INSILICO HOMOLOGY MODELING OF RIBULOSE-1,5-
BISPHOSPHATE CARBOXYLASE PROTEIN IN GRACILARIA 
EDULIS

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ABSTRACT
Seaweeds are the subset of a larger group known as the algae. It has been widely used in many countries as a source of food and additionally has biomedical and industrial applications such as cosmetic, paint, crop, textile, paper, rubber, and building industries. The marine algae are essential to nature and directly valuable to humans since they have antioxidant, antimicrobial, antiviral, anticancer, anticoagulant and heme agglutinating properties. Moreover, nitrogen containing seaweeds are generally useful as good nutritive source for plants used as fertilizers. Further their applications extend in industrial level as weight reducer, raw materials for fuel production and useful adsorbent for waste water treatment. Moreover, these seaweeds have many beneficial effects and hence could be developed into potential products for biomedical and industrial applications. In this research study we found out the potential mutation gene and to perform protein sequence analysis, protein modeling using Bioinformatics tools. There are three steps involved in our present research investigation. The protein primary structure analysis, Secondary structure analysis and protein three dimensional structure prediction.

KEYWORDS: Seaweeds, Marine algae, antioxidant, antimicrobial, antiviral, anticancer, anticoagulant, Homology Modeling.
INTRODUCTION
Pharmacologist, microbiologist, botanists and natural products chemist are combing the earth for phytochemicals and leads that could be developed for treatment of various disease. Seaweeds have evolved the ability to synthesize compounds that help them defend against a wide variety of predators. These predators have beneficial effects and used to treat human disease such secondary metabolites are highly varied in structure. Many are aromatic substance, most of which are phenols or their oxygen substituted derivatives.

Secondary metabolites are the chemicals produced by plants for which no role has yet been found in growth and photosynthesis. Many thousands have been identified in several major classes. Humans are so flavorings or recreational drugs. Secondary metabolites are classified on the basis of chemical structure (e.g. having rings, containing a sugar), composition (containing nitrogen or not), their solubility in various solvents, or the pathway by which they are synthesized (e.g. phenyl propanoid, which produced tannins). A simple classification includes the terpenes (made from mevalonic acid: composed almost entirely of carbon and hydrogen), Phenolics (made from simple sugars, containing benzene ring, hydrogen and oxygen), and nitrogen containing compounds.

Many secondary metabolites have signaling functions. They influence the activities of other cells, control the metabolic activities and co-ordinate the development of sea weeds. Many of them are antibiotics, i.e., they inhibit the existence of competing species in the surrounding of their producer, thus safe guarding its ecological niche. Many compounds have an outstanding role in medicine. They are used either directly or after chemical modification. Some products have psycho pharmacological effects and morphine or mescaline are even counted among the hard drugs. During the last 20 to 30 years the analysis of secondary seaweed products has progressed a lot. Clinical researches have confirmed the efficacy of several seaweeds in the treatment of liver disease. Traditional medicinal or folk medicine practice is based on the use of plant and plant extracts. It is also known as botanical medicines, medical herbalism herbology or phototherapy.[1]

All seaweed chemical compounds are divided into primary metabolites, such as sugars and fats, found in all plants, and secondary metabolites compounds not essential for basic function found in a smaller range of plants, have medicinal properties. The function of secondary metabolites are varied. Some secondary metabolites are toxin used to predation and others are pheromones used to attract insects for pollination. Phytoalexins protect against
Shoba et al. World Journal of Pharmacy and Pharmaceutical Sciences

bacterial and fungal attacks. There is up regulation and down regulation in their biochemical paths in response to other local mix of herbivores; pollinators and microorganism. The chemical profile of a single plant may vary over time as it reacts to changing condition. It is the secondary metabolites and pigments that can have therapeutics actions in human and which can be refined to produce drugs.

Seaweeds are rich in secondary metabolites such as tannins and alkaloids. These phytochemicals have antiviral, antibacterial, antifungal and anti-helminthic properties are used for medicines. Alkaloids contain a ring with nitrogen. Many alkaloids have effects on the central nervous system. Poly phenol also known as phenolics contain phenol rings. Tannin, isoflavones, phytoestrogen are some of the important phytochemicals which gives colours and fragrances to the plants. Terpenoids are the built up from terpene building blocks and glycosides which consists of glucose moiety are bioactivity substance present in seaweeds. Many consumers believe that natural medicines are safe because they are ‘natural’. In 2002 the U.S. National center for complementary and Alternative Medicine of the National Institute of health began funding clinical trials into the effectiveness of herbal medicine.

In 2010 survey of 1000 plants, 356 had clinical trials published evaluating their pharmacological activity. Many herbs have shown positive results in-vitro, animal model or small-scale clinical test. In this line seaweeds are large subset of a group known as algae. They are marine algae, and ecologically and biologically Important. They maintain the ecosystem stability. The Seaweeds grow attached to rocks, shell, or any other solid objects. Sometimes they grow in floating condition at the water edges or scatter on the shore. The polysaccharides of seaweeds are used in food, fodder, manure, medicine, cosmetic, paint, crop, textile, paper, rubber, and building Industries. In addition they are used in food industries and also utilized as animal feed. In spite of its various uses the seaweeds are nowadays known for its Antioxidant activity and other related biological activities like, antibacterial, antiviral, Antitumor, anticoagulant and fibrinolytic properties etc. Seaweeds are macroscopic marine subset algae there are about 10,000 species of seaweeds, of which 6000 red algae (Roddophytae) are, 2000 are brown algae and 2000 are green algae. In Asia, seaweeds have been consumed as a vegetable. Japanese eat 1.4kg /person/day. In recent years scientist have started looking towards seaweeds for the production of new drug from natural products. These products are also increasingly being used in medical and biomedical research. For many years chemical preservatives have been used in food to act as
antimicrobials or anti-oxidants or both. These chemical compounds are hazards to health and cause asthma and cancer and suspected to be neurotoxin. Now seaweeds are used as a safer food preservative.\(^7\)

**Types of Seaweeds**

Based on the colour of their pigmentation, the seaweeds are broadly classified in to various categories: Chlorophyceae (Green), Phaeophyceae (brown), Rhodophyceae (red).

**Components of seaweed**

Seaweed is having many secondary metabolites, which have antioxidant, antimicrobial and antitumor agents. The secondary metabolites includes flavinoids – which plays a role in removal of toxin from skin. Alkaloids and phenolic compounds – a powerful free radical scavenging agent.\(^8\) It has minerals like, chromium, cobalt, copper, silicon, sodium, potassium, phosphorus, nickel etc. It has vitamin A, vitamin B-Complex and vitamin C which are powerful antioxidant. It has an omega-3-fattyacid and omega-6-fatty acid which prevents the coronary heart disease. It has different composition of amino acid like, alanine arginine, threonine and serine which are the building block of our body and act as an effective immune booster. It has high carbohydrates content which boost physical stamina and nucleic acid that improves mental focus. 24- Hydroperoxy-24-vinyl-cholesterol was isolated from, Sargassum ringgoldianum and S. horneri. It is the first isolation of this sterol from the plant sources.

Methanol extracts of Green algae belonging to the phyla Volvocophyta Chlorophyta and Charophyta which are freshwater habitats revealed the presence of D-norandrostane-16-carboxylic acid, β-sitosterol and trans-phytol. The unsaturated fatty acids were found in larger proportion (54–94%) than the saturated fatty acids (6–40%). The C15:0 and C16:0 were the most commonly occurring fatty acids, followed by C18:1, C19:1, C15:3, and C17:3 acids. These algae resembled green seaweeds in their fatty acid composition. They displayed a significant phytotoxic activity but non-significant cytotoxic, insecticidal, and antitumor activities.\(^9\) Small amounts of D-sorbitol were extracted from 15 species of brown seaweeds. Sorbitol phosphate was also isolated and identified.\(^10\)

Seaweeds also have antimicrobial activity against human pathogens like viruses, fungi and yeast. Extract of seaweeds belong to chlorophyceae (ulvalactua, Halimedia gracilis) Rhodophyceae (gracilaria edulis, hyprea musiformis) and phaeophyceae (Turbinaria conoides, Sargassum myricystum) families are effective to human pathogen like Eschericha
coli, pseudomonas aeruginosa, staphylococcus aureus Klebsiella pneumonia and Enterococcus faecalis. Seaweeds like sargassum wightii Gracilaria edulis, which has nutritional value also, have antimicrobial properties.\[11\] Petroleum ether and methanol extract of codium decorticatum show the presence of secondary metabolites like saponins, photosterol, alkaloids and glycosides and anti-bacterial activities against gram positive bacteria such as streptococcus pneumonia, staphylococcus aureus and gram negative bacteria such as E.coli, K.Pneumoniae, salmonella typhi, pseudomonas aeruginosa.\[12\]

**Gracilaria edulis**

Gracilaria, an agar yielding red seaweed, is geographically distributed between 50°North latitude and 50°South latitude. It is collected from its natural grounds or from waters where it is cultivated and then processed into food, bacteriological or industrial agar. The biggest producer of Gracilaria is Chile, followed by the Philippines and South Africa. Japan is the largest producer of agar as well as its largest consumer; in 1984, its production was 2,440 tonnes.\[13\]

Because of the increasing demand for agarophytes, cultivation of Gracilaria has become more important during the last few years. It is being successfully cultivated in ponds in China, Taiwan and, more recently, Indonesia.\[14\] But Gracilaria culture in tropical, open water systems is still experimental. However, positive results have been reported from the West Indies.\[15\]

**MATERIALS AND METHODS**

NCBI-PMC, NCBI – Pubmed, NCBI – OMIM, Protparm, CPH 3.0 model server, Discovery studio software.

**Literature collection**

The molecular details (literature) of Gracilaria edulis protein (ribulose-1,5-bisphosphate carboxylase) were collected using online digital libraries and literature databases like OMIM, NCBI – PUBMED and PUBMED Central database.

**Sequence retrieval system**

The protein sequence of ribulose-1, 5-bisphosphate carboxylase protein sequence was retrieved from the NCBI FASTA format-protein, in order to perform the protein sequence analysis and modeling.
Primary sequence analysis
The primary sequence analysis of ribulose-1, 5-bisphosphate carboxylase protein was determined using EXPASY – Compute P/Mw tool in order to identify the physiochemical properties of the input protein sequence.

Secondary sequence analysis
The Secondary sequence analysis of ribulose-1, 5-bisphosphate carboxylase protein was determined using EXPASY – Gor tool in order to identify the functional properties of the input protein sequence.

3Dimensional structure prediction
The ribulose-1, 5-bisphosphate carboxylase – peptide sequences were converted into 3D structure using protein homology modeling software -CPH 3.0 model servers.

Molecular visualization tool
The predicted 3D structure was viewed with help of molecular visualization tools like Discovery studio.

Protein structure validation
The predicted modeled peptide structures were validated using Assessment of Ramachandran plot server – RAPPER.

> AAL46501.1 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit, partial (chloroplast) [Gracilaria edulis]
RYESGVIPYAKMGMGWDNYAKDKTDILALFRVSPQPGVDPVEASAAVAGESSTATWTVVVTDLLLTACDLYRACKAYKDAVPNTTDQYFAFIAYDIDLFEEGSIANLTASIIGNVFGFKAVKALARLEDMRIPVAYLKTFFGQPATGVLVERERMDKGRPFLGATVKPKJGLSGLKYGRVYEGLKGGLDFLKDDENINSQPMRVRKFLYSMEGVNRSAATGEVKHYMNVTAAATMEDMYERAEFKQLGTIVIMDLVIGYTAIQTGMEVWARKNDMILHLHRAGNSTYSRQKHIHMNFRVICKWMRMAVHDHHTAGTVVESKLEPGMLMIQFYNTLTTLHLIDLPSQIFSEQUASLRKTPVASCCHGQMRLQLLDLLNVDVQLFQGGTIGHPDGIQAGATANRVALEAMVLARNERDYaEGPQILRDAAKTCGPLQTAADWKIDTNYSTTDATDFVEETPTANV

Figure: 1 Protein FASTA sequence.
Figure 1.1: The primary structure analysis Compute PI/Mw tool.

Figure 1.2: The Secondary structure analyses.
Figure 1.3: PDB structure database.

Figure 2: Protein Modeling server – 3 Dimensional structure of (ribulose-1, 5-bisphosphate carboxylase) protein structure prediction.
Discovery studio software – cartoon model view and amino acids residues color
ASP, GLU bright red CYS, MET yellow LYS, ARG blue SER, THR orange PHE, TYR mid blue ASP, GLU cyan GLY light grey LEU, VAL, ILE green ALA dark grey TRP pink HIS pale blue PRO flesh.

![Figure 3: Validation – Rapper server (ribulose-1, 5-bisphosphate carboxylase).](image)

**Evaluation of residues**
Residue [A 52: SER] (-130.67, -72.64) in Allowed region
Residue [A 53: THR] (-143.13, -16.69) in Allowed region
Residue [A197: ASN] (-128.96, -83.77) in Allowed region
Residue [A 202: MET] (-170.72, 116.57) in Allowed region
Residue [A 284: ARG] (-80.74, 52.30) in Allowed region
Residue [A 392: GLY] (-82.87, -71.53) in Allowed region
Residue [A 456: PHE] (-129.89, 61.51) in Allowed region
Residue [A 466: PHE] (-134.26, 59.87) in Allowed region
Residue [A 326: GLY] (-174.20, 91.96) in Outlier region
Number of residues in favoured region (~98.0% expected): 456 (98.1%)

CONCLUSION
Comparative ("homology") modeling approximates the 3D structure of a target protein for which only the sequence is available, provided an empirical 3D "template" structure is available with >30% sequence identity. Homology modeling can produce high-quality structural models when the target and template are closely related, which has inspired the formation of a structural genomics consortium dedicated to the production of representative experimental structures for all classes of protein folds. From the literature studies it shows that the ribulose-1, 5-bisphosphate carboxylase protein is one of the responsible anticancer proteins present in Gracilaria edulis seeweeds. Hence, it has been taken for various studies like protein primary structure analysis, Secondary structure analysis and protein three dimensional structure predictions. A comprehensive study of protein may be further used in research for discovering a drug to prevent the molecular cause of disease.

REFERENCES


