ज्ञान-विज्ञान विमुक्तये

Final Report of Major Research Project
Funded by the
UNIVERSITY GRANTS COMMISSION (UGC)
NEW DELHI

(UGC F. No 42-471/2013 (SR) Dated-22nd March, 2013)

ISOLATION OF BIOACTIVE COMPOUNDS FROM SELECTED SEAWEEDS OF MANDAPAM COAST, TAMILNADU

WITH COURAGE AND FAITH

Submitted by

Dr. K. Kolanjinathan, M.Sc., M.Phil., Ph.D.,

Assistant Professor & Principal Investigator,
Division of Microbiology,
Faculty of Science,
Annamalai University,
Annamalai Nagar-608 002.
Tamil Nadu, India.

Annexure –III

UNIVERSITY GRANTS COMMISSION (UGC)
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

Statement of expenditure in respect of Major Research Project

1.	Name of the principal Investigator	:	Dr. K. Kolanjinathan
2.	Dept. of principal investigator University / College	:	Assistant professor, Dept. of Microbiology, Faculty of Science, Annamalai University, Annamalainagar – 608 002, Tamilnadu, India. Cell: +91 Email: drkolanji@gmail.com
3.	UGC approval letter no & Date	:	UGC F. No 42-471/2013 (SR) Dated-22nd March, 2013 & 25th May 2016
4.	Title of the project	:	**Isolation of bioactive compounds from selected seaweeds of Mandapam coast, Tamilnadu.**
5.	Date of Implementation	:	01.04.2017
6.	a. Tenure of the project	:	From 01.04.2017 to 31.03.2017

b. Details of Expenditure

S. no Col 1	Head Col 2	Amount approved (Rs.) Col 3	Amount received in I Installment (Rs.) Col 4	Amount received in II installment (Rs.) Col 5	Total amount released (Rs.) (Col 6= col 4+5)	Expenditure incurred so far (Rs.) Col 7	Balance (Rs.) Col 8= Col 6-7
1.	Books & journal	25,000	25,000	---	25,000	24,924	+76
2.	Equipments	1,50,000	1,50,000	---	1,50,000	1,40696.05	+9303.95
3.	Project fellow	5,28,000	2,64,000	2,04,960	4,68,960	4,68,920	+40
4.	Travel/fieldwork	30,000	15,000	12,000	27,000	27,000	----
5.	Chemical & glassware	1,50,000	75,000	60,000	1,35,000	1,34,794	+206
6.	Contingency	60,000	30,000	24,000	54,000	54,000	---
7.	Hiring services	---	---	---	---	---	---
8.	Overhead charges	73,800	73,800	---	73,800	73,800	---
9.	Additional grand for contingency/ hiring	---	---	---	---	---	----
	Total	**10,16,800**	**6,32,800**	**3,00,960**	**9,33,760**	**9,24,134.05**	**9625.95**

c. Staff

Date of appointment: Annexure – VI (Enclosed)

S.no	Item	From	To	Amount approved (Rs.)	Expenditure incurred (Rs.)
1.	Honorarium to PI (Retired teachers) @ Rs.18,000/- p.m	Nil	Nil	Nil	Nil
2.	Project fellow M.Manigandan	14.04.2013	31.03.2017	5,28,000	4,68,920

1. It is certified that the appointment(s) have been made in accordance with the terms and condition laid down by the commission: Yes
2. It as a result of checks or audit objective, same regularly is noticed, later date action will be taken to refund, adjust or regularize the objected amounts.
3. Payment @ revised rates shall be made with arrears on the availability of additional funds.
4. This is certified that the grant of **Rs.**9,33,760 (**Rupees** Nine lakh thirty three thousand seven hundred and sixty only) received out of Rs. 10,16,800 (Rupees Ten lakh sixteen thousand eight hundred) from the University Grants Commission under the scheme of support for Major Research Project entitled "**Isolation of bioactive compounds from selected seaweeds of Mandapam coast, Tamilnadu."** Vide UGC letter no. UGC F. No 42-471/2013 (SR) Dated-22nd March, 2013 and **Rs.**9,24,134.05 (Rupees Nine lakh twenty four thousand one hundred thirty four rupees and five paisa only) has been utilized for the purpose for which it was sanctioned and in accordance with the term and conditions laid down by the University Grants Commission.

SIGNATURE OF THE CO-INVESTIGATOR

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

REGISTRAR

Annexure - IV

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

STATEMENT OF EXPENDITURE INCURRED ON FIELD WORK

1. Details of T.A & D.A bill of the principal Investigator: Dr. K. Kolanjinathan and Project fellow Mr. M. Manigandan
(Period from 12/04/2013 to 13/09/2015)

S.no	Date of journey	Voucher No.	From	To (Purpose)	Amount Rs.
1.	12/04/2013	-	Adhirampattinam to Chidambaram, Chidambaram to Adhirampattinam	External expert TA/DA bill	Rs. 752.50
2.	27/06/2014 to 29/06/2014	23	Chidambaram	Mandapam	Rs. 6940
3.	27/06/2014 to 29/06/2014	-	Mandapam	Sea (Sample collection)	Rs. 9300
4.	28/03/2015 to 29/03/2015	-	Mandapam	Sea (Sample collection)	Rs. 5800
5.	12/09/2015 to 13/09/2015	-	Mandapam	Sea (Sample collection)	Rs. 7100
				Total	**Rs. 29892.50**

ANNAMALAI UNIVERSITY

DEPARTMENT OF MICROBIOLOGY

UGC Major Research Project

Principal Investigator : Dr. K. Kolanjinathan

Assistant professor in Microbiology

Statement of Expenditure

Abstract

T.A. & D.A. bill of the Principal Investigator and Junior Research Fellow

S.no	Particulars	Amount Rs.
1.	T.A. & D.A. bill of the external expert for project fellow interview (12/04/2013)	Rs. 752.50
2.	T.A. & D.A bill of the Principal Investigator & junior research Fellow (Period from 27/06/2014 to 29/06/2014)	Rs. 16240
3.	T.A. & D.A bill of the Principal Investigator & junior research Fellow (Period from 28/03/2015 to 29/03/2015)	Rs. 5800
4.	T.A. & D.A bill of the Principal Investigator & junior research Fellow (Period from 12/09/2015to 13/09/2015)	Rs.7100
	Total	**Rs.29892.50**

Restricted amount : - 2892.50

Grand total : 27,000/-

Certified that the above expenditure is in accordance with the UGC norms for Major Research Projects.

SIGNATURE OF THE CO-INVESTIGATOR

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

REGISTRAR

Annexure - V

UNIVERSITY GRANTS COMMISSION

BAHADUR SHAH ZAFAR MARG

NEW DELHI – 110 002

UTILIZATION CERTIFICATE

Certified that an amount **Rs.9,33,760** (Rupees Nine lakh thirty three thousand seven hundred and sixty only) received from the University Grants Commission under the scheme of support for Major Research Project entitled "Isolation of bioactive compounds from selected seaweeds of Mandapam coast, Tamilnadu" vide UGC letter F. No. 42-471/2013 (SR) dated 22nd March 2013 and **Rs.**9,24,134.05 (Rupees Nine lakh twenty four thousand one hundred thirty four rupees and five paisa only) has been utilized for the purpose for which it was sanctioned and in accordance with the terms and conditions as laid down by the University Grants Commission.

SIGNATURE OF THE
CO-INVESTIGATOR

SIGNATURE OF THE
PRINCIPAL INVESTIGATOR

STATUTORY AUDITOR
(Seal)

REGISTRAR
(Seal)

Annexure-VI

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUPPLYING THE INFORMATION IN RESPECT OF THE STAFF APPOINTED UNDER THE SCHEME OF MAJOR RESEARCH PROJECT

UGC File No. F. 42-471/2013 (SR) **Years: 2013**

TITLE OF THE PROJECT: "Isolation of bioactive compounds from selected seaweeds of Mandapam coast, Tamilnadu"

1	Name of the Principal Investigator	**Dr. K. KOLANJINATHAN**				
2	Name of the University/College	Annamalai University				
3	Name of the Research Personnel appointed	**M. MANIGANDAN**				
4	Academic qualification	**S. no**	**Qualification**	**Year**	**Marks**	**(%)**
		1	M.Sc.	May 2011	7.76/10 OGP	77.6
		2	M.Phil.	August 2014	7.97/10	79.7
		3	Ph.D.	-	-	-
5	Date of Joining	14-04-2013				
6	Date of Birth of Research Personnel	12-06-1987				
7	Amount of HRA, if drawn	-				
8	Number of Candidate applied for the post	Three candidates				

CERTIFICATE

This is to certify that all the rules and regulations of UGC major research project outlined in the guidelines have been followed. Any lapses on the part of the university will liable to terminate of said UGC project.

PRINCIPAL INVESTIGATOR **HEAD OF THE DEPT** **REGISTRAR**

Annexure- VIII

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002
FINAL REPORT OF THE WORK DONE ON THE MAJOR RESEARCH PROJECT

1.	Project report No. 1st/ 2nd/ 3rd /final	Final report
2.	UGC Reference No. & Date	F.No.42-471/2013 (SR) 22.03.2013
	UGC extension letter No. & Date	F.No.42-471/2013 (SR) 25.05.2016
3.	Period of the project:	01.04.2013 to 31.03.2017
4.	Title of the research Project	Isolation of bioactive compounds from selected seaweeds of Mandapam coast, Tamilnadu
5.	(a) Name of the Principal Investigator	Dr. K. Kolanjinathan
	(b) Department	Department of Microbiology
	(c) University/ college where work has progressed	Annamalai University
6.	Effective date of starting of the project	01.04.2013
	Grants approved and expenditure incurred during the period of the report	
	a. Total amount approved Rs.	**Rs.10,16,800/-**
	b. Total expenditure Rs.	**Rs.9,33,760 /-**
	Report of the work done: (please attach a separate sheet)	
	i. Brief objective of the project	Enclosed (Appendix-I)
	ii. work done so far and results achieved and publication, if any, resulting from the work (give details of the papers and names of the journals in which it has been published or accepted for publication)	List of publication enclosed (Appendix-II)
	iii. Has the progress been according to original plan of work and towards achieving objectives if not, state reasons	Yes, progress achieved as per work plan
	iv. Please indicate the difficulties, if any, experienced in implanting the project	Nil
	v. Please indicate the approximate time by which the project work is likely to be completed. A summary of the work done for the period (Annual basis) may please be sent to the commission on a separate sheet.	Not applicable

	vi. If the project has been completed, please enclose a summary of the findings of the study. One bound copy of the final report of the work done may also be sent to University Grants Commission	Yes, completed, Final report enclosed (Appendix-II)
	vii. Any other information which would help in evaluation of work done on the project. At the completion of the project, the first report should indicate the output, such as	
	a) Manpower trained –	The project fellow was trained in various aspects Viz., Specimen collection/ microbial cultures collection, identification, antimicrobial activity
	b) Ph.D., awarded –	Nil
	c) Publication of results –	Yes, 3 papers published (Appendix-III)
	d) Other impact, if any -	Isolation of bioactive compound from marine seaweeds, Potential uses of selected marine macroalgae or seaweeds from Mandapam coastal regions

SIGNATURE OF THE CO-INVESTIGATOR

SIGNATURE OF THE PRINCIPAL INVESTIGATOR

REGISTRAR

Annexure-IX

UNIVERSITY GRANTS COMMISSION
BAHADUR SHAH ZAFAR MARG
NEW DELHI – 110 002

PROFORMA FOR SUBMISSION OF INFORMATION AT THE TIME OF FINAL REPORT OF THE WORK DONE ON THE PROJECT

1	UGC Reference No. & Date	F. No.47-471/2013 (SR) Dated – 22nd March, 2013
2	Name of the Principal Investigator	**Dr. K. Kolanjinathan**
3	Address	**Office**: Assistant Professor Division of Microbiology Faculty of Science Annamalai University Annamalai Nagar – 608002 **Residential**: Plot no:13, Sri Sakthi Nagar extension, Gandhi nagar post, Vadakkuthu Panchayat, Kurinchipadi (tk), Neyveli- 607 308.
4	UGC approval letter no. and date	F. No. 42-471/2013 (SR) dated 22.03.2013
	UGC extension letter no. and date	F. No. 42-471/2013 (SR) dated 25.05.2016
5	Date of Implementation	01.04.2013
6	Title of the project	**"Isolation of bioactive compounds from selected seaweeds of Mandapam coast, Tamilnadu"**
7	Tenure of the Project	Three years from 01.04.2013 to 31.03.2016
8	Total grant allocated	Rs. 10,16,800
9	Total grant received	**Rs. 9,33,760**
10	Final expenditure	**Rs.9,24,134.05**
11	Objective of the Project	Enclosed (Appendix - I)
12	Whether objectives were achieved (Give detail)	Yes, enclosed in report (Appendix-V)
13	Achievement from the project	yes, enclosed (Appendix-II)
14	Summary of the findings (In 500 words)	Yes enclosed (Appendix-III)

15	Contribution to the society (give details)	Yes, enclosed (Appendix-IV)
16	Whether any Ph.D., Enrolled/Produced out of the project	Nil
17	No of publications out of the project (please attach)	Yes, three papers published (Appendix-V)

CO-INVESTIGATOR **PRINCIPAL INVESTIGATOR**

REGISTRAR

Appendix I

OBJECTIVES OF THE PROJECT

1. To Collect and identify selected seaweeds
2. To Prepare crude extracts from the selected seaweeds
3. To test antibacterial activity by disc diffusion method and minimum inhibitory concentration method
4. To test antifungal activity by disc diffusion method and minimum inhibitory concentration method
5. To Separate and identify bioactive compounds from most promising seaweeds using
    a) column chromatography (CC)
    b) Thin layer chromatography (TLC)
    c) Gas chromatography- Mass spectrometry (GC-MS)
    d) Infrared spectroscopy (Fourier Transform)
    e) Ultraviolet–visible spectroscopy
    f) ^{1}H Nuclear Magnetic Resonance
    g) ^{13}C Nuclear Magnetic Resonance

Appendix II

ACHIEVEMENTS FROM THE PROJECT

- Finding of the most promising bioactive compound containing seaweed.
- Usage of the bioactive compound against pathogenic bacteria and fungi.
- Finding the effective concentration in controlling the pathogens by the disc diffusion and minimal inhibitory concentration (MIC)
- Revealing the role of seaweed in medicinal value.
- Standardizing the natural way of controlling pathogenic organism.

Appendix III

SUMMARY OF THE FINDINGS

The seaweeds *viz*., *Ulva reticulata, Ulva lactuca, Caulerpa racemosa, Hypnea musciformis, Hypnea valentiae, Gracilaria corticata, Gracilaria edulis, Gelidiella acerosa, Sargassum myriocystum* and *Padina gymnospora* were collected from Mandapam coast of Tamil Nadu, India. Collected seaweeds were dried and fine powdered using mixer grinder. Seaweeds crude extracts were prepared using various solvents such as methanol, acetone, chloroform, hexane and ethyl acetate in Soxhlet apparatus. Prepared extracts were dried and stored in a refrigerator at 4ºC for further use. Bacterial and fungal pathogens were collected from the MTCC, Chandigarh, India. The antimicrobial activity of the seaweed crude extracts against the collected bacterial and fungal pathogen were determined by disc diffusion method and the minimum inhibitory concentration of the extracts were screened by broth dilution method. Ampicillin (5mg/ml) and Flucanozole (5mg/ml) were used as positive controls for bacteria and fungi respectively. The disc containing 5% DMSO alone used as blind control. Among the ten seaweeds tested, the highest mean zone of inhibition was observed in methanol extract of *Gracilaria corticata* against all tested bacterial and fungal pathogens and the highest minimum inhibitory concentration (MIC) was obtained in methanol extract of *Gracilaria corticata* against all tested bacterial and fungal pathogens. The antimicrobial compound present in the crude extract of *G. corticata* was isolated and identified using the chromatographic techniques.

Appendix IV

15. CONTRIBUTION TO THE SOCIETY

Production of bioactive compounds from selected seaweeds against for controlling human pathogenic microorganisms. The wonderful highly available natural biomass is an excellent source to meet out the growing demand of antimicrobial agents is achieved to some extent in this project. Exploring marine sources as natural drug is a novel approach of this project. After a detailed pharmacological study the isolated marine bioactive compound diszoprogesterone, hexadecane and 2,4 dimethylcyclopentanol from the *Gracilaria corticata* may be used a effective drug against the tested bacterial and fungal pathogens.

Appendix V

WHETHER OBJECTIVES WERE ACHIEVED (GIVE DETAIL)

FINDINGS OF THE PRESENT UGC MAJOR RESEARCH PROJECT

12.1. INTRODUCTION

Seaweeds

Seas and oceans represent a big store for beneficial algae. It is a real fact that the importance of marine organisms as a source of new substances is growing. With marine species comprising approximately a half of the total global biodiversity, the sea offers an enormous resource for novel compounds, and it has been classified as the largest remaining reservoir of natural molecules to be evaluated for drug activity. A very different kind of substances have been obtained from marine organisms among other reasons because they are living in a very exigent, competitive and aggressive surrounding very different in many aspects from the terrestrial environment, a situation that demands the production of quite specific and potent active molecules.

Marine algae are one of the largest producers of biomass in the marine environment (Bhadury and Wright, 2004). They produce a wide variety of chemically active metabolites in their surroundings, potentially as an aid to protect themselves against the other settling organisms. These active metabolites, also known as biogenic compounds, such as halogenated compounds, alcohols, aldehydes, terpenoids are produced by several species of marine macro and microalgae and have antibacterial, antialgal, antimacrofouling and antifungal properties which are effective in the prevention of biofouling and have other likely uses, as in therapeutics (Smit, 2004).

Seaweeds are the eukaryotic organisms that live in salty water and are recognized as a potential source of bioactive natural products. Seaweeds have been used since ancient times as food, fodder, fertilizer and as source of medicine. Today, seaweeds are the raw materials for

many industrial productions like agar, algin and carrageenan but they continue to be widely consumed as food in Asian countries (Mishra *et al*., 1993). In the sea, 3 types of plants occur and they are phytoplanktons, seaweeds or marine algae and seagrasses. Phytoplanktons are microscopic and free floating forms, they are the primary producers of the sea. Seaweeds or marine algae are macroscopic, attached or freely floating plants. They form one of the important marine living renewable resources. They are primitive plants without any true root, stem and leaves. They belong to the division of Thallophyta in plant kingdom. Marine algae are classified into four groups namely Chlorophyceae (green algae), Phaeophyceae (brown algae), Rhodophyceae (red algae) and Cyanophyceae (blue-green algae) based on the type of pigments, morphological, anatomical and reproductive structures.

PHARMACOLOGICAL PROPERTIES OF SEAWEEDS

Seaweeds represent a potential source of antimicrobial substances due to their diversity of secondary metabolites with antiviral, antifungal, antibacterial and antifungal activities (Del Val *et al*., 2001). The antibacterial activity of seaweeds was generally assayed using extracts in various organic solvents *viz*., acetone, methanol-toluene, petroleum ether and chloroform-methanol. Several extractable compounds, such as cyclic polysulfides and halogenated compounds are toxic to microorganisms and therefore, responsible for the antibiotic activity of some seaweeds (Wrattens and Faulkner, 1976). Similarly seaweeds are known for their protein diversity i.e. lectins, hexose oxidase, bromoperoxidase, chitinase, *etc* (Groen *et al*., 1997) but little attention has been given to their potential as antimicrobial agents.

