference with carbohydrate-hydrolyzing enzymes. Although the activities were lower than those of the corresponding reference compounds, the results suggest the biological potential of *H. clathratus* as a source of bioactive marine metabolites.

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Data availability

Data will be made available from corresponding authors upon reasonable request.

Compliance with Ethical Standards

Declaration of competing 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 article.

Ethical Statement

There are no human or animal subjects involved in this study, and informed consent is not applicable.

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