Screening of Seaweeds and Seaweed-associated bacteria from the Goan coast for Omega-3-fatty acid

A dissertation for

Course code and course title: MIC-651 Discipline-specific dissertation

Credit:16

Submitted in partial fulfillment of

Master Degree

MSc. In Microbiology

by

SARVASVI SANTOSH DEVIDAS

22P0420018

ABC ID 651854083492

PRN: 201905729

Under the supervision of

DR. LATA GAWADE

Microbiology,

School Of Biological Sciences and Biotechnology

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Date: April 2024

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DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "Screening of Seaweeds and Seaweed Associated Bacteria from the Goan Coast for Omega-3-Fatty Acid" is based on the results of investigations carried out by me at the School of Biological Sciences and Biotechnology (Microbiology programme), Goa University under the supervision Dr. Lata Gawade and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations / experimental or other findings given in the dissertation.

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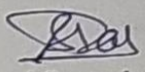

Sarvasvi Santosh Devidas

22P0420018

Microbiology

School Of Biological Sciences and Biotechnology

Date: 08/04/2024

Place: Goa University

COMPLETION CERTIFICATE

This is to certify that the dissertation report **"Screening of Seaweeds and Seaweed-associated Bacteria from the Goan Coast for Omega-3-Fatty Acid"** is a bonafide work carried out by **Ms. Sarvasvi Santosh Devidas** under my supervision in partial fulfillment of the requirements for the award of the degree of (MSc. Microbiology) in the Microbiology discipline at the School of Biological Sciences and Biotechnology /Dept of Microbiology, Goa University.

Gawade.

Dr. Lata Gawade
Microbiology,
Goa University

Date: 8/4/24

8/4/24

Dean of School of Biological Sciences
& Biotechnology
Goa University, Goa-403206

School/ dept stamp

Date: 8/4/24

Place: Goa University

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PREFACE

This report has been prepared as a part of my Dissertation, as part of my Master's degree. I embark on a journey to introduce and demonstrate a novel idea for harnessing the potential of marine ecosystems, particularly focusing on the rich diversity of algae. As a resident of Goa with coastal state blessed with abundant marine life, I have witnessed how the local population underestimates seaweed as a food source. However, my research efforts aim to shed light on the untapped nutritional wealth of algae, which are rich in nutrients, micronutrients, polysaccharides and lipids. Given the threat of climate change, the concentration of lipids in algae may vary, presenting both challenges and opportunities.

However, I firmly believe that these algae have enormous potential for income through research into their diverse uses. In this report, I focus on the detection of algae and algae-associated bacterial lipids and amino acids. This research serves as a springboard for obtaining valuable nutrients such as omega-3 fatty acids from algae and associated bacteria.

With this dissertation, I hope to contribute to a broader understanding of marine ecosystems and to pave the way for innovative approaches to sustainable resource use.

In this project, I have taken different types of seaweed and isolated associated bacteria and further screened for omega-3 fatty acids and amino acids. In the results, the concentrations of omega-3 fatty acids found in each seaweed species and its associated bacteria are presented.

ACKNOWLEDGMENT

I want to thank my guide from the bottom of my heart, Dr. Lata Gawade, for her guidance throughout my project.

I am grateful to Dr. Lakshangy Charya, Programme Director, Microbiology, School of Biological Science and Biotechnology for extending necessary facilities.

I would like to extend gratitude to all my teachers, Dr. Sandeep Garg, Dr Milind Naik, Dr. Judith Noronha, Dr. Trupti Asolkar, and Dr. Bhakti Salgaonkar.

Special thanks to the non-teaching staff Mr. Surendra Velip, Mrs. Robertina Fernandes, Mr Lakshman sir, Mr. Bhagwant Karpe, and Mr Domingos Dias for their constant help while carrying out this work.

My sincere thanks to Manaswi, Nikita, Bhavana, Kareena, Rachel, Apurva, Saloni, Aarzo, Tanaya, and all my friends for their constant support and help.

I would like to thank my parents and Guardian. No words would be adequate to express my gratitude for their immeasurable understanding and encouragement. Lastly, I would like to thank the almighty who kept blessing me and took me on the path of success.

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ABBREVIATIONS USED

Entity	Abbreviation
ALA	α-linolenic acid
ARA	Arachidonic acid
CGAR	Compound Annual Growth Rate
DHA	Docosahexaenoic acid
EPA	Ecosapentaenoic acid
GC-FID	-Gas Chromatography with Flame Ionization Detection
GCMS	Gas chromatography Mass Spectrometry
GLC	Gas-liquid Chromatography
GSC	Gas-solid chromatography
HUFA	Highly Unsaturated Fatty Acid
MUFA	Monounsaturated Fatty Acid
O3FA	Omega-3 Fatty Acid
PUFA	Polyunsaturated Fatty Acid
RF-	Retention factor
SAM	S-adenosyl methionine
SFA	Saturated Fatty acid
TF	Triphenyl formazan
TLC	Thin Layer Chromatography
TTC	2,3,5-Triphenyl tetrazolium chloride
UFA	Unsaturated Fatty Acid

ABSTRACT

Seaweeds and their associated bacteria have become a focus in the search for vegan sources of omega-3 fatty acids. Our goal in this study was to find out if seaweeds along the Goan coast contain omega-3 fatty acids and if bacteria are linked to them. The goals were to collect seaweeds from the intertidal regions of Goa, isolate bacteria associated with seaweeds, screen for omega-3 fatty acids, and compare the biochemical composition of the bacteria and seaweeds, including lipids and amino acids. According to our hypothesis, the biochemical makeup of seaweeds varies according to environmental factors, which may cause variations in the associated bacteria and, possibly, in the qualitative and quantitative production of omega-3 fatty acids. The market's lack of vegan omega-3 fatty acids emphasizes the significance of looking into alternate sources, such as bacteria linked to seaweed and algae. Analytical methods like TTC screening, TLC and Iodine Value test and instrumental methods like Gas chromatography were employed to screen for omega-3 fatty acids and ascertain the concentration and existence of these vital nutrients. Lipid and amino acid profiles of seaweeds and related bacteria were compared using biochemical analyses. The results of this investigation advance our knowledge towards the innovation and marine life.

1. INTRODUCTION

1.1. BACKGROUND

1.1.1 Omega-3 Fatty Acid

Omega-3 fatty acids (OM3FAs) are unsaturated fatty acids that have at least one double bond between the third and fourth omega-end carbon. The three most important omega-3 polyunsaturated fatty acids are α-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) (Krupa et al., 2023). These fatty acids can be obtained from plant sources, fish, fish products, seeds, nuts, green leafy vegetables, and beans (Shahidi & Ambigaipalan, 2018). Vegan omega-3 fatty acids are derived from plant sources like walnuts, flaxseeds, chia seeds, hemp seeds, edamame, seaweed, and algae, as well as other green leafy vegetables and beans as shown in Table 1.1 and Table 1.2. While ALA is found in plants, DHA and EPA are found in algae and fish, with marine algae and phytoplankton being primary sources of omega-3 fatty acids. Fish that consume these algae accumulate DHA and EPA (Cholewski et al., 2018).

Table 1.1 ALA Content in the fish oil

Common name	Omega (g)
Herring, sardines	1.3-2
Mackerel	1.1-1.7
Salmon	1.1.-1.9
halibut	0.60-1.12

Tuna	0.21-1.1
Pollock	0.17-0.24
Cod	0.45

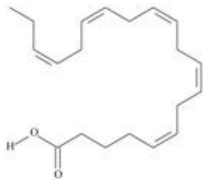
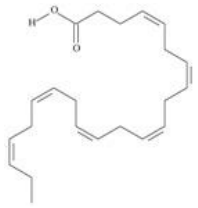
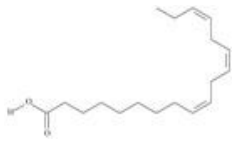
Table1.2: ALA content in Vegan sources

Common name	Omega %
Kiwifruit	63
Shiso	61
Chia	58
Linseed or flexseed	53-59
Fig	47.7

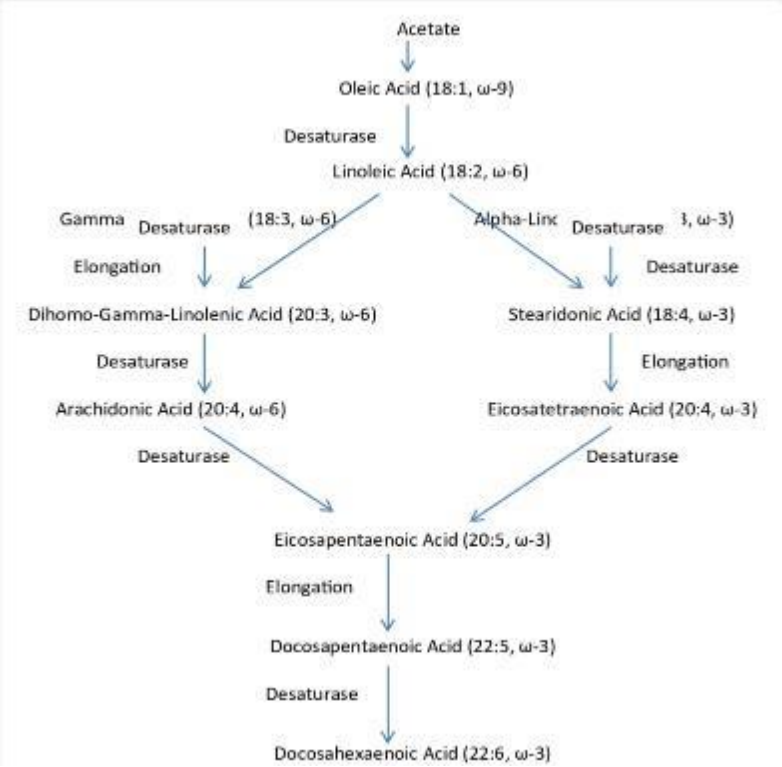
(Rocha et.al 2021) (Saini et al., 2023)

