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# A COMPREHENSIVE STUDY ON BIOLOGICAL ACTIVITES OF *Halimeda copiosa* AND ITS *in silico* ANALYSIS

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## ABSTRACT

**This research was aimed to study the phytochemical composition and biological activities of *Halimeda copiosa* extracts, focusing on their antioxidant, anti-microbial, antidiabetic, and anti-inflammatory properties. The plant extracts were prepared using methanol and ethyl acetate, with the methanol extract showing a higher concentration of bioactive compounds. Various antioxidant activity assays yielded $IC_{50}$ values of 97.26 mg $mL^{-1}$ (total antioxidant capacity), 872.38 mg $mL^{-1}$ (DPPH), 385.91 mg $mL^{-1}$ (reducing power), and 592.02 mg $mL^{-1}$ (hydrogen peroxide scavenging activity). The extract demonstrated significant antimicrobial activity against five microorganisms, as well as strong *in vitro* amylase and glucosidase inhibitory activities with $IC_{50}$ values of 4.30 and 9.11 mg $mL^{-1}$, respectively, and an anti-inflammatory $IC_{50}$ value of 587.5 mg $mL^{-1}$. Additionally, bioinformatics studies on the ribosomal protein L5 indicated potential antioxidant and pro-apoptotic activities. This study is the first to confirm these activities in *Halimeda copiosa*, suggesting its potential as a source for novel therapeutic agents.**

**Keywords:** Antidiabetic, anti-inflammatory, antimicrobial, antioxidant, *Halimeda copiosa*, *in silico* studies

## INTRODUCTION

Seaweeds are marine photosynthetic algae found abundantly in all the oceans. There are three recognised phyla of seaweeds: Rhodophyta, Phaeophyta, and Chlorophyta (Abdel-Kareem and ElSaied, 2022). Seaweed extracts of green, red, and brown algae reportedly possess antioxidant anti-inflammatory, antidiabetic, and anticancer properties (Kumar *et al.,* 2021). The species of green algal genus *Halimeda* (Caulerpales, Halimedaceae) are identified as crucial components of both Pacific reefs and tropical Atlantic (Hillis-Colinvaux 1980). A few branches of the novel calcareous algae species *Halimeda copiosa* were rediscovered in June 1964 at a depth of 35 m, after it was initially discovered in Jamaica in 1961. *H. copiosa* is microscopically close to the species *H. opuntia* L. *(*Lamouroux*);* but differs in single holdfast and flattened branching. The thinner nodal structure and its uniform raised midrib distinguish *H. copiosa* from *H. gracilis* Harvey *v. opuntioides* Borgesen. *Halimeda* species are large and ubiquitous, about 30 m long found only under deep fore-reef slopes (Goreau and Graham, 1967). *Halimeda* species depict enormous pharmacological activities like antioxidant, anticoagulant, antiviral, antihelminthic, antibacterial,

antifungal, anticancer and anti-inflammatory (Sravya *et al.*, 2023; Mukta, 2024) and yet the *H. copiosa* properties are meagrely reported.

*H. copiosa* (taxonomy ID: 170408) consists nine proteins (*https://www.ncbi.nlm.nih.gov/protein?term=txid170408 [Organism]*. Of these, GenBank: ACN23297 - Ribosomal protein L5, partial (chloroplast) exhibit antioxidant activity as well as the ability to suppress cell division and promote death in early stages of embryogenesis (Schreiner *et al.*, 2022). The perusal of literature reveals no report related to the pharmacological activities of *H. copiosa* and the present study is the first report regarding pharmacological potential of this rare marine macroalgae. In order to comprehensively understand the potential medical applications of *H. copiosa*, the present study was aimed to assess the phytochemicals and some pharmacological activities of methanolic extract of *H. copiosa*. Further, *in silico* structural analysis of protein was carried out by using various bioinformatics tools.

## MATERIALS AND METHODS

### *Samples collection and extract preparation*

The algal samples were obtained from Rameshwaram in Tamil Nadu (India). After cleaning the samples under running water, the samples were air-dried before homogenization into a fine powder and then kept in airtight bottles in a refrigerator. Using the Soxhlet extraction procedure, the crude sample extract was extracted (Supardy *et al.*, 2011). All the algal extracts underwent preliminary phytochemical examination using the standard techniques (Supardy *et al.*, 2011).

### *Antioxidant activity*

**DPPH radical scavenging activity:** DPPH radical scavenging activity was conducted as per Supardy *et al.* (2011). Prism 5.0 was used to compute the $IC_{50}$ value. The percentage of inhibition was determined by using the equation: [(Abs of control - Abs of test)/ Abs of control] × 100.

**Hydrogen peroxide radical scavenging activity:** For the estimation of hydrogen peroxide radical scavenging activity the method of Ruch *et al.* (1989) was followed. For this, 200 µL sample was combined with 0.6 mL hydrogen peroxide (prepared in phosphate buffer). The absorbance was measured in comparison to a blank solution. Graph Pad Prism 5.0 was used to calculate the $IC_{50}$ value and the percent inhibition calculated.