The extracts and active constituents of various algae have been shown to have *in vitro* antibacterial activity against Gram positive and Gram negative bacteria. The production of antimicrobial activities was considered to be an indicator of the capacity of the seaweeds to synthesize bioactive secondary metabolites (Nair *et al*., 2007). There are numerous reports of compounds derived from macroalgae with a broad range of biological activities, such as

antibacterial, antifungal, antiviral, antitumoral, anticoagulant and antifouling. Compounds with cytostatic, antiviral, antihelminthic, antifungal and antibacterial activities have been detected in green, brown and red algae. Also, considering their great taxonomic diversity, investigations related to the search of new biologically active compounds from algae can be seen as an almost unlimited field (Athukorola *et al*., 2006).

The various red algae particularly *Corallina officinalis*, *Corallina rubens* and *Alsidium helminthocorton* were employed as vermifuges in ancient times. Dulse is a laxative and also used to reduce fever. Several red algae such as *Chondrus crispus*, *Gracilaria* sp., *Gelidium* sp. and *Pterocladia* sp. have been used to treat various stomach and intestinal disorders. The stipes of *Laminaria cloustoni* have been used aiding child birth by distending the uterus during labour. A number of marine algae have been found to have anticoagulant and antibiotic properties. *Carrageenan* was used in ulcer therapy and alginates are found to prolong the rate of activity of certain drugs. Species of *Sargassum* were used for cooling and blood cleaning. *Hypnea musciformis* was employed as vermifuge or worm expelling agent and *Centroceras clavulatum* as cathartic agent. The iodine rich seaweeds such as *Asparagopsis taxiformis* and *Sarconema furcellatum* can be used for controlling goitre disease caused by the enlargement of thyroid gland. Many bioactive compounds can also be obtained from seaweeds. The fuel gas for domestic use can be produced from the brown algae *Sargassum* sp.

SEAWEEDS IN INDIA

Seaweeds occur in the intertidal, shallow and deep waters of the sea upto 180 m depth and also in estuaries and backwaters. They grow on dead corals, rocks, stones, pebbles, other substrates and as epiphytes on seagrasses. Several species of green, brown and red algae with luxuriant growth occur along the Southern Tamil Nadu Coast from Rameswaram to Kanyakumari covering 21 islands of Gulf of Mannar. In Gujarat coast, seaweeds occur abundantly in Okha, Dwarka, Porbandar, Veraval, Diu and Gopnath areas. Rich seaweed beds

are present at Mumbai, Ratnagiri, Goa, Karwar, Varkala, Vizhinjam, Visakhapatnam and coastal lakes of Pulicat and Chilka. Seaweeds also occur abundantly in Lakshadweep, Andaman and Nicobar Islands. More than 10,000 species of marine algae have been reported all over the world. In India, about 220 genera and 740 species of marine algae were recorded of which 60 species are of economic value. In Mandapam area 180 species of seaweeds are growing, of which about 40 species are economically important.

It is estimated from the seaweed resources, survey conducted so far by the Central Marine Fisheries Research Institute, National Institute of Oceanography and other research organizations at different maritime states of India and Lakshadweep that the total standing crop of seaweeds in the intertidal and shallow waters is 91339 tonnes (wet wt.) consisting of 6000 tonnes of agar yielding seaweeds, 16000 tonnes of algin yielding seaweeds, remaining edible and other seaweeds. The standing crop of seaweeds in deep waters (5 to 22 m depths) from Dhanushkodi to Kanyakumari was estimated at 75373 tonnes (wet wt.) in an area of 1863 sq. km. The biomass of economically important seaweeds of Gulf of Mannar was estimated at 8445 tonnes (wet wt).

Marine algae are not only the primary and major producers of organic matter in the sea, but they also exert profound effects on the density and distribution of other inhabitants of the marine environment. An understanding of the wide range of behavioral relationships that exist among organisms would provide us with clues to substances of biomedical interest. Marine secondary metabolites are organic compounds produced by microbes, sponges, seaweeds and other marine organisms. The host organisms biosynthesizes these compounds as non-primary or secondary metabolites to protect themselves and to maintain homeostasis in their environment. Some of these secondary metabolites offer avenues for developing cost effective, safe and potent drugs. Nearly 50 lakhs species available in the sea are virtually untapped sources of secondary metabolites. Those compounds already isolated from seaweeds are

providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammation (Elena *et al*., 2001) apart from their potential ecological/industrial significances such as controlling reproduction, settlement/biofouling and feeding deterrents (Selvin, 2002).

Tamil Nadu has a geographical extent of 1, 30,058 sqm. It can be divided into two divisions namely the Eastern coastal plains and hills of North and East, which is endowed with the varied coastal habitat like mangroves, corals, seaweeds, seagrass beds, salt marshes, mud flats, sand dunes etc. The coast of Tamil Nadu bears luxuriant growth of seaweeds. More than two hundred species of seaweeds have been found in this area. Indian seaweed industries depend on this coastline for raw materials regarding production of agar and sodium alginate. They are consumed in the form of soups as well as salads. The intake of seaweeds in the diet is said to prevent hair loss in men and women. It is also consumed by pregnant and lactating mothers because of their rich iron content. They are called the medical food of the 21st century (Isnansetyo and Kamei, 2003).

OBJECTIVES OF THE PROJECT

1. To Collect and identify selected seaweeds
2. To Prepare crude extracts from the selected seaweeds
3. To test antibacterial activity by disc diffusion method and minimum inhibitory concentration method
4. To test antifungal activity by disc diffusion method and minimum inhibitory concentration method
5. To Separate and identify bioactive compounds from most promising seaweeds using
    a) column chromatography (CC)
    b) Thin layer chromatography (TLC)
    c) Gas chromatography- Mass spectrometry (GC-MS)

d) Infrared spectroscopy (Fourier Transform)

e) Ultraviolet–visible spectroscopy

f) ^{1}H Nuclear Magnetic Resonance

g) ^{13}C Nuclear Magnetic Resonance

12.2. MATERIALS AND METHODS

12.2.1. COLLECTION OF SEAWEEDS

The seaweeds *viz*., *Ulva reticulata, Ulva lactuca, Caulerpa racemosa, Hypnea musciformis, Hypnea valentiae, Gracilaria corticata, Gracilaria edulis, Gelidiella acerosa, Sargassum myriocystum* and *Padina gymnospora* were collected from Mandapam coast of Tamil Nadu, India (Fig.1) that is situated in $9^{o}17'$N latitude and $79^{o}07'$E longitude and having 9 m MSL in Tamil Nadu. The seaweeds were taxonomically identified at the Centre for Advanced Studies in Marine Biology, Annamalai University.

12.2.2. PREPARATION OF SEAWEED EXTRACTS

The collected seaweeds samples were cleaned and the necrotic parts were removed. The seaweeds washed with tap water to remove any associated debris and shade dried at room temperature (28 ± 2^{o}C) for 5-8 days or until they are brittle easily by hand. After completely drying, the seaweed materials (1.0 kg) were ground to a fine powder using electrical blender. Forty gram of powdered seaweeds were extracted successively with 200 ml of solvents (methanol, acetone, chloroform, hexane and ethyl acetate) in Soxhlet extractor until the extract was clear. The extracts were evaporated to dryness by reduced pressure using rotary vacuum evaporator and the resulting pasty form extracts were stored in a refrigerator at 4°C for future use.

12.2.3. COLLECTION OF TEST BACTERIAL CULTURES

Eleven different bacterial cultures of Gram positive and Gram negative bacteria were procured from Microbial Type Culture Collection (MTCC), Chandigarh.

12.2.3.1. Gram positive bacteria

a) Staphylococcus aureus (MTCC 3160)

b) Streptococcus epidermidis (MTCC 889*)*

c) Streptococcus pyogenes (MTCC 1926*)*

d) Bacillus subtilis (MTCC 1427)

e) Bacillus cereus (MTCC 7417).

12.2.3.2. Gram negative bacteria

a) *Escherichia coli* (MTCC 1195)

b) *Pseudomonas aeruginosa* (MTCC7093)

c) *Vibrio cholerae* (MTCC 3904)

d) *Salmonella typhi* (MTCC 3215)

e) *Klebsiella pneumoniae* (MTCC 4032)

f) Enterobacter aerogenes (MTCC 6804).

12.2.4. COLLECTION OF TEST FUNGAL CULTURES

Six different fungal isolates were used in this present study. The fungal cultures were procured from Microbial Type Culture Collection (MTCC), Chandigarh.

a) *Aspergillus flavus* (MTCC 1883)

b) *Aspergillus niger* (MTCC 4285)

c) *Aspergillus fumigatus* (MTCC 4964)

d) *Candida albicans* (MTCC 7315)

e) *Candida glabrata* (MTCC 3983).

12.2.5. CULTURES MAINTENANCE AND INOCULUM PREPARATION

12.2.5.1. Maintenance of test bacterial cultures

The test bacterial isolates were sub-cultured and maintained on Nutrient agar slants and stored in refrigerator at 4°C.

12.2.5.2. 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.

12.2.5.3. Maintenance of test fungal cultures

The test fungal isolates were sub-cultured and maintained on Sabouraud's dextrose agar slants and stored in refrigerator at 4°C.

12.2. 5.4. Fungal inoculum preparation

Fungal inoculum was prepared by inoculating a loopful of test organisms in 5 ml of Sabouraud's dextrose broth and incubated at 28°C for 2 days (yeasts) and 3 days (moulds) till a moderate turbidity was developed. The turbidity was matched with 0.5 Mc Farland standards and then used for the determination of antifungal activity.

12.2.6. DISC PREPARATION

12.2.6.1. Preparation of algal disc for antibacterial activity

6 mm diameter discs were prepared using sterile Whatmann No.1 filter paper. The seaweeds crude extracts (5 mg/ml) obtained using solvents (methanol, acetone, chloroform, hexane and ethyl acetate) were mixed with 1ml of 5% Dimethyl sulfoxide (DMSO). The discs were impregnate with 20 µl of different solvent extracts to check their antibacterial activity. The Ampicillin (5 mg/ml) was used as positive control and the 5% DMSO was used as a blind control.

12.2.6.2. Preparation of algal disc for antifungal activity

6 mm diameter discs were prepared using sterile Whatmann No.1 filter paper. The seaweeds crude extracts (5 mg/ml) obtained using solvents (methanol, acetone, chloroform,

hexane and ethyl acetate) were mixed with 1ml of 5% Dimethyl sulfoxide (DMSO). The discs were impregnated with 20 µl of different solvent extracts of seaweeds to check their antifungal activity. The Flucanozole (5mg/ml) was used as positive control and the 5% DMSO was used as a blind control.

12.2.7. ANTIBACTERIAL ASSAY

12.2.7.1. Disc diffusion method

The antibacterial activity of seaweed extracts were determined by disc diffusion method. Petriplates were prepared by pouring 20 ml of Mueller Hinton agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 ml of standardized inoculum suspension was poured and uniformly spreaded. The excess inoculum was drained and the plates were allowed to dry for five minutes. After drying, the discs with extract were placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the agar surface. The Ampicillin (5 mg/ml) was used as positive control and the 5% DMSO was used as a blind control in these assays. The plates were incubated at 37°C for 24 hours. The zone of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times for concordance.

12.2.7.2. Minimum inhibitory concentration for bacteria

Minimum inhibitory concentration (MIC) of the seaweed extracts against bacterial isolates was tested in Mueller Hinton broth by broth macro dilution method. The seaweed extracts were dissolved in 5% DMSO to obtain 128mg/ml stock solutions. 0.5 ml of stock solution was incorporated into 0.5 ml of Mueller Hinton broth for bacteria to get a concentration of 64, 32, 16, 8, 4, 2 and 1 mg/ml for seaweeds extracts and 50 µl of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and devoid of seaweed extracts. The culture tubes were incubated at 37^{o}C for 24 hours. The lowest concentration, which did not show any growth of tested

organism after macroscopic evaluation was determined as minimum inhibitory concentration (MIC).

12.2.8. ANTIFUNGAL ASSAY

12.2.8.1. Disc diffusion method

The antifungal activity of seaweed extracts were determined by disc diffusion method. Petri plates were prepared by pouring 20 ml of Sabouraud's dextrose agar and allowed to solidify for the use in susceptibility test against bacteria. Plates were dried and 0.1 ml of standardized inoculum suspension was poured and uniformly spreaded. The excess inoculum was drained and the plates were allowed to dry for five minutes. After drying the discs with extract were placed on the surface of the plate with sterile forceps and gently pressed to ensure contact with the agar surface. The flucanozole (5mg/ml) was used as positive control and the 5% DMSO was used as a blind control in these assays. The plates were incubated at 28°C for 48 hours (yeasts) and 72 hours (molds). The zone of inhibition was observed and measured in millimeters. Each assay in these experiments was repeated three times for concordance.

12.2.8.2. Minimum inhibitory concentration for fungi

Minimum inhibitory concentration (MIC) of the seaweed extracts against fungal isolates was tested in Sabouraud's dextrose broth by broth macro dilution method. The seaweed extracts were dissolved in 5% DMSO to obtain 128 mg/ml stock solutions. 0.5 ml of stock solution was incorporated into 0.5 ml of Sabouraud's dextrose broth for fungi to get a concentration of 64, 32, 16, 8, 4, 2 and 1 mg/ml for seaweeds extracts and 50 µl of standardized suspension of the test organism was transferred on to each tube. The control tube contained only organisms and devoid of seaweed extracts. The culture tubes were incubated at 28^{o}C for 48 hours (yeasts) and 72 hours (moulds). The lowest concentration, which did not show any growth of tested organism after macroscopic evaluation was determined as minimum inhibitory concentration (MIC).

12.2.9. ANALYSIS OF BIOACTIVE COMPONENTS FROM PROMISING SEAWEED

12.2.9.1. Column Chromatography

The methanol extract of the dried powder of *Gracilaria corticata* was adsorbed onto silica gel by triturating in a mortar and allowed to dry. The column (2cm × 25cm) was packed with a solution of silica gel with benzene using the wet slurry method. This involves preparing a solution of silica gel, with benzene in this case, in a beaker and subsequently adding this upto the column till it is about three-fourths filled. The solution was stirred for dispersal and quickly added to the column before the gel settles. A ball of wool was pushed into the column to settle a top of the packed silica gel. A substantial amount of methanol was poured continuously into the column and allowed to drain but prevented from reaching the cotton wool. The collected quantity was poured back into the column. Periodically, a piece of rubber tubing was used to agitate the column to allow for the escape of trapped air bubbles. About 20 fractions are eluted and collected in dry glass bottles. The column fractions were again tested with TLC chromatogram and the Rf values were determined. The fractions with similar Rf value were combined together and kept for bioautography screening

12.2.9.2. Thin Layer Chromatography (TLC)

Thin Layer Chromatography was carried out to monitor the course of the reaction and purity of the product. The melting points were recorded in open capillaries and are uncorrected.

12.2.9.3. Gas Chromatography - Mass Spectrometry (GC-MS)

Mass spectrum of CD6 was recorded on a Varian GC-MS-Saturn 2200 mass spectrometer, capillary column (carbon) Vf5MS of 30 m length, 0.25 mm internal

diameter and 0.25 µm film thickness. Temperature of column ranges from 50°C to 280^0C (10^0C/min) and injector temperature was maintained at 250°C.

The sample was diluted with HPLC grade $CDCl_3$ and injected in split injection mode in the ration of 1 ml or less. The methanol extract of *Gracilaria corticata* was analyzed using Gas chromatography. It has operated in the electron ionization (EI) mode at 1.88EV.

12.2.9.4. Fourier Transforms Infrared Spectroscopy (FTIR)

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plants extract. The IR Affinity was incorporated with an auto dryer that electrolytically removes the moisture inside the interferometer using a solid polymer electrolyte membrane. One milligram of sample was dissolved in 10 ml of water and the spectra were recorded at 200–400 nm range. The infrared spectra were recorded on Shimadzu IR-470 model. The spectra were scanned in the 400 to 4000 cm-1 range. The spectra were obtained using potassium bromide pellet technique. Potassium bromide was dried under vacuum at 100°C for 48 h and 100 mg of KBr with 1 mg of sample was taken to prepare KBr pellet. The spectra were plotted as intensity versus wave number.

12.3. EXPERIMENTAL RESULTS

The bioactivity of ten different marine seaweeds crude extracts *viz*., *Ulva reticulata*, *Ulva lactuca*, *Caulerpa racemosa*, *Hypnea musciformis*, *Hypnea valentiae*, *Gracilaria corticata*, *Gracilaria edulis*, *Gelidiella acerosa*, *Sargassum myriocystum* and *Padina gymnospora* were evaluated against pathogenic bacteria (*Staphylococcus aureus, Streptococcus pyogenes, Streptococcus epidermidis, Bacillus subtilis, Bacillus cereus, Escherichia coli, Pseudomonas aeruginosa, Vibrio cholerae, Salmonella typhi, Klebsiella pneumoniae* and *Enterobacter aerogenes*), fungi (*Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Candida albicans* and *Candida glabrata*). Five different solvents *viz*., methanol, acetone, chloroform, hexane and ethyl acetate were used for the extraction of seaweeds. The seaweeds crude extracts were evaporated to dryness and the resulting pasty form seaweeds crude extracts were used for antibacterial and antifungal activity. The results of the present findings were given below.

12.3.1. Antibacterial activity of crude seaweeds extracts

Ulva reticulata

The antibacterial activity of marine seaweed crude extracts of *Ulva reticulata* was investigated against Gram positive and Gram negative bacteria and the results were given in Table-1. The methanol crude extract of *Ulva reticulata* (5.0 mg/ml) showed highest mean zone of inhibition against the Gram positive cocci *Streptococcus pyogenes* (17± 0.6 mm) and in Gram negative bacteria *Klebsiella pneumoniae* (15 ± 0.6 mm). No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin (5 µg) showed zone of inhibition was ranging from 16 ± 0.8 mm to 22 ± 0.8 mm against the tested bacterial pathogens. The

lowest MIC (2 mg/ml) value of methanol crude extract was recorded against *Staphylococcus aureus, Bacillus subtilis*, *Bacillus cereus, Klebsiella pneumoniae* and *Enterobacter aerogenes.*

Table 1: Antibacterial activity of crude extracts of *Ulva reticulata*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Staphylococcus aureus	15±0.3	15±0.2	12±0.3	11±0.4	14±0.2	18±0.5	2	4	16	16	4	4
Streptococcus pyogenes	17±0.6	14±0.8	12±0.3	11±0.7	15±0.6	20±0.3	4	8	16	32	8	8
Streptococcus epidermidis	14±0.6	14±0.7	12±0.5	11±0.2	13±0.8	16±0.8	4	8	16	32	8	8
Bacillus subtilis	13±0.6	12±0.5	11±0.2	10±0.8	11±0.3	21±0.6	2	4	8	16	4	8
Bacillus cereus	12±0.5	12±0.7	11±0.6	10±0.6	11±0.6	19±0.5	2	4	8	16	8	8
Escherichia coli	14±0.8	14±0.8	12±0.5	11±0.4	13±0.5	19±0.3	4	8	16	32	16	4
Pseudomonas aeruginosa	12±0.3	13±0.7	10±0.2	11±0.5	12±0.5	20±0.7	4	8	16	32	8	4
Vibrio cholera	11±0.6	11±0.3	10±0.5	11±0.7	12±0.4	18±0.5	8	16	32	32	16	16
Salmonella typhi	11±0.3	12±0.6	11±0.6	10±0.6	10±0.3	21±0.6	8	16	32	64	32	16
Klebsiella pneumoniae	15±0.6	13±0.8	10±0.5	10±0.4	12±0.9	22±0.8	2	4	16	32	8	8
Enterobacter aerogenes	13±0.3	14±0.4	11±0.5	9±0.4	12±0.5	19±0.4	2	2	16	32	8	8

Mean ± SD, *Ampicillin (5 µg)

Ulva lactuca

The antibacterial activity of marine seaweeds crude extracts of *Ulva lactuca* was investigated against Gram positive and Gram negative bacteria and the results were given in the Table-2. The *Ulva lactuca* methanol crude extract (5.0 mg/ml) showed maximum zone of inhibition against the Gram positive bacilli *Bacillus cereus* (18 ± 0.3 mm) and Gram negative bacteria *Enterobacter aerogenes* (17 ± 0.7 mm). No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin (5 µg) showed zone of inhibition was ranging from 14 ± 0.8 mm to 22 ± 0.8 mm against the tested bacterial pathogens. The lowest MIC (1 mg/ml) value of methanol crude extract was recorded against *Klebsiella pneumoniae.*

Caulerpa racemosa

The antibacterial activity of marine seaweed crude extracts of *Caulerpa racemosa* was investigated against Gram positive and Gram negative bacteria and the results were given in the Table-3. The methanol crude extract of *Caulerpa racemosa* (5.0 mg/ml) showed maximum zone of inhibition against the Gram positive bacilli *Bacillus cereus* (16 ± 0.6 mm) and Gram negative bacteria *Enterobacter aerogenes* (16 ± 0.3 mm). No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin (5 µg) showed zone of inhibition was ranging from 14 ± 0.8 mm to 22 ± 0.8 mm against the tested bacterial pathogens. The lowest MIC (1 mg/ml) value of methanol crude extract was recorded against *Staphylococcus aureus, Bacillus subtilis* and *Enterobacter aerogenosa.*

Hypnea musciformis

The antibacterial activity of marine seaweed crude extracts of *Hypnea musciformis* was investigated against Gram positive and Gram negative bacteria and the results were given in the Table-4. The methanol crude extract of *Hypnea musciformis* (5.0 mg/ml) showed highest mean zone of inhibition against the Gram positive cocci *Streptococcus epidermidis*

(16 ± 0.4 mm) and Gram negative bacteria *Pseudomonas aeruginosa* (15 ± 0.8 mm). No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin (5 µg) showed zone of inhibition was ranging from 14 ± 0.8 mm to 22 ± 0.8 mm against the tested bacterial pathogens. The lowest MIC (1 mg/ml) value of methanol crude extract was recorded against *Klebsiella pneumoniae*.

Table-2: Antibacterial activity of crude extracts of *Ulva lactuca*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal Inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Staphylococcus aureus	13±0.6	15±0.3	11±0.4	12±0.7	12±0.3	18±0.5	2	4	16	32	8	4
Streptococcus pyogenes	16±0.3	15±0.5	12±0.3	10±0.6	13±0.5	20±0.3	4	8	32	64	8	8
Streptococcus epidermidis	14±0.3	15±0.7	13±0.5	11±0.5	14±0.4	16±0.8	4	8	16	32	8	8
Bacillus subtilis	17±0.2	15±0.4	13±0.4	12±0.3	11±0.6	21±0.6	2	4	8	16	4	8
Bacillus cereus	18±0.3	16±0.2	11±0.5	10±0.4	11±0.5	19±0.5	2	4	8	16	8	8
Escherichia coli	17±0.2	16±0.4	14±0.6	12±0.3	12±0.4	19±0.3	2	4	8	16	4	4
Pseudomonas aeruginosa	15±0.6	15±0.5	13±0.5	11±0.2	12±0.3	20±0.7	4	8	16	32	8	4
Vibrio cholera	12±0.2	13±0.3	14±0.4	13±0.4	11±0.5	18±0.5	8	16	32	64	32	16
Salmonella typhi	15±0.3	14±0.4	11±0.3	11±0.6	11±0.6	21±0.6	16	16	64	64	32	16
Klebsiella pneumoniae	16±0.6	16±0.6	15±0.2	11±0.3	13±0.7	22±0.8	1	2	8	16	4	8
Enterobacter aerogenes	17±0.7	15±0.3	14±0.5	11±0.5	14±0.8	19±0.4	2	2	8	16	4	8

Mean ± SD, * positive control- Ampicillin (5 µg)

Table-3: Antibacterial activity of crude extracts of *Caulerpa racemosa*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal Inhibitory Concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Staphylococcus aureus	15±0.3	13±0.3	12±0.5	11±0.3	12±0.5	18±0.5	1	2	8	16	4	4
Streptococcus pyogenes	14±0.2	13±0.2	11±0.6	10±0.5	12±0.3	20±0.3	2	4	16	32	8	8
Streptococcus epidermidis	13±0.4	13±0.4	10±0.5	10±0.3	12±0.2	16±0.8	4	4	16	32	8	8
Bacillus subtilis	14±0.3	13±0.3	12±0.9	10±0.4	12±0.5	21±0.6	1	2	8	16	4	8
Bacillus cereus	16±0.6	13±0.5	10±0.5	9±0.3	11±0.4	19±0.5	4	8	32	32	16	8
Escherichia coli	14±0.3	12±0.4	11±0.5	10±0.4	11±0.5	19±0.3	2	4	16	32	8	4
Pseudomonas aeruginosa	15±0.4	13±0.3	11±0.3	10±0.3	13±0.3	20±0.7	2	4	16	16	8	4
Vibrio cholera	11±0.3	10±0.5	9±0.2	9±0.5	10±0.3	18±0.5	4	8	32	64	16	16
Salmonella typhi	12±0.6	12±0.4	10±0.2	9±0.3	11±0.5	21±0.6	4	4	16	32	8	16
Klebsiella pneumoniae	15±0.5	14±0.5	11±0.3	9±0.4	13±0.3	22±0.8	2	4	16	16	8	8
Enterobacter aerogenes	16±0.3	14±0.3	11±0.5	10±0.3	12±0.2	19±0.4	1	2	8	16	4	8

Mean ± SD, * positive control- Ampicillin (5 µg)

Table-4: Antibacterial activity of crude extracts of *Hypnea musciformis*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Staphylococcus aureus	15±0.2	14±0.3	12±0.2	12±0.3	14±0.3	14±0.5	4	8	16	32	8	4
Streptococcus pyogenes	15±0.3	15±0.6	12±0.5	11±0.3	13±0.6	20±0.3	2	4	8	16	4	8
Streptococcus epidermidis	16±0.4	15±0.3	13±0.4	11±0.6	14±0.5	16±0.8	2	4	16	32	8	8
Bacillus subtilis	14±0.5	13±0.4	12±0.3	10±0.5	12±0.4	21±0.6	2	4	8	16	8	8
Bacillus cereus	14±0.6	14±0.5	12±0.5	12±0.4	12±0.3	19±0.5	2	4	16	32	16	8
Escherichia coli	15±0.7	14±0.5	12±0.3	12±0.5	13±0.4	19±0.3	4	8	16	32	8	4
Pseudomonas aeruginosa	15±0.8	15±0.6	12±0.2	12±0.4	14±0.6	20±0.7	4	8	16	32	16	4
Vibrio cholerae	12±0.4	11±0.6	11±0.3	10±0.3	11±0.3	18±0.5	8	16	32	64	32	16
Salmonella typhi	12±0.6	13±0.3	11±0.2	12±0.6	12±0.3	21±0.6	8	16	32	64	32	16
Klebsiella pneumoniae	13±0.5	12±0.5	11±0.4	10±0.5	12±0.5	22±0.8	1	2	8	16	4	8
Enterobacter aerogenes	14±0.4	14±0.4	13±0.6	11±0.5	11±0.7	19±0.4	2	4	8	16	8	8

Mean ± SD, * positive control- Ampicillin (5 µg)

Hypnea valentiae

The antibacterial activity of marine seaweed crude extracts of *Hypnea valentiae* was investigated against Gram positive and Gram negative bacteria and the results were given in the Table-5. The *Hypnea valentiae* methanol crude extract (5.0 mg/ml) showed maximum zone of inhibition against the Gram positive bacilli *Bacillus cereus* (19 ± 0.5 mm) and Gram negative bacteria *Escherichia coli* (18 ± 0.6 mm). No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin (5 µg) showed zone of inhibition was ranging from 14 ± 0.8 mm to 22 ± 0.8 mm against the tested bacterial pathogens. The lowest MIC (1 mg/ml) value of methanol crude extract was recorded against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*

Gracilaria corticata

The antibacterial activity of marine seaweed crude extracts of *Gracilaria corticata* was investigated against bacteria and the results were given in the Table-6. The methanol crude extract of *Gracilaria corticata* (5.0 mg/ml) showed highest mean zone of inhibition against the Gram positive cocci *Streptococcus pyogenes* (20 ± 0.4 mm) and Gram negative bacteria *Klebsiella pneumoniae* (19 ± 0.5 mm). No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin (5 µg) showed zone of inhibition was ranging from 14 ± 0.8 mm to 22 ± 0.8 mm against the tested bacterial pathogens. The lowest MIC (1mg/ml) value of methanol crude extract was recorded against *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus epidermidis, Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Enterobacter aerogenes.*

Gracilaria edulis

The antibacterial activity of marine seaweed crude extracts of *Gracilaria edulis* was investigated against Gram positive and Gram negative bacteria and the results were given in the Table-7. The *Gracilaria edulis* methanol crude extract (5.0 mg/ml) showed maximum

zone of inhibition against the Gram positive cocci *Staphylococcus aureus* (19 ± 0.4 mm) and Gram negative bacteria *Enterobacter aerogenes* (17 ± 0.5 mm). No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin (5 µg) showed zone of inhibition was ranging from 16 ± 0.8 mm to 22 ± 0.8 mm against the tested bacterial pathogens. The lowest MIC (1mg/ml) value of methanol crude extract was recorded against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and *Klebsiella pneumonia.*

Table-5: Antibacterial activity of crude extracts of *Hypnea valentiae*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal Inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Staphylococcus aureus	17±0.6	17±0.5	15±0.7	14±0.3	16±0.6	20±0.5	1	2	8	16	4	4
Streptococcus pyogenes	16±0.4	16±0.6	16±0.5	14±0.3	17±0.3	22±0.3	2	4	8	16	4	8
Streptococcus epidermidis	18±0.6	15±0.4	16±0.5	15±0.5	17±0.2	18±0.8	2	4	8	16	8	8
Bacillus subtilis	15±0.3	17±0.5	17±0.4	13±0.4	17±0.4	23±0.6	1	2	8	8	4	8
Bacillus cereus	19±0.5	17±0.3	14±0.5	14±0.3	18±0.5	21±0.5	1	1	4	8	4	8
Escherichia coli	18±0.6	16±0.6	15±0.4	14±0.7	15±0.6	21±0.3	2	4	8	16	4	4
Pseudomonas aeruginosa	16±0.3	18±0.7	14±0.3	13±0.6	17±0.5	22±0.7	1	2	8	8	4	4
Vibrio cholerae	16±0.5	15±0.3	15±0.4	13±0.3	13±0.3	20±0.5	4	8	16	32	8	16
Salmonella typhi	17±0.4	14±0.2	13±0.5	12±0.2	13±0.4	23±0.6	4	8	32	64	16	16
Klebsiella pneumoniae	15±0.3	14±0.5	11±0.4	11±0.3	13±0.3	22±0.8	1	2	8	16	4	8
Enterobacter aerogenes	15±0.2	15±0.4	13±0.6	12±0.4	14±0.5	19±0.4	2	4	16	16	8	8

Mean ± SD, * positive control- Ampicillin (5 µg)

Table-6: Antibacterial activity of crude extracts of *Gracilaria corticata*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal Inhibitory Concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Staphylococcus aureus	19±0.3	18±0.3	13±0.4	12±0.4	12±0.6	18±0.5	1	1	4	8	2	4
Streptococcus pyogenes	20±0.4	16±0.5	12±0.3	9±0.5	12±0.5	20±0.3	1	2	8	8	4	8
Streptococcus epidermidis	18±0.6	17±0.3	14±0.5	11±0.6	14±0.3	16±0.8	1	2	8	16	4	8
Bacillus subtilis	19±0.5	18±0.3	14±0.3	13±0.5	16±0.4	21±0.6	1	1	4	4	2	8
Bacillus cereus	18±0.2	15±0.8	15±0.4	14±0.3	16±0.5	19±0.5	1	1	4	8	2	8
Escherichia coli	18±0.3	14±0.6	16±0.6	13±0.3	16±0.3	19±0.3	2	2	8	8	4	4
Pseudomonas aeruginosa	18±0.5	14±0.3	15±0.3	14±0.4	15±0.3	20±0.7	2	4	8	16	8	4
Vibrio cholerae	13±0.4	10±0.4	10±0.4	10±0.6	12±0.4	18±0.5	4	8	32	64	16	16
Salmonella typhi	18±0.6	17±0.4	14±0.5	14±0.4	15±0.6	21±0.6	4	4	16	32	8	16
Klebsiella pneumoniae	19±0.5	17±0.6	14±0.4	11±0.5	16±0.5	22±0.8	1	2	4	8	2	8
Enterobacter aerogenes	18±0.3	17±0.7	15±0.5	14±0.4	16±0.6	19±0.4	1	1	4	4	2	8

Mean ± SD, * positive control- Ampicillin (5 µg)

Table-7: Antibacterial activity of crude extracts of *Gracilaria edulis*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal Inhibitory Concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Staphylococcus aureus	19±0.4	18±0.5	15±0.4	12±0.6	15±0.5	18±0.5	1	2	8	16	4	4
Streptococcus pyogenes	17±0.3	15±0.3	13±0.5	12±0.3	13±0.3	20±0.3	2	4	8	16	4	8
Streptococcus epidermidis	16±0.5	15±0.2	13±0.5	12±0.4	14±0.4	16±0.8	2	4	16	32	8	8
Bacillus subtilis	15±0.6	14±0.6	11±0.5	11±0.5	12±0.5	21±0.6	1	2	8	8	4	8
Bacillus cereus	16±0.4	15±0.5	12±0.3	11±0.2	14±0.3	19±0.5	1	2	8	16	4	8
Escherichia coli	15±0.6	14±0.4	12±0.7	11±0.6	13±0.5	19±0.3	2	4	16	16	8	4
Pseudomonas aeruginosa	14±0.4	14±0.6	13±0.5	12±0.3	13±0.3	20±0.7	4	4	16	32	8	4
Vibrio cholerae	13±0.4	12±0.3	11±0.2	11±0.5	12±0.4	18±0.5	8	16	32	32	32	16
Salmonella typhi	15±0.4	14±0.5	12±0.3	11±0.6	13±0.4	21±0.6	8	16	32	64	32	16
Klebsiella pneumoniae	16±0.2	15±0.5	13±0.5	12±0.5	14±0.3	22±0.8	1	2	8	16	4	8
Enterobacter aerogenes	17±0.5	15±0.3	12±0.6	11±0.3	14±0.3	19±0.4	2	4	16	32	8	8

Mean ± SD, * positive control- Ampicillin (5 µg)

Gelidiella acerosa

The antibacterial activity of marine seaweed crude extracts of *Gelidiella acerosa* was investigated against Gram positive and Gram negative bacteria and the results were given in the Table-8. The methanol crude extract of *Gelidiella acerosa* (5.0 mg/ml) showed highest mean zone of inhibition against the Gram positive cocci *Staphylococcus aureus* (14 ± 0.6mm) and Gram negative bacteria *Enterococcus aerogenes* (16 ± 0.2 mm). No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin (5 µg) showed zone of inhibition was ranging from 14 ± 0.8 mm to 22 ± 0.8 mm against the tested bacterial pathogens. The lowest MIC (1 mg/ml) value of methanol crude extract was recorded against *Enterobacter aerogenes.*

Sargassum myriocystum

The antibacterial activity of marine seaweed crude extracts of *Sargassum myriocystum* was investigated against Gram positive and Gram negative bacteria and the results were given in the Table-9. The methanol crude extract of *Sargassum myriocystum* (5.0 mg/ml) showed highest mean zone of inhibition against the Gram positive cocci *Streptococcus pyogenes* (16 ± 0.3 mm) and Gram negative bacteria *Pseudomonas aeruginosa* (15 ± 0.6 mm). No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin (5 µg) showed zone of inhibition was ranging from 14 ± 0.8 mm to 20 ± 0.8 mm against the tested bacterial pathogens. The lowest MIC (1 µg/ml) value of methanol crude extract was recorded against *Staphylococcus aureus, Bacillus subtilis* and *Klebsiella pneumoniae.*

Padina gymnospora

The antibacterial activity of marine seaweed crude extracts of *Padina gymnospora* was investigated against Gram positive and Gram negative bacteria and the results were

given in the Table-10. The methanol crude extract of *Padina gymnospora* (5.0 mg/ml) showed highest mean zone of inhibition against the Gram positive cocci *Staphylococcus aureus* (15 ± 0.3 mm) and Gram negative bacteria *Enterobacter aerogenes* (15 ± 0.4 mm). No zone of inhibition was seen in DMSO blind control and the positive control Ampicillin (5µg) showed zone of inhibition were ranging from 14 ± 0.8 mm to 22 ± 0.8 mm against the tested bacterial pathogens. The lowest MIC (1 mg/ml) value of methanol crude extract was recorded against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

Table-8: Antibacterial activity of crude extracts of *Gelidiella acerosa*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal Inhibitory Concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Staphylococcus aureus	14±0.6	12±0.6	11±0.6	10±0.2	10±0.3	18±0.5	2	4	16	32	8	4
Streptococcus pyogenes	13±0.5	11±0.5	10±0.5	9±0.2	12±0.4	20±0.3	4	8	16	32	8	8
Streptococcus epidermidis	13±0.4	11±0.5	10±0.3	9±0.4	11±0.3	14±0.8	2	4	8	16	4	8
Bacillus subtilis	12±0.2	11±0.4	11±0.2	10±0.3	11±0.2	21±0.6	4	8	32	64	16	8
Bacillus cereus	12±0.2	11±0.5	10±0.3	9±0.5	9±0.2	19±0.5	4	8	32	64	16	8
Escherichia coli	12±0.2	11±0.4	10±0.4	9±0.6	8±0.5	17±0.3	2	4	16	32	8	4
Pseudomonas aeruginosa	14±0.3	13±0.3	10±0.3	9±0.3	11±0.3	20±0.7	4	4	16	32	8	4
Vibrio cholerae	12±0.6	9±0.5	9±0.5	9±0.5	8±0.4	16±0.5	4	8	32	64	16	16
Salmonella typhi	13±0.5	12±0.6	10±0.6	9±0.6	11±0.2	21±0.6	4	8	32	64	16	16
Klebsiella pneumoniae	15±0.4	13±0.2	11±0.3	10±0.4	9±0.3	22±0.8	2	4	16	32	8	8
Enterobacter aerogenes	16±0.2	15±0.3	12±0.4	10±0.3	12±0.2	19±0.4	1	2	8	16	4	8

Mean ± SD, * positive control- Ampicillin (5 µg)