1.1.2 Structure and Biosynthesis Pathway of Omega-3 Fatty Acids

Table no 1.3: Structure of Omega-3 fatty acid

Ecosapentaenoic acid (EPA)/timnodonic acid	C20:5*n*-3	$C_{20}H_{30}O_2$	
Docosahexaenoic acid (DHA) /cervonic acid	C22:6*n*-	$C_{22}H_{32}O_2$	
α-linolenic acid	C18:3n-3	C18H30O2	

(Cholewski et al., 2018)



(Khan.et.al 2015)

Fig 1.1: Biosynthesis pathway of Omega-3 fatty acid

The biosynthesis of omega-3 fatty acids begins with the de novo synthesis of short-chain fatty acids, typically oleic acid, from acetate. Most organisms are capable of de novo fatty acid synthesis. The oleic acid undergoes a series of desaturation and elongation reactions to form longer chain fatty acids. (Jump et al., 2012)

1.1.3 Health Benefits of Omega-3 Fatty Acids:

Omega 3 fatty acids are beneficial for various aspects of our health, including heart, brain, eye, joint, skin, and pregnancy. They can help lower triglyceride levels, decrease blood pressure, reduce blood clotting, and improve overall heart health. Furthermore, they are associated with a lower risk of heart disease and stroke. DHA, a major component of brain tissue, plays a crucial role in cognitive function and development. Omega 3 fatty acids have been linked to improved mood, reduced symptoms of depression and anxiety, and a lower risk of age-related cognitive decline. DHA is also essential for the proper functioning of the retina of the eye. Age-related muscular degeneration (AMD) has been linked to a decreased risk of adequate consumption of Omega 3 fatty acids. Because of their anti-inflammatory qualities, omega-3 fatty acids can aid in lowering inflammation all over the body. This may help control long-term inflammatory diseases like inflammatory bowel disease and rheumatoid arthritis.

Omega 3 fatty acids can reduce joint pain and stiffness associated with conditions like rheumatoid arthritis and osteoarthritis, improving overall joint function and mobility. EPA and DHA play an important role in maintaining healthy skin cell membranes, promoting hydration, and reducing inflammation. This can benefit skin conditions like eczema and psoriasis. During pregnancy, Omega 3 fatty acids are vital for fetal brain and eye development. Adequate intake may reduce the risk of preterm birth and support healthy infant growth and development.t. (Rocha et al., 2021)

1.1.4 Demands in market for Vegan source of Omega-3 Fatty Acid

Global Non-Fish Omega-3 Supplements Market Outlook (2023 to 2033) A newly released Non-Fish Omega-3 Supplements Market Analysis Report by Fact. MR announces that global sales of non-fish omega-3 supplements were $212.9 million in 2022. With a projected growth of 9.4% from 2023-2033, the market is expected to reach a valuation of $569.2 million by the end of the forecast period. Non-fish omega-3 capsules are expected to generate significant sales with a projected CAGR of over 9% from 2023 to 2033. Report Features and Details: Size of the global omega-3 supplements market excluding fish (2022) $212.9 million. Global non-fish omega-3 supplements market size (2033) $569.2 million. CAGR of Global Non-Fish Omega-3 Supplements Market (2023). until 2033) 9.4%U.S. Non-Fish Omega-3 Supplements Market CAGR (2023 to 2033) 9.2% (Physicians Committee for Responsible Medicine, n.d.)

1.1.5 Seaweeds

Seaweeds, which are known as macroalgae, have been recognized for their nutritional and medicinal value for centuries. They are rich in diverse bioactive compounds such as polysaccharides, proteins, vitamins, minerals, and polyunsaturated fatty acids (PUFAs), including omega-3 fatty acids (OFAAs).

Seaweed is any marine algae that develops along shorelines and can be red, green, or brown. Usually, seaweeds use "holdfasts," which are roots-like structures that tie them to the ocean floor or other solid surfaces. The roots of seaweeds are only places of attachment; they do not absorb

nutrients like those of higher plants do. Many types of seaweed are edible and valuable to humans commercially. Some are used as fertilizers and as sources of polysaccharides.

Seaweeds often form dense growths on rocky shorelines or gather in shallow water. Near the seaside, where the water is no deeper than 50 meters (165 feet), many have a clearly defined zonation. Fucus and kelps are examples of brown algae (class Phaeophyceae) that are frequently found as seaweeds. They are not found in tropical waters but are extensively spread in colder climates. The kelps are among of the largest algae; some Macrocystis and Nereocystis species found in the Pacific and Antarctic are more than 33 meters (100 ft). Another type of kelp that is common along the Pacific and Atlantic coasts is laminaria. In the Gulf Stream and Sargasso Sea, free-floating clumps of Gulfweed, or Sargassum, are widespread.

Seaweeds in the red alga (Rhodophyta) division include laver (Porphyra), dulse (Palmaria palmata), Gelidium, and Chondrus. Along the rocky Atlantic coast, the lowest half of the zone exposed at low tide is covered in a variety of species of Chondrus, including Irish moss (C. crispus). One of the comparatively rare species of green algae (division Chlorophyta) seaweeds is Ulva species, also referred to as sea lettuce.

As there is a growing need to evaluate new food sources that do not put undue pressure on terrestrial ecosystems, seaweeds present a promising alternative for the food market as a source of fatty acids (FA). Despite containing a low total lipid concentration, seaweeds have a significant amount of essential unsaturated fatty acids (UFA) that are crucial for human welfare. Seaweeds synthesize ω-3 and ω-6, polyunsaturated fatty acids (PUFA), and highly unsaturated fatty acids (HUFA), as well as monounsaturated fatty acids (MUFA), which have already been demonstrated to play an important role in human metabolism (essential fatty acids—EFA). These fatty acids are

involved in cell growth and metabolic pathways, unlike Saturated Fatty acids, which mainly serve as energy sources. (Dhargalkar & Kavlekar, 2004)

The lipidic profile of seaweeds differs between species FA concentration and profile differ among the variation of biotic and abiotic parameters, as well as with genetic characteristics from each algae. Hence, the life cycle of each seaweed can also influence the FA content and characterization of algae. As compared to terrestrial plants, seaweeds present a wider variety of metabolites with important biological properties, as well as higher abundances of highly unsaturated fatty acids, namely the ω-3 EPA & DHA and the ω-6 ARA, being particularly important for the introduction of such macronutrients in food webs. Hence, seaweeds present a high potential not only as a direct food product but also for technological applications that can use their biological compounds to produce functional foods.

Lipids are of varied groups with structural, functional, storage, signaling, and transcription factor activities and characteristics, needed for numerous metabolic processes. Lipid accumulation and EPA concentration of respective seaweed are given in Table 1.4

Table 1.4 Lipids accumulation of some Seaweeds.

Seaweed	Species	Lipids g/100 g	EPA (%)
Porphyra/Pyropia spp. (China)	Red algae	1.0 ± 0.2	10.4 ± 7.46
Ascophyllum nodosum	Brown algae	3.62 ± 0.17	7.24 ± 0.08
Bifurcaria bifurcata	Brown algae	6.54 ± 0.27	4.09 ± 0.08
Durvillaea antarctica	Brown algae	0.8 ± 0.1	4.95 ± 0.11

Fucus vesiculosus	Brown algae	3.75 ± 0.20	9.94 ± 0.14
Himanthalia elongata	Brown algae	<1.5	7.45
Caulerpa lentillifera	Green algae	1.11 ± 0.05	0.86
Codium fragile	Green algae	1.5 ± 0.0	2.10 ± 0.00
Ulva lactuca	Green algae	1.27 ± 0.11	0.87 ± 0.16

1.1.6 Amino Acid Content

Amino acids that may function as antioxidants in biology include taurine, valine, leucine, and isoleucine found in a variety of seaweeds. The majority of seaweed species contain large amounts of acidic amino acids like aspartic acid or glutamic acid, which make up the majority of essential amino acids (El-Beltagi et al., 2022). Although threonine, tryptophan, sulfur amino acids (methionine and cysteine), lysine, or histidine-limiting amino acids were thought to make up algal proteins, their overall amounts are higher than in terrestrial plants (El-Beltagi et al. 2022c). Additionally, the synthesis of nitrogenous low molecular weight compounds and hormones—both significant biological processes—requires amino acids. Because amino acids play specific physiological roles, they can be used to treat certain disorders. Methionine supplements, for instance, can benefit individuals with multiple sclerosis. Although seaweed proteins are low in certain essential amino acids, these seaweeds can be added to cereal foods like pasta to improve the amino acid composition (Dawczynski et al. 2007,).

Macroalgal organisms like *Chlorella sp. Dunaliella tertiolecta, Aphanizomenon flosaquae,* and *Spirulina plantensis* are frequently utilized as human food sources because of their high protein

content or nutritive quality. Several algae species contain abundant amounts of endogenous (threonine, serine, aspartic acid, proline, glutamic acid, or glycine) and exogenous (histidine, lysine, isoleucine, methionine, phenylalanine, leucine, valine, or threonine) amino acids. has a high concentration of glutamic acid, serin or alanine, and Sargassum vulgare has a high concentration of methionine. *Ulva australis* has taurine or histidine, *Himanthalia elongata* (sea spaghetti), and *Palmaria palmata* (dulse). 2022).

1.1.7 Seaweed Associated Bacteria

Microalgae, commonly referred to as seaweeds, are an integral part of the marine ecosystem, providing a habitat for diverse microbial communities. Bacteria, in particular, play a critical role in the health, growth, and ecology of seaweeds by forming a dynamic and multifaceted association. This relationship contributes to nutrient cycling, defense mechanisms, and the overall well-being of seaweed populations.

Different types of bacteria are associated with seaweed, each contributing uniquely to their ecological functions. Notable bacterial groups include Bacteroidetes, Firmicutes, Proteobacteria, Actinobacteria, and Cyanobacteria. These bacteria interact with seaweed through complex mechanisms, including biofilm formation, nutrient exchange, and chemical signaling.

One significant aspect of these bacterial associations is their lipid metabolism, which influences the biochemical composition of seaweed and the surrounding marine environment. Several associated bacteria produce lipids that encompass a diverse range of compounds, such as fatty acids, glycolipids, and Polyunsaturated fatty acids like Omega 3 and Omega 6 fatty acids.

Bacteria that thrive on seaweed contain amino acids crucial for their metabolic processes and cellular growth. Nitrogen metabolism relies heavily on glutamate, which acts as a precursor for other amino acids. Seaweed-associated bacteria, particularly those involved in nitrogen fixation and recycling, may play a role in producing glutamate.

Alanine, an amino acid involved in energy metabolism, can be synthesized by bacteria using different biosynthetic pathways. Seaweed-associated bacteria may produce alanine as a metabolic byproduct or as part of their cellular process.

Aspartate is essential in the urea cycle and protein synthesis. Bacteria associated with cwierz may contribute to aspartate production through amino acid synthesis and catabolic pathways.

Serine, a non-essential amino acid, serves as a precursor for other amino acids like glycine and cysteine. It also plays a significant role in the biosynthesis of lipids and nucleotides.

Threonine, an essential amino acid, is crucial for protein synthesis and acts as a precursor for other amino acids such as isoleucine and methionine.

Cysteine, a semi-essential amino acid, is involved in protein structure, redox signaling, and the synthesis of antioxidants such as glutathione.

Methionine, an essential amino acid, serves as a precursor for the synthesis of proteins and other vital molecules, including S-adenosyl methionine (SAM), which is a key methyl donor in numerous cellular processes.

1.1.8 2,3 5 Triphenyl Tetrazolium Chloride Test

Under reducing conditions, the colorless compound 2,3,5-Triphenyl tetrazolium chloride (TTC) turns red. A strong correlation was found between the presence of EPA in Gram-negative bacteria and the reduction of TTC to the red triphenyl formazan (TF) when TTC was added to both liquid and solid-state fermentation treatments. For color response and cell growth in liquid cultures and agar plates, incubation in 0.1 percent w/v TTC was ideal. TTC was converted to TF by bacteria that make EPA. (Estupiñán & co. 2020); Razak et al. (2013).

1.1.9 Bligh and Dyer method for extraction of Lipid

In 1959, the method of extraction by E.G. Bligh and W.J. Dyer was used to come up with an efficient way of studying lipid deterioration in frozen fish. In their procedure, they relied on Folch's methodology (Folch-Pi et al., 1957) while reducing the quantity of chloroform and methanol as major reagents in the first stage of extraction. A biological sample is homogenized with a monophasic solution containing chloroform/methanol/water. Next, phase separation is initiated by adding chloroform and water. Finally, lipids are extracted from the chloroform phase. (Bligh and Dyer, 1959)

1.1.10 Thin Layer Chromatography

A widely utilized chromatographic technique known as thin-layer chromatography (TLC) is employed for the separation and analysis of a variety of compounds, including lipids and amino acids. The process involves coating a flat support, such as a glass plate or plastic sheet, with a thin

layer of adsorbent material, typically silica gel or alumina. The sample mixture is then applied to this stationary phase, and the plate is subsequently developed by allowing a mobile phase (solvent) to move through the stationary phase via capillary action. As the mobile phase progresses, it carries the sample components along, resulting in separation based on variations in their partition coefficients between the mobile and stationary phases.

The migration of compounds on the TLC plate is characterized using a crucial parameter known as the retention factor (Rf value). To calculate the Rf value, the distance covered by the compound is divided by the distance covered by the solvent front (which is the total distance traveled by the solvent) (Kowalska & Sherma, 2007).

1.1.11 Iodine Value Test to measure Unsaturation of Fatty Acids

The total amount of double bonds found in fats and oils is expressed as the iodine value. (Gharby and others, 2017). It is stated as the quantity of iodine grams that, in 100 grams of fats or oils, will react with the double bonds. Saturated and unsaturated fatty acids are both present in fat and oil. Halogens new chemicals are formed when put over the unsaturated fatty acid double bonds.

When oil and iodine monochloride or monobromide react in the dark, the iodine joins the chain of fatty acids that contain double bonds. The amount of double bonds present determines how much iodine is consumed. By adding extra potassium iodide, the amount of wasted iodine can be ascertained.

Next, using starch as an indicator, sodium thiosulphate is titrated with the sample. The degree of unsaturation and the oil's vulnerability to oxidative rancidity are indicated by the iodine value.

1.2 HYPOTHESIS

The biochemical content of different seaweed varies differently based on the environmental parameters hence associated bacteria also may vary therefore producing different Omega-3-Fatty Acid both qualitatively and quantitatively.

Different types of seaweeds and its associated bacteria may be an efficient source of omega-3 fatty acids.

1.2 AIM AND OBJECTIVE

Aim:

To screen Seaweeds and Seaweed-associated bacteria from the Goan coast For Omega-3-Fatty Acids.

Objective:

- Collection of seaweeds from the Goan coast and isolation of seaweed-associated bacteria.
- Screening of seaweed samples and its associated bacteria for omega-3 fatty acids.
- To estimate and compare the biochemical content of seaweed and seaweed-associated bacteria, eg. Lipids, and amino acids.

2. LITERATURE REVIEW

- There is a great demand for vegan omega-3 fatty acids, especially in international markets, but PUFA (omega-3 fatty acid) is mostly found in fish, which is becoming less abundant. This area's first examination of the diversity of culturable microorganisms living in the non-vent sediments of the Mid-Atlantic Ridge (MAR) revealed a high diversity of Gram-positive strains and good squalene production by an uncommon strain of *Bacillus* sp. In strain *Shewanella sp*., MAR089 yielded the highest amount of EPA ever recovered. 441 MAR. associated Vibrio sp. with North Sea sponges. In contrast to other bacteria associated with tropical Caribbean marine sponges, strain NSP560 produced significant amounts of EPA and no PUFAs were detected. A *photobacterium* sp. The first description of strain MA665, which was isolated from the North Sea coast, was published. It was found to be easily cultured in atmospheric conditions and to contain significant amounts of EPA, up to 25% of total fatty acids (TFA) (or 106 mg g-1 in dried cells). According to Zhang (2011b).
- "Repurposing our findings on algae as a potential source for foods, nutraceuticals and pharmaceuticals." This study elaborate upon the potential of algae in the development of nutraceuticals and pharmaceuticals, with a focus on their therapeutic properties such as antioxidant, anti-inflammatory and anti-cancer properties. In addition, challenges and opportunities in the further development of pharmaceuticals and nutraceuticals and their integration into various industries are discussed. Future research should focus on efficient extraction and purification techniques, toxicity analysis, clinical efficacy, mode of action and interactions with regular diet. (Baghel et al., 2023)
- "Nutritional and Digestive Health Benefits of Seaweed in Advances in Food and Nutrition Research" Seaweed, a popular Asian delicacy and source of essential dietary hydrocolloids,

provides numerous health benefits due to its unique marine environment. It is rich in nutrients such as fiber, ω-3 fatty acids, essential amino acids, and vitamins A, B, C, and E. This chapter discusses the nutritional value and functional effects of seaweed, particularly in promoting digestive health. (Rajapakse & Kim, 2011)

- Most algae such as brown algae have been examined for omega-3 fatty acids and omega-6 fatty acids. (0.79% to 7.78% Omega 3 and Omega 6 content) (Peñalver et al., 2020) Microbial production of omega-3 polyunsaturated fatty acids: Current status and future prospects: In this review, the nutritional aspects are commercially used sources of polyunsaturated omega-3 fatty acids (PUFAs) from plants, microalgae, macroalgae, and thraustochytrids are discussed. It highlights the increasing interest in natural dietary sources of n-3 PUFAs due to the increase in chronic diseases. Among plant sources, seed oils from chia (*Salvia hispanica*), flax (*Linum usitatissimum*) and garden cress (*Lepidium sativum*) are now generally considered to be responsible for increasing α-linolenic acid (ALA) in the diet. The study also addresses issues related to the oxidative stability and bioavailability of n-3 PUFAs and future prospects in these areas. (Saini et al., 2021b)
- "A review of potential oil-containing microorganisms and their role in the biodiesel and omega-3 fatty acid-based industries" Oil-containing microorganisms accumulate over 20% of lipids based on cell dry weight and synthesize fatty acids from short to long chains. These oils are used in the production of biodiesel or nutraceuticals. Microalgae, bacteria, seaweeds, and yeasts are involved in biodiesel production, while thraustochytrids, fungi, and some microalgae produce omega-3 fatty acids. This review article presents their expertise in the production of biodiesel and omega-3 fatty acids. (Patel et al., 2020b)

- "Impacts of climate change on marine ecosystems" This review examines the complex interactions between algae and associated bacteria, focusing on their role in marine biogeochemical cycling. The diversity of bacteria associated with different algae species, their metabolic capabilities, and their effects on nutrient cycling and ecosystem function are discussed. . (Doney et al., 2012)
- “The Role of Bacteria in Seaweed Growth: A Review” This review provides insights into the mutual relationships between algae and bacteria, with an emphasis on the role of bacteria in promoting the growth and development of seaweeds. Various mechanisms by which bacteria influence the physiology and productivity of algae are discussed. (Mata et al., 2010) Algae, a group of photosynthetic organisms, are critically important in aquatic ecosystems as they contribute to global primary production and provide food and shelter. They have a diverse range of microorganisms, including epiphytic bacteria, which play an important role in the health and defense of the host. This relationship suggests a holobiontic interaction between macroalgae and epiphytic bacteria, similar to that observed in corals. Understanding the role of bacteria in algal communities could help develop effective marine management strategies. (Egan et al., 2013)
- "Antimicrobial effects of compounds from seaweed". Metabolites produced by algae help defend against various environmental conditions. These substances have antibacterial, antiviral, antiprotozoal, and antifungal properties. Macroalgae are easy to cultivate and could be a valuable source of chemicals for novel drugs that could combat emerging diseases or pathogenic microbes with multidrug resistance. Chemicals with strong antibacterial effects obtained from green, brown, and red algae include polysaccharides, fatty acids, phlorotannins, pigments, lectins, alkaloids, terpenoids, and halogenated

compounds. Here, the major antimicrobial chemicals identified in macroalgae are discussed along with some of their most promising applications. (Pérez et al., 2016)

- A review of the phytochemical and potential properties of seaweeds and their recent applications is presented. Seaweeds are used as a source of highly bioactive secondary metabolites that have the potential to be important medicinal ingredients. Also, a great deal of progress has been made in the study of the biological activity of specific seaweed compounds, with a focus on the composition and uses of these compounds for the nutrition of humans and animals. Seaweeds are used as fertilizers, energy, medicines, cosmetics, and in the biosynthesis of alginate and agar in industrial settings. They are also consumed as animal feed. Minerals, vitamins, phenols, polysaccharides, sterols, and a number of other bioactive substances are primarily responsible for the health benefits of seaweed. The compounds in question exhibit properties that may be beneficial against cancer, inflammation, diabetes, bacteria, and antioxidants. Information regarding the function of seaweeds in industrial, biochemical, pharmaceutical, and nutritional applications as well as their effects on human health are the goals of this review. El-Beltagi and associates. (2022).
- "The Seaweed Aquaculture Evolution Road : Cultivation Technologies And The Industry 4.O". Seaweeds, also known as marine macroalgae, are autotrophic organisms that generate a wide range of intriguing compounds. Seaweeds originated in Asian nations and later spread to Europe, South America, North America, and Australia, where they are now considered an important source of nutrition. Edible seaweeds have been discovered to have high dietary fiber, lipid, and protein content. They also contain

a multitude of bioactive compounds with potential uses in medicine, cosmetics, and nutraceuticals. Although harvesting and cultivating seaweeds has been documented since antiquity, aquaculture has grown as knowledge of seaweeds and their valuable compounds has advanced. The cultivation techniques differ between offshore and onshore. In integrated multi-trophic aquaculture (IMTA), seaweeds can also be employed. (Poza-García et al. 2020).

- Large-celled marine heterokonts known as *thaustochytrids* are categorized as oleaginous microorganisms because they produce ω-3-fatty acids, which include docosahexaenoic (DHA) and eicosapentaenoic (EPA). The use of microbial DHA and EPA for human health is growing quickly, and numerous clinical studies have been conducted to confirm their effectiveness. The culture of thraustochytrids depends on the advancement of sophisticated isolation and identification methods. *Thraustochytrids* are also amenable to different production strategies that increase omega-3 oil output because of their high proportion of lipid biomass. The extraction yields of DHA and EPA have been increased by utilizing advanced analytical instruments and making modifications to the current lipid extraction methods. These marine protists can also yield other metabolites, including extracellular polysaccharides, carotenoids, and enzymes. The biotechnological potential of *thraustochytrids* will be further enhanced by methods like metabolic engineering, which includes gene cloning, searching for more diverse isolates with rapid growth rates, and cultivating the organisms on alternative, less expensive carbon sources. (Gupta et al., 2012)
- Fisheries scientists frequently utilize gas chromatography with flame ionization detection (GC-FID) to measure fish fatty acid content. This investigation confirmed the widely used

technique for measuring omega-3 fatty acids (DHA and EPA) in warm-water fish, selayang, both raw and cooked, using GC-FID in preparation for a later Weibull model evaluation of EPA and DHA retention. A high-polarity capillary GC HP-88 column (60 m length, 0.25 mm ID, 0.2 μm DF) was used to separate the EPA and DHA, and the entire run time was 45.87 minutes. According to ICH criteria, the method's linearity, precision, accuracy, specificity, and sensitivity were validated. Furthermore, the technique demonstrated a high recovery rate (>95%) and good precision (RSD ≤ 2%) with overall RSDs for both falling below 0.001%. (Alinafiah et al., 2021)

3. METHODOLOGY

3.1 SAMPLE COLLECTION

The several varieties of seaweeds were sampled from the intertidal zones of Cacra and Anjuna beaches in Goa during low tide. A zip-lock bag was used to collect the samples and brought to laboratory within two hours of collection. They were cleaned with autoclaved sea water to eliminate any remaining sand and debris.

3.2 SEAWEED IDENTIFICATION:

We used a seaweed manual book to identify the gathered seaweeds. (Dhargalkar & Kavlekar, 2004)

3.3ISOLATION OF SEAWEED-ASSOCIATED BACTERIA:

Epiphytes were extracted by immersing 1 gram of seaweed in 9 ml of sterile seawater, vertexing for 2 minutes, and serially diluting to a 10–4 dilution. From the last two dilutions, 0.1 ml aliquots were spread out on Zobell Marine Agar in duplicate. The inoculated Zobell Marine Agar plates were incubated at room temperature for 48 hours. Individually, different colonies were selected and then streaked on Zobell Marine Agar to form single colonies. Isolates were further purified before being routinely subcultured on Zobell Marine Agar. (Karthick & Mohanraju, 2018)

3.4 GRAM STAINING:

A bacterial smear from a 24-hour-old culture was prepared on a clean, grease-free slide. The slide was air-dried and heat-fixed. It was inundated with Gram's crystal violet for one

minute. Drain and flood the slide with Gram's iodine solution for 1 minute. The slide was drained, and decolorization was achieved by rinsing with 95% ethanol for 30 seconds. The slide was cleaned with water, air-dried, and examined under the oil immersion lens of the microscope. (Paray et al., 2023)

3.5 OXIDASE TEST:

A loopful of bacterial isolate that had been isolated for 24 hours was rubbed onto a Whatman filter strip after a few drops of oxidase reagent had been applied to it in the dark. Purple coloration in the rubbed region indicates positive outcomes. (Tankeshwar & Tankeshwar, 2023)

3.6 CATALASE TEST:

A drop of hydrogen peroxidase reagent was applied to a grease-free slide after a loopful of a culture that had been growing for 24 hours. Positive outcomes are indicated by the production of effervescence. (Khatoon et al., 2022)

3.7 2,3,5-TRIPHENYL TEST TETRAZOLIUM CHLORIDE

A 0.1% TTC (2,3,5-triphenyl tetrazolium chloride test) solution was added to the 1% autoclaved Zobell Marine Broth. The bacterial suspension was then added to the TTC tubes. After being vortexed and incubated for a whole day, a color shift was noticed. Positive outcomes are indicated by the color red. (Estupiñán et al., 2020), (Razak et al., 2013)

3.8 BLIGH AND DYER METHOD FOR EXTRACTION OF SEAWEED-ASSOCIATED BACTERIAL LIPID.

Following an initial 72-hour shaking, bacterial isolates were added to Zobell Marine Broth. We used a UV spectrophotometer to measure the cell density. Centrifuging culture broth for 15 minutes at 10,000 rpm produced a thick cell pellet of 1g was taken following optimal growth. Chloroform: Vertexing was done after adding methanol (2:1) to the cell pellet tubes. In order to break down the bacterial cell wall, the tubes were submerged in an ice bath and subjected to two minutes of 20-Hz sonication. For ten minutes, the cell was centrifuged at 5000 rpm once again. Phase separation was seen by keeping the tubes motionless for three to four hours. The organic phase is separated into a glass vial and stored in an oven at 30 °C for 12 hours once the phases are distinct. (Bligh & Dyer, 1959) (Kumari et al., 2011) (Saini et al., 2021)

3.9 BLIGH AND DYER METHOD FOR EXTRACTION OF SEAWEED LIPID

A sample of seaweed was collected, and its wet weight was recorded. Seaweed was dried for 72 hours at 45C in an oven. A measured amount of dry seaweed was ground into a powder using pastel. A centrifuge tube was filled with 1g of dried seaweed powder, and then 1:2 chloroform: methanol was added. Allow the tubes to sit at room temperature for half an hour.

We used sonication to rupture the cell wall. Tubes were centrifuged at 10,000 rpm for 10 minutes following sonication. Following the removal of supernatant and chloroform, the cell pellet was mixed with methanol (1:2) and centrifuged once more for ten minutes at 10,000 rpm. After combining the two supernatant, 0.9% saline was added. at room temperature for one hour. After 1 hr organic phase was extracted in a glass vial and allowed to evaporate the chloroform. (Ren et al., 2021)

3.10 THIN LAYER CHROMATOGRAPHY FOR SEAWEED-ASSOCIATED BACTERIAL LIPID AND SEAWEED LIPID

TLC sheets were cut into strips(8x3). Solvent system was prepared using Acetone: Butanol: Acetic Acid: Distilled water (14:14:4:8). Using capillary drops of bacterial lipid was placed onto the TLC sheet for 5 times. TLC sheet was placed into the solvent system and observed the run. TLC sheets was removed after the solvent reached to rf point. Rf point was marked. TLC sheet then kept in iodine chamber for 5 mins. bands were marked and rf value was calculated by dividing its distance travelled by the component & distance travelled by solvent front. (“QUANTITATIVE THIN LAYER CHROMATOGRAPHY” Joseph C. Touchstone (School of Medicine, University of Pennsylvania), Ed. John Wiley &Amp; Sons, New York/London/Sydney/Toronto, 1973. 330 Pp,” 1974) . (Gayathri, 2014)

3.11 THIN LAYER CHROMATOGRAPHY FOR AMINO ACIDS DETECTION

The TLC sheets were cut into strips (8x3). The solvent system was composed of hexane, diethyl ether, and acetic acid (40:10:1). Using capillary drops, bacterial lipid was applied

to the TLC sheet five times. The TLC sheet was immersed in the solvent solution and monitored during the run. TLC sheets were removed after the solvent reached the RF point. The reference point was noted. The TLC sheets were sprayed with 1% ninhydrin. Dark purple color bands were marked, and the RF value was determined by by dividing its distance travelled by the component & distance travelled by solvent front.. (Hussein et al., 2021) (Gayathri, 2014)

3.12 IODINE VALUE TEST FOR DETECTION OF OMEGA-3- FATTY ACIDS.

In a 250-ml conical flask, 0.25 ml of lipid was added first, followed by 2.5 ml of carbon tetrachloride and 2.5 ml of Wij solution, which were thoroughly mixed. The flask was sealed with a stopper cap coated with potassium iodide. Incubated in the dark for 30 minutes. To distribute potassium iodide into the flask, the stopper cap was cleaned with 10 ml of distilled water. With vigorous shaking, the material was titrated against 0.1N sodium thiosulfate. Following a minor shift in color from dark brown to light, 1 ml of 1% starch solution was added. Then it is titrated again with 0.1N sodium thiosulfate until the color changes to milky white. The readings were recorded, and the lipid's iodine value was computed using the formula given below. (Firestone, 1994)

$$\text{Iodine Value} = \frac{(V1\text{-}V2) \times N \times 1.269}{\text{Sample weight}}$$

V1= Blank Titre value
V2= Sample Titre Value
N= 0.1N

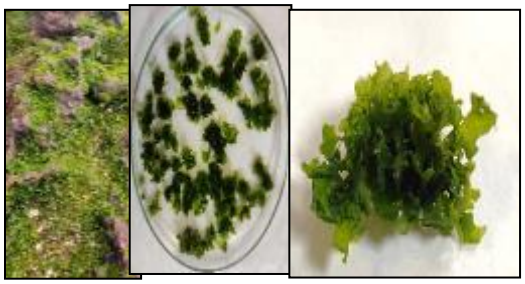
4 . RESULT

AND

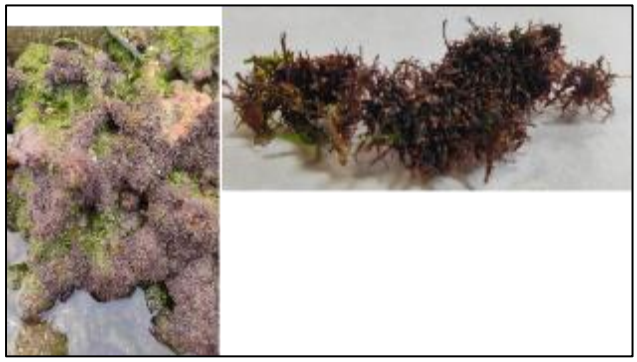
DISCUSSION

4.1. COLLECTION OF SEAWEED

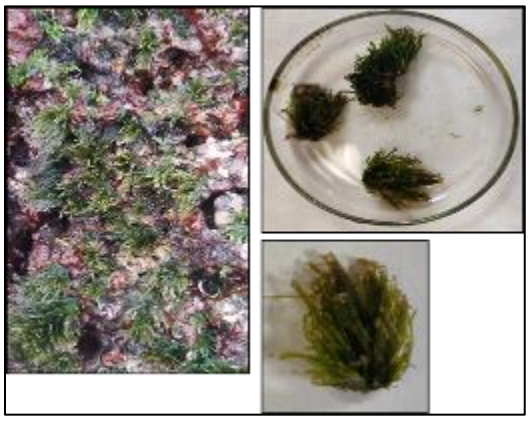
In order to analyze the lipid and amino acid composition of seaweed and seaweed-associated bacteria, samples of seaweed were collected from the intertidal region of Goa. Two sampling sites were selected, Cacra and Anjuna, from where a total of 8 different types of seaweeds were collected. As shown in Figure 4.1 Identification of these seaweeds was done according to the guidelines outlined in Seaweed - A Field Manual..



Ulva lactuca (AN1SD)



Chondracanthus (AN2SD)



*Chaetomorpha gracilis (*AN3SD*)*

*Padina pavonica (*AN4SD*)*

*Sargassum spp.(*AN5SD*)*

Enteromorpha spp. (AN6SD)

Green algae(*Trentepohila*) (AN7SD)

Sargassum mangarevense SDLG

Figure 4.1 : Collected seaweed from Cacra and Anjuna intertidal regions.

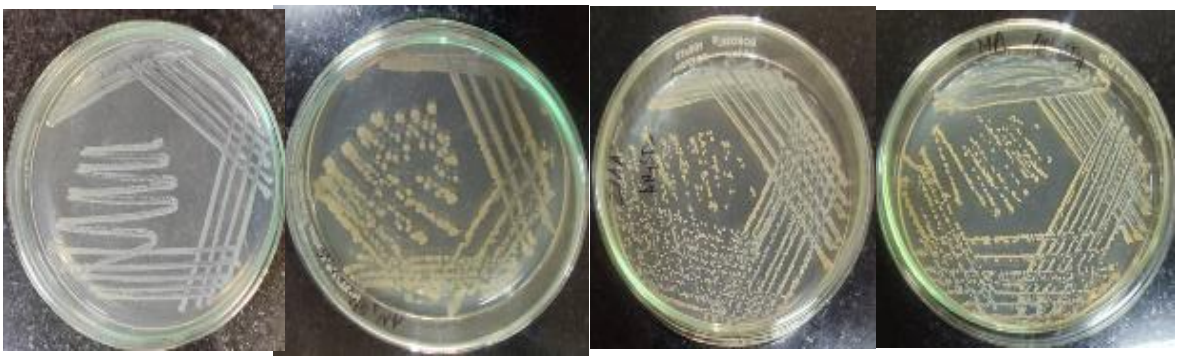
4.2. ISOLATION OF SEAWEED-ASSOCIATED BACTERIA

To extract epiphytes, seaweed was immersed in sterile seawater and vortexed for 2 minutes and serially diluted. From the last two dilutions, aliquots were spread on Zobell Marine Agar, incubated for 48 hours, and colonies were selected and purified for subculture. Different coloured and non colored colonies were observed with different morphology, Number of colonies selected are presented in Table 4.1. Isolates are presented in Figure 4.2

Table 4.1: Collected seaweed and seaweed associated bacteria

Sample No.	Name of the Seaweed	No. of bacteria isolated
SDLG	*Sargassum mangarevense*	8
AN1SD	*Ulva lactuca*	7
AN2SD	*Chondracanthus*	4
AN3SD	*Chaetomorpha gracilis*	2
AN4SD	*Padina pavonica*	4
AN5SD	*Sargassum spp.*	5
AN6SD	*Enteromorpha spp.*	4
AN7SD	*Green algae(Trentepohila)*	4

SDLG1 SDLG4 SDLG6



AN2SD3 AN3SD2 AN7SD 2 AN7SD 4

Figure 4.2: Isolated bacterial culture from seaweed

4.3 GRAM STAINING

Further Gram staining was performed to distinguish seaweed-associated bacteria into gram-positive and gram-negative as shown in figure 4.3. Detail characteristics are explained in Table 4.2

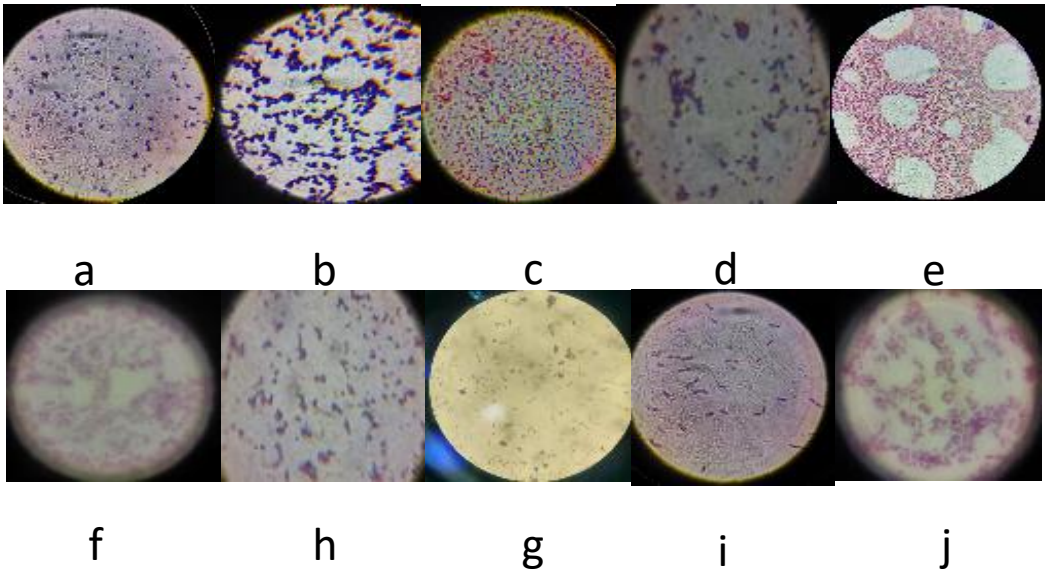


Figure 4.3: Gram character of bacterial isolates

Table 4.2: Gram character of bacterial isolates

Number	Organism	Gram Character
1	SDLG1 (a)	+VE Cocci
2	SDLG2	+VE Cocci
3	SDLG3	+VE short rods
4	SDLG4(b)	+VE Cocci
5	SDLG5	+VE Cocci
6	SDLG6(c)	-VE Cocci in chains
7	SDLG7	+VE Cocci
8	SDLG8	+VE Cocci bunches
9	S6c1	+VE rods
10	S10c1	+VE short rods
11	S1c8	+VE long rods
12	S4c1	+VE long rods
13	S1c4	+VE Cocci
14	AN1SD1	+ short rods
15	AN1SD2	-VE cocci

16	AN1SD3	+VE cocci
17	AN1SD4	-VE long rods
18	AN1SD5	-VE short rods
19	AN1SD6	-VE cocci in chain
20	AN2SD1	-VE rods
21	AN2SD2	+VE rods
22	AN2SD3	+VE cocci
23	AN3SD1	+VE cocci
24	AN3SD2	+VE cocci in chains
25	AN4SD1	-VE rods
26	AN4SD2	-VE long rods in bunches
27	AN4SD3	+VE short rods
28	AN4SD4	+VE cocci
29	AN5SD1	-VE cocci
30	AN5SD2	-VE long rods in bunches
31	AN5SD3	+VE rods
32	AN5SD4	+VE short rod in bunches

33	AN5SD5	+VE short rods
34	AN6SD1	-VE cocci
35	AN6SD2	+VE rods
36	AN6SD3	+VE short rods
37	AN6SD4	-VE cocci
38	AN7SD1	-VE rods
39	AN7SD2	+VE cocci
40	AN7SD3	+VE long rods in bunches
41	AN7SD4	-VE rods

4. 4 CATALASE AND OXIDASE TEST

Catalase and oxidase tests were performed using 3% hydrogen peroxide. Bubble formation indicates catalase positive as shown in figure 4.4a. Bubbles are produced, it indicates the presence of a catalase enzyme that converts hydrogen peroxide to water and oxygen.

An oxidase test was performed using tetra-methyl- p-phenylenediamine dihydrochloride reagent. Purple coloration indicates positive results as shown in Figure 4.4b Results of Catalase test and oxidase test are presented in Table 4.3.

Figure 4.4: a. Catalase test and b. Oxidase test

Table 4.3: Result of oxidase and catalase test shown by the bacterial cultures.

Number	**Organism**	**Oxidase**	**Catalase**
1	SDLG1	-	+
2	SDLG2	-	-
3	SDLG3	-	-
4	SDLG4	-	-
5	SDLG5	-	-
6	SDLG6	-	-
7	SDLG7	+	+
8	SDLG8	-	+
9	S6c1	-	-
10	S10c1	-	+
11	S1c8	-	+

12	S4c1	-	+
13	S1c4	-	+
14	AN1SD1	-	-
15	AN1SD2	+	+
16	AN1SD3	-	-
17	AN1SD4	-	+
18	AN1SD5	-	-
18	AN1SD6	-	+
19	AN2SD1	+	+
20	AN2SD2	-	+
21	AN2SD3	-	-
22	AN2SD4	+	+
23	AN3SD1	-	+
24	AN3SD2	-	+
26	AN4SD2	-	-
27	AN4SD3	+	+
28	AN4SD4	+	+
29	AN5SD1	-	-
30	AN5SD2	-	-
31	AN5SD3	-	+
32	AN5SD4	+	-
33	AN5SD5	+	-
34	AN6SD1	-	-

35	AN6SD2	-	+
36	AN6SD3	-	-
37	AN6SD4	-	-
38	AN7SD1	+	-
39	AN7SD2	+	-
40	AN7SD3	-	+

4.5 2,3 5 TRIPHENYL TETRAZOLIUM CHLORIDE TEST

Red coloration of tubes indicates TTC positive and no color change indicates TTC negative as shown in figure 4.5. Detail list of TTC positive and TTC negative bacterial isolates are presented in table 4.4. Based on the test organisms were selected for omega-3 fatty acid detection and extraction.

Figure 4.5: TTC test result of seaweed associated bacteria.

Table 4.4: Results of TTC test by bacterial cultures

Number	Organism	RESULT	Number	Organism	RESULT
1	SDLG1	+++	21	AN2SD3	-
2	SDLG2	+	22	AN2SD4	+
3	SDLG3	-	23	AN3SD1	+
4	SDLG4	++	24	AN3SD2	++
5	SDLG5	-	26	AN4SD2	-
6	SDLG6	++	27	AN4SD3	-
7	SDLG7	-	28	AN4SD4	-
8	SDLG8	-	29	AN5SD1	-
9	S6c1	-	30	AN5SD2	-
10	S10c1	-	31	AN5SD3	-
11	S1c8	-	32	AN5SD4	-
12	S4c1	-	33	AN5SD5	-

13	S1c4	-	34	AN6SD1	-
14	AN1SD1	-	35	AN6SD2	-
15	AN1SD2	++	36	AN6SD3	-
16	AN1SD3	-	37	AN6SD4	-
17	AN1SD4	+	38	AN7SD1	+
18	AN1SD5	-	39	AN7SD2	+++
18	AN1SD6	+	40	AN7SD3	+
21	AN2SD3	-			
22	AN2SD4	+			
23	AN3SD1	+			

4.6 BLIGH AND DYER METHOD FOR LIPID EXTRACTION

Lipid was extracted from 7 different seaweeds using Bligh and Dyer method. Seaweed was dried and crushed to powder as shown in Figure 4.6 and Figure 4.7. Lipid was extracted from the organic phase and kept in the oven at 30^0C to evaporate the chloroform. The lipid was stored in a glass vail for further analysis. From 1g of seaweed approximately 0.5 ml of lipid was extracted from lipid.

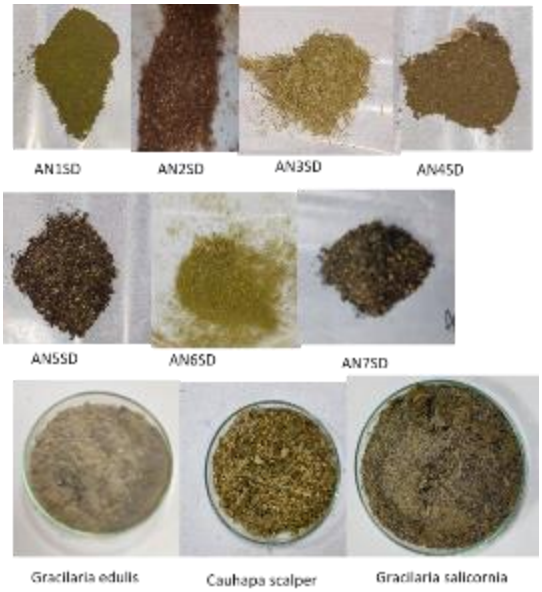


Figure 4.6: Seaweeds powder used for extraction

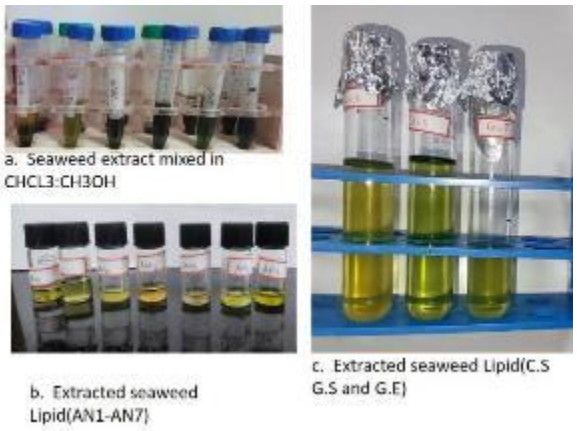


Figure 4.7: Seaweed lipid extraction lipid and extracted lipid

Based on TTC results 11 bacterial cultures as shown in Figure 4.8 and figure 4.9 were selected for lipid extraction. From 2 g of cell pellet 0.5ml of lipid was extracted from organic phase.

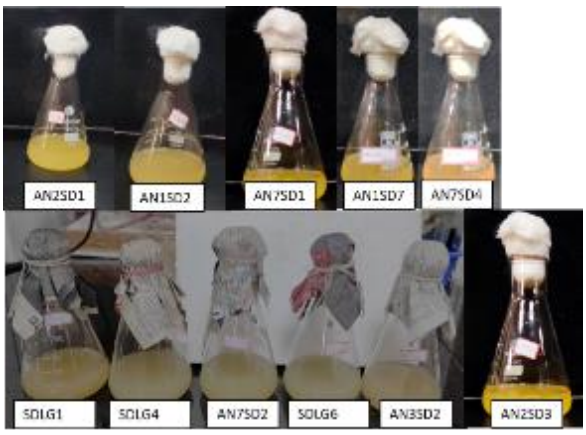


FIGURE 4.8: TTC positive seaweed-associated Bacterial cultures used for lipid extraction

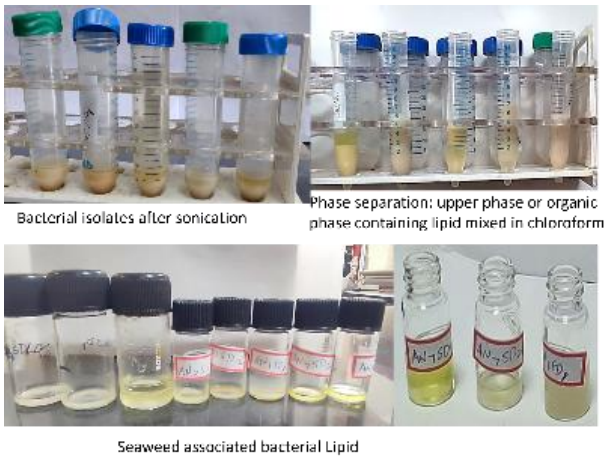


FIGURE 4.9 : Seaweed associated bacterial lipid extraction and extracted lipid

4.7 THIN LAYER CHROMATOGRAPHY

Presence of omega-3 fatty acids derivatives in the lipid extracted from seaweed-associated bacteria and seaweed was observed by thin-layer chromatography. The bands observed on the TLC plate were compared to those found on omega 3 fatty acid standard TLC plates Subsequently, the observed bands were subjected to Rf value calculations. As shown in Figure 4.10 and Table 4.5

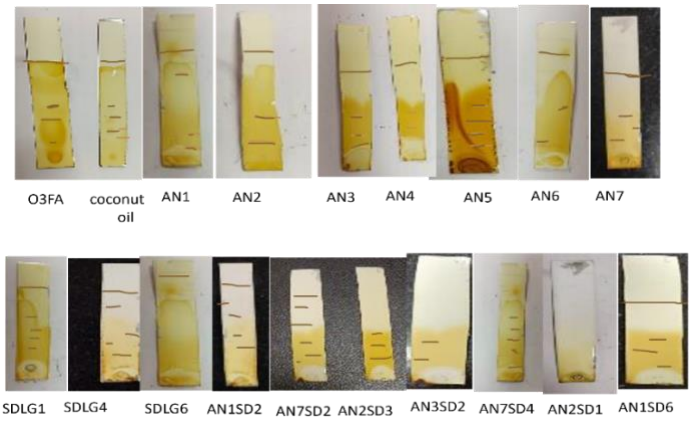


Figure 4.10: Seaweed and seaweed associated bacterial Lipid TLC sheets showing bands.

Table 4.5: Result of Thin layer chromatography for seaweed lipid

SPOTS	**AN1**		**AN2**		**AN3**		**AN4**		**AN5**	
Solvent front	5.6		5.9		6.5		6.6		6.2	
S1	1.6	0.285	1.9	0.322	1.9	0.292	3	0.45	0.9	0.14
S2	3.3	0.589	4.4	0.745	4.2	0.646	3.9	0.59	1.5	0.241
S3	4.3	0.767							4.5	0.725
S4									4.6	0.741

AN6		AN7		OIL		O3FA	
6.9		6.5		5.9		5.9	
1.2	0.1739	1.1	0.16	2.5	0.423	2.1	0.355
3.7	0.536	3.6	0.55	3.1	0.525	2.6	0.44
		4.2	0.646	4.1	0.694	3.2	0.54

Extracted lipid was separated on TLC plate. Rf value of sample closer to Rf value of standard omega-3-fatty acid was compared. Darker band were shown by Gracilaria spp indicating high amount PUFA. Best presented in Table 4.6

Table 4.6: Result of Thin layer chromatography for seaweed-associated bacteria lipid

SPOTS	SDLG1 Rf		SDLG4 Rf		SDLG6 Rf		AN1SD2 Rf	
Solvent front	6.3		6.7		6.5		7	
S1	2.1	O.33	1.5	0.22	1.4	0.215	1	0.142

S2	2.5	0.396	2.4	0.358	1.6	0.246	1.4	0.2
S3	3.3	0.523	4.1	0.611	5.6	0.861	1.6	0.228
S4	3.5	0.555			6.1	0.938	5.6	0.86
S5							6.4	0.914

AN7SD2		AN7SD4		AN3AS2		AN1SD2	AN1SD6	
Rf		Rf		Rf			Rf	
7.1		6.4		5.9		-	5	
0.9	0.126	1.5	0.23	2.2	0.372		4.2	0.84
1.2	0.169	1.6	0.25	3.9	0.661		4.	0.8
1.5	0.211	3.5	0.546				3.5	0.7
5.3	0.746	5.2	0.812					
6	0.845							

Table 4.7 Comparision of sample RF value with Standard RF of omega 3 fatty acid tablet

Omega 3 fatty acid Rf value	AN2	AN4	AN6	AN7	SDLG1	SDLG4	AN7SD4
0.355(DHA)	0.322				0.33	0.45	
0.44(EPA)		0.45					
0.54(ALA)			0.53	0.55	0.55		0.54

4.8: THIN LAYER CHROMATOGRAPHY FOR AMINO ACID DETECTION

Different amino acid present in seaweed and seaweed associated bacterial extract was detected using TLC as shown in Figure 4.11. Rf value of seaweed associated bacterial amino acids was calculated as presented in Table 4.7. Rf value of amino acids from seaweed extract is shown in Table 4.8 and 4.9 Darker bands indicates high content of amino acids presented AN5,SDLG1,SDLG4 and AN3SD2.

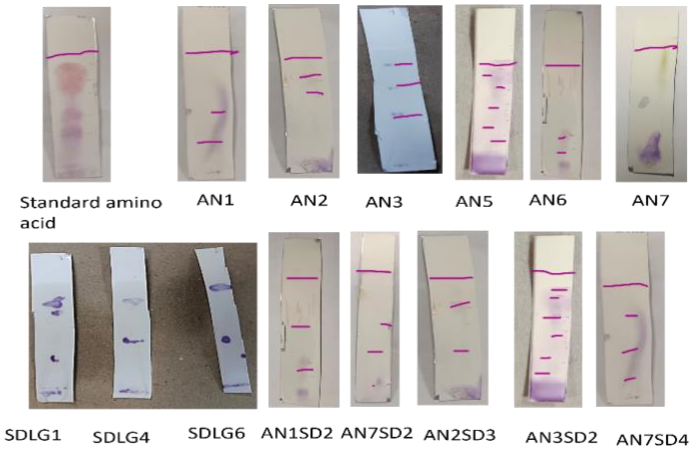


Figure 4.11: Seaweed and seaweed associated bacterial amino acid TLC sheets showing bands

Table 4.8: Result of Thin layer chromatography for Standard amino

STD AMINO ACID	RF VALUE Spot	Rf	AMINO ACIDS
RF	6.4		
R1	2.3	0.35	GLYCINE
R2	2.4	0.37	PROLINE
R3	2.5	0.39	HISTIDINE
R4	2.7	0.42	SERINE
R5	3	0.46	THERONINE
R6	3.4	0.53	VALINE
R7	4.1	0.64	CYSTINE
R8	4.2	0.65	PROLINE
R9	5.2	0.81	LEUCINE
R10	5.6	0.87	TRYPTOPHAN

Table 4.9: Result of Thin layer chromatography for seaweed Amino acid

RF	AN1		AN2		AN3		AN4	AN5		AN6		AN7	
	Spot	Rf	Spot	Rf	Spot	Rf		Spot	Rf	Spot	Rf	Spot	Rf
RF	6.3		6.2		6.6		-	6.1		6.6		6.3	
R1	0.9	0.14	3.6	0.57	1	0.15		1	0.16	2	0.303	1.6	2.5
R2	5	0.79	4.6	0.73	1.3	0.196		2.2	0.36	3.5	0.53	2.1	0.33
R3					1.5	0.227		2.6	0.43	4.1	0.6		
R4					1.6	0.24		4	0.655				
R5								5	0.81				

Table 4.10: Result of Thin layer chromatography for seaweed-associated bacterial amino acid

RF	SDLG1		SDLG4		SDLG6		AN1SD2	
	Spot	Rf	Spot	Rf	Spot	Rf	Spot	Rf
RF	6.3		6.7		6.9		7	
R1	0.5	0.079	1.	0.14	1.5	0.21	2.8	0.4

R2	1.9	0.3	2.3	0.34	4.6	0.66	5	0.7
R3	2.7	0.42	4.1	0.61	5.5	0.79		
R4	5.9	0.936						

AN7SD2	AN7SD4		AN3AS2		AN1SD2		AN1SD6
	Spot	Rf	Spot	Rf	Spot	Rf	
-	6.7		5.9		5		-
	1.5	0.22	2.2	0.37	3.5	7	
	2.1	0.313	3.9	0.66	4.	0.8	
	4.2	0.62			4.2	0.84	

Table 4.11 : Comparision of sample amino acid found in seaweed and seaweed associated bacteria with Standard amino acid results.

Amino Acids	Rf of STD	AN1	AN5	AN6	AN7	SDLG1	SDLG4	SDLG6	AN1SD2	AN7SD4	AN3SD2	AN1SD2

Glycine	0.35		0.36		0.33		O.34					
Proline	0.37										0.37	
Histidine	0.39											
Serine	0.42		0.43			0.42			O.40			
Theronine	0.46											
Valine	0.53			0.53								
Cystine	0.64									0.62		
Proline	0.65		0.65					0.66			0.66	
Leucine	0.81	0.79				0.79		0.79				0.8

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Algae and algae-associated bacteria are rich in amino acids. Bacterial isolate number SDLG1, AN1SD2 has an Rf value closer to serine and leucine, SDLG4 has an Rf value closer to glycine, SDLG6, AN3SD2 has an Rf value closer to proline and leucine, AN7SD4 has a closer value of

cysteine. Algae species such as *Ulva lactuca* are rich in leucine, *Enteromorpha* sp is rich in glycine, serine and proline, some green algae are also rich in glycine.

4.9 IODINE VALUE TEST

High value of iodine value represent presence of high number of double bond present in the lipid. End titration giving white milky colour precipitate indicate saturation. Refer Figure 4.12 Using the iodine value formula unsaturation value or iodine value was calculated as shown in Table 4.11. Seaweed associated bacterial lipid and seaweed lipid showing higher iodine value was compared with standard omega 3 fatty acids iodine value. As shown in Table 4.12

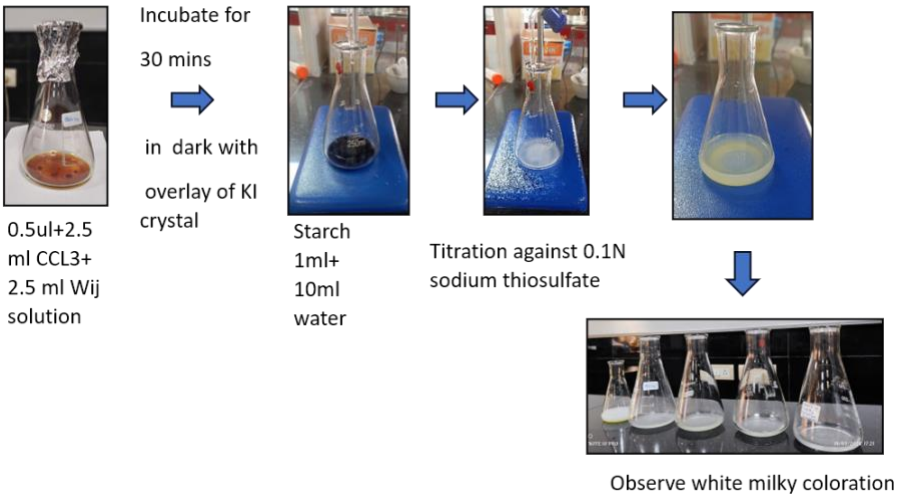

Figure 4.12: Iodine value test for Seaweed and seaweed associated bacteria

Table 4.12: Result of Iodine value test to detect Unsaturation of fatty acid by seaweed and seaweed associated bacterial lipid

Blank Value = 6.5

Sample	**Titre Value**	**Iodine Value g/ml**
SDLG1	3.5	7.61
SDLG4	3.2	8.37
SDLG6	2.1	11.16
AN1SD2	3.1	8.629
AN1SD7	2	11.42
AN2SD1	4.2	5.83
AN2SD3	2.3	10.65
AN3SD2	4	6.345
AN7SD1	3	8.883
AN7SD2	6	12.69

AN7SD4	1	13.95
AN1	3	8.8
AN2	2	11.42
AN3	2.6	9.89
AN4	9	-6.34
AN5	5.2	3.17
AN6	2.3	7.71
AN7	3.2	5.78
G.E	1.5	12.69
G.S	2.3	10.65
C.S	4.6	4.8
BLANK(OIL)	3.7	7.10
O3FA	1.5	12.69

Omega-3 Fatty acid has iodine value of 12.69. Among seaweed species *Gracilaria edulis has* similar value of 12.69. *Gracilaria salicornia* has 10.69 and *Chondracanthus* with a value 11.42. Theses values are closer to omega-3 fatty acid values.

4.10 DISCUSSION

Table 4.13. Comparative study of Literature review results and present study results of omega-3 fatty acid

Sr. No	**Lipid extracted**	**Omega-3 Fatty Acid component**	**Method**	**Organism/source**	**Reference**
1	1ml/g	ALA and DHA	Folch method	Flexseed	(Lane et al., 2021)
2	0.35ml/g	DHA and EPA	Bligh and Dyer method	Vegetable oil	(Dhanya et al., 2020)
3	0.6ml/g	DHA and EPA	Bligh and Dyer method	*Schizochytrium spp*.	(Dhanya et al., 2020)
4	2ml/g	EPA and DHA	GC-FID and GC-MS	*Vibrio spp.*	(Estupiñán et al., 2020)
5	25ml/g	DHA	CH3CL-MeOH	Green algae	(Shukla et.al.,2011)
6	1.27 ml/g	EPA	Folch Method	*Ulva Lactuca*	(Dhargalkar & Kavlekar, 2004)

7	3ml/g	EPA	Folch Method	*Fucus vesiculosus*	(Dhargalkar & Kavlekar, 2004)
8	0.5ml/g	DHA	TLC and Iodine value test	*Chondracanthus*	Present Study
9	0.7ml/g	ALA	TLC and Iodine test	*Enteromorpha spp. and* Green algae*(Trentepohila*	Present Study
10	0.5ml/g	EPA	TLC and Iodine test	*Padina pavonica*	Present Study
11	0.2 ml/g	EPA and ALA	TLC and Iodine test	SDLG1	Present Study
12	0.2 ml/g	DHA	TLC and Iodine test	SDLG4	Present Study
13	0.2g/ml	ALA	TLC and Iodine test	AN7SD4	Present Study
14	1ml/g	Omega-3-Fatty Acid	Iodine Test	*Gracilaria edulis* and *Chondracanthus*	Present Study

Table 4.14. Comparative study of Literature review results and present study results of Amino acids

Sr. No	Amino acid	Method	Organism/species	Reference

1.	Histidine, isoleucine, Leucine, Lysine, methionine, Phenylalinine	HPLC	*Ulva Lactuca*	(Mohammed et al., 2021)
2.	Histidine, Isoleucine, Tyrosine, Valine, Leucine, Lysine, Methionine, Phenylalinine	HPLC	*U. rigida* and *U.pertusa*	(Mohammed et al., 2021)
3.	Histidine, Isoleucine, Thronine, Valine, Leucine, Methionine, Phenylalinine	TLC	*Gracilaria spp.* And *P .palmata*	(Sun et al., 2021)
4.	Glutamate, Alanine, Serine, Glycine	HPLC	*Vibrio spp.*	(Singh & Reddy, 2014)
5.	Glutamate, Aspartase, Lysine, Arginine.	HPLC	Proteobacteria	(Tumbarski et al., 2018)
6.	Glycine, Serine, Proline.	TLC	*Padina pavonica*	Present Study
7.	Glycine,Proline ,Leucine	TLC	SDLG4, SDLG6	Present Study
8.	Cysteine	TLC	AN7SD2	Present Study
9.	Leucine	TLC	SDLG1, AN3SD2, AN1SD2	Present Study

SUMMARY

Seaweed are diverse in marine habitat. Goan coastal area is suitable for seaweed population due to environmental parameters and nutrient requirements. With growing market there is lack of vegan omega-3- fatty acid in market. Seaweed is a good source of vegan omega-3 fatty acids because it has a good content of lipids and amino acids necessary for human health In this study different types of seaweed namely *Sargassum mangarevense Ulva lactuca, Chondracanthus Chaetomorpha gracilis, Padina pavonica, Sargassum spp. Enteromorpha spp. and* Green algae*(Trentepohila)* were collected from intertidal zones of Cacra and Anjuna beaches in Goa. Forty Seaweed associated bacteria were isolated by serial dilution method and later screened for biochemical test like catalase and oxidase. Using TTC screening 11 bacterial isolates were selected for lipid extraction. Among seaweed Green algae such as *Gracilaria edulis* has iodine value 12.69 g and *Gracilaria salicornia*- 10.69g *Chondracanthu* has 11.42g indicating high polyunsaturated bond. On TLC they show close Rf value to standard omega 3 fatty acid. *Chondracanthu*s shows close Rf value 0.3 for DHA. *Padina pavonica,* is rich in EPA as dark band observed on TLC plate with Rf value 0.4 and *Enteromorpha spp.* And Green algae*(Trentepohila)* rich in ALA with Rf value 0.5 Eleven bacteria isolated from seaweed showed good results for TTC, indicating EPA production Isolate SDLG1, SDLG4 and AN7SD1 had shown high amount of PUFA with iodine value approximately 12g/ml and TLC with Rf value for EPA 0.4, DHA 0.3 and ALA 0.5. .Similar method used for the lipid extraction of seaweed associated bacteria. Bacterial isolate number SDLG1, AN1SD2 has an Rf value closer to serine and leucine, SDLG4 has an Rf value closer to glycine, SDLG6, AN3SD2 has an Rf value closer to proline and leucine, AN7SD4 has a closer value of cysteine. Algae species such as *Ulva lactuca* are rich in leucine, *Enteromorpha* sp is rich in glycine, serine and proline, some green algae are also rich in glycine.

CONCLUSION

Seaweed is a good source of vegan omega-3 fatty acids because it has a good content of lipids and amino acids necessary for human health. Seaweeds are best cultivated at temperature 27-32C or below and Goan coastal temperature its favourable for seaweed. They produces little low concentration of lipid but they are rich in omega-3 fatty acid. Qualitatively *Gracilaria edul*is and *Gracilaria salicornia, Chondracanthu, Padina pavonica, Enteromorpha spp. and* Green algae*(Trentepohila* produces EPA,DHA and ALA in moderate concentration. They show high unsaturation of fatty acids indicating presence of PUFA. Quantitatively 1g of seaweed powder gives more than 0.5ml lipid as compared to seaweed associated bacteria giving 0.2ml lipid. Green algae present dark band for EPA on TLC which shows high amount of EPA , bacterial isolate SDLG1 isolated from *Sargassum spp* showed high amount of DHA (dark band). Compared to non-vegan sources and other vegan sources seaweeds have high amounts of omega-3 fatty acids. Seaweed-associated bacteria are rich in amino acids. Bacterial isolate number SDLG1, AN1SD2 has an Rf value closer to serine and leucine, SDLG4 has an Rf value closer to glycine, SDLG6, AN3SD2 has an Rf value closer to proline and leucine, AN7SD4 has a closer value of cysteine. *Ulva lactuca* are rich in leucine, *Enteromorpha* sp is rich in glycine, serine and proline, some green algae are also rich in glycine. Quantitatively darker band presented by *Sargassum spp* produces high amount of serine and proline and isolate AN3SD2 isolated from *Chaetomorpha spp* produces high amount of proline.

Current work has good potential for vegan omega-3 fatty acid production in both Western and Indian markets. It can be economical and cost-effective. As consumer demand for vegan alternatives continues to grow, the results of this research have significant implications for health and sustainability. And they can promote environmental sustainability and diversification of food sources for an entire year.

FUTURE PROSPECT

- To check antimicrobial and antitumor activities of seaweed and bacterial lipids.
- Pigment extraction from pigmented seaweed-associated bacteria for antimicrobial and antifungal, antitumor activities

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APPENDIX

APPENDIX -I

(MEDIA)

1. Zobell Merine Broth**:**

Composition	**Grams/Liter**
Peptone	5.000
Yeast extract	1.000
Ferric citrate	0.100
Sodium chloride	19.450
Magnesium chloride	8.800
Sodium sulphate	3.240
Calcium sulphate	1.800
Potassium chloride	0.550
Sodium bicarbonate	0.160
Potassium bromide	0.080
Strontium chloride	0.034
Boric acid	0.022
Sodium silicate	0.004
Sodium fluorate	0.0024
Ammonium nitrate	0.0016
Disodium phosphate	0.008
pH(25^0C)	7.6=/-0.2

40.25 g suspended in 1000ml filter sterilized sea water. Then autoclave the media at 15 Ibs pressure for 121^0C for 15 mins.

2. Zobell Merine Broth:

Composition	**Grams/Liter**
Peptone	5.000
Yeast extract	1.000
Ferric citrate	0.100
Sodium chloride	19.450
Magnesium chloride	8.800
Sodium sulphate	3.240
Calcium sulphate	1.800
Potassium chloride	0.550
Sodium bicarbonate	0.160
Potassium bromide	0.080
Strontium chloride	0.034
Boric acid	0.022
Sodium silicate	0.004
Sodium fluorate	0.0024
Ammonium nitrate	0.0016
Disodium phosphate	0.008
pH(25^0C)	7.6=/-0.2

55.25 g suspended in 1000ml filter sterilized sea water. Then autoclave the media at 15 Ibs pressure for 121^0C for 15 mins. Well mixed and pour in sterile petri plate.

3. Zobell marine broth supplemented with TTC

Composition	**Grams**
Zobell marine broth	4.025
TTC	1
Distilled water	100ml

APPENDIX -II

(REAGENTS)

0.1%Starch solution

Composition	**Grams**
Starch	1
Distilled water	100ml

0.1 N Sodium Thiosulfate (pipette**)**

Composition	**Grams**
Sodium Thiosulfate	2.5
Distilled water	100ml

Note: Add 2.5g of sodium thiosulfate in 80 ml of distilled water heat for some time and make100ml final volume

Ninhydrin solution

Composition	**Grams**
Ninhydrin powder	1.5
Butanol	100ml
Acetic acid	3ml

Wij solution

Composition	**Grams**
Iodine crystal	1.3
Glacial acetic acid	100ml

0.1 % TTC

Composition	**Grams**
TTC	1
Distilled water	100ml

Strips for Oxidase test:

Cut the filter paper into small strips, autoclave it then dipped into oxidase test reagent i.e. tetra-methyl- p-phenylenediamine dihydrochloride reagent., and dry it .

0.9% NaCl Solution

Composition	**Grams**
NaCl	0.9
Distilled water	100ml

TLC Solvent for lipid separation

Composition	**Milliliter**
Acetone	14
Butanol	14
Acetic Acid	4
Distilled water	8

TLC Solvent for Amino acid separation

Composition	**Milliliter**
Hexane	40
Diethyl ether	10
Acetic Acid	1