Percentage of inhibition = [(Absorbance of control - Absorbance of sample)/ Absorbance of control)] × 100

**Reducing power assay:** The reducing power assay was conducted as per the method of Oyaizu (1986). Initially, a solution of sample extract and ascorbic acid was prepared in phosphate buffer. To this mixture, potassium ferricyanide (1% w/v) was added, and the sample was incubated at 50°C for 30 min. The mixture was then centrifuged at 3000 rpm for 10 min to obtain supernatant. Subsequently, 0.1% ferric chloride solution and distilled water were added to the supernatant. The absorbance was measured at 700 nm using a UV-Vis spectrophotometer (Elico India Ltd., model 371), with higher absorbance indicating greater reducing power. Results were expressed as ascorbic acid equivalents per gram dry weight of extract, demonstrating its potential antioxidant activity.

**Total antioxidant capacity of extract:** The total antioxidant capability was calculated in terms of ascorbic acid mM equivalent after the absorbance of extract was measured spectophotomertically at 695 nm (Pavithra and Banu, 2017).

### *Antimicrobial activity*

The antibiotic activity was evaluated with respect to the following bacterial strains: *Salmonella typhi* (MTCC 733), *Pseudomonas aeruginosa* (MTCC 1688), *Escherichia coli* (MTCC 722), *Staphylococcus aureus* (MTCC 96), and *Bacillus subtilis* (MTCC 121). The microbial type culture

collections (MTCC) were procured from the Institute of Microbial Technology in Chandigarh (India). The above bacteria were raised in nutrient broth for liquid culture; and in nutrient agar for solid culture. The overnight culture of each bacterium was inoculated on sterile nutrient broth and incubated for 24 h at 37°C till it reached the logarithmic phase. Antimicrobial activity of extracts was screened by disc diffusion method (Bauer *et al.,* 1966). Muller Hinton agar (MHA) [Himedia, Mumbai] was used for measuring *in vitro* antibacterial activity of extracts. To prepare the MHA plates, 15 mm of molten medium were poured into sterile petriplates. For this, a sterile 6 mm disc as impregnated with the extracts at a concentration of 40 mg $disc^{-1}$. These disc were placed on the surface of medium and the extract was allowed to diffuse into medium for 5 min. The plates were then incubated at 37°C for 24 h and the inhibition zones that developed around the disc were measured (mm) using a transparent ruler.

### *Antidiabetic activity*

***In vitro* amylase inhibitory assay:** For this, porcine pancreatic α-amylase was prepared by dissolving 27.5 mg in 100 mL of 20 mM phosphate buffer (pH 6.9) containing 6.7 mM sodium chloride. A 1% (w/v) starch solution was prepared by dissolving 1 g starch in phosphate buffer. Plant extracts at concentrations of 2, 4, 8, 10, and 15 μg $mL^{-1}$ were mixed with 200 μL enzyme solution and incubated at 37°C for 20 min. Then 100 μL starch solution was added, and incubated once again for 10 min. The reaction was stopped by adding 200 μL of 3,5-dinitrosalicylic acid (DNSA) band the solution was heated in a boiling water bath for 5 min. After cooling, the solution was diluted, and absorbance measured at 540 nm using a UV-Vis spectrophotometer (Keerthana *et al.,* 2013).

***In vitro* glucosidase inhibition assay:** For α-glucosidase inhibition assay (Kim *et al.,* 2005), 1 mg α-glucosidase was dissolved in 100 mL phosphate buffer (pH 6.8). The plant extracts were added to the α-glucosidase enzyme solution and incubated at 37°C for 20 min. Then 100 μL of 3 mM p-nitrophenyl-α-D-glucopyranoside (p-NPG) was added, and the mixture incubated for additional 10 min. The reaction was terminated with 2 mL of 0.1 M sodium carbonate, and the amount of nitrophenol released was measured spectrophotometrically at 405 nm. Acarbose served as a positive control for both the assays. The $IC_{50}$ values, representing the concentration of plant extract required to inhibit 50% enzyme activity, were calculated to determine the antidiabetic potential of extracts (Banerjee *et al.,* 2017).

### *Anti-inflammatory activity*

For anti-inflammatory activity of plant extract, the inhibition of albumin denaturation was estimated as per Mizushima *et al.* (1968) and Sakat *et al.* (2010) with slight modifications. The test concentrations of extracts and 1% aqueous solution of bovine albumin fraction were mixed well and incubated at 37ºC for 20 min, and then heated to 51ºC for 20 min. The turbidity of test extracts was measured at 660 nm using UV-visible spectrophotometer. The following formula was used to determine the percentage inhibition of protein denaturation:

Percentage inhibition = (Absorbance of test sample – Absorbance of control) x 100/ Absorbance of control

### *In silico analysis of protein structure*

**Retrieval of sequences and structure prediction:** The Genbank database, from NCBI (*https://www.ncbi.nlm.nih.gov*) was used to obtain the protein FASTA sequences. The ExPASy-ProtParam programme was used to examine the protein main structures (Gasteiger *et al.,* 2005). SOPMA (Self Optimised Prediction Method with Alignment) was employed to predict secondary structures (Combet *et al.,* 2000). The proteins' tertiary structures were predicted using the Swiss model (Arnold *et al.,* 2006).

**Functional characterization:** The physiochemical characteristics of proteins, including their theoretical isoelectric point (pI), molecular weight, total number of positive and negative residues, extinction coefficient, instability index (Ii), aliphatic index (Ai), and grand average of hydropathy (GRAVY), were obtained from Expasy Protparm server (Gasteiger *et al.,* 2005). The SOSUI tool

determines if a protein is transmembrane or soluble in nature (Gomi *et al.*, 2004). The prediction technique TMHMM, which is based on a hidden Markov model, was used to verify the protein topology (Möller *et al.*, 2001).

Ramachandran plot analysis was used to evaluate the overall stereo-chemical properties of proteins (Ramachandran *et al.*, 1963). PROCHECK (Laskowski *et al.*, 1996) and ProSA-web servers (Wiederstein *et al.*, 2007) were used to validate the structure models that were acquired from the two software packages. Swiss PDB Viewer (*https://swissmodel.expasy.org/*) was used to generate figure representations and conduct structural analysis (Guex and Peitsch, 1997). The multiple sequence alignment as well as phylogenetic tree was constructed by using SMART-BLAST (*https://blast.ncbi.nlm.nih.gov/smartblast/?LINK_LOC=BlastHomeLink*).

## RESULTS AND DISCUSSION

### *Phytochemical analysis*

The methanolic extract of *Halimeda copiosa* revealed the presence of five phytochemicals *viz*., flavonoids, tannins, alkaloids, steroids, and carbohydrates. In contrast, ethyl acetate extract showed only three phytochemicals *viz*., carbohydrates, oils and resins, and steroids. Notably, flavonoids, tannins, and alkaloids were exclusive to the methanolic extract, while oils and resins were only found in ethyl acetate extract. Neither extract contained phenols, saponins, or arthroquinone. The presence of flavonoids and other polyphenolic compounds in the methanolic extract is significant, as these phytochemicals are well-documented for their antioxidant properties. Research has shown that flavonoids can scavenge free radicals, thereby contribute to the reduced oxidative stress and lower risk of chronic diseases such as cancer (Tavares *et al.*, 2023). The findings align with Singkoh *et al.* (2023) who reported bioactive compounds like phenols and tannins in *H. macroloba*, suggesting that similar compounds in *H. copiosa* may also enhance its antioxidant potential. In contrast, the ethyl acetate extract's lower diversity of phytochemicals limits its overall health benefits as compared to the methanolic extract. The presence of oils and resins, along with steroids and carbohydrates, indicates that while some bioactive compounds are retained, the potential for antioxidant activity is not as pronounced as in the methanol extract. This observation supports the findings of Latifah *et al*. (2020) who noted that steroid and terpenoid compounds predominated in their analysis of *H. macroloba*, suggesting that solvent choice significantly impacts the extraction of bioactive compounds.

Interestingly, the absence of phenols and saponins in both extracts may indicate specific characteristics of *H. copiosa* as compared to the other species in *Halimeda* genus, as noted in studies like those of Gazali *et al.* (2023), who studied phytochemical activity in *H. tuna*. Overall, present results underscore the importance of solvent selection in phytochemical extraction and suggest that *H. copiosa* has significant potential as a natural source of antioxidants, particularly through its methanolic extract.

### *Antioxidant activity*

The structural characteristics of algae and cyanobacteria could be beneficial additions to the diet. Apart from nutritional value, they are rich in naturally occurring antioxidants and antibacterial agents, and possess high amounts of physiologically active compounds (Frazzini *et al.*, 2022). The human body needs natural antioxidants to be in equilibrium to protect itself from the damages caused by free radicals. The antioxidants have the ability to deactivate the production of highly volatile free radicals and halt/slow down oxidation reactions (Martemucci *et al.*, 2022). Secondary metabolites in plants like phenyl propanoid, antakuinon, flavonoids, saponins, alkaloids, phenol molecules, etc. are known radical scavengers (Roy *et al.*, 2022). These antioxidants play a crucial role in premature aging, prevent cancer, and maintain food product’s quality (Ali *et al.*, 2021).

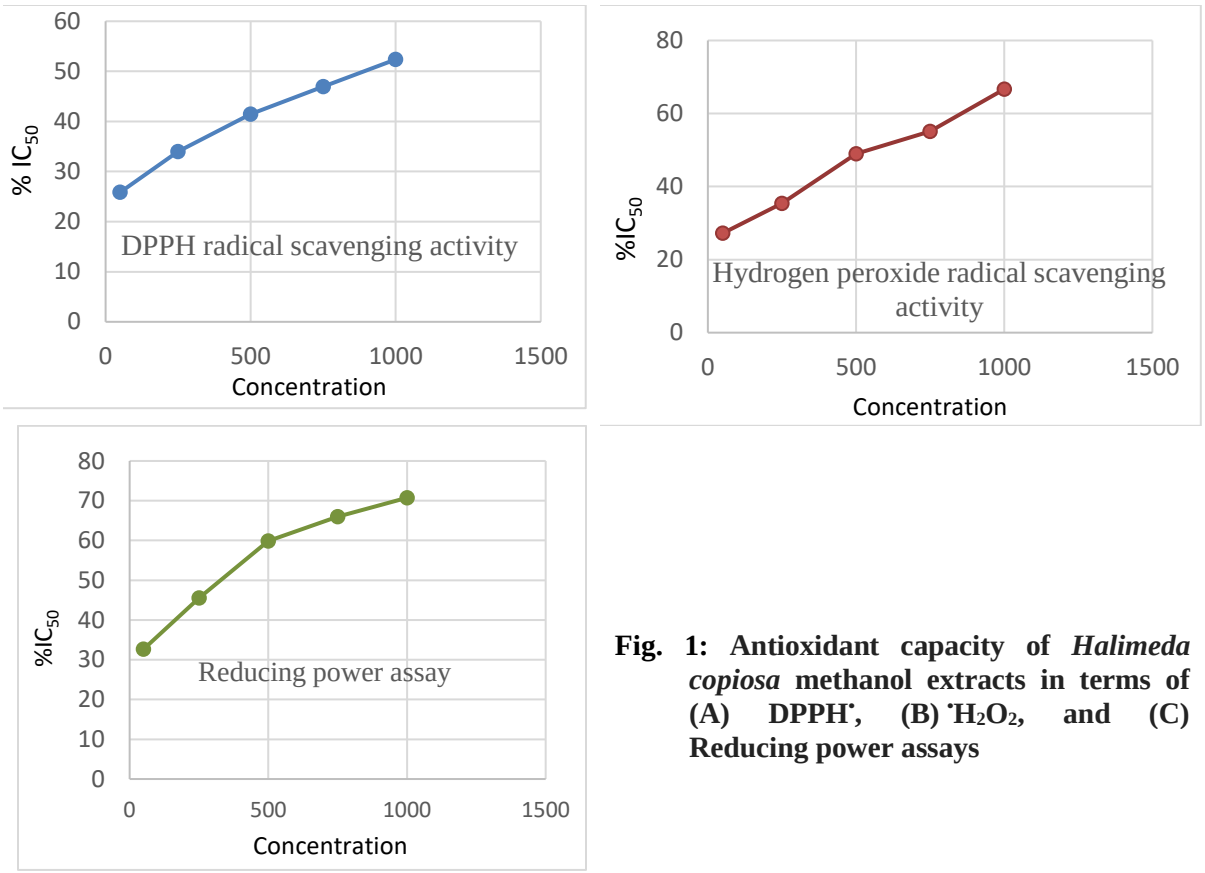


**Fig. 1: Antioxidant capacity of *Halimeda copiosa* methanol extracts in terms of (A) DPPH˙, (B) $H_2O_2$, and (C) Reducing power assays**

In present study, *in vitro* antioxidant activity of *Halimeda copiosa* has been reported for first time by assessing its DPPH radical scavenging activity, hydrogen peroxide radical scavenging activity, reducing power potential (Fig. 1). The study showed higher DPPH radical scavenging activity than hydrogen peroxide and reducing power assay; whereas *H. copiosa* methanolic extract showed strong percentage of inhibition with $IC_{50}$ values of 872.4, 529.0, and 385.9 and total antioxidant capacity was 97.26 was 97.26 µg $mL^{-1}$. In present report the inhibition percentage of *H. copiosa* gradually increased with increase in concentration. Widowati *et al.* (2021) studied the antioxidant activity for the extracts of *H. macroloba*, *G. salicornia*, *H. asperi*, and *H. gracilis* and found varied activity in these test species. Further, they observed that *G. salicornia*, and *H. gracilis* had increased activity with increase in concentration. Nazarudin *et al.* (2022) reported that the methanolic extract of *H. tuna* had free radical scavenging activity which increased with increase in concentration. Further, the methanolic extract of *H. tuna* @ 200 µg $mL^{-1}$ demonstrated 56.29 to 63.91% inhibition in DPPH radical scavenging activity.

***Antimicrobial activity***

*Escherichia coli*, *Salmonella typhi*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* were used to assess the antibacterial activity of *H. copiosa* extracts. The methanolic extracts of *H. coipiosa* did not show any inhibitory action against *S. aureus* and *B. subtilis* at 30 µg $mL^{-1}$ level; and maximum inhibitory activity was observed against *S. typhi* at 60 µg $mL^{-1}$ level (Fig. 2). The study revealed higher inhibition across different extract concentrations against test bacteria. In control, the inhibitory zone measured 23 mm, while at 30 µg $mL^{-1}$ concentration, *E. coli* showed least activity, with mean inhibitory zone of 8 mm. Fig. 3 illustrates inhibitory effect against all test bacteria at variable concentrations. It was evident that *H. copiosa* has broader function as an antibiotic which were 10-15 mm likewise showing efficacy against all bacterial types. It is believed that the bacterial cell membrane is harmed by inhibition mechanism of steroid compounds.

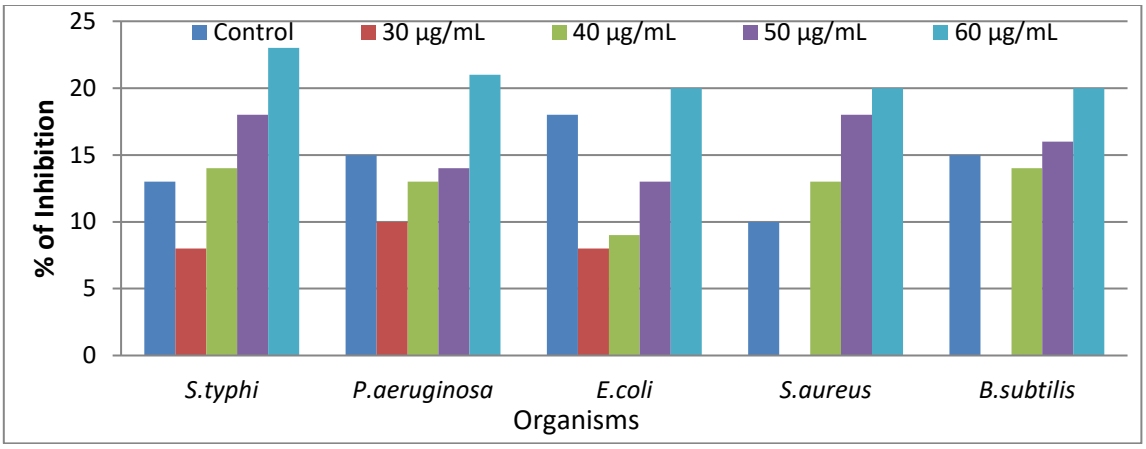


**Fig. 2: Antimicrobial activity of *Halimeda copiosa* extract against *E. coli, S. typhi, B. subtilis, P. aeruginosa,* and *S. aureus***

**Fig. 3: The antimicrobial activities of *H. copiosa* against *E. coli, S. typhi, B. subtilis, P. aeruginosa, and S. aureus***

The antimicrobial properties of crude extract of *H. macroloba* and *H. gracilis* have previously been determined with *H. macroloba* showing lesser activity than *H. gracilis* against *Stenotrophomonas maltophilia* and *Vibrio harveyi* (Basir *et al.*, 2020). *S. aureus* was not susceptible to the antibacterial activity of *H. gracilis*. *S. aureus* and *E. coli* showed greater susceptibility to the antibacterial activities of crude extracts of *H. macroloba* (Basir *et al.*, 2020). Latifah *et al.* (2020) studied the antibacterial activity of *H. macroloba* against three Gram negative bacteria *Aeromonas hydrophilla*, *V. harveyi* and *V. parahaemolyticus* and found that it inhibited *A. hydrophilla* and *V. parahaemolyticus*.

***Antidiabetic activity***

*In vitro* and *in vivo* studies have shown that polyphenols of green seaweeds are beneficial in the treatment and prevention of type 2 diabetes mellitus (Widowati *et al.*, 2021). The methanolic extract of *H. copiosa* exhibited inhibitory effect against *in vitro* amylase and *in vitro* glucosidase (Fig. 4). The tested concentrations of *H. copiosa* extract exhibited variable inhibition of α-amylase and glucosidase. The 2 mg $mL^{-1}$ (40.90) showed lowest inhibition with $IC_{50}$ of 4.3%, while 15 mg $mL^{-1}$ concentration (82.57) caused highest inhibition in α-amylase. Similarly, the concentration of 2 mg $mL^{-1}$ (33.33) depicted lowest inhibition with $IC_{50}$ value of 9.11% for this enzyme. The concentration of 15 mg $mL^{-1}$ (61.36) caused highest inhibition in glucosidase. Seaweeds play a major role in antidiabetic activity (Erinati *et al.*, 2022). *Halimeda tuna* has antidiabetic activity (Mohanapriya *et al.*, 2016). The methanolic extract of *H. tuna* depicted higher inhibition in α-amylase

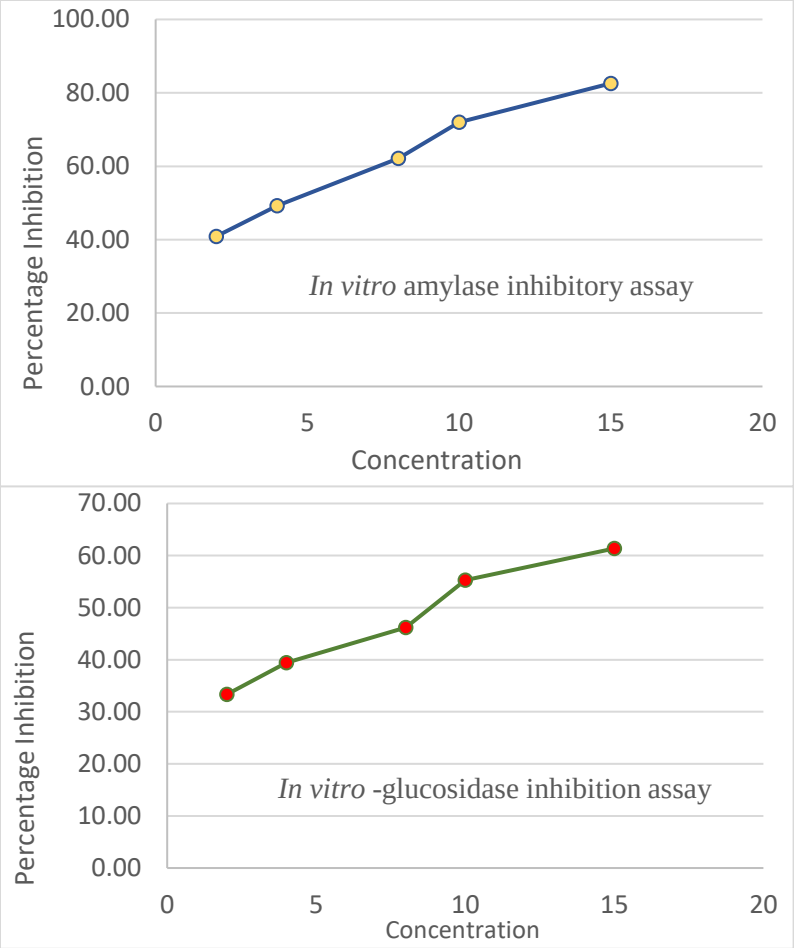


**Fig. 4: Antidiabetic activity of *Halimeda copiosa* (A) *In vitro* amylase (B) *In vitro* glucosidase**

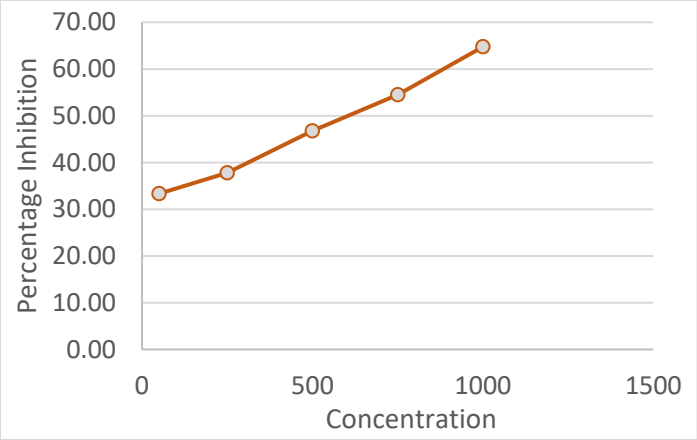


**Fig. 5: Anti-inflammatory activity of *Halimeda copiosa***

activity at 10 mg $mL^{-1}$ (46.42) and higher inhibition in glucosidase activity at 2.5 mg $mL^{-1}$ (61.10) [Gazali *et al.*, 2023].

***Anti-inflammatory activity***

The inhibition rate of albumin denaturation increased progressively with increase in the concentration of methanolic extract of *H. copiosa*. Notably, at 1000 mg $mL^{-1}$, maximum inhibition was observed, while at 50 mg $mL^{-1}$ concentration lowest inhibition, with $IC_{50}$ value of 587.5% was observed. The present study reports anti-inflammatory activity in the methanolic extract of *H. copiosa* for the first time and the results indicate the inhibition ranging from 30-65%. Thanh *et al.* (2021) observed three high polar lipid fractions of 32.57 to 41.661.95 µg $mL^{-1}$ concentration in the extracts of *Halimeda incrassate.*

Sabari Anandh *et al.* (2022) prepared Sidha pharmacopeia from *H. gracilis* after determining its antioxidant, anti-inflammatory, and anti-cancer properties. *H. copiosa* in present study showed anti-inflammatory and anti-diabetic properties which can be exploited in pharmaceutical or medicinal research.

***Structural prediction***

Protein FASTA sequences were sourced from NCBI Genbank database. The theoretical pI, molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index, and grand average hydropathy (GRAVY) were computed for physico-chemical characterisation using Expasy ProtParam server. The outcomes were displayed in Table 2. The partial (chloroplast) molecular weight of ribosomal protein L5 in present work was 8649.74 kDa. Isoelectric point (pI) is the pH of solution at which the net charge of a protein's surface amino

**Table 2: Ribosomal protein L5, partial (chloroplast) primary structure prediction, using Expasy's Protparam tool**

| Accession No. | Protein | Length | Mol. Wt | pI | -R | +R | EC | II | AI | GRAVY |
|---|---|---|---|---|---|---|---|---|---|---|
| ACN23297 | Ribosomal protein L5, partial (chloroplast) | 74 | 8649.74 | 4.55 | 10 | 4 | 8480 | 61.99 | 79.05 | -0.457 |

Note: Mol. Wt = Molecular weight (Da), pI = Isoelectric point, -R = No. of negative residues, +R = No. of positive residues, EC = Extinction coefficient at 280 nm, II = Instability index, AI = Aliphatic index, GRAVY = Grand average hydropathy,

acids equal zero (Gasteiger *et al.,* 2005). The protein's calculated pI value was 4.55. In order to ensure the stability of purified protein, it is crucial to use the computed pI value to screen potential buffering systems for the purification of protein of interest (Gasteiger *et al.,* 2005). Furthermore, Table 2 reveals a total of 10 negatively charged residues [-R(Asp + Glu)] and 4 positively charged residues [+R (Arg + Lys)]. With regard to the molar extinction coefficient of tyrosine, tryptophan, and cystine (cysteine does not absorb appreciably at wavelengths >260 nm, while cystine does), the ExPASy-ProtParam tool computes the extinction coefficient (EC) at wavelength 280 nm with EC values of 8480 (Gasteiger *et al.,* 2005). Is is possible to optimise the purification process of target proteins by using the EC value of protein (Gasteiger *et al.,* 2005). The protein stability in a test tube can be estimated by measuring the instability index. The statistical examination of 12 unstable and 32 stable proteins showed that some dipeptides are much more common in unstable proteins than they are in stable ones (Guruprasad *et al.*,1990). A protein is considered stable if its instability index is < 40; conversely, a value > 40 indicates that the protein is unstable Gasteiger *et al.* (2005). Since proteins have instability index (II) of 61.99, so they are unstable. The proportion volume filled by aliphatic side chains (leucine, isoleucine, valine, and alanine) determines a protein's aliphatic index. It might be advantageous for the rise in globular protein's thermostability (Gasteiger *et al.,* 2005). For proteins, the AIs were 79.05. The protein GRAVY value in this study was -0.457. A protein's hydrophilic character and potential for improved protein-water interaction are indicated by a higher negative GRAVY score Gasteiger *et al.* (2005).

**Table 3: Prediction of ribosomal protein L5, partial (chloroplast) secondary structures**

| Protein | Alpha helix (Hh) | Extended strand (Ee) | Beta turn (Tt) | Random coil (Cc) |
|---|---|---|---|---|
| Ribosomal protein L5, partial (chloroplast) | 12.16% | 16.22% | 13.51% | 58.11% |

SOPMA's secondary structure prediction, ribosomal protein L5 partial (chloroplast), showed that α-helix, extended strand, β-turn, and random coil were more common (Table 3). Window width: 17, similarity threshold: 8 and the number of states: 4 were default parameters used to estimate the secondary structure. Random coil showed dominance over other coil types, and TMHMM v.2.0 and SOSUI projected that ribosomal protein L5, partial (chloroplast) proteins was a soluble protein. The ribosomal protein L5, partial (chloroplast) amino acid sequence (length: 74 AA) was predicted by SOSUI to be a soluble protein with 0 transmembrane helices (Fig. 6) and an average hydrophobicity of -0.456757.

```
# WEBSEQUENCE length: 74
# WEBSEQUENCE No. of predicted TMHs:  0
# WEBSEQUENCE Exp number of AAs in TMHs: 0.00035
# WEBSEQUENCE Exp number, first 60 AAs:  0.00032
# WEBSEQUENCE Total prob of N-in: 0.06943
WEBSEQUENCE TMHMM2.0 outside 1 74
```

Swiss model, a homology modelling programme, was used to model the protein's three-dimensional structure. Fig. 7 displays the results of using Swiss PDB Viewer to visualise the final modelled structures.

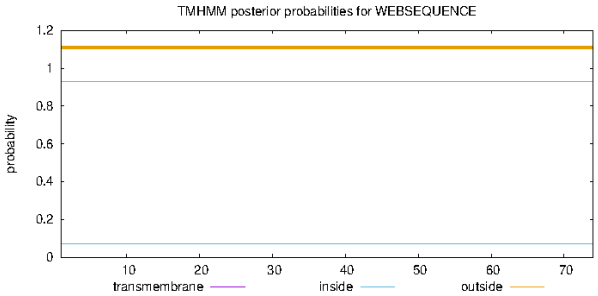


**Fig. 6: TMHMM of ribosomal protein L5, partial (chloroplast)**

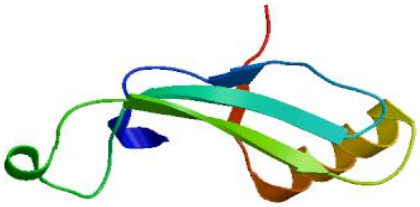

**Fig. 7: 3D structure of ribosomal protein L5, partial (chloroplast)**

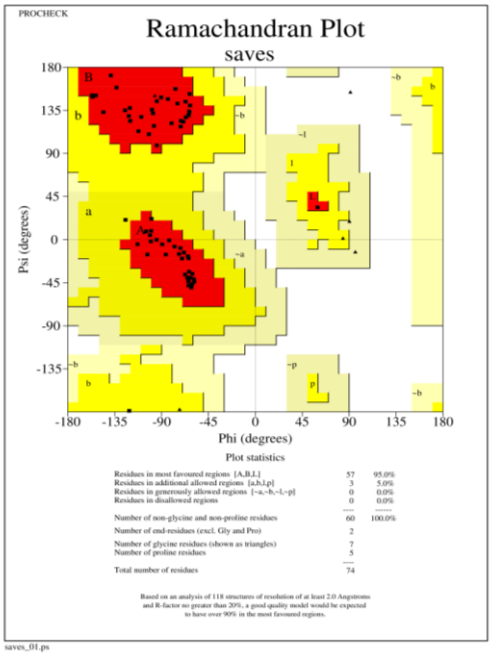


**Fig. 8: Ramachandran plot for Ribosomal protein L5, partial (chloroplast):** The allowed zones are represented by yellow regions, while the most allowed regions are indicated by red regions. Other residues are shown as squares, and glycine is represented by triangles.

Ramachandran plot and PROVE plot was created to validate the produced structure. Fig. 8 provided a summary of the Phi and PSi distribution of Ramachandran map produced by non-glycine, non-proline residues.

Using Ramachandran map computations produced by the PROCHECK programme, the stereo-chemical quality of the projected model and the precision of the protein were assessed following the refinement model procedure. Fig. 8 displayed the evaluation of model that Swiss model predicted. The plotted ones are the following main chain parameters: overall G factor, peptide bond planarity, poor non-bonded contacts, main chain hydrogen bond energy, C-alpha chirality, and Ramachandran plot quality. The residues were categorised in Ramachandran plot analysis based on their quadrangle regions. The graph outcome showed that 95.0% of the residues in the permitted region of the modelled structure for ribosomal protein L5, partial (chloroplast). These numbers from the Ramachandran plot indicated that the projected models are of high quality. The atoms in the modellar structure of ribosomal protein L5, partial (chloroplast) were calculated using the PROVE plot, and the specific results are displayed in Fig. 8.

The ProSA-web server was also used to assess the NTDs' modelled structures (Wiederstein and Sippl, 2007). ProSA-web provides a score known as the "z-score" that represents the "degree of nativeness" of the modelled protein structures. The quality of modelled protein structure is indicated by the z-score value; a big negative z-score suggests native fold, whereas scores closer to positive values imply problematic or incorrect models (Wiederstein and Sippl, 2007). The model structure's strong quality was further supported by the RMS Z score of -3.82. It displayed similarities between five species, ranging from 66 to 62%. Table 4 and Fig. 9 display the evolutionary tree of *Halimeda copiosa*'s partial (chloroplast) ribosomal protein L5. Since certain proteins found in *H. copiosa* have been shown to have significant and pharmacological potency, the plant can be exploited to address a variety of health issues as well as be utilised in research on drug creation and pharmaceutical discoveries.

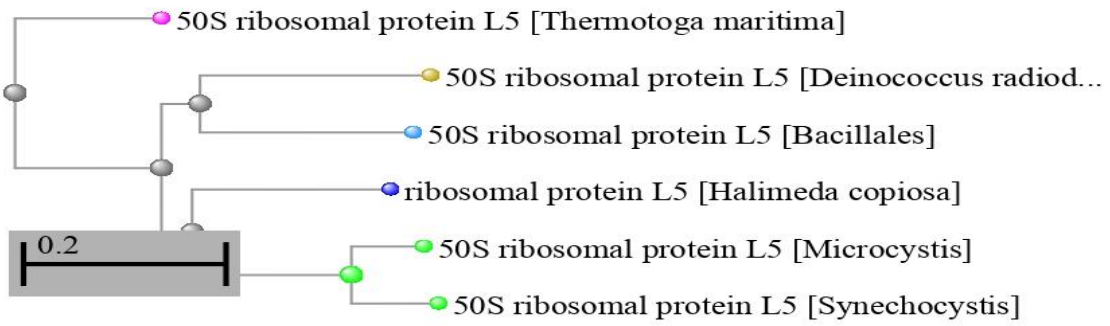


**Fig. 9: Phylogeny of ribosomal protein L5, partial (chloroplast) protein**

**Table 4: Phylogeny of ribosomal protein L5, partial (chloroplast) protein**

| Description | Accession No. | Identity |
|---|---|---|
| 50S ribosomal protein L5 [*Microcystis*] | WP_002734750.1 | 66.20% |
| 50S ribosomal protein L5 [*Synechocystis*] | WP_190596113.1 | 65.28% |
| 50S ribosomal protein L5 [*Deinococcus radiodurans*] | WP_010886968.1 | 63.89% |
| 50S ribosomal protein L5 [*Bacillales*] | WP_003225809.1 | 61.11% |
| 50S ribosomal protein L5 [*Thermotoga maritima*] | WP_004081811.1 | 62.50% |

**Conclusion:** The study's findings indicate that in comparison to ethyl acetate the phytochemical content of seaweed *Halimeda copiosa* is higher in methanol. By using the *in vitro* antioxidant, antimicrobial, antidiabetic and antiinflammatory activities were demonstrated by the methanolic extract of *H. copiosa*. Further, the homology study for the protein ribosomal protein L5, partial (chloroplast) were analysed. Deeper and thorough analysis can provide an optimised view of the properties of this algae. The study proved that the genus *Halimeda* be thoroughly screened for its bioactive compounds and applied in medicines.

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