Table-9: Antibacterial activity of crude extracts of *Sargassum myriocystum*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal Inhibitory Concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Staphylococcus aureus	15±0.4	15±0.2	10±0.6	9±0.5	13±0.3	18±0.5	1	2	8	16	4	4
Streptococcus pyogenes	16±0.3	14±0.4	4±0.5	10±0.3	14±0.2	20±0.3	2	4	16	32	8	8
Streptococcus epidermidis	14±0.5	15±0.6	10±0.3	9±0.3	10±0.4	16±0.8	2	4	8	16	4	8
Bacillus subtilis	15±0.6	14±0.6	10±0.2	9±0.4	12±0.6	21±0.6	1	2	8	16	4	8
Bacillus cereus	15±0.3	12±0.3	10±0.2	9±0.6	11±0.6	19±0.5	1	2	4	8	2	8
Escherichia coli	14±0.3	13±0.2	9±0.4	8±0.3	10±0.2	19±0.3	4	8	16	32	8	4
Pseudomonas aeruginosa	15±0.6	14±0.4	9±0.3	8±0.2	11±0.3	20±0.7	4	8	32	32	16	4
Vibrio cholerae	10±0.4	10±0.3	7±0.5	7±0.4	8±0.4	18±0.5	8	8	64	64	32	16
Salmonella typhi	14±0.6	11±0.2	9±0.3	7±0.5	10±0.5	21±0.6	8	16	64	64	32	16
Klebsiella pneumoniae	14±0.4	11±0.4	9±0.5	8±0.3	10±0.4	22±0.8	1	2	8	8	4	8
Enterobacter aerogenes	14±0.3	12±0.5	9±0.4	8±0.3	10±0.2	19±0.4	2	4	16	16	8	8

Mean ± SD, * positive control- Ampicillin (5 µg)

Table-10: Antibacterial activity of crude extracts of *Padina gymnospora*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal Inhibitory Concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Staphylococcus aureus	15±0.3	13±0.3	10±0.5	10±0.4	11±0.3	18±0.5	1	2	8	16	4	4
Streptococcus pyogenes	14±0.2	13±0.4	10±0.3	10±0.5	11±0.4	20±0.3	2	4	16	32	8	8
Streptococcus epidermidis	13±0.3	12±0.2	10±0.5	9±0.3	11±0.5	16±0.8	2	4	16	32	8	8
Bacillus subtilis	12±0.5	12±0.5	8±0.4	9±0.2	11±0.4	21±0.6	1	2	8	16	4	8
Bacillus cereus	13±0.4	12±0.4	11±0.5	8±0.2	13±0.5	19±0.5	2	4	16	32	8	8
Escherichia coli	13±0.3	12±0.3	10±0.6	7±0.7	11±0.3	19±0.3	2	4	16	32	8	4
Pseudomonas aeruginosa	14±0.5	12±0.6	9±0.3.	8±0.6	11±0.2	20±0.7	1	2	8	16	4	4
Vibrio cholerae	10±0.3	10±0.4	9±0.4	7±0.3	10±0.5	18±0.5	4	8	16	32	8	16
Salmonella typhi	12±0.2	13±0.5	11±0.5	7±0.2	12±0.3	21±0.6	4	8	32	64	16	16
Klebsiella pneumoniae	14±0.3	13±0.4	11±0.3	7±0.3	10±0.5	22±0.8	1	2	8	16	4	8
Enterobacter aerogenes	15±0.4	13±0.3	10±0.2	9±0.5	12±0.4	19±0.4	1	2	4	8	4	8

Mean ± SD, * positive control- Ampicillin (5 µg)

12.3.2. Antifungal activity of crude extracts of seaweeds

Ulva reticulata

The antifungal activity of marine seaweed crude extracts of *Ulva reticulata* was investigated against fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans* and *Candida glabrata*) and the results were given in Table-11. The methanol crude extract of *Ulva reticulata* (5 mg/ml) showed highest mean zone of inhibition against *Aspergillus flavus* (15 ± 0.6 mm). No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole (100 units/disc) showed zone of inhibition was ranging from 12 ± 0.7 mm to 17 ± 0.6 mm against the tested fungal pathogens. The lowest MIC (4 mg/ml) value of methanol crude extract was recorded against *Candida albicans* and *Candida glabrata.*

Ulva lactuca

The antifungal activity of marine seaweed crude extracts of *Ulva lactuca* was investigated against fungal isolates and the results were recorded in Table-12. The *Ulva lactuca* methanol extract (5 mg/ml) showed highest mean zone of inhibition against *Candida glabrata* (15 ± 0.5 mm). No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole (100 units/disc) showed zone of inhibition was ranging from 10 ± 0.6 mm to 17 ± 0.6 mm against the tested fungal pathogens. The lowest MIC (4 mg/ml) value of methanol crude extract was recorded against *Candida albicans* and *Candida glabrata.*

Caulerpa racemosa

The antifungal activity of marine seaweed crude extracts of *Caulerpa racemosa* was investigated against fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans* and *Candida glabrata*) and the results were given in Table-13.

The methanol extract of *Caulerpa racemosa* (5 mg/ml) showed highest mean zone of inhibition against *Aspergillus niger (*13 ± 0.5 mm). No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole (100 units/disc) showed zone of inhibition was ranging from 10 ± 0.6 mm to 17 ± 0.6 mm against the tested fungal pathogens. The lowest MIC (2 mg/ml) value of methanol crude extract was recorded against *Aspergillus niger.*

Table-11: Antifungal activity of crude extracts of *Ulva reticulata*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimal Inhibitory Concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Aspergillus flavus	15±0.6	12±0.3	10±0.3	8±0.3	10±0.5	17±0.6	8	8	32	32	16	8
Aspergillus niger	10±0.5	10±0.5	9±0.4	8±0.3	9±0.6	15±0.4	8	16	32	64	32	8
Aspergillus fumigatus	12±0.3	10±0.4	8±0.5	7±0.5	8±0.3	16±0.7	8	8	32	32	16	8
Candida albicans	11±0.4	11±0.4	8±0.4	8±0.5	10±0.4	14±0.7	4	8	16	32	8	4
Candida glabrata	11±0.6	10±0.5	9±0.3	8±0.6	10±0.3	15±0.6	4	8	16	32	16	4

*** Flucanozole- positive control, Mean± SD.**

Table-12: Antifungal activity of crude extracts of *Ulva lactuca*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimum inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Aspergillus flavus	14±0.5	12±0.3	10±0.4	8±0.3	10±0.3	17±0.6	8	8	16	32	16	8
Aspergillus niger	11±0.5	10±0.4	9±0.3	8±0.6	8±0.5	15±0.4	8	16	32	32	16	8
Aspergillus fumigatus	13±0.3	11±0.5	10±0.3	8±0.3	8±0.5	16±0.7	8	8	16	32	16	8
Candida albicans	14±0.6	13±0.3	10±0.3	9±0.5	10±0.5	14±0.7	4	8	16	16	8	4
Candida glabrata	15±0.5	13±0.6	9±0.5	9±0.3	11±0.4	15±0.6	4	8	16	32	16	4

*** Flucanozole- positive control, Mean± SD.**

Table-13: Antifungal activity of crude extracts of *Caulerpa racemosa*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimum inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Aspergillus flavus	12±0.7	12±0.3	10±0.6	9±0.4	11±0.6	17±0.6	4	4	16	32	8	8
Aspergillus niger	13±0.5	12±0.6	10±0.3	8±0.6	11±0.5	15±0.4	2	4	8	16	4	8
Aspergillus fumigatus	10±0.5	10±0.3	9±0.5	8±0.4	9±0.3	16±0.7	4	8	16	32	8	8
Candida albicans	12±0.5	11±0.5	9±0.3	7±0.4	9±0.6	12±0.7	4	4	16	32	8	4
Candida glabrata	11±0.5	10±0.4	9±0.5	9±0.5	9±0.6	15±0.6	4	8	16	32	8	4

*** Flucanozole- positive control, Mean ± SD.**

Hypnea musciformis

The antifungal activity of marine seaweed crude extracts of *Hypnea musciformis* was investigated against fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans* and *Candida glabrata*) and the results were given in Table-14. The methanol extract of *Hypnea musciformis* (5 mg/ml) showed highest mean zone of inhibition against *Candida albicans* (13 ± 0.5 mm). No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole (100 units/disc) showed zone of inhibition was ranging from 10 ± 0.6 mm to 17 ± 0.6 mm against the tested fungal pathogens. The lowest MIC (4 mg/ml) value of methanol crude extract was recorded against *Candida albicans.*

Hypnea valentiae

The antifungal activity of marine seaweed crude extracts of *Hypnea valentiae* was investigated against fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans* and *Candida glabrata*) and the results were given in Table-15. The *Hypnea valentiae* methanol crude extract (5 mg/ml) showed highest mean zone of inhibition against *Aspergillus flavus* (14 ± 0.5 mm). No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole (100 units/disc) showed zone of inhibition was ranging from 10 ± 0.6 mm to 17 ± 0.6 mm against the tested fungal pathogens. The lowest MIC (4 mg/ml) value of methanol crude extract was recorded against *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Candida glabrata.*

Gracilaria corticata

The antifungal activity of marine seaweed crude extracts of *Gracilaria corticata* was investigated against fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans* and *Candida glabrata*) and the results were given in Table-16.

The methanol crude extract of *Gracilaria corticata* (5 mg/ml) showed highest mean zone of inhibition against *Aspergillus flavus* (16 ± 0.6 mm). No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole (100 units/disc) showed zone of inhibition was ranging from 10 ± 0.6 mm to 17 ± 0.6 mm against the tested fungal pathogens. The lowest MIC (2 mg/ml) value of methanol crude extract was recorded against *Candida albicans.*

Table-14: Antifungal activity of crude extracts of *Hypnea musciformis*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimum inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Aspergillus flavus	12±0.3	10±0.7	9±0.3	8±0.5	9±0.5	17±0.6	16	16	32	64	32	8
Aspergillus niger	11±0.5	10±0.8	7±0.3	7±0.4	8±0.6	15±0.4	8	16	32	32	16	8
Aspergillus fumigatus	11±0.3	10±0.6	8±0.5	7±0.7	8±0.4	16±0.7	8	8	16	32	16	8
Candida albicans	13±0.5	11±0.3	7±0.5	8±0.3	9±0.3	14±0.7	4	8	16	16	8	4
Candida glabrata	12±0.6	10±0.3	7±0.3	7±0.4	10±0.5	15±0.6	8	8	16	32	16	4

*** Flucanozole- positive control, Mean± SD.**

Table-15: Antifungal activity of crude extracts of *Hypnea valentiae*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimum inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Aspergillus flavus	14±0.5	12±0.3	9±0.5	9±0.6	11±0.3	17±0.6	4	8	16	16	8	8
Aspergillus niger	12±0.7	12±0.6	10±0.6	9±0.6	12±0.6	15±0.4	4	4	8	8	8	8
Aspergillus fumigatus	12±0.4	11±0.3	10±0.3	10±0.6	12±0.2	16±0.7	8	16	16	32	16	8
Candida albicans	11±0.6	10±0.5	8±0.5	8±0.3	9±0.3	14±0.7	4	4	8	16	8	4
Candida glabrata	11±0.3	11±0.6	9±0.3	7±0.7	9±0.5	5±0.6	4	8	16	16	8	4

*** Flucanozole- positive control, Mean± SD.**

Table-16: Antifungal activity of crude extracts of *Gracilaria corticata*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimum inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Aspergillus flavus	16±0.6	15±0.5	12±0.6	11±0.7	13±0.4	17±0.6	8	16	32	32	16	8
Aspergillus niger	15±0.7	15±0.4	13±0.7	12±0.8	14±0.6	15±0.4	4	8	16	16	8	8
Aspergillus fumigatus	15±0.5	14±0.6	14±0.6	13±0.6	14±0.7	16±0.7	8	8	16	32	16	8
Candida albicans	14±0.8	14±0.4	12±0.7	11±0.7	10±0.4	14±0.7	2	4	8	16	8	4
Candida glabrata	14±0.5	13±0.6	10±0.3	10±0.4	11±0.6	15±0.6	4	8	16	32	8	4

*** Flucanozole- positive control, Mean± SD.**

Gracilaria edulis

The antifungal activity of marine seaweed crude extracts of *Gracilaria edulis* was investigated against fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans* and *Candida glabrata*) and the results were given in Table-17. The *Gracilaria edulis* methanol extract (10 mg/ml) showed highest mean zone of inhibition against *Aspergillus niger* (15 ± 0.6 mm). No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole (100 units/disc) showed zone of inhibition was ranging from 10 ± 0.6 mm to 17 ± 0.6 mm against the tested fungal pathogens. The lowest MIC (2 mg/ml) value of methanol extract was recorded against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Candida glabrata*.

Gelidiella acerosa

The antifungal activity of marine seaweed crude extracts of *Gelidiella acerosa* was investigated against fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans* and *Candida glabrata*) and the results were given in Table-18. The methanol crude extract of *Gelidiella acerosa* (5 mg/ml) showed highest mean zone of inhibition against *Aspergillus fumigatus* (12 ± 0.6 mm). No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole (100 units/disc) showed zone of inhibition was ranging from 10 ± 0.6 mm to 17 ± 0.6 mm against the tested fungal pathogens. The lowest MIC (4 mg/ml) value of methanol crude extract was recorded against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Candida glabrata*.

Sargassum myriocystum

The antifungal activity of marine seaweed crude extracts of *Sargassum myriocystum* was investigated against fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans* and *Candida glabrata*) and the results were given in Table-19.

The methanol crude extract of *Sargassum myriocystum* (5mg/ml) showed highest mean zone of inhibition against *Aspergillus flavus* (14 ± 0.6 mm). No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole (5mg/ml) showed zone of inhibition was ranging from 10 ± 0.6 mm to 18 ± 0.6 mm against the tested fungal pathogens. The lowest MIC (2 mg/ml) value of methanol crude extracts was recorded against *Candida albicans*.

Table-17: Antifungal activity of crude extracts of *Gracilaria edulis*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimum inhibitory concentration (mg/ml)					
	Methanol	**Acetone**	**Chloroform**	**Hexane**	**Ethyl acetate**	**Positive control**	**Methanol**	**Acetone**	**Chloroform**	**Hexane**	**Ethyl acetate**	**Positive control**
Aspergillus flavus	14±0.3	13±0.6	12±0.6	10±0.6	10±0.7	17±0.6	2	4	8	16	4	8
Aspergillus niger	15±0.6	12±0.3	11±0.3	10±0.6	10±0.5	15±0.4	2	4	8	16	8	8
Aspergillus fumigatus	11±0.7	10±0.4	11±0.4	9±0.3	9±0.6	16±0.7	4	8	16	32	16	8
Candida albicans	14±0.4	11±0.6	11±07	9±0.7	10±0.7	14±0.7	2	4	8	16	8	4
Candida glabrata	13±0.5	12±0.7	10±0.6	8±0.4	11±0.6	15±0.6	2	4	8	16	8	4

*** Flucanozole- positive control, Mean± SD.**

Table-18: Antifungal activity of crude extracts of *Gelidiella acerosa*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimum inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Aspergillus flavus	9±0.6	10±0.7	9±0.6	10±0.5	8±0.3	17±0.6	4	8	16	32	8	8
Aspergillus niger	10±0.8	11±0.4	9±0.7	10±0.4	9±0.3	14±0.4	4	4	16	16	8	8
Aspergillus fumigatus	12±0.6	10±0.5	10±0.4	9±0.6	9±0.6	16±0.7	4	8	16	32	8	8
Candida albicans	11±0.5	10±0.3	8±0.5	8±0.6	8±0.6	12±0.7	8	8	16	32	16	4
Candida glabrata	8±0.2	11±0.6	10±0.6	10±0.5	9±0.3	15±0.6	4	8	16	16	8	4

*** Flucanozole- positive control, Mean± SD.**

Table-19: Antifungal activity of crude extracts of *Sargassum myriocystum*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimum inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Aspergillus flavus	14±0.6	12±0.6	11±0.6	10±0.6	11±0.6	17±0.6	8	16	32	32	16	8
Aspergillus niger	12±0.3	11±0.4	10±0.4	11±0.7	10±0.7	15±0.4	4	8	16	16	8	8
Aspergillus fumigatus	13±0.6	11±0.7	11±0.6	9±0.8	10±0.3	16±0.7	8	8	16	32	16	8
Candida albicans	11±0.7	10±0.6	10±0.6	8±0.6	9±0.6	14±0.7	2	4	8	16	8	4
Candida glabrata	12±0.6	10±0.8	8±0.6	8±0.7	8±0.8	15±0.6	4	8	16	32	8	4

*** Flucanozole- positive control, Mean± SD.**

Padina gymnospora

The antifungal activity of marine seaweed crude extracts of *Padina gymnospora* was investigated against fungal pathogens (*Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Candida albicans* and *Candida glabrata*) and the results were given in Table-20. The methanol crude extract of *Padina gymnospora* (5mg/ml) showed highest mean zone of inhibition against *Aspergillus niger* (12 ± 0.6 mm). No zone of inhibition was seen in DMSO blind control and the positive control Flucanozole (100 units/disc) showed zone of inhibition was ranging from 10 ± 0.6 mm to 17 ± 0.6 mm against the tested fungal pathogens. The lowest MIC (2 mg/ml) value of methanol crude extract was recorded against *Aspergillus fumigatus.*

Table-20: Antifungal activity of crude extracts of *Padina gymnospora*

Microorganisms	Zone of inhibition (mm) at 5 mg/ml						Minimum inhibitory concentration (mg/ml)					
	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control	Methanol	Acetone	Chloroform	Hexane	Ethyl acetate	Positive control
Aspergillus flavus	10± 0.6	9±0.7	10±0.6	8±0.5	8±0.6	17±0.6	4	4	16	32	8	8
Aspergillus niger	12±0.6	10±0.5	11±0.3	9±0.4	10±0.4	15±0.4	4	8	16	16	8	8
Aspergillus fumigatus	9±0.6	9±0.5	9±0.6	8±0.6	10±0.5	16±0.7	2	4	16	32	8	8
Candida albicans	10±0.3	8±0.4	10±0.6	9±0.5	8±0.7	14±0.7	4	4	16	32	8	4
Candida glabrata	11±0.7	8±0.6	9±0.5	8±0.6	9±0.6	15±0.6	4	8	16	32	16	4

*** Flucanozole- positive control, Mean± SD.**

12.3.3. GC – MS ANALYSIS OF BIOACTIVE COMPOUNDS

Among the extracts obtained from ten seaweeds, *Gracilaria corticata* showed highest antibacterial and antifungal activity. Hence, *Gracilaria corticata* was selected for compound identification by using GC–MS analysis. This study reveals the presence of following nine different compounds. The details of the components are enlisted in the Table 21. The GC-MS chromatogram for the components is also given in the Figure.

Table - 21: Components identified in the *Gracilaria corticata* sample by GC-MS analysis

S.No	RT	Name of the components	Molecular	MW	Peak Area %
1	10.11	Hexadecane	$C_{16}H_{34}$	226	14.37
2	11.59	1, 10 – Decanediol	$C_{10}H_{22}O_2$	174	4.02
3	11.66	2 – Heptanone, 5- methyl	$C_8H_{16}O$	128	1.72
4	14.92	2,4 – Dimethylcyclopentanol	$C_7H_{14}O$	114	5.17
5	17.27	Pentanal, 2 – methyl -	$C_6H_{12}O$	100	4.60
6	20.79	(2S, 3S) – (-) – 3 - Propyloxiranemethanol	$C_6H_{12}O_2$	116	1.72
7	23.09	3,4 – Hexanediol, 2,5 – dimethyl -	$C_8H_{18}O_2$	146	2.87
8	24.45	Octane, 3, 4, 5, 6 – tetramethyl -	$C_{12}H_{26}$	170	3.45
9	28.63	Diazoprogesterone	$C_{21}H_{30}N_4$	338	62.07

RT – Retention Time; MW – Molecular weight

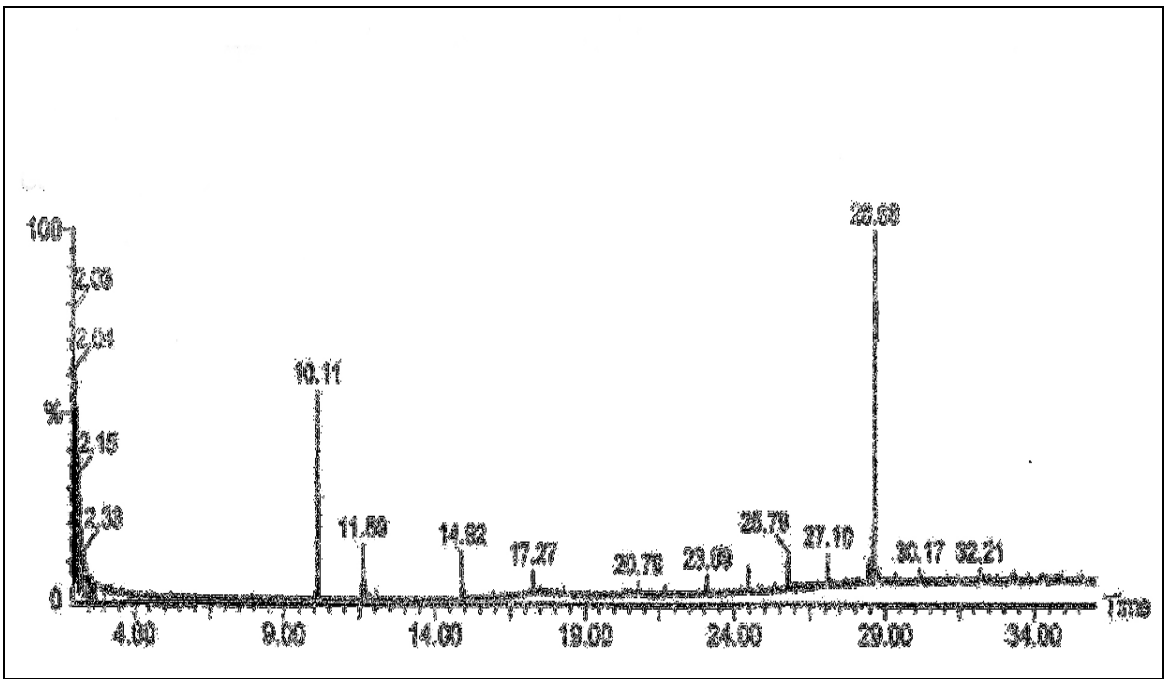


Figure 1a: GC-MS Chromotogram of *Gracilaria corticata* sample

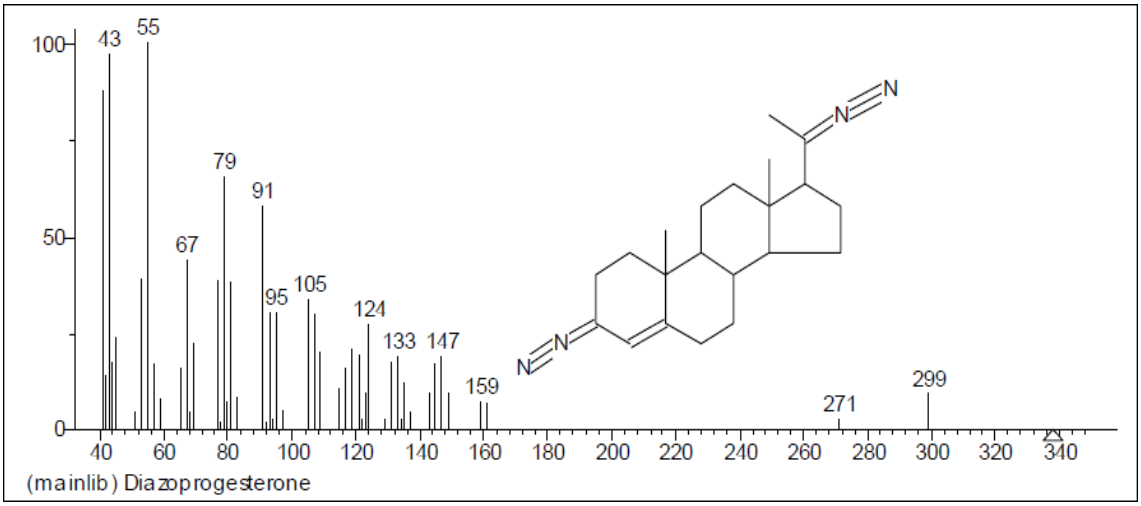


Figure 1b: compound structure of Diazopogestrone

12.3.4. FT-IR analysis

The average spectra obtained for the *Gracilaria corticata* was obtained in the range of 400 – 4000 cm-1 wave number region presented in the table 22 and figure 2. The spectra were normalized with respect to the band at ~3265.46 cm^{-1} (alkynes). The bands observed at~ 3022.45 corresponds to Alkenes with =C-H stretch. The band at ~2920.23 corresponds to Alkanes and Alkyls with C-H stretch. The C≡N stretching band at ~2345.44 is associated with Nitriles. The band observed at ~1730.15 cm-1 is due to the C=O stretch of Aldehydes. The N-H bend at Amides was observed with the band ~1516.05. The band observed at ~1217.08 showed C-O stretch (alcohol). The peak value with band at ~ 761.23 is strongly associated with C-Cl stretch of Alkyl halides and 671.23 showed C-Br stretch.

Figure 2: Fourier Transform Infrared Spectroscopy (FTIR)

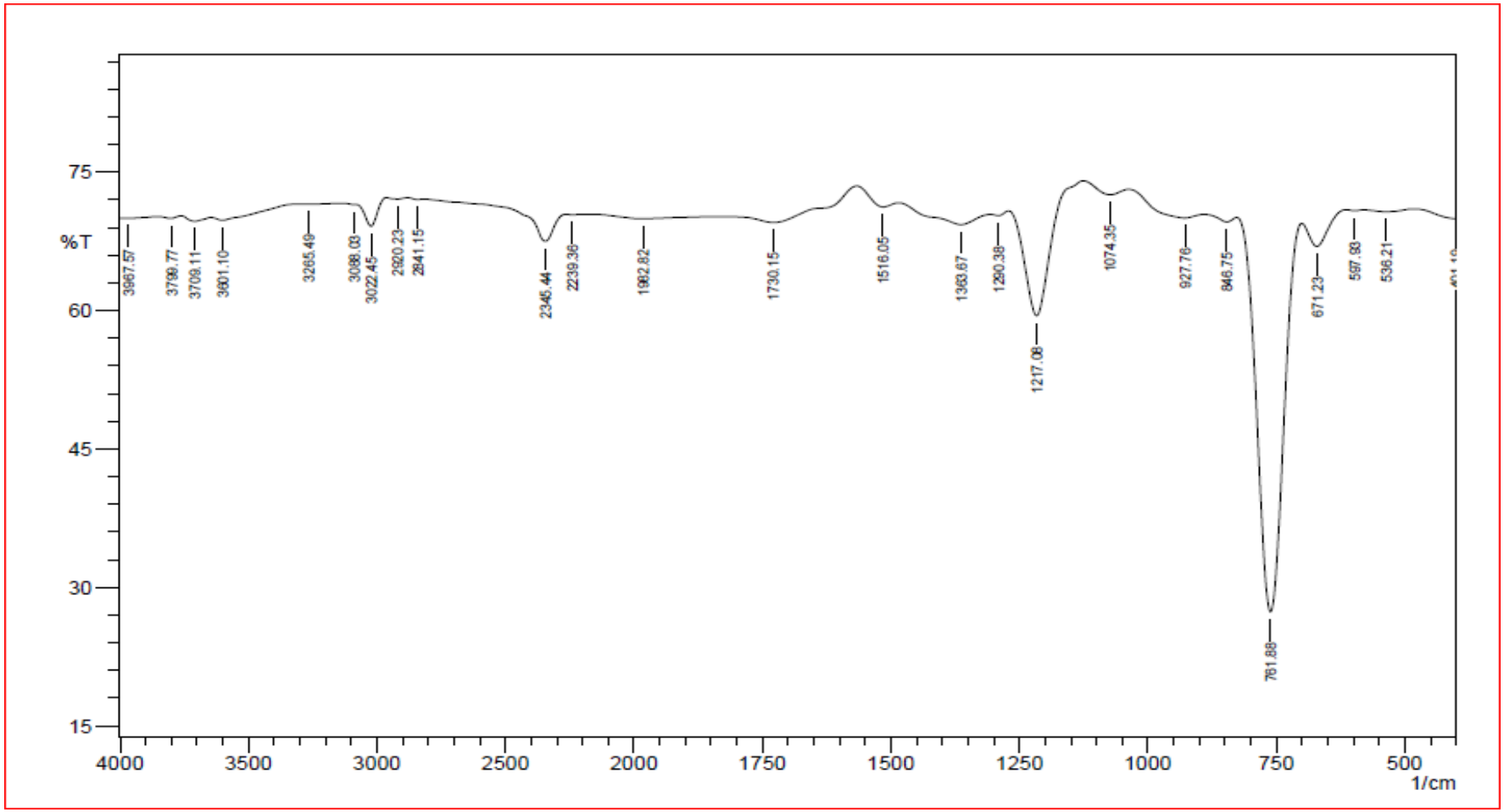


Table 22: Fourier Transform Infrared Spectroscopy (FTIR)

S. No	Peak	Class of Compounds	Frequency Assignment
1.	671.23	Alkyl halides	C-Br stretch
2.	761.23	Alkyl halides	C-Cl stretch
3.	1217.08	Alcohols	C-O stretch
4.	1516.05	Amides	N-H bend
5.	1730.15	Aldehydes	C=O Stretch
6.	2345.44	Nitriles	C≡N stretch
7.	2920.23	Alkanes and alkyls	C-H stretch
8.	3022.45	Alkenes	=C-H stretch
9.	3265.49	Alkynes	≡C-H stretch

12.3.5. UV- Visible spectroscopy

This absorption spectroscopy uses electromagnetic radiations between 190 nm to 800 nm and is divided into the ultraviolet (UV, 190-400 nm) and visible (VIS, 400-800 nm) regions. Most absorption bands of organic compounds are due to electronic transitions from fundamental levels n or Π on the excited levels Π*. Absorption bands for these transitions fall in the 200 nm – 700 nm region, these transitions require the presence, in the molecule, of an unsaturated group which has Π electrons. Table 23 showed the UV- visible electron transition with class of compounds, and the Table 24 showed the typical absorptions of simple chromophores.

N → Π* transitions have low molar absorptivity (ϵ~10 – 100 L/mol·cm)

Π → Π* transitions have high molar absorptivity (ϵ-1.000 -10.00 L/mol·cm)

The solvent could influence the position of absorption bands.

With increasing polarity n → Π* transitions are shifter to lower wavelengths (blue shift). This shift is due to unpaired electrons (orbital energy decreases n)

With increasing polarity Π → Π* transitions are often shifter to higher wavelengths (red shift). This is caused by attractive polarization forces between the solvent and absorbent, which determine the decrease of ground and excited states energy. This decrease is greater for excited state than for fundamental state, so the difference in energy between the two levels decreases, resulting in a shift of the absorption band to higher wave numbers (red shift).

Table 23: UV- visible electron transition with class of compounds

Electron transition	Class of compounds
σ→σ*	Alkanes
σ→π*	Carbonyl compounds
π →π*	Alkenes, carbonyl compn, alkyne etc.
n→σ*	Oxygen, nitrogen, sulfur and halogen compounds
n →π*	Carbonyl compounds

Table 24: Typical absorptions of simple chromophores

Class	Transition	Wavelength maximum (nm)
R- CHO	π →π*	190
	n →π*	290
R2CO	π →π*	180
	n →π*	280
RCOOH	n →π*	205
RCOO R’	n →π*	205
H_2O	σ→σ*	183
C-C a C-H, CH4	σ→σ*	170, 173
C-X, CH3OH, CH3NH2, CH3I	n→σ*	180-260, 187, 215, 258
C=N	n→σ*, n→π*	190, 300
N=N	n→π*	340
C=S	n→π*	500
NO_2	n→π*	420-450
N=O	n→π*	630-700

Table 25: UV- Visible spectroscopy of the isolated compound

S.no	Wavelength range	Absorptive range	Electron transition	Class of compounds
1.	666.5	1.359	n →π*	Carbonyl compounds
2.	609.5	0.347		
3.	534	0.512		
4.	503	0.683		
5.	406.5	4		
6.	270	4		
7.	250.5	3.181		
8.	231	2.545		
9.	219.5	1.818		
10.	207	1.308		

Figure 3: UV- Visible spectroscopy

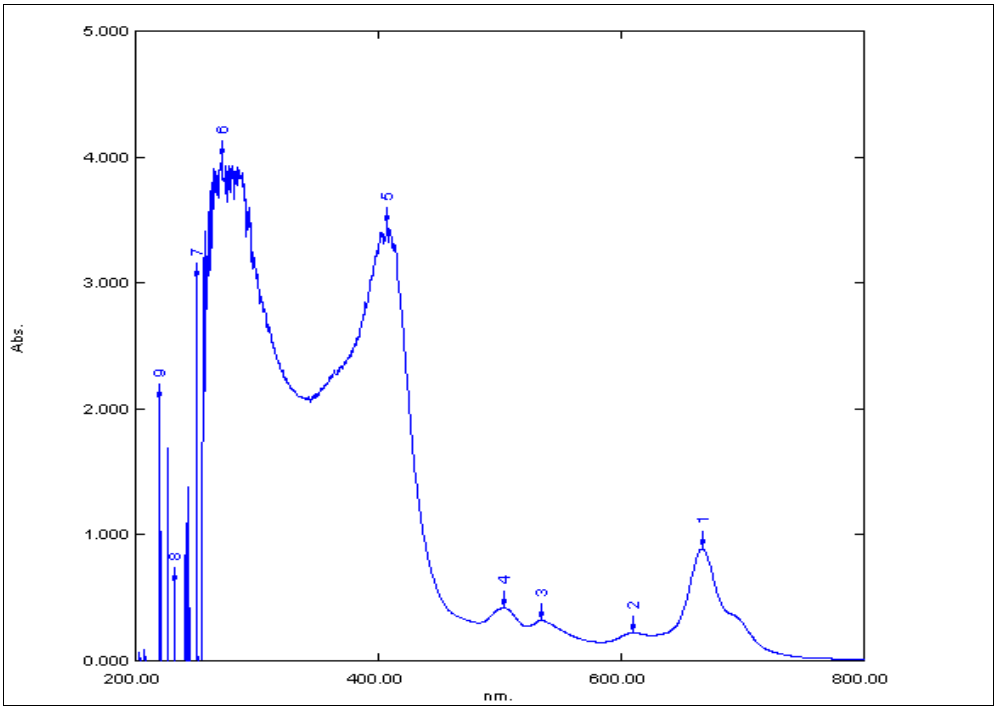


Table 25 showed UV- Visible spectroscopy of the isolated compound with carbonyl class of compounds. The maximum wavelength range was absorbed between 220 to 450 nm ranges with the maximum absorption range of 4000 L/mol·cm. Figure 3 shows Π → Π* transitions of high molar absorptivity (ϵ-1.000 -10.00 L/mol·cm). The electron transmission range of the tested compound showed **n →π***.

12.3.6. NMR spectra of the isolated compound

The NMR spectra (400 MHz, DMSO) for the isolated compound was subjected to NMR analysis ^{1}H and ^{13}C NMR. From the ^{13}C -NMR analysis totally 16 signals were observed. ^{13}C Chemical Shifts of Carbonyl Groups (δ in ppm). Results revealed the chemical shift was observed from δ 14.14 ppm to δ 24.97 ppm showed Aldehyde C – I type of carbon, δ 25.48 ppm to δ 34.14 ppm showed R_3CH type of carbon. δ 39.38 ppm and δ 51.48 ppm showed RCH_2NH_2, RCH_2O and RCH_2Cl type of carbon.

From the ^{1}H NMR spectra, –OH side chain at δ 0.788, δ 1.181, δ 1.530 ppm was observed with aromatic –CH at δ 3.593 - δ 7. 201ppm.

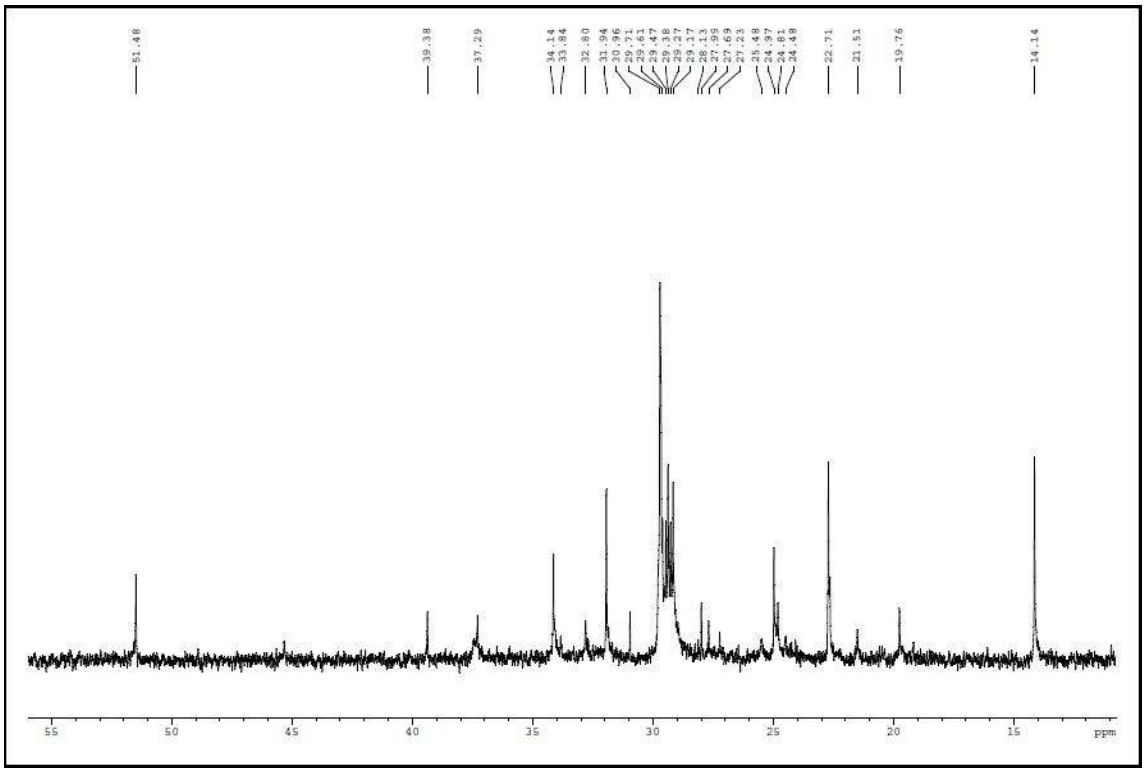


Figure- 4: ^{13}C NMR spectrum of the isolated compound

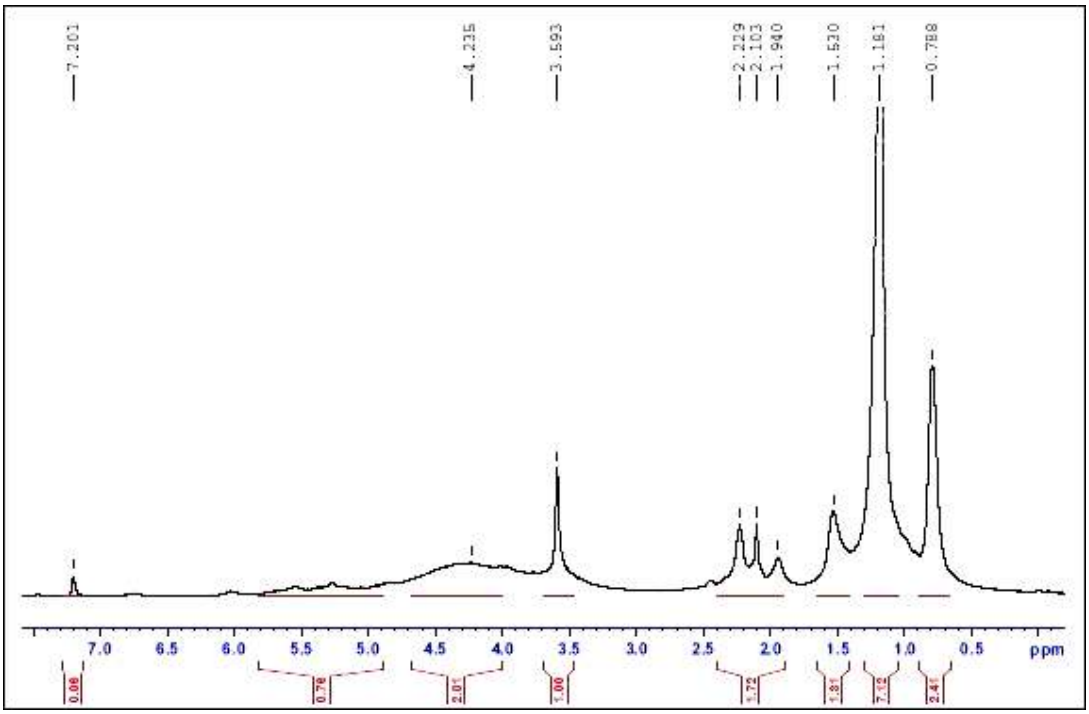


Figure-5: ^{1}H NMR spectrum of the isolated compound

Plate-1: Photos of Collected Seaweeds

Ulva reticulata

Gracilaria corticata

Ulva lactuca

Gracilaria edulis

Caulerpa racemosa

Gelidiella acerosa

Hypnea musciformis

Sargassum myriocystum

Hypnea valentiae

Padina gymnospora

Plate-2: Antibacterial activity of methanol extract of *Gracilaria corticata* against Gram positive bacteria

Staphylococcus aureus

Bacillus cereus

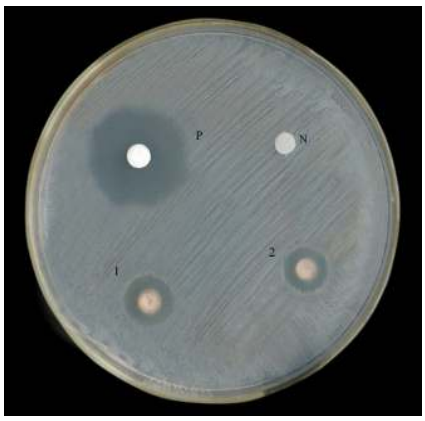
Streptococcus pyogenes

Bacillus subtilis

Streptococcus epidermidis

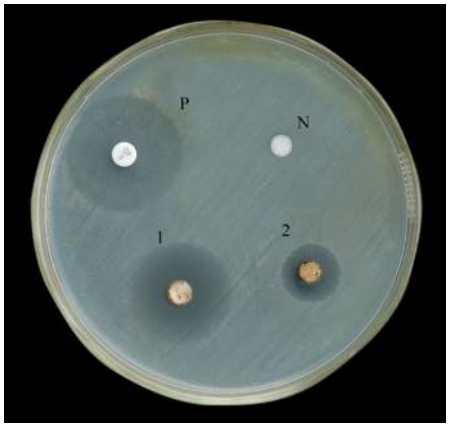
1-2.5mg/ml of extract, 2-5mg/ml of extract, P-Positive control, N-Negative (blind) control

Plate-4: Antibacterial activity of methanol extract of *Gracilaria corticata* against Gram negative bacteria



Escherichia coli ***Pseudomonas aeruginosa***

Vibrio cholerae *Salmonella typhi*

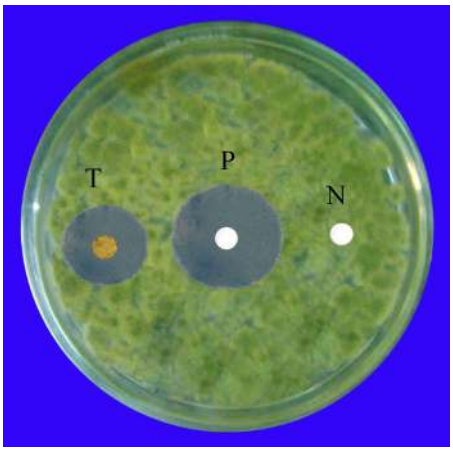


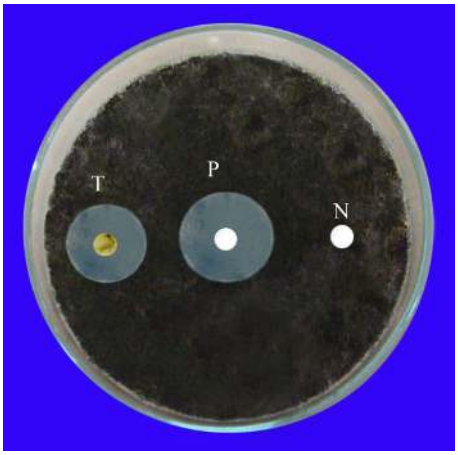
Klebsiella pneumoniae *Enterobacter aerogenes*

1-2.5mg/ml of extract, 2-5mg/ml of extract, P-Positive control, N-Negative (blind) control

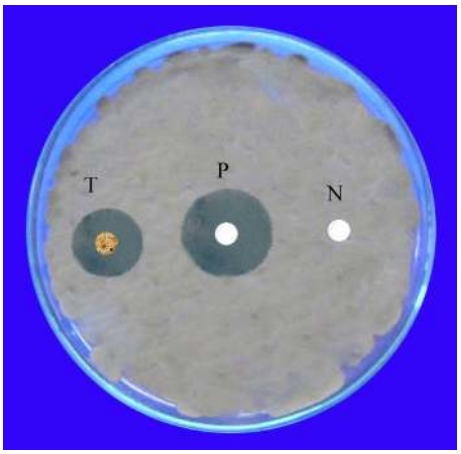
Plate-5: Antifungal activity of Methanol extract of

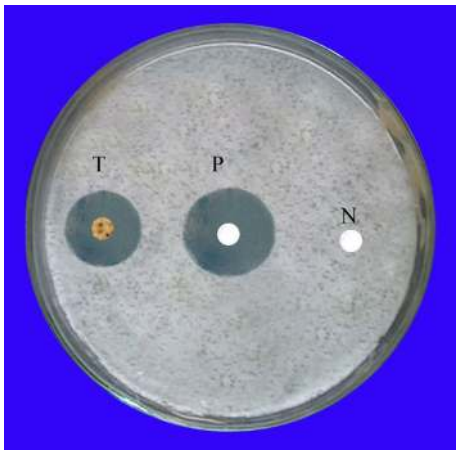
Gracilaria corticata



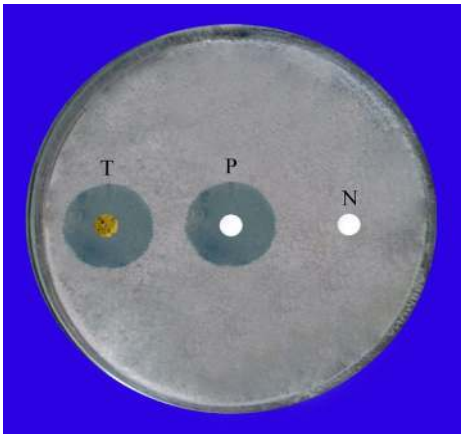


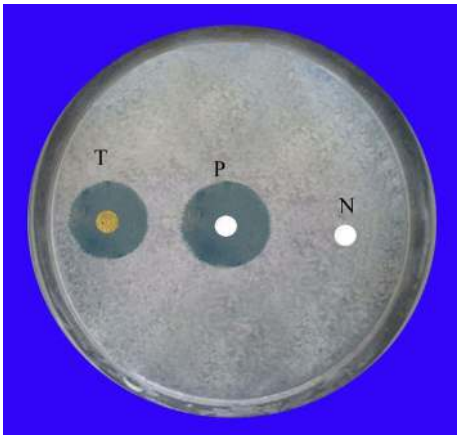
Aspergillus flavus *Aspergillus niger*





Aspergillus fumigatus ***Saccharomyces cerevisiae***





Candida albicans ***Candida glabrata***

T-Tested, P-Positive control, N-Negative (blind) control

12.4. DISCUSSION

Seaweeds are the eukaryotic organisms that lives in salty water of the ocean and recognized as a potential source of bioactive natural products. They contain compounds ranging from sterols, terpenoids to brominated phenolics, which show bioactivity against microorganisms. Seaweeds are rich and varied source of bioactive natural products and have been studied as potential biocidal and pharmaceutical agents. In recent years, there are numerous reports of macroalgae derived compounds that have a broad range of biological activities such as antibacterial, antifungal, antiviral, antineoplastic, antifouling, antiinflammatory, antitumoric, cytotoxic and antimitotic activities (Bhosale *et al*., 2002). Presently seaweeds constitute commercially important marine renewable resources which are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammations (Elena *et al*., 2001).

It is a real fact that the importance of marine organisms as a source of new substances is growing. With marine species comprising approximately a half of the total global biodiversity, the sea offers an enormous resource for novel compounds, and it has been classified as the largest reservoir of natural molecules to be evaluated for drug activity. A very different kind of substances have been obtained from marine organisms among other reasons because they are living in a very exigent, competitive, aggressive surrounding and very different in many aspects from the terrestrial environment, a situation that demands the production of quite specific and potent active molecules.

Although terrestrial biodiversity is the foundation of the pharmaceutical industry, the oceans have enormous biodiversity and potential to provide novel compounds with commercial value (Hay, 1996; Smit, 2004). In the current study, we screened for antibacterial, antifungal and larvicidal activity of selected marine seaweeds. Overuse of antibiotics and the ability of bacteria to acquire resistance to the drugs, means there is a

constant search for new classes of antibiotics with novel structures that are effective against human pathogens.

12.4.1. ANTIBACTERIAL ACTIVITY OF MARINE SEAWEEDS AGAINST PATHOGENIC BACTERIA

In the present study, antibacterial activity of five different solvents *viz*., methanol, acetone, chloroform, hexane and ethyl acetate extracts of seaweeds were evaluated against pathogenic bacteria. Among the extracts of five solvents tested, the methanol extract showed the greatest inhibition diameters against Gram positive and Gram negative bacterial isolates. These results are in agreement with the observations of Karabay-Yavasoglu *et al*. (2007), Taskin *et al*. (2007) and Kandhasamy and Arunachalam (2008), who reported that extracts prepared with methanol showed the best activity.

The results from the present study showed that the Gram positive bacteria are more susceptible than Gram negative bacteria on seaweeds extracts which was also supported by earlier works with different species of seaweeds indicating that the more susceptibility of Gram positive bacteria to the algal extracts was due to the differences in their cell wall structure and their composition (Taskin *et al*., 2001; Tuney *et al*., 2006). Further in Gram negative bacteria, the outer membrane acts as a barrier to many environmental substances including antibiotics and the presence of thick murine layer in the cell wall also prevents the entry of the inhibitors (Tortora *et al*., 2001). Further mild differences between the results of the present investigation and results of similar studies on different species of related seaweeds (Kendrick and Walker, 1991; Caceres *et al*., 2000) may be due to the production of bioactive compounds related to the seasonal variations, methods, organic solvents used for extraction of bioactive compounds and differences in assay methods.

The overall antimicrobial activity assessed from the below results indicated the presence of active constituents in the extractions of seaweeds which showed better

antimicrobial activity against pathogens used. Hence they can be considered as potential sources of bioactive compounds acting as lead molecules for the investigation of natural antibiotics.

In the present study, antibacterial activity of extracts obtained from marine seaweeds *Ulva reticulata* and *Ulva lactuca* were investigated against Gram positive and Gram negative bacteria. The methanol crude extract of *Ulva reticulata* (5.0 mg/ml) showed highest mean zone of inhibition (15 ± 0.6 mm) against the Gram positive *Streptococcus pyogenes* followed by *Staphylococcus aureus* (13 ± 0.3 mm), *Streptococcus epidermidis* (12 ± 0.6 mm), *Bacillus subtilis* (11 ± 0.6 mm) and *Bacillus cereus* (10 ± 0.5 mm). For Gram negative bacteria, maximum zone of inhibition was recorded in methanol crude extract of *Ulva reticulata* against *Klebsiella pneumoniae* (13 ± 0.6 mm) followed by *Escherichia coli* (12 ± 0.8 mm), *Enterobacter aerogenes* (11 ± 0.3 mm), *Pseudomonas aeruginosa* (10 ± 0.3 mm), *Vibrio cholerae* (9 ± 0.6 mm) and *Salmonella typhi* (9 ± 0.3 mm). The minimum zone of inhibition obtained from the hexane crude extract of seaweed *Ulva reticulata* against bacterial pathogens was comparatively very less when compared to the other solvent extracts. The minimum inhibitory concentration (MIC) value of *Ulva reticulata* against bacteria was ranged between 2.50 mg/ml to 80 mg/ml. The lowest MIC (2.50 mg/ml) value was recorded against *Staphylococcus aureus, Bacillus subtilis*, *Bacillus cereus, Klebsiella pneumoniae* and *Enterobacter aerogenes.*

The *Ulva lactuca* methanol crude extract (5.0 mg/ml) showed maximum zone of inhibition (16 ± 0.3 mm) against the Gram positive bacilli *Bacillus cereus* followed by *Bacillus subtilis* (16 ± 0.2 mm), *Streptococcus pyogenes* (14 ± 0.3 mm), *Staphylococcus aureus* (13 ± 0.6 mm) and *Streptococcus epidermidis* (12 ± 0.3 mm). For Gram negative bacteria, highest zone of inhibition was recorded in methanol crude extract of *Ulva lactuca* against *Enterobacter aerogenes* (15 ± 0.7 mm) followed by *Klebsiella pneumoniae* (15 ± 0.6

mm), *Escherichia coli* (15 ± 0.2 mm), *Pseudomonas aeruginosa* (13 ± 0.6 mm) and *Vibrio cholerae* (10 ± 0.2 mm). The minimum zone of inhibition obtained from the hexane crude extract of seaweed *Ulva lactuca* against bacterial pathogens was comparatively very less when compared to the other solvent extracts. The Minimum inhibitory concentration (MIC) value of *Ulva lactuca* against bacteria was ranged between 1.25 mg/ml to 80 mg/ml. The lowest MIC (1.25 mg/ml) value was recorded against *Klebsiella pneumoniae.*

Mtolera and Semesi (1996) screened the antibacterial activity of the extracts of six marine green algae against three bacterial species *viz*., *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* using a disc assay method. The crude extract of *Ulva pertusa* was more active against *Staphylococcus aureus* and *Bacillus subtilis* but less active against *Escherichia coli* (Chengkui and Junfu, 1984; Glombitza, 1979).

Joseph Selvin and Aaron Premnath Lipton (2004) collected the secondary metabolites of seaweeds *Ulva fasciata* and *Hypnea musciformis* from Southeast and Southwest coast of India and tested the biotoxicity potential of collected seaweeds. Both species showed potent activity in antibacterial, brine shrimp cytotoxicity, larvicidal, antifouling and ichthyotoxicity assays. The green alga *Ulva fasciata* exhibited broad spectrum antibacterial activity whereas the red algae *Hypnea musciformis* showed narrow spectrum antibacterial activity.

Roberta Paulert *et al*. (2007) studied the antibacterial activity of cell-wall polysaccharides and crude extracts from the seaweed *Ulva fasciata*. The antibacterial activity was assessed by agar diffusion assay and by means of the broth dilution method for estimating the minimum inhibitory concentration (MIC). The following human bacterial strains were tested: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Micrococcus luteus* and two plant pathogens: *Xanthomonas campestris* and *Erwinia carotovora*. Both methanol soluble and insoluble (remaining fraction) extracts were active against *Pseudomonas aeruginosa*, *Xanthomonas campestris* and *Erwinia carotovora.*

The highest activity of extracts was observed against *Erwinia* (MIC 1 mg/ml). The methanol extracts showed maximum activity than the other solvent extracts. The results of the present study also reported that methanol extract showed maximum activity than the other solvents.

Vallinayagam *et al*. (2009) screened the antibacterial activities of two important seaweeds namely *Ulva lactuca* and *Gracilaria edulis* against human bacterial pathogens like *Staphylococcus aureus, Vibrio cholerae, Shigella dysentriae, Shigella boydii, Salmonella paratyphi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* The maximum activity was recorded from the extract of *Gracilaria edulis* against *Staphylococcus aureus* and minimum by *Ulva lactuca* against *Pseudomonas aeruginosa*. In the present study also similar type of results were obtained with respect to antibacterial activity of *Gracilaria edulis* against *Staphylococcus aureus* (17 mm).

Karthigai Devi *et al*. (2009) evaluated the antibacterial activity of commonly occurring green algae *Codium adherens*, *Ulva reticulata* and *Halimeda tuna* by agar diffusion method. Seven different solvents namely *viz*., acetone, methanol, chloroform, diethyl ether, ethyl acetate, ethanol and petroleum ether were used for extraction. The zone of inhibition was compared and the ethanol extract showed the better result for the other extracts. Some extracts found more effective than the commercial medicine. The maximum antibacterial activity was noted in ethanol extracts which showed activity against *Staphylococcus* sp. and the minimum was recorded in methanol extracts against *Escherichia coli*, *Staphylococcus* sp., *Proteus* sp., *Streptococcus* sp. and *Enterococcus* sp. The findings of the present study showed that the methanol extract recorded better results than other extracts and the results of the present study was not parallel with the above results, this might be due to the difference in the solvent employed.

In this present result, the antibacterial activity of extracts obtained from marine seaweed *Caulerpa racemosa* was studied against five Gram positive bacteria and six Gram

negative bacteria. The methanol crude extract of *Caulerpa racemosa* (5.0 mg/ml) showed maximum zone of inhibition (14 ± 0.6 mm) against the Gram positive bacilli *Bacillus cereus* followed by *Staphylococcus aureus* (13 ± 0.3 mm), *Bacillus subtilis* (12 ± 0.3 mm), *Streptococcus pyogenes* (12 ± 0.2 mm) and *Streptococcus epidermidis* (11 ± 0.4 mm). For Gram negative bacteria, highest mean zone of inhibition was recorded in methanol crude extract of *Caulerpa racemosa* against *Enterobacter aerogenes* (14 ± 0.3 mm) followed by *Klebsiella pneumoniae* (13 ± 0.5 mm), *Pseudomonas aeruginosa* (13 ± 0.4 mm), *Escherichia coli* (12 ± 0.3 mm), *Salmonella typhi* (10 ± 0.6 mm) and *Vibrio cholerae* (9 ± 0.3 mm). The minimum zone of inhibition obtained from the hexane crude extract of seaweed *Caulerpa racemosa* against bacterial pathogens was comparatively very less when compared to the other solvent extracts. The minimum inhibitory concentration (MIC) value of *Caulerpa racemosa* against bacteria was ranged between 1.25 to 80 mg/ml. The lowest MIC (1.25 mg/ml) value was recorded against *Staphylococcus aureus, Bacillus subtilis* and *Enterobacter aerogenes.*

Mtolera and Semesi (1996) investigated the antimicrobial activity of the extracts of six marine green algae against three bacterial species *viz*., *Staphylococcus aureus*, *Bacillus subtilis* and *Escherichia coli* using a disc assay method. The crude extract of *Caulerpa racemosa* was inactive against all the tested organisms. The antimicrobial activity shown by *Caulerpa racemosa* in their study may be attributed to caulerpin or caulerpein (Doty and Santos, 1970; Paul *et al*., 1987), or flexin and trifarin (Blackman and Wells, 1978) or by caulerpanyene (Amico *et al*., 1978). The inactivity shown by an extract of *Caulerpa racemosa* suggests that the species might be lacking the above mentioned active substance.

Thirumaran and Anantharaman (2007) reported that antibacterial activity of marine macroalga *Caulerpa scalpeliformis* from Gulf of Mannar coast, the maximum activity was

noted in methanol extract against *Salmonella typhi, Micrococcus* sp. and *Shigella boydii.* The results of their study were similar with the findings of our study.

Srivastava *et al*. (2010) collected the extract from two species of seaweed samples namely *Caulerpa racemosa* and *Grateloupia lithophila* which were from different locations and screened for its antimicrobial activity. Extracts of methanol, ethanol, butanol, acetone, chloroform and dichloromethane were tested against selected human pathogens. Both the seaweeds collected had shown moderate antibacterial activity with <15 mm of zone of inhibition. Out of many solvents used, butanolic extract has shown significant activity. The results of the present study revealed the solvent methanol as a best one for extracting antibacterial compounds from the seaweed *Caulerpa racemosa* and the findings of the present study was in contrast to their study because they used butanol as a solvent.

Lavanya and Veerapan (2011) tested the *in vitro* antibacterial activity of six selected marine algae as an alternative to commonly used antibiotics. Extracts of seaweed samples namely *Codium decorticatum, Caulerpa scalpelliformis, Gracilaria crassa, Acanthophora spicifera, Sargassum wightii* and *Turbinaria conoides* were selected for antibacterial activity using solvents *viz*., acetone, methanol, chloroform, diethyl ether, ethyl acetate, hexane and aqueous were tested against selected human pathogens such as *Vibrio parahaemolyticus*, *Salmonella* sp., *Shewanella* sp*., Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. All the seaweed extracts have shown moderate antibacterial activity, out of which only methanolic extract has shown significant activity. The results of the present study also showed that methanol is the best solvent for extracting antimicrobial compounds.

In the present investigation, the antibacterial activity of extracts obtained from marine seaweeds *Hypnea musciformis* and *Hypnea valentiae* were investigated against Gram positive bacteria and Gram negative bacteria. The methanol crude extract of *Hypnea musciformis* (5.0

mg/ml) showed highest mean zone of inhibition (14 ± 0.4 mm) against the Gram positive cocci *Streptococcus epidermidis* followed by *Staphylococcus aureus* (14 ± 0.2 mm), *Streptococcus pyogenes* (13 ± 0.3 mm), *Bacillus cereus* (12 ± 0.6 mm) and *Bacillus subtilis* (12 ± 0.5 mm). For Gram negative bacteria, maximum zone of inhibition was recorded in methanol crude extract of *Hypnea musciformis* against *Pseudomonas aeruginosa* (13 ± 0.8 mm) followed by *Escherichia coli* (13 ± 0.7 mm), *Enterobacter aerogenes* (13 ± 0.4 mm), *Klebsiella pneumoniae* (11 ± 0.5 mm), *Salmonella typhi* (10 ± 0.6 mm) and *Vibrio cholerae* (10 ± 0.4 mm). The minimum zone of inhibition obtained from the hexane crude extract of seaweed *Hypnea musciformis* against bacterial pathogens was comparatively very less when compared to the other solvent extracts. The minimum inhibitory concentration (MIC) value of *Hypnea musciformis* against bacteria was ranged between 1.25 mg/ml to 80 mg/ml. The lowest MIC (1.25 mg/ml) value was recorded against *Klebsiella pneumoniae*.

The *Hypnea valentiae* methanol crude extract (5.0 mg/ml) showed maximum zone of inhibition (15 ± 0.5 mm) against the Gram positive bacilli *Bacillus cereus* followed by *Streptococcus epidermidis* (14 ± 0.6 mm), *Staphylococcus aureus* (13 ± 0.6 mm), *Bacillus subtilis* (13 ± 0.3 mm) and *Streptococcus pyogenes* (12 ± 0.4 mm). For Gram negative bacteria, highest zone of inhibition was recorded in methanol extract of *Hypnea valentiae* against *Escherichia coli* (14 ± 0.6 mm) followed by *Salmonella typhi* (13 ± 0.4 mm), *Klebsiella pneumoniae* (13 ± 0.3 mm), *Enterobacter aerogenes* (13 ± 0.2 mm), *Vibrio cholerae* (12 ± 0.5 mm) and *Pseudomonas aeruginosa* (12 ± 0.3 mm). The minimum zone of inhibition obtained from the hexane crude extract of seaweed *Hypnea valentiae* against bacterial pathogens was comparatively very less when compared to the other solvent extracts. The minimum inhibitory concentration (MIC) value of *Hypnea valentiae* against bacteria was ranged between 1.25 mg/ml to 80 mg/ml. The lowest MIC (1.25 mg/ml) value was recorded

against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.*

Chemical composition and energy values of *Hypnea musciformis* have been studied by Dhargalkar *et al.* (1980). Ascorbic and dehydroascorbic acid contents of this seaweed have been reported by Qasim and Barkati (1985). A preliminary survey of its saturated and unsaturated fatty acids has also been made (Qasim, 1986; Shameel, 1990; Hayee-Memon *et at.,* 1992). Some of these fatty acids have shown inhibitory effect on *Mycobacterium tuberculosis* (Marolia *et al.,* 1982).

Joseph Selvin and Aaron Premnath Lipton (2004) collected the secondary metabolites of seaweeds *Ulva fasciata* and *Hypnea musciformis* and tested the biotoxicity potential of collected seaweeds. Both species showed potent activity in antibacterial, brine shrimp cytotoxicity, larvicidal, antifouling and ichthyotoxicity assays. The green alga *Ulva fasciata* exhibited broad spectrum antibacterial activity whereas the red algae *Hypnea musciformis* showed narrow spectrum antibacterial activity.

Bansemir *et al*. (2006) investigated the antibacterial activities of extracts from 26 algal species prepared with dichloromethane against five fish pathogenic bacteria. He reported that the most active algal species was *Asparagopsis armata* against all tested bacteria. The dichloromethanolic extracts of *Halopitys incurvus* and *Ceramium rubrum* showed their highest activity against the marine bacterium *Pseudomonas anguilliseptica*, while the extract of *Plocamium cartilagineum* showed a weak activity on these five fish-pathogenic bacteria. The extract methanol - dichloromethane (1:1) of *Hypnea musciformis* inhibited the growth of Gram positive strains (*Bacillus cereus*, *Bacillus subtilis* and *Micrococcus luteus*) to the extent of 66.0% at 30°C, whereas all the Gram positive bacteria were susceptible at 20°C (Selvin and Lipton, 2004).

Taskin *et al.* (2007) reported that the highest activity was shown on *Escherichia coli* and *Enterococcus faecalis* by a methanolic extract of *Corallina officinalis*. Kandhasamy and Arunachalam (2008) observed that methanolic extract of *Hypnea musciformis* was active on the *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Enterococcus faecalis* bacteria. The findings of our results are in parallel with their results, with respect to the methanolic extracts of *Hypnea* spp.

Etahiri *et al.* (2003) mentioned that the methanolic extract of *Hypnea musciformis* and *Gelidium latifolium* showed an inhibition on the bacterium *Staphylococcus aureus*. The methanolic extract of the seaweed *Hypnea musciformis* exhibited strong antibacterial activity against the Gram positive and seven Gram negative bacteria (Siddqiui *et al.*, 1993). The results of the present study also showed the methanol extract of *Hypnea musciformis* as a efficient solvent for extracting bioactive compounds from the seaweed and the results of the present study closely related to the research findings of Siddqiui *et al.* (1993) and Etahiri *et al.* (2003).

In another study carried out by Salvador *et al*. (2007), the extracts that were prepared from fresh and lyophilized samples of algae *Padina pavonica* which was collected during the four seasons and *Hypnea musciformis* which was collected only in summer season were screened for antimicrobial activities and the activities were compared. Bioactivities of the extracts showed differences depending on the season that samples were collected and the type of samples by which the extracts have been prepared. *Hypnea musciformis* extract that was prepared from samples which were collected showed higher inhibitory activity against Gram positive bacteria than Gram negative bacteria. The results of the present study showed that seaweed extract of *Hypnea musciformis* had maximum inhibitory activity against Gram positive bacteria and the results obtained from the study of Salvador *et al*. (2007) was similar to the present study.

Aseer Manilal *et al*. (2009) analyzed the bioactivity from crude extract of fresh and dried samples prepared from different polar and non polar solvents. Of these, four species of red algae (*Asparagopsis taxiformis, Laurencia ceylanica, Laurencia brandenii, Hypnea valentiae*) were found to be highly active. Among the pathogens tested, shrimp pathogenic *Vibrio* sp. was the most susceptible organisms. In the present study also methanol was found to be the best solvent for extracting antimicrobial metabolites from dried samples.

In the present study, antibacterial activity of extracts obtained from marine seaweeds *Gracilaria corticata* and *Gracilaria edulis* were investigated against Gram positive and Gram negative bacteria. The methanol crude extract of *Gracilaria corticata* (5.0 mg/ml) showed highest mean zone of inhibition (18 ± 0.4 mm) against the Gram positive cocci *Streptococcus pyogenes* followed by *Bacillus subtilis* (17 ± 0.5 mm), *Staphylococcus aureus* (17 ± 0.3 mm), *Streptococcus epidermidis* (16 ± 0.6 mm) and *Bacillus cereus* (16±0.2 mm) . For Gram negative bacteria, the maximum zone of inhibition was recorded in methanol crude extract of *Gracilaria corticata* against *Klebsiella pneumoniae* (17 ± 0.5 mm) followed by *Enterobacter aerogenes* (17 ± 0.3 mm), *Salmonella typhi* (16 ± 0.6 mm), *Pseudomonas aeruginosa* (16 ± 0.5 mm), *Escherichia coli* (16 ± 0.3 mm) and *Vibrio cholerae* (11 ± 0.4 mm). The minimum inhibitory concentration (MIC) values of *Gracilaria corticata* against bacteria was ranged between 1.25 to 80 mg/ml. The lowest MIC (1.25 mg/ml) value was recorded against *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus epidermidis, Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae* and *Enterobacter aerogenes.*

The *Gracilaria edulis* methanol crude extract (5.0 mg/ml) showed maximum zone of inhibition (17 ± 0.4 mm) against the Gram positive cocci *Staphylococcus aureus* followed by *Streptococcus pyogenes* (15 ± 0.3 mm), *Streptococcus epidermidis* (14 ± 0.5 mm), *Bacillus cereus* (14 ± 0.4 mm) and *Bacillus subtilis* (13 ± 0.6 mm). For Gram negative bacteria, highest zone of inhibition was recorded in methanol crude extract of *Gracilaria edulis* against

Enterobacter aerogenes (15 ± 0.5 mm) followed by *Klebsiella pneumoniae* (14 ± 0.2 mm), *Escherichia coli* (13 ± 0.6 mm), *Pseudomonas aeruginosa* (13 ± 0.4 mm), *Salmonella typhi* (13 ± 0.4 mm) and *Vibrio cholerae* (11 ± 0.4 mm). The minimum zone of inhibition obtained from the hexane crude extract of seaweed *Hypnea valentiae* against bacterial pathogens was comparatively very less when compared to the other solvent extracts. The minimum inhibitory concentration (MIC) value of *Gracilaria edulis* against bacteria was ranged between 1.25 to 80 mg/ml. The lowest MIC (1.25 mg/ml) value was recorded against *Staphylococcus aureus, Bacillus subtilis, Bacillus cereus* and *Klebsiella pneumoniae.*

Invariably, seaweeds have been proven to be potent source of antimicrobial compounds. Ethanolic extract of eight species of seaweeds belonging to the groups of Chlorophyta, Pheophyta and Rhodophyta exhibited broad spectrum antibacterial and antifungal activities (De-Campos *et al*., 1998). The algal extracts such as *Padina gymnospora, Sargassum wightii* and *Gracilaria corticata* were active against Gram positive and Gram negative bacteria (Rao *et al*., 1991). Based on the present findings, it could be inferred that the bioassay guided fractionation and purification may come up with potent antibacterial compounds. The above findings are in line with our research.

Oranday *et al*. (2004) screened polar and non polar extracts of four species of marine seaweeds for antibacterial properties against seven microorganisms by the disc diffusion method. The non polar extracts of *Sargassum fluitans* and polar extracts of *Gracilaria tikvahiae* inhibited the growth of microorganisms. The findings of the present study showed the polar solvent, methanol as an effective one for the extraction of antimicrobial compounds from the seaweed *Gracilaria edulis* and the results of our study showed similarity to the above research.

Santhanam Shanmugapriya *et al*. (2008) collected fourteen seaweeds and tested against ten human pathogenic bacteria using the well diffusion test in the Casitone agar

medium. Of these, seven species were determined to be highly bioactive and screened against the multiresistant pathogens. In their study, methanol:toluene (3:1) was found to be the best solvent for extracting the antimicrobial principles from fresh algae. The findings of their study revealed that the tested seaweed *Gracilaria corticata* was highly active against Gram negative bacteria than Gram positive bacteria. The present study showed that the seaweed *Gracilaria corticata* was highly active against Gram positive bacteria than Gram negative bacteria. Due to the extraction of *Gracilaria corticata* using methanol:toluene mixture, the nature of antibacterial activity might be varied.

Vallinayagam *et al*. (2009) screened the antibacterial activity of seaweed *Gracilaria edulis* against human bacterial pathogens *Staphylococcus aureus, Vibrio cholerae, Shigella dysenteriae, Shigella boydii, Salmonella paratyphi, Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* The maximum activity was recorded from the extract of *Gracilaria edulis* against *Staphylococcus aureus* only. In our study also, same type of results were obtained.

Subba Rangaiah *et al*. (2010) tested the antimicrobial activity of seaweed *Gracilaria corticata* collected from different coastal regions against clinical and phytopathogens. For microbiological testing of the seaweed extracts, agar well diffusion method was used. The zone of inhibition was measured for all the different crude algal extracts (chloroform, ethanol, methanol and water) against six strains of Gram positive and Gram negative bacteria. Crude extracts revealed a wide range of antimicrobial activity against tested pathogens. Seaweed extracts in different solvents exhibited different antimicrobial activities. In case of *Gracilaria corticata*, maximum inhibition was noticed with methanol and minimum with chloroform extracts. In this present research, the methanol extract of *Gracilaria corticata* showed maximum inhibitory activity against Gram positive and Gram negative bacteria, hexane extract of *Gracilaria corticata* showed minimum zone of inhibition against bacterial

isolates. The findings of Subba Rangaiah *et al*. (2010) were similar to the present study in case of Gram positive bacteria.

Renuka Bai (2010) evaluated different organic solvent extracts *viz*., acetone, chloroform, diethyl ether, ethanol and methanol for antibacterial activity, employing Gram negative (*Klebsiella pneumoniae* and *Pseudomonas aeruginosa*) and Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacteria. The ethanol extract of the alga was found to be active against *Pseudomonas aeruginosa* and *Bacillus subtilis*. In the present study, the methanol extract showed higher activity against Gram positive and Gram negative bacteria. The zone of inhibition against *Pseudomonas aeruginosa* and *Bacillus subtilis* by methanol extract were 16 mm and 17 mm respectively.

Lavanya and Veerapan (2011) investigated the *in vitro* antibacterial activity of six selected marine algae which have been selected and their extracts have been tested as an alternative to commonly used antibiotics. Extracts of six seaweed samples namely *Caulerpa scalpelliformis, Gracilaria crassa* and *Sargassum wightii* were selected for antibacterial activity extract using solvents *viz*., acetone, methanol, chloroform, diethyl ether, ethyl acetate, hexane and aqueous were listed against selected human pathogens such as species *Vibrio parahaemolyticus*, *Salmonella* sp., *Shewanella* sp*., Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Pseudomonas aeruginosa* and *Proteus mirabilis*. All the seaweeds extracts have shown moderate antibacterial activity, out of which methanolic extract alone showed significant activity. The results of the present study also showed methanol as a best solvent among the five different tested solvents for extracting antimicrobial compounds.

In the present study, the antibacterial activity of extracts obtained from marine seaweed *Gelidiella acerosa* was investigated against Gram positive and Gram negative bacteria. The methanol crude extract of *Gelidiella acerosa* (5.0 mg/ml) showed highest mean

zone of inhibition (11 ± 0.6 mm) against the Gram positive cocci *Staphylococcus aureus* followed by *Streptococcus pyogenes* (11 ± 0.5 mm), *Streptococcus epidermidis* (11 ± 0.4 mm), *Bacillus subtilis* (10 ± 0.2 mm) and *Bacillus cereus* (10 ± 0.2 mm). For Gram negative bacteria, maximum zone of inhibition were recorded in methanol crude extract of *Gelidiella acerosa* against *Enterococcus aerogenes* (14 ± 0.2 mm) followed by *Klebsiella pneumoniae* (13 ± 0.4 mm), *Pseudomonas aeruginosa* (12 ± 0.3 mm), *Salmonella typhi* (11 ± 0.5 mm), *Vibrio cholerae* (10 ± 0.6 mm) and *Escherichia coli* (10 ± 0.2 mm). The minimum inhibitory concentration (MIC) value of *Gelidiella acerosa* against bacteria was ranged between 1.25 to 80 mg/ml. The lowest MIC (1.25 mg/ml) value was recorded against *Enterobacter aerogenes.*

Gelidium acerosa is very popular in developing countries on account of improved knowledge on secondary metabolites, and it has been investigated as a source of medicinal agents. It is also proved that the phytochemicals have antimicrobial activities and therefore used for the treatment of various bacterial and fungal infections (Okigbo and Omadamiro, 2006). Nowadays there is an urgent need for discover of new antimicrobial compounds with chemicals structure as effective drug against microbial infections (Iwu *et al*., 1999), so *Gelidium acerosa* is one of the red algae, which with more components of phytochemicals and strong antimicrobial activity has been taken for the study.

Recently Hebsibah Elsie *et al*. (2011) compared the antimicrobial activity of ethanol and acetone extracts of *Gelidiella acerosa*. The ethanol extract of *Gelidiella acerosa* showed maximum activity against pathogens like *Staphylococcus aureus*, minimum activity in *Klebsiella pneumoniae*, moderate activity against *Micrococcus luteus* and *Bacillus cereus*, no activity against bacteria like *Micrococcus luteus* and *Escherichia coli.* The acetone extract showed a minimum activity against *Micrococcus luteus* and *Staphylococcus aureus* and maximum activity against the *Bacillus cereus* and no activity was seen against *Escherichia*

coli, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. In the present investigation, methanol extract showed maximum antibacterial activity followed by acetone extract against *Staphylococcus aureus*.

In the present investigation, the antibacterial activity of extracts obtained from marine seaweed *Sargassum myriocystum* was evaluated against Gram positive and Gram negative bacteria. The methanol crude extract of *Sargassum myriocystum* (5.0 mg/ml) showed highest mean zone of inhibition (14 ± 0.3 mm) against the Gram positive cocci *Streptococcus pyogenes* followed by *Bacillus subtilis* (13 ± 0.6 mm), *Staphylococcus aureus* (13 ± 0.4 mm), *Bacillus cereus* (13 ± 0.3 mm) and *Streptococcus epidermidis* (12 ± 0.5 mm). For Gram negative bacteria, maximum zone of inhibition was recorded in methanol crude extract of *Sargassum myriocystum* against *Pseudomonas aeruginosa* (13 ± 0.6 mm) followed by *Klebsiella pneumoniae* (13 ± 0.4 mm), *Enterobacter aerogenes* (13 ± 0.3 mm), *Salmonella typhi* (12 ± 0.6 mm), *Escherichia coli* (12 ± 0.3 mm) and *Vibrio cholerae* (8 ± 0.4 mm). The minimum inhibitory concentration (MIC) value of *Sargassum myriocystum* against bacteria was ranged between 1.25 to 80 mg/ml. The lowest MIC (1.25 mg/ml) value was recorded against *Staphylococcus aureus, Bacillus subtilis* and *Klebsiella pneumoniae.*

The marine seaweeds have been proven to be potent source of antimicrobial compounds. Ethanolic extract of eight species of seaweeds belonging to the groups of Chlorophyta, Pheophyta and Rhodophyta exhibited broad spectrum antibacterial and antifungal activities (De-Campos *et al*., 1998). The algal extract *Sargassum wightii* was active against Gram positive and Gram negative bacteria (Rao *et al*., 1991). Based on the present findings, it could be inferred that the bioassay guided fractionation and purification may come up with potent antibacterial compounds.

Oranday *et al*. (2004) screened polar and non polar extracts of four species of marine seaweeds for antibacterial properties against seven microorganisms by the diffusion method.

The extract of *Sargassum fluitans* inhibited the growth of more than four microorganisms. The findings of the present study also showed solvent were an effective one for the extraction of antimicrobial compounds from the seaweed *Sargassum myriocystum.*

Subba Rangaiah *et al*. (2010) investigated the antimicrobial activity of seaweed *Sargassum ilicifolium* against clinical and phytopathogens by agar well diffusion method. The zone of inhibition was measured for all the different crude algal extracts (chloroform, ethanol, methanol and water) against six strains of Gram positive and Gram negative bacterial organisms that cause diseases and disorders in man, animals and plants. Crude extracts revealed a wide range of antimicrobial activity against tested pathogens. Seaweed extracts in different solvents exhibited different antimicrobial activities. Among the various solvents used for seaweed extractions, maximum inhibition was noticed with ethanol extract and minimum with chloroform crude extract. In the present study, five different solvents (methanol, acetone, chloroform, hexane and ethyl acetate) were used for the seaweed *Sargassum myriocystum* extraction. Among the solvent used, methanol extract showed maximum zone of inhibition against Gram positive and Gram negative bacteria and minimum zone was seen in hexane extract. The finding of the present study was completely differed from their research because of the difference in solvent usage.

Vijayabaskar and Shiyamala (2011) determined the antibacterial activities of brown marine algae *Sargassum wightii* and *Turbinaria ornate* collected from the Gulf of Mannar biosphere reserve. The methanol extract of both seaweeds were tested against various Gram positive and Gram negative human pathogenic microbes. Their finding envisages that methanol extracts of *Sargassum wightii* and *Turbinaria ornate* could be utilized as a good source of antimicrobial agent in pharmaceutical industry. The findings of the present study also showed the methanol extract of *Sargassum wightii* as a good source of antimicrobial agent.

In the present study, the antibacterial activity of extracts obtained from marine seaweed *Padina gymnospora* was tested against Gram positive and Gram negative bacteria. The methanol crude extract of *Padina gymnospora* (5.0 mg/ml) showed highest mean zone of inhibition (13 ± 0.3 mm) against the Gram positive cocci *Staphylococcus aureus* followed by *Streptococcus pyogenes* (12 ± 0.2 mm), *Bacillus cereus* (11 ± 0.4 mm), *Streptococcus epidermidis* (11 ± 0.3 mm) and *Bacillus subtilis* (10 ± 0.5 mm). For Gram negative bacteria, maximum zone of inhibition was recorded in methanol crude extract of *Padina gymnospora* against *Enterobacter aerogenes* (13 ± 0.4 mm) followed by *Klebsiella pneumoniae* (13 ± 0.3 mm), *Pseudomonas aeruginosa* (12 ± 0.5 mm), *Escherichia coli* (11 ± 0.3 mm), *Salmonella typhi* (10 ± 0.2 mm) and *Vibrio cholerae* (8 ± 0.3 mm). The minimum inhibitory concentration (MIC) value of *Padina gymnospora* against bacteria was ranged between 1.25 to 80 mg/ml. The lowest MIC (1.25 mg/ml) value was recorded against *Staphylococcus aureus, Bacillus subtilis, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

In the study carried by Salvador *et al*. (2007), the extracts that were prepared from fresh and lyophilized samples of algae *Padina pavonica* and were screened for its antibacterial activity. *Padina pavonica* extract that was prepared from samples which were collected in winter showed highest inhibitor activity against Gram positive bacteria when compared to Gram negative bacteria. The results of the present research showed that seaweed extract of *Padina gymnospora* showed maximum inhibitory activity against Gram positive bacteria and the findings obtained from the study of Salvador *et al*. (2007) was well matched with the present study.

Subba Rangaiah *et al*. (2010) investigated the antimicrobial activity of seaweed *Padina tetrastromatica* by agar well diffusion method. The zone of inhibition was measured for all the different crude algal extracts (chloroform, ethanol, methanol and water) against six strains of Gram positive and Gram negative bacteria. Seaweed extracts in different solvents

exhibited different antimicrobial activities. The various solvents used for seaweed extractions, maximum inhibition were noticed with ethanol extracts and minimum with chloroform crude extracts. In the present study, five different solvents (methanol, acetone, chloroform, hexane and ethyl acetate) were used for the seaweed *Padina gymnospora* extraction. Among the solvent used, methanol extract showed maximum zone of inhibition against Gram positive and Gram negative bacteria and minimum zone was seen in hexane extract. The finding of the present research doesn't show any similarity with their research because of the change in solvent of this study.

Manivannan *et al*. (2011) evaluated the antimicrobial activity of *Turbinaria conoides*, *Padina gymnospora* and *Sargassum tenerrimum* against human bacterial pathogens. The antimicrobial activity of the seaweed extract was determined by disc diffusion method. The methanol extract showed better results than others. Strong antibacterial inhibition was noted by them in methanol extract of *Padina gymnospora* against *Bacillus subtilis*. The results of our findings are also in coincidence with the above findings.

12.4.2. ANTIFUNGAL ACTIVITY OF MARINE SEAWEEDS AGAINST PATHOGENIC FUNGI

In the present study, antifungal activity of extracts obtained from marine seaweeds *Ulva reticulata* and *Ulva lactuca* was investigated against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata*. The methanol crude extract of *Ulva reticulata* (10 mg/ml) showed highest mean zone of inhibition (13 ± 0.6 mm) against *Aspergillus flavus* followed by *Aspergillus fumigatus* (10 ± 0.3 mm), *Candida glabrata* (9 ± 0.6 mm), *Candida albicans* (9 ± 0.4 mm), *Aspergillus niger* (8 ± 0.5 mm) and *Saccharomyces cerevisiae* (7 ± 0.6 mm). The minimum inhibitory concentration (MIC) value of *Ulva reticulata* against fungi was ranged between 4

mg/ml to 64 mg/ml. The lowest MIC (4 mg/ml) value was recorded against *Candida albicans* and *Candida glabrata.*

The *Ulva lactuca* methanol extract (10 mg/ml) showed highest mean zone of inhibition (13 ± 0.5 mm) against *Candida glabrata* followed by *Candida albicans* (12 ± 0.6 mm)*, Aspergillus flavus* (12 ± 0.5 mm)*, Aspergillus fumigatus* (11 ± 0.3 mm)*, Aspergillus niger* (9 ± 0.5 mm) and *Saccharomyces cerevisiae* (8 ± 0.2 mm). The minimum inhibitory concentration (MIC) value of *Ulva lactuca* against fungi was ranged between 4 mg/ml to 32 mg/ml. The lowest MIC (4 mg/ml) value was recorded against *Candida albicans* and *Candida glabrata.*

Roberta Paulert *et al*. (2007) studied the antifungal activity of cell wall polysaccharides and crude extracts from the seaweed *Ulva fasciata* against filamentous fungi and yeast. The antifungal activity was assessed by agar diffusion assay and by means of the broth dilution method for estimating the minimal inhibitory concentration (MIC). MIC was determined for the fungi *Colletotrichum lindemuthianum* (plant pathogen), *Trichophyton mentagrophytes* and *Microsporum canis* (dermatophyte pathogens). The methanol insoluble extract inhibited the growth of *Trichophyton mentagrophytes* at concentration of 2 mg/ml. In this present study, the minimum inhibitory concentration (MIC) value of *Ulva reticulata* and *Ulva lactuca* against fungi were ranged between 4 mg/ml to 64 mg/ml. The lowest MIC (4 mg/ml) value was recorded against *Candida albicans* and *Candida glabrata.*

In the present study, antifungal activity of extracts obtained from marine seaweeds *Caulerpa racemosa* was investigated against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata*. The methanol extract of *Caulerpa racemosa* (10 mg/ml) showed highest mean zone of inhibition (11 ± 0.5 mm) against *Aspergillus niger* followed by *Aspergillus flavus* (10 ± 0.7 mm)*, Saccharomyces cerevisiae* (10 ± 0.5 mm), *Candida albicans* (10 ± 0.5 mm)*, Candida*

glabrata (9 ± 0.5 mm) and *Aspergillus fumigatus* (8 ± 0.5 mm). The minimum inhibitory concentration (MIC) value of *Caulerpa racemosa* against fungi was ranged between 4 mg/ml to 32 mg/ml. The lowest MIC (4 mg/ml) value was recorded against *Aspergillus niger*.

Mtolera and Semulkuesi (1996) screened the antimicrobial activity of the extracts of six marine green algae against the yeast, *Candida albicans* using a disc assay method. Of the six species tested, *Valonia aegrophila* extract was most active against all the tested organisms, and its extract was even more active against *Candida albicans* than Penicillin G. The extract of *Ulva pertusa* was not active against *Candida albicans*. The extract of *Caulerpa mexicana* was inactive against all the tested organisms. The findings of their study was in contrast with their research because in this present study *Caulerpa racemosa* showed inhibitory activity against the tested fungal pathogens. The antimicrobial activity shown by *Caulerpa racemosa* in their study may be attributed tocaulerpin or caulerpein (Doty and Santos, 1970; Paul *et al*., 1987), or flexin and trifarin (Blackman and Wells, 1978) or by caulerpanyene (Amico *et al*., 1978). The inactivity shown by an extract of *Caulerpa racemosa* suggests that the species might be lacking the substances mentioned.

In the present study, antifungal activity of extracts obtained from marine seaweeds *Hypnea musciformis* and *Hypnea valentiae* were investigated against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata*. The zone of inhibition of *Hypnea musciformis* extracts against fungal pathogens was ranged between 5 mm to 11 mm at 10 mg/ml. The methanol extract of *Hypnea musciformis* (10 mg/ml) showed highest mean zone of inhibition (11 ± 0.5 mm) against *Candida albicans* followed by *Candida glabrata* (10 ± 0.6 mm), *Saccharomyces cerevisiae* (10 ± 0.4 mm), *Aspergillus flavus* (10 ± 0.3 mm), *Aspergillus niger* (9 ± 0.5 mm) and *Aspergillus fumigatus* (9 ± 0.3 mm). The minimum inhibitory concentration (MIC) value of

Hypnea musciformis against fungi was ranged between 4 mg/ml to 64 mg/ml. The lowest MIC (4 mg/ml) value was recorded against *Candida albicans.*

The *Hypnea valentiae* methanol crude extract (10 mg/ml) showed highest mean zone of inhibition (12 ± 0.5 mm) against *Aspergillus flavus* followed by *Aspergillus niger* (10 ± 0.7 mm)*, Aspergillus fumigatus* (10 ± 0.4 mm)*, Candida albicans* (9 ± 0.6 mm), *Saccharomyces cerevisiae* (9 ± 0.5 mm) and *Candida glabrata* (9 ± 0.3 mm). The minimum inhibitory concentration (MIC) value of *Hypnea valentiae* against fungi was ranged between 4 mg/ml to 32 mg/ml. The lowest MIC (4 mg/ml) value was recorded against *Aspergillus flavus*, *Aspergillus niger*, *Candida albicans* and *Candida glabrata.*

Rossana Aguiar Cordeiro *et al*. (2006) screened a protein fraction, rich in lectin, obtained from the red seaweed *Hypnea musciformis* was assessed for antifungal potential against the human pathogen yeasts *Candida albicans* and *Candida guilliermondii*. It was capable of inhibiting the growth of *Candida guilliermondii* but it showed only a discrete inhibition against *Candida albicans* irrespective of the tested concentrations. In the present study, the view of the above researcher has become true as the extracts showed strong inhibitory effect towards the tested fungal pathogens.

In the present study, antifungal activity of extracts obtained from marine seaweeds *Gracilaria corticata* and *Gracilaria edulis* were investigated against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata*. The methanol crude extract of *Gracilaria corticata* (10 mg/ml) showed highest mean zone of inhibition (14 ± 0.6 mm) against *Aspergillus flavus* followed by *Aspergillus fumigatus* (14 ± 0.5 mm)*, Aspergillus niger* (13 ± 0.7 mm)*, Candida albicans* (12 ± 0.8 mm), *Candida glabrata* (12 ± 0.5 mm) and *Saccharomyces cerevisiae* (11 ± 0.6 mm). The minimum inhibitory concentration (MIC) value of *Gracilaria corticata* against

fungi was ranged between 1mg/ml to 16mg/ml. The lowest MIC (1mg/ml) value was recorded against *Candida albicans*, *Aspergillus niger* and *Aspergillus flavus.*

The *Gracilaria edulis* methanol extract (10 mg/ml) showed highest mean zone of inhibition (13 ± 0.6 mm) against *Aspergillus niger* followed by *Aspergillus flavus* (13 ± 0.3 mm)*, Candida albicans* (12 ± 0.4 mm)*, Aspergillus fumigatus* (11 ± 0.7 mm), *Candida glabrata* (11 ± 0.5 mm) and *Saccharomyces cerevisiae* (10 ± 0.6 mm). The minimum inhibitory concentration (MIC) value of *Gracilaria edulis* against fungi was ranged between 2 mg/ml to 32 mg/ml. The lowest MIC (2mg/ml) value was recorded against *Aspergillus niger*, *Aspergillus flavus*, *Candida albicans* and *Candida glabrata*.

Oranday *et al*. (2004) screened polar and non polar extracts of four species of marine seaweeds for antifungal properties against seven microorganisms by the disc diffusion method. The polar extracts of *Gracilaria tikvahiae* inhibited the growth of more than four microorganisms. The findings of the present study showed that the polar solvent as an effective one for the extraction of antimicrobial compounds from the seaweed *Gracilaria edulis* and the results of the present study was similar with the studies of Oranday *et al*. (2004).

Subba Rangaiah *et al*. (2010) showed that the seaweed extracts in different solvents exhibited different antimicrobial activities. In case of *Sargassum ilicifolium, Padina tetrastromatica,* of the various solvents used for seaweed extractions, maximum inhibition was noticed with ethanol extracts and minimum with chloroform crude extracts while in case of *Gracilaria corticata*, maximum inhibition was noticed with methanol and minimum with chloroform extracts. Antifungal activity of all the crude extractions of *Gracilaria corticata* showed maximum activity against *Rhizopus stolonifer.* The results of the present findings showed that the extracts of seaweed *Gracilaria* spp. have inhibitory activity against *Candida albicans*, from this; the presence of antifungal activity of *Gracilaria* spp. was proved.

In the present study, antifungal activity of extracts obtained from marine seaweed *Gelidiella acerosa* was investigated against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata*. The methanol crude extract of *Gelidiella acerosa* (10 mg/ml) showed highest mean zone of inhibition (10 ± 0.6 mm) against *Aspergillus fumigatus* followed by *Saccharomyces cerevisiae* (9 ± 0.6 mm), *Candida albicans* (9 ± 0.5 mm), *Aspergillus niger* (8 ± 0.8 mm), *Aspergillus flavus* (7 ± 0.6 mm) and *Candida glabrata* (6 ± 0.2 mm). The minimum inhibitory concentration (MIC) value of *Gelidiella acerosa* against fungi was ranged between 4 mg/ml to 32 mg/ml. The lowest MIC (4 mg/ml) value was recorded against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Candida glabrata*.

Hebsibah Elsie *et al*. (2011) compared the antimicrobial activity of ethanol and acetone extracts of *Gelidiella acerosa*. In fungi, ethanol extract of *Gelidium acerosa* showed maximum activity against *Candida albicans*, *Candida tropicalis*, *Aspergillus niger* and minimum zone formation in *Aspergillus flavus*. The maximum activity of acetone extract was seen in *Candida albicans* and minimum activity was against *Aspergillus flavus*. In this present investigation, methanol extract showed maximum inhibitory concentration against fungal pathogens and next to methanol extract, acetone extract showed more inhibitory activity. In the study of Hebsibah Elsie *et al*. (2011) similar inhibitory activity was also obtained from the methanolic and acetone extracts of *Gelidium acerosa.*

In the present study, antifungal activity of extracts obtained from marine seaweed *Sargassum myriocystum* was investigated against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata*. The methanol crude extract of *Sargassum myriocystum* (10 mg/ml) showed highest mean zone of inhibition (12 ± 0.6 mm) against *Aspergillus flavus* followed by *Aspergillus fumigatus* (11 ± 0.6 mm), *Candida glabrata* (10 ± 0.6 mm), *Aspergillus niger* (10 ± 0.3 mm),

Candida albicans (9 ± 0.7 mm) and *Saccharomyces cerevisiae* (8 ± 0.2 mm). The minimum inhibitory concentration (MIC) value of *Sargassum myriocystum* against fungi was ranged between 2 mg/ml to 32 mg/ml. The lowest MIC (2 mg/ml) value was recorded against *Candida albicans*.

Oranday *et al*. (2004) screened the extracts of four species of marine seaweed for antifungal properties against seven microorganisms by the disc diffusion method. The non polar extract of *Sargassum fluitans* and polar extracts of *Gracilaria tikvahiae* inhibited the growth of more than four microorganisms. The findings of the present study also showed that the seaweed *Sargassum myriocystum* has the antimicrobial activity and the results of the present study was similar with the findings of the above scientists.

Seaweed extracts in different solvents exhibited different antimicrobial activities. In case of *Sargassum ilicifolium* and *Padina tetrastromatica,* various solvents were used for seaweeds extraction. All the crude extracts exhibited minimum activity against *Candida albicans* and no activity against *Saccharomyces cerevisiae.* The results of the present findings also showed that the seaweed extracts of *Sargassum myriocystum* had showed low inhibitory activity against *Candida albicans* and *Saccharomyces cerevisiae*, and the above research of Subba Rangaiah *et al*. (2010) was similar with the present study.

In the present study, antifungal activity of extracts obtained from marine seaweeds *Padina gymnospora* was investigated against *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Saccharomyces cerevisiