



INVITRO AND INSILCO STUDIES OF *GRACILARIA EDULIS* AGAINST CLINICAL PATHOGENS

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Abstract: The rising prevalence of antibiotic resistance necessitates exploring novel bioactive compounds from natural sources. This study investigates the antimicrobial potential of *Gracilaria edulis*, a marine red alga, through in vitro and in silico approaches. In vitro, bioactive compounds were extracted from *Gracilaria edulis* which using Chromatography technique and tested against clinical pathogens, including Gram-positive and Gram-negative bacteria, and fungi, using agar well diffusion method. In silico studies identified potential bioactive compounds and predicted their interactions with microbial enzymes and proteins via molecular docking simulations. Results demonstrated that *Gracilaria edulis* extracts exhibited substantial antimicrobial activity with notable inhibition zones and low Minimum Inhibitory Concentration (MIC) values. In silico analysis supported these findings, showing strong binding interactions between the compounds and microbial proteins. This study highlights *Gracilaria edulis* as a promising source of novel antimicrobial agents, integrating in vitro and in silico methods to pave the way for algae-based therapeutics against clinical pathogens.

Keywords - *Gracilaria edulis*, antimicrobial activity, in vitro, in silico, clinical pathogens, molecular docking, gas chromatography, phytochemical analysis, Secondary Metabolism, bioactive compounds.

I. INTRODUCTION

The increasing prevalence of antibiotic-resistant clinical pathogens has become a significant global health concern, driving the need for novel antimicrobial agents derived from natural sources. Marine organisms, particularly algae, are rich in bioactive compounds with potential therapeutic applications. *Gracilaria edulis*, a marine red algae, has gained attention for its diverse bioactive properties, including antimicrobial effects. However, the antimicrobial potential of *Gracilaria edulis* against clinical pathogens has not been extensively explored. This study aims to evaluate the antimicrobial activity of *Gracilaria edulis* using both in vitro and in silico approaches. The in vitro analysis involves extracting bioactive compounds and testing their efficacy against a range of clinical pathogens, while in silico studies focus on identifying and predicting the interactions of these compounds with key microbial enzymes and proteins. By integrating these methodologies, we aim to provide a comprehensive understanding of the antimicrobial capabilities of *Gracilaria edulis* and explore its potential as a source of new antimicrobial agents.

The red algae *Gracilaria edulis* is a valuable source of bioactive compounds with potential applications in medicine and biotechnology. In this study, we aim to explore the phytochemical composition and bioactivity of *G. edulis*, with a focus on its antibacterial and antifungal properties. The collected samples of *G. edulis* will be prepared and bioactive compounds will be extracted using methanol as the solvent and maceration as the extraction method. To further investigate the biological activities of these extracts, we will employ antibacterial and antifungal assays.

To purify the bioactive compounds, column chromatography will be utilized on ground, fine-particle seaweed samples. The identification and characterization of these compounds will be conducted using advanced spectroscopy techniques, including Gas Chromatography-Mass Spectrometry (GC-MS) and Nuclear Magnetic Resonance (NMR) spectroscopy. By providing a detailed analysis of the phytochemical composition of *G. edulis*, this research aims to contribute to the broader field of microbiology.

Additionally, computer-aided drug design (CADD) techniques such as molecular docking, virtual screening, and lead optimization will be employed to optimize these bioactive compounds for potential drug development. The objective is to develop new molecules that can specifically bind to biological targets, potentially leading to therapeutic agents that are more effective and less toxic than existing treatments. This research will also provide insights into the metabolic processes within *G. edulis* and the roles of specific compounds in these processes. Furthermore, optimizing the cultivation of *G. edulis* for various applications, including drug discovery and biotechnology, is another significant goal of this study.

II. METHODS AND MATERIALS

2.1 Sample Collection

The sample of *Gracilaria edulis* was collected from the Devipattinam coastline (Figure 1) located at latitude 9.473649° and longitude 78.896112°, at Madharasa Street, Devipattinam, Tamil Nadu 623514, India on February 26th, 2023 at 12:19 PM. The *Gracilaria edulis* (Figure 2) was found growing in association with *Kappaphycus alvarezii* seaweed raft, at a depth of 30 cm in the sea. The seaweed was collected from zip lock bags. After collecting the seaweed sample, it was washed in distilled water (Salunke, M. et al., 2022). The collected algae were dried in the shade at room temperature for six days in a dark room. The shade-dried seaweeds were completely dry (Figure 3). The mosses were ground into fine particles using a 550W mixer grinder and the powdered sample was kept for later use.



Figure 1: Sample Collected from Devipattinam Seashore.



Figure 2: *Gracilaria edulis*

Figure 3: Shade dried seaweed (*Gracilaria edulis*)



2.2 Preparation of Seaweed Sample Extract

The maceration method involved soaking 30g of ground seaweed sample in 180ml of methanol at a 1:6 (w/v) biomass/solvent ratio and storing it in a hot air oven at 37°C for five days. After complete solvent evaporation, the crude extract was obtained in paste form (Salunke, M. et al., 2022). Maceration is a common method used in natural product extraction, as it is simple, inexpensive, and can be used with a wide range of solvents. In this study, methanol was chosen as the solvent for its ability to extract a broad range of compounds from the seaweed sample. The 1:6 sample-to-solvent ratio was selected to ensure adequate extraction of the targeted compounds (Figure 4). To maintain a consistent temperature throughout the extraction process, the samples were stored in a hot air oven at 37°C. After five days, the solvent was evaporated using a rotary evaporator, leaving behind a concentrated paste. This paste was then weighed and stored at -20°C until further use in chemical analysis or biological assays.

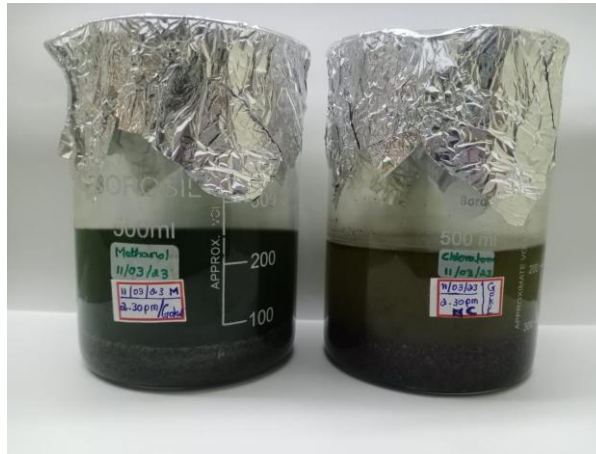


Figure 4: Maceration method (Methanol extract)

2.3 Determination of Antimicrobial Activity from *Gracilaria edulis*:

2.3.1 Disk Preparation

In flexible antibiotic studies, sterile discs are employed to determine the susceptibility of microorganisms to different antibiotics, wherein each disc is impregnated with a specific antibiotic and then placed on a culture plate to assess the efficacy of the drug against the organism being tested. The solvent extracts of *Gracilaria edulis* (Methanol) (Kolanjinathan, K., et al., 2014) were mixed with 1ml of Dimethyl sulfoxide (DMSO). The discs were impregnated with 30 μ l, 40 μ l, 50 μ l, 60 μ l of different solvent extracts of sea weeds at four different concentrations ranging 30mg/ml, 40mg/ml, 50mg/ml and 60mg/ml to check their antibacterial activity and antifungal activity. The sterile discs which contain 5% DMSO were act as a blind control and the Hi-media antibiotic disk (Gentamicin 10) act as a positive control for bacterial and the sterile discs were impregnated with Fluconazole 30mg/ml act as positive control for fungi (Kolanjinathan, K., et al., 2014).

2.3.2. Collection of Test Microbial Cultures

The clinical samples (*Pseudomonas sp.*, *Bacillus sp.*, *E. coli*, *Staphylococcus sp.*, *Salmonella sp.*, *Aspergillus sp.*, *Candida sp.*, *Alternaria sp.*, *Trychophyton sp.*) were collected from the Frontline Hospital, Chatram bus stand, Trichy. Then it was isolated by performing serial dilution and spread plate in UTI agar medium, Nutrient agar, Rose Bengal agar and incubated for 24 hours at 37°C the organisms were then differentiated through selective and differential media and confirmed through biochemical tests.

2.4 Determination of Antibacterial Activity from *Gracilaria edulis*

2.4.1 Bacterial Inoculum Preparation

Bacterial inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Nutrient broth and incubated at 37°C for 3-5 hours till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standards and then used for the determination of antibacterial activity (Kolanjinathan, K., et al., 2014).

2.4.2 Disk Diffusion Method

The antibacterial activity of *Gracilaria edulis* extract was determined by Disc diffusion method proposed by (Bauer et al. 1966 (86). A bacterial suspension was spread on Mueller-Hinton (pH 7.4) agar1 using a cotton swab. The Mueller Hinton agar plates were prepared and inoculated with test bacterial organisms by spreading the bacterial inoculum on the surface of the media. The discs containing *Gracilaria edulis* extracts (Methanol) at four different concentration (30mg/ml, 40mg/ml, 50mg/ml and 60mg/ml) was placed on the surface of the Mueller Hinton agar plates. The Sterile discs which contain 5% DMSO were act as a blind control and the Hi-media antibiotic disk (Gentamicin 10) act as a positive control for bacterial. The plates were incubated at 37°C for 24 hours. The antibacterial activity was assessed by measuring the diameter of the zone of inhibition in (mm) (Bauer et al. 1966).

2.5 Determination of Antifungal Activity from *Gracilaria edulis*

2.5.1 Disk Diffusion Method

The antifungal activity of *Gracilaria edulis* extract was determined by Disc diffusion method proposed by (Bauer et al. 1966 (86). A fungal suspension was spread on Rose Bengal agar (pH 7.4) using a cotton swab. The Rose Bengal agar plates were prepared and inoculated with test fungal organisms by spreading the fungal inoculum on the surface of the media. The discs containing *Gracilaria edulis* extracts (Methanol) at 10 mg/ml was placed on the surface of the Mueller Hinton agar plates. The paper discs which contain 5% DMSO were act as a blind control and the paper discs containing Fluconazole 30mg/ml act as appositve control for fungi. The plates were incubated at 23°C for 2 days. The antifungal activity was assessed by measuring the diameter of the zone of inhibition in (mm) (Bauer et al. 1966)

2.6 Phytochemical Screening

The methanolic extract was subjected to preliminary phytochemical screening, for evaluation of major phytoconstituents such as alkaloids, flavonoids, triterpenoids, tannins. Phytochemical screening of the extract was performed using the following reagents and chemicals.

2.6.1 Phytochemical Analysis of Secondary Metabolites

2.6.1.1 Test for Alkaloids

To 1ml of the extract, 1ml of Dragendroff's reagent (potassium bismuth iodine solution) was added. An orange red precipitate indicates the presence of alkaloids (Chaves, O. S et al., 2017).

2.6.1.2 Test for Flavonoids

Shinoda Test was performed to confirm the presence of flavonoids. Pieces of magnesium ribbon and HCl concentrated were mixed with aqueous crude plant extract a pink color observed after few minutes indicate the presence of flavonoid (Mahesh, A. R. et al., 2013).

2.6.1.3 Test for Terpenoids

0.5ml of the extract was mixed with 2ml of chloroform and concentrated sulphuric acid (3ml) which from a layer. A reddish-brown coloration of the interfere showed the presence of terpenoids. (Yusuf, A. Z. et al., 2014).

2.6.1.4 Test of Tannins

To 1ml of the extract 1ml of ferric chloride solution was added, formation of dark blue or greenish black product showed the presence of tannins (Mailloa, M. N. et al., 2013).

2.6.1.5 Test for Carbohydrates

To 2ml of Barfoed's reagent was added to 2 ml of the Plant extract, mixed & kept a in boiling water bath for 1 min. red precipitate formed indicates the presence of monosaccharides (UC, R., & NAIR, V. M. G. et al., 2013).

2.6.1.6 Test for Glycosides

To 1ml of extract, 1ml of alpha naphthol and chloroform was added along the sides and observed for the development of violet color which indicated the presence of glycosides (Gurucharan, D. N. et al., 2012).

2.6.1.7 Test for Anthraquinone

1ml of benzene and 1ml of 10% ammonia was added with 300µl of extract. Presence of anthraquinone was observed by the formation of pink, red or violet color in the lower phase of ammonia (Nagalingam, S. et al., 2012).

2.6.1.8 Test for Quinine

To the extract, freshly prepared FeSO₄ solution (1mL) and ammonium thiocyanate were added then conc. H₂ SO₄ was added drop by drop. The deep red color indicated the presence of quinine (Sharma, T. et al., 2020).

2.6.1.9 Test for Phenol

A small amount of the ethanolic extract was taken with 1 mL of water in a test tube and 1 to 2 drops of Iron III chloride (FeCl₃) was added. A blue, green, red or purple color is a positive test (Iqbal, E. et al., 2015).

2.6.1.10 Test for Inulin

To the few ml of extract, added 1ml of alpha-naphthol solution and 0.5 ml sulphuric acid, a brownish red color formed indicates the presence of inulin. (Kumar et al., 2013)

2.7 Separation of Phytochemical

2.7.1 Constituents Column Chromatography

Column chromatography is frequently used by organic chemists to purify liquids/solids. An impure sample is loaded onto a column of adsorbent, such as silica gel or alumina. An organic solvent or a mixture of solvents flows down through the column. Components of the sample separate from each other by partitioning between the stationary packing material (silica or alumina) and the mobile eluent. Molecules with different extents, and therefore move through the column at different rates. The eluent is collected as fractions. Fractions are typically analyzed by thin layer chromatography to see if separation of the components was successful.

2.7.2 Materials used

A column size of 45cm×3 cm (length and diameter) was used. The absorbent used in the column was silica gel (Acme's mesh: 60-120µ).

2.7.3 Packing of the Column

A piece of wire as used to add a plug of cotton to the bottom of the column. There should be enough cotton that the silica would not fall out) of the column. However, too much cotton or cotton packed too tightly would prevent the eluent from dripping at an acceptable rate. The column was clamped to a ring stand and added enough silica to fill the curved portion of the column. Placed a pinch clamp on the tubing, and then filled the column 1/4 to 1/3 full with the initial eluent. (The composition of eluent was often changed as the separation proceeds).

Prepared a slurry of silica in the initial eluent by pouring dry silica into a beaker of eluent (add a volume of silica gel, such as 20g, to approximately double the volume of eluent, 40ml). The slurry was poured in to the column immediately to maximize the amount of silica that goes into the column instead of remaining behind in the beaker. The pinch clamp was removed to allow solvent to drip into a clean flask. The column was tapped on the side of the column with a rubber stopper or tubing to help the silica settle uniformly. A Pasteur pipette was used, and carefully the sample was added to the column.

Silica was allowed to settle while eluent was continuing to drip into the flask. A long Pasteur pipette was used, and carefully the sample was added to the column. The eluent was drained from the column until no sample remains above the surface of the silica. 1ml of eluent was used to rinse container and pipette. This milliliter of sample was added to the silica. The above step was repeated two or three times to completely transfer the sample onto the silica gel. Once sample was rinsed onto the silica, eluent was added carefully to the top of the column. The eluent was added as necessary. The eluent collected prior to the elution of sample could be recycled. The composition of the eluent could be changed as the column progressed. If the eluent composition was to be changed, always start with least polar solvent/mixture and change to the more polar solvent/mixture.

2.7.4 Ratio of the solvent

The *Gracilaria edulis* (30g) was purified by column chromatography on a silica gel column. The column was eluted with Methanol (100%). The selected purified fractions were then subjected to GC-MS analysis to estimate phytochemical composition.

2.8 GAS CHROMATOGRAPHY WITH MASS SPECTROMETRY (GC-MS) Analysis

GC-MS was performed at Satyabhama Institute of Science and Technology, Centre for Ocean Research analyses were performed using an Agilent 6890B gas chromatograph fitted with an HP-5MS fused silica column (5% phenyl methyl polysiloxane 30m x 0.25 mm id., film thickness 0.25µm) that was interfaced with an Agilent mass selective detector 5973N (Agilent Technologies, USA) and operated by HP Enhanced Chem Station software. For GC-MS detection, an electron ionization system with an ionization energy of 70 eV was used. Helium was used as the carrier gas at a flow rate of 1 ml/min. The injector and transfer line temperatures were set at 250 and 285°C, respectively. The column temperature was initially kept at 10°C for 1 minute and was then gradually increased to 250°C at a rate of 5°C/min; finally, it was raised to 285°C at a rate of 1°C/min. A diluted sample in hexane (20% solution) of 2.0µl was injected in split mode at a ratio of 1:50. The chromatographic conditions and column used for GC analyses (Agilent 5973N gas chromatograph with FID detector) were the same as those for GC-MS analyses. The identity of the components was selected by comparison on the HP-5MS column and GC-MS spectra from the Wiedly7Nist data by co-injection with authentic compounds (Sigma, Aldrich, Fluka). Quantification of the components was performed on the basis of their GC peak areas on the HP-5MS column.

2.9 Docking Studies

2.9.1 Protein preparation

The target protein β -ketoacyl-acyl carrier protein synthase III (FabH) (PDB ID ID: 1ZOW) obtained from the PDB database. The non-standard residues (residues other than amino acids) were first removed from the target proteins using DS client, and the resulting protein structures were processed using the _protein preparation wizard 'of Maestro, Schrodinger. Energy minimization of the resulting modelled protein was carried out by using Maestro 9.0.111 protein preparation wizard (Schrodinger, LLC, 2008, New York, NY) where a brief relaxation was performed using an all-atom constrained minimization with the Improve (Impact Refinement module), to alleviate steric clashes; bond orders were assigned and hydrogen 's was added. The energy minimization was carried out at the default cut off (Root Mean Square Deviation) RMSD value of 0.30 Å using OPLS[°] 2001 force field. (Brindha et al., 2009)

2.9.2 Ligand Preparation

The 3D coordinates for the ligands were generated using Ligprep Module of Schrodinger Software in Maestro 9.0.111 (Schrodinger, NY) using a force field OPLS 2005. Five low energy conformers were generated per ligand which resulted from plant compounds (TETRAPENTACONTAN, DOTRIACONTANE, METHYLHEPTADECANE, Dotriacontane, HEXATRIACONTANE, TETRACOSANE, 3 Tetratriacontane, Tritriacontane, 4-PYRIDINECARBOXYLIC ACID, 2-PYRIDINECARBOXYLIC ACID). Schrodinger utilities were used to remove salts, neutralize and ionize compounds at the physiological pH 7.0 ± 2.0. Default settings of Leather face protonation, tautomer states and specified chirality 's were retained before energy minimization, to result in a low energy conformer. The ligand poses that pass the initial screens were subjected to energy minimization on precompiled Van der Waals and electrostatic grids and pass through filters for the initial geometric and complementary fit between ligands and the receptor – beta ketoacyl carrier protein synthase III. (Friesner RA et al., 2006).

2.9.3. Active Site

Identification and characterization of binding site is the key step in structure-based drug design (Laskowski et al., 1996). The active site region of the protein is identified by FT site (Ngan et al., 2012). This server analytically furnishes the area and the volume at the probable active site of each pocket to envisage the binding site.

2.9.4. Receptor Grid Generation

The co-crystallized ligand was differentiated from the active site of receptor. The grid was defined around the co-crystallized ligand. The atoms were scaled by van der Waals radii of 1.0 Å with the partial atomic charge less than 0.25 defaults. The functionally important sites were defined as an enclosing box at the centroid of the workspace (co-crystallized) ligand was allowed to dock into the active site. No constraints were defined. (Glide version 6.6, 2005).

2.9.5. Docking

Docking has become a promising method for identifying active lead/active compounds and has combined with the pipeline of drug discovery in most pharmaceutical companies. Glide module has been used for all the docking protocol. Among 3 small molecules of plant compounds that compounds have been used for screening and get fewer toxic compounds from the hits. The ligands were processed with the LigPrep program to assign the suitable protonation states at physiological pH= 7.2 ± 0.2 . Conformer generation was carried out with the ConfGen torsional sampling and Ligand docking used OPLS_2005 force field. The van der Waals radii were scaled using a default scaling factor of 0.80 and default partial cut-off charge of 0.15 to decrease the penalties.

III. RESULTS AND DISCUSSION

3.1 Anti-Bacterial Assay

The Methanol fractions of *Gracilaria edulis* algae were subjected to antibacterial assay against both Gram positive and Gram-negative bacteria namely, *Staphylococcus*, *Pseudomonas*, *E. coli*, *Salmonella* and *Bacillus*. This study observed and compared the antimicrobial activity of methanol extracts of *G. edulis* leaves on both Gram positive and Gram-negative bacteria. Gentamicin was used as a positive control whereas n-Methanol was used as negative control. The results of antibacterial assay are shown in (Table 1). The methanol fractions of *G. edulis* showed inhibitory effect against all of the bacteria used in the study (Fig.5,6,7,8 & 9). The ranges of zone were from 10-25mm.

The bacterial activity of different fractions of *G. edulis* extract varied on different types of bacteria. In this study, *Staphylococcus* showed highest sensitivity with zone of inhibition range from 18-25mm (Plate) whereas *Pseudomonas* showed lowest sensitivity with zone of inhibition range from 13-17. (Plate).

Similarly, described the antibacterial effects of the crude extract of *G. edulis* against *S. aureus* and *Escherichia coli* (Kasanah, et al., 2019). Moreover, reported the antibacterial activity against bacterial fish pathogens like *Vibrio fluvialis* and *Vibrio compbelii*. Again, confirmed the methanol extract of *G. edulis* to have remarkable inhibitory activity toward *Bacillus subtilis*, *S. aureus*, *E. coli* and *Pseudomonas fluorescens* (Asgar, A., et al., 2021).



Figure 5: (*Pseudomonas sp.*)



Figure 6: (*Bacillus sp.*)



Figure 7: (*Salmonella sp.*)



Figure 8: (*E. coli*)



Figure 9: (*Staphylococcus sp.*)

Table1: Antibacterial Test

S. No.	Pathogens	Zone of Inhibition (mm)				Positive discs (Gentamicin)
		30µg	40µg	50µg	60µg	
1.	<i>Staphylococcus sp.</i>	-	18	24	25	29
2.	<i>Pseudomonas sp.</i>	-	13	15	15	22
3.	<i>E. coli</i>	-	15	16	16	24
4.	<i>Salmonella sp.</i>	-	15	17	19	18
5.	<i>Bacillus sp.</i>	10	19	23	24	27

(-) = No Zone of Inhibition

3.2. Anti-Fungal Activity

Activity of Methanol extracts of *G. edulis* on selected fungal strains ere also evaluated. Fluconazole was used as a positive control for antifungal activity whereas n Methanol was used as negative control. The results of antifungal assay are shown in (Table 2). A fraction of *G. edulis* showed inhibitory effect against the 4 fungal strains used in this study (Fig). The ranges of zone were from 10-13mm. Highest activity was found against *Aspergillus nigar* (10-13mm).

Furthermore, the sensitivity and susceptibility of the microbes to the plant extracts varied. In particular, the fungal strains were highly sensitive and susceptible to the seaweed extracts than the bacterial strains. Varying degrees of solubility of the active constituents with the solvents might be a reason behind the difference in efficacy of different solvents fractions. It was observed that the antimicrobial effect of seaweed extract varies from one plant to another in different researches carried out in different regions of the world. This may be due to many factors such as, the effect of climate, soil composition, age and vegetation cycle stage, on the quality, quantity and composition of extracted product, different bacterial strains (Masotti et al., 2003). Moreover, different studies found that the type of solvent has an important role in the process of extracting.

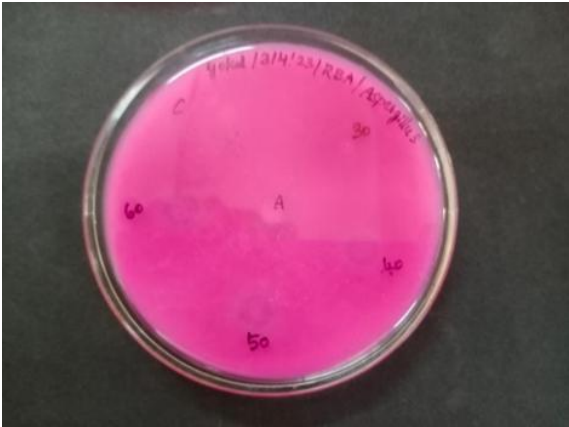


Figure 10: (*Aspergillus sp.*)



Figure 11: (*Trichophyton sp.*)

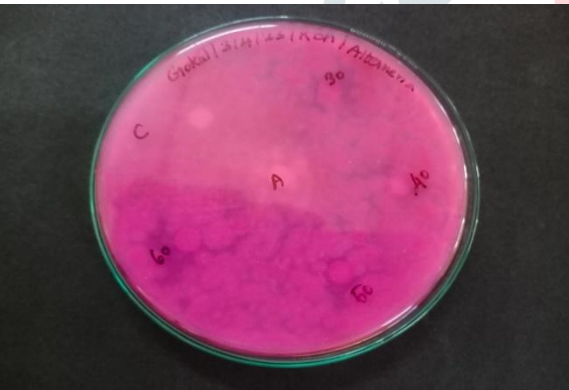


Figure 12: (*Alternaria sp.*)

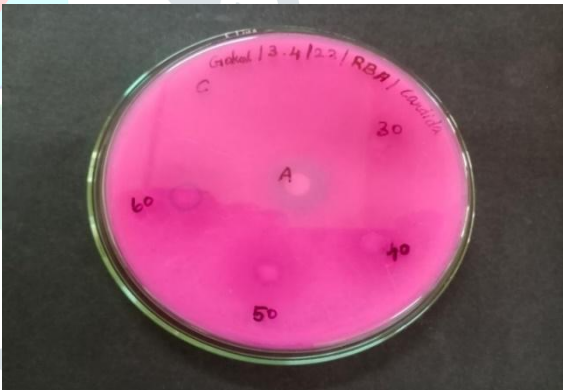


Figure 13: (*Candida sp.*)

Table 2: Anti-Fungal Test

S. No.	Pathogens	Zone of Inhibition (mm)				Positive discs (Gentamicin)
		30µg	40µg	50µg	60µg	
1.	<i>Alternaria</i>	-	-	-	-	-
2.	<i>Aspergillus</i>	10	13	13	12	13
3.	<i>Trichophyton</i>	10	10	12	13	14
4.	<i>Candida</i>	-	10	10	11	14

(-) = No Zone of Inhibition

3.3 Phytochemical Analysis

Shows, the phytochemical analysis of crude extracts of marine seaweed extract. From the table it is very evident that they are good sources of secondary metabolites and possess protein and amino acid content. This may be a good sign that these Seaweeds may be used as a protein supplement. The rich level of protein in the seaweeds studied indicates an interesting possibility of exploiting this natural marine resource in raw or processed form to alleviate the chronic problem of protein deficiency in developing countries, such as India. Several chemical compounds including (Alkaloids, Terpenoids, Carbohydrates, Glycosides, Quinine, Phenol, Inulin) have been detected in the Seaweeds (Figure14; Table 3).

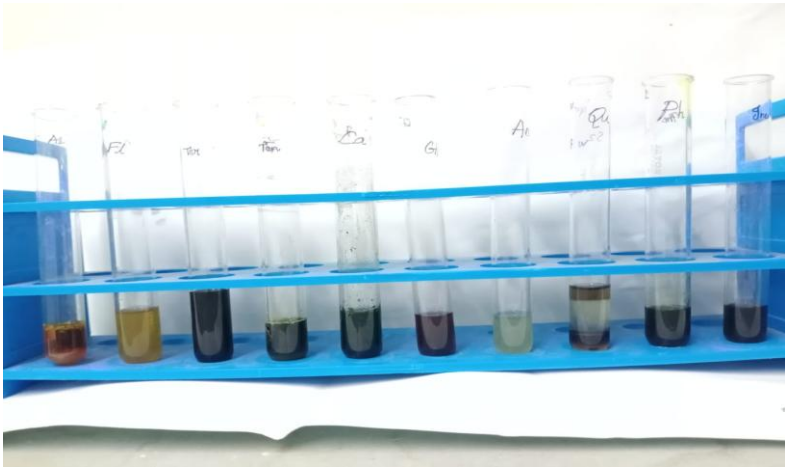


Figure 15. (Phytochemical analysis - alkaloids, flavonoids, Anthraquinone, Tannins, Terpenoids, Carbohydrates, Glycosides, Quinine, Phenol, Inulin.)

Table 3: Phytochemical Test

S. No	Phytochemical Tests	Results
1.	Alkaloids	+
2.	Flavonoids	-
3.	Terpenoids	+
4.	Tannins	-
5.	Carbohydrates	+
6.	Glycosides	+
7.	Anthraquinone	-
8.	Quinine	+
9.	Phenol	+
10.	Inulin	+

(+) = Present, (-) = Absent

3.4. PHYTOCHEMICAL PROFILING BY GC-MS

The GC-MS study of *Gracilaria edulis* seaweeds has shown many phytochemicals which contributes to the medicinal activity of the seaweed (Table 4). The major compounds which present Lupenone-20(29)-EN-3-YLACETATE, Cholest-4-en-24-ol-3-one, Saline, diethylheptyloxyoctadecyloxy-. The other compounds like Hexadecenoic Acid, 1,2,3 propanetriyl ester, 9-Octadecenoic acid, 1,2,3-propanetriyl, 1-Heptacosanol, Androst-5-en-3 olm, alpha-Tocopherylacetate, Silane and diethylheptyloxyoctadecyloxy- in the *Gracilaria edulis*.

The results revealed the presence of various secondary metabolites of therapeutical importance. The major phytochemicals found were Alkaloids, Terpenoids, carbohydrates, Glycosides, Quinine, Phenol and Inulin. Numerous phytochemical investigations of seaweed in this genus confirm the presence of Alkaloids, Terpenoids, Carbohydrates, Glycosides, Quinine, Phenol and Inulin. Various studies have confirmed that these extracts or active substances that were isolated from the genus *Gracilaria* have multiple pharmacologica activities.

Table 4: GC-MS Compound Peak Table

Peak Report TIC				
Peak#	R.Time	Area	Area%	Name
1	33.916	88724	0.47	HEXADECANOIC ACID, 1-(HYDROXYM
2	36.391	100102	0.53	9-Octadecenoic acid, 1,2,3-propanetriyl ester
3	36.820	151893	0.81	1-Heptacosanol
4	36.948	215335	1.14	1-Heptacosanol
5	38.136	372670	1.98	Androst-5-en-3-ol, 4,4-dimethyl-, (3.beta.)-
6	38.510	129738	0.69	.alpha.-Tocopheryl acetate
7	38.630	153376	0.81	9-OCTADECENOIC ACID (Z)-, 2-[(TRIME
8	38.862	477684	2.53	LUP-20(29)-EN-3-YL ACETATE
9	40.595	731264	3.88	Cholest-4-en-24-ol-3-one
10	41.530	16432008	87.16	Silane, diethylheptyloxyoctadecyloxy-
		18852794	100.00	

3.5 Docking Studies

Bacterial β -ketoacyl-ACP synthase (KAS) enzymes are important in the elongation steps of fatty acid biosynthesis (Heath et al. 2001; Khandekar et al. 2003). KAS I (FabB) and KAS II (FabF) are involved in the condensation of malonyl-ACP with a growing acyl-ACP chain to form β -ketoacyl-ACP, which is a substrate for β -ketoacyl- ACP reductase (FabG). KAS III (FabH) catalyzes the initiation of fatty acid biosynthesis by condensing malonyl-ACP with acetyl-CoA.

Staphylococcus aureus (S. aureus) is a Gram-positive human pathogen that causes diseases in humans, including skin infections, scalded-skin syndrome, and toxic shock syndrome. The bacterium is also the leading cause of nosocomial infections. S. aureus-induced disease is often suppurated and causes extensive tissue destruction and necrosis. Infections caused by S. aureus are often resistant to antibiotic treatments, and can reoccur even years after apparently successful therapy (Lowy 1998). There are few antibiotics on the market to combat S. aureus resistance and these are starting to fail; therefore, there is a growing need to develop new anti-bacterial agents against S. aureus.

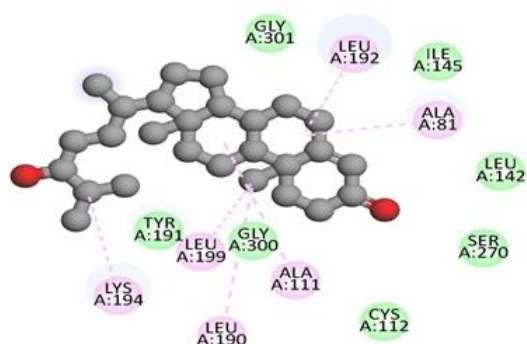
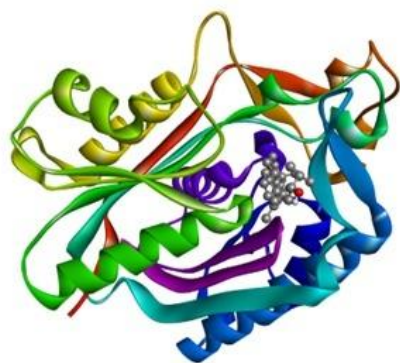
Because they are essential enzymes for bacteria and differ significantly from human fatty acid synthase (FAS), various bacterial FabHs have been studied as potential anti-bacterial. Substrate specificity of the various FabH enzymes appears to be the determining factor in the biosynthesis of branched- or straight-chain fatty acids of the type II fatty acid synthase. Consistent with this notion, FabH purified from Gram-negative and Gram-positive bacteria, despite their overall similar catalytic mechanism, have displayed significantly different substrate specificities (Khandekar et al. 2003). For example, Streptococcus pneumonia, a Gram-positive bacterium, is able to utilize both straight- and branched-chain (C4–C6) acyl CoA primers (Khandekar et al. 2001), while E. coli, a Gram-negative organism FabH, utilizes primarily short straight-chain acyl CoAs—preferentially acetyl-CoA

The bacterial beta-Ketoacyl-ACP synthase III is an attractive drug target for antibacterial pathogens. Binding energies of the protein-ligand(drug) interactions are important to describe how fit the drug binds to the target macromolecule. We applied the docking procedures, to dock seaweed compounds to dock with beta-Ketoacyl-ACP synthase III into the binding pocket of this enzyme. Our docking simulation resulted in a very close target protein structure, which supports our findings. There are two compounds highly interect with beta-Ketoacyl-ACP synthase III and high potent inhibitory activity. 42 | P a g e Interestingly, residues seem to play a central role in the with beta-Ketoacyl-ACP synthase III binding site. The amino acid residues LYS194, TYR191, LEU199, GLY300, ALA111, LEU190, ALA81, LEU192, ILE145 were involved in the active site of beta-Ketoacyl-ACP synthase III.

The amino acid residue LYS194, TYR191, LEU199, GLY300, ALA111, LEU190, ALA81 was involved in interaction with CHLOEST-4-EN-24-OL-3-ONE in the active site of beta-Ketoacyl-ACP synthase III. The length of hydrogen bond formed 1.811 Å, 2.1 Å, 2.3,3.1 Å,2.4 Å,2.12 Å,2.89 Å AND 2.5 Å and also IC50 value of this compound have 1.21(μm). (Figure)

The amino acid residues LEU109, LEU199, ALA81were involved in interactions with alpha—tocopheryl acetate in the active site of beta-Ketoacyl-ACP synthase III. The length of hydrogen bonds formed 1.884 Å, 1.934 Å and 1.89 and also IC50 value of this compound have 4.72(μm). (Figure 16)

So, in this result, the compound CHLOEST-4-EN-24-OL-3-ONE and alpha tocopheryl acetate compounds better than seaweed compounds from insilico activity. Hence CHLOEST-4-EN-24-OL-3-ONE compound become a good lead compound as well as an inhibitor for beta-Ketoacyl-ACP synthase III.



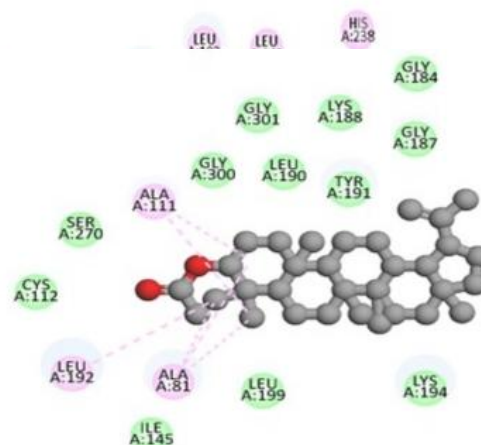
(A) Fabh_Cholest-4-en-24-ol-3-one (7.8 Kcal/Mol)
 (B) Fabh_alpha-Tocopheryl acetate (-7.5 Kcal/Mol)
 (C) Fabh_LUP-20(29)-EN-3-YL ACETATE (-7.3 Kcal/Mol)

Figure 16

IV. CONCLUSION

Medicinal plants are considered the most important features in field of Indian medicine. The medicinal plants play a vital role in the traditional medicines such as Ayurveda, Unani, Siddha and Homeopathy. Based on this the present study was done to identify the antimicrobial activity of the leaves extract of *Gracilaria edulis* against the common Gram positive and Gram-negative pathogens which includes *Pseudomonas*, *Staphylococcus*, *Bacillus*, *Salmonella* and *E. coli*. It was then compared with the marine organisms of same species so that the difference in their nature and constituents could be identified and used in the future prospects of Drug discovery.

In this study, a methanol extract of *Gracilaria edulis* against *Staphylococcus* showed highest zone of inhibition range from 18-25 mm whereas salmonella showed lowest sensitivity with zone of inhibition range 15-18mm. A methanol extract of *Gracilaria*



edulis showed inhibitory effect against the 4 fungal strains used in this study. Highest zone of inhibition was found against *Trichophyton* (10-14mm) and lowest zone of inhibition was found against *Alternaria* (no zone of inhibition)

The GC-MS studies of *Gracilaria edulis* seaweeds has shown many phytochemicals which contributes to the medicinal activity of the seaweed. The major compounds which present Lupenone-20(29)-EN-3-YLACETATE (Figure 16. (A)), Cholest 4-en-24-ol-3-one, Saline, diethylheptyloxyoctadecyloxy-. The other compounds like Hexadecenoic Acid, 1,2,3-propanetriyl ester, 9-Octadecenoic acid, 1,2,3-propanetriyl, 1 Heptacosanol, Androst-5-en-3-olm, alpha-Tocopherylacetate (Figure 16. (A)), Silane and diethylheptyloxyoctadecyloxy- in the *Gracilaria edulis*.

The present study was analyzed binding affinity of seaweed compounds against beta Ketoacyl-ACP synthase III *Staphylococcus aureus* and its sequence similar to gram negative bacteria and gram-positive bacteria. In this enzyme not present in human and animals. So, its attractive drug target for drug designs bacterial infections.

A virtual screening of suitable compounds tetrapentacontane, dotriacontane, hexatriacontane, 3-methylheptadecane, dotriacontane, tetracosane, tetratriacontane, tritriacontane, 4-pyridinecarboxylic acid, 2-pyridinecarboxylic acid were screened from seaweed compounds and the best hits compounds such as 4-pyridinecarboxylic acid, 2 pyridine carboxylic acid were identified based on the docking score and hydrogen bond interactions.

These compounds bind with active site region in beta-Ketoacyl-ACP synthase III *Staphylococcus aureus* enzyme and which will be useful in designing new inhibitor and its analogs for beta-Ketoacyl-ACP synthase III *Staphylococcus aureus* and also

gram negative and gram-positive bacteria pathogens. The novel molecular entities have the potential as leads compounds used to designing anti-bacterial molecules in short span of time. As whole results throw light for future development of more potent and drug like inhibitors for beta-Ketoacyl-ACP synthase III *Staphylococcus aureus* enzymes gram negative and gram-positive pathogens.

To find out the antimicrobial activity Methanol extracts of Seaweed of *Gracilaria edulis* were prepared. The pharmacogenetic, phytochemical and antimicrobial activity were identified through the standard procedures. All the solvents showed good zone of inhibition and it is found to be fit as Ayurvedic pharmacopeia of India. It has been confirmed that the antimicrobial activity is due to the presence of the phytochemical components such as Alkaloids, Terpenoids, Tannins, glycoside, Proteins, Lignin and Inulin. It is also determined that the marine organisms are more resistant than the clinical species.

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REFERENCES

- [1] Salunke, M., Wakure, B., & Wakte, P. (2022). High-resolution liquid chromatography mass spectrometry (HR-LCMS) and ¹H NMR analysis of methanol extracts from marine seaweed *Gracilaria edulis*. *Natural Product Research*, 38(8), 1441–1444. <https://doi.org/10.1080/14786419.2022.2146906>
- [2] Kolanjinathan, K., & Saranraj, P. (2014). Pharmacological efficacy of marine seaweed *Gracilaria edulis* extracts against clinical pathogens. *Global Journal of Pharmacology*, 8(2), 268-274.
- [3] Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol*. 1966 Apr;45(4):493-6. PMID: 5325707.
- [4] Chaves, O. S., Teles, Y. C. F., Monteiro, M. M. D. O., Mendes Junior, L. D. G., Agra, M. D. F., Braga, V. D. A., ... & Souza, M. D. F. V. D. (2017). Alkaloids and phenolic compounds from *Sida rhombifolia* L. (Malvaceae) and vasorelaxant activity of two indoloquinoline alkaloids. *Molecules*, 22(1), 94.
- [5] Mahesh, A. R., Ranganath, M. K., & Harish Kumar, D. R. (2013). Enrichment of flavonoids from the methanolic extract of *Boerhaavia diffusa* roots by partitioning technique. *Research Journal of Chemical Sciences* ISSN, 2231, 606X.
- [6] Yusuf, A. Z., Zakir, A., Shemau, Z., Abdullahi, M., & Halima, S. A. (2014). Phytochemical analysis of the methanol leaves extract of *Paullinia pinnata* linn. *Journal of pharmacognosy and phytotherapy*, 6(2), 10-16.
- [7] Mailoa, M. N., Mahendradatta, M., Laga, A., & Djide, N. (2013). Tannin extract of guava leaves (*Psidium guajava* L) variation with concentration organic solvents. *International Journal of Scientific and Technology Research*, 2(9), 106-110.
- [8] UC, R., & NAIR, V. M. G. (2013). Phytochemical analysis of successive reextracts of the leaves of *Moringa oleifera* Lam. *International Journal of Pharmacy and pharmaceutical sciences*, 5, 629-634.
- [9] Gurucharan, D. N., Swamy, K. K., Onkarappa, R., Shobha, K. S., & Gautham, S. A. (2012). Antifungal metabolites of *Bulbophyllum nilegiriensis* wt. from Western Ghats of Karnataka, India. *Natural Products: an Indian Journal*, 9(4).
- [10] Nagalingam, S., Sasikumar, C. S., & Cherian, K. M. (2012). Extraction and preliminary phytochemical screening of active compounds in *Morinda citrifolia* fruit. *Asian journal of pharmaceutical and clinical research*, 5(2), 179-181.
- [11] Sharma, T., Pandey, B., Shrestha, B. K., Koju, G. M., Thusa, R., & Karki, N. (2020). Phytochemical screening of medicinal plants and study of the effect of phytoconstituents in seed germination. *Tribhuvan University Journal*, 35(2), 1-11.
- [12] Iqbal, E., Salim, K. A., & Lim, L. B. (2015). Phytochemical screening, total phenolics and antioxidant activities of bark and leaf extracts of *Goniolanthus velutinus* (Airy Shaw) from Brunei Darussalam. *Journal of King Saud University-Science*, 27(3), 224-232.
- [13] Kumar, K., Parida, M., & Katiyar, V. K. (2013). Short term traffic flow prediction for a nonurban highway using artificial neural network. *Procedia-Social and Behavioral Sciences*, 104, 755-764.
- [14] Brindha V, Saravanan A and Manimekalai R. Drug designing for ring finger protein 110 involved in adenocarcinoma (human breast cancer) using casuarinin extracted from *Terminalia arjuna*. *Ind. J. Sci. Technol*, 2009; 2: 22–26.
- [15] Friesner RA, Murphy RB, Repasky MP, Frye LL, Greenwood JR, Halgren AT, Sanschagrin CP, Mainz DT. Extra precision glide: docking and scoring incorporating a model of hydrophobic enclosure for protein–ligand complexes, *J. Med. Chem*. 2006; 49: 6177–6196.
- [16] Laskowski, R. A., Rullmann, J. A. C., MacArthur, M. W., Kaptein, R., & Thornton, J. M. (1996). AQUA and PROCHECK-NMR: programs for checking the quality of protein structures solved by NMR. *Journal of biomolecular NMR*, 8, 477-486.
- [17] Ngan CH, Hall DR, Zerbe B, Grove LE, Kozakov D, Vajda S.FTSite: high accuracy detection of ligand binding sites on unbound protein structures. *Bioinformatics*. 2012. *Bioinformatics*. 2012.15; 28(2):286-7.
- [18] Kasanah, N., Amelia, W., Mukminin, A., Triyanto, & Isnansetyo, A. (2019). Antibacterial activity of Indonesian red algae *Gracilaria edulis* against bacterial fish pathogens and characterization of active fractions. *Natural product research*, 33(22), 3303-3307.
- [19] Asghar, A., Tan, Y. C., Shahid, M., Yow, Y. Y., & Lahiri, C. (2021). Metabolite profiling of malaysian *Gracilaria edulis* reveals eplerenone as novel antibacterial compound for drug repurposing against MDR bacteria. *Frontiers in microbiology*, 12, 653562.

- [20] Masotti, V., Juteau, F., Bessière, J. M., & Viano, J. (2003). Seasonal and phenological variations of the essential oil from the narrow endemic species *Artemisia molinieri* and its biological activities. *Journal of agricultural and food chemistry*, 51(24), 7115-7121.
- [21] Khandekar, S.S., Daines, R.A., and Lonsdale, J.T. 2003. Bacterial β -ketoacyl-acyl carrier protein synthases as targets for antibacterial agents. *Curr. Protein Pept. Sci.* 4: 21–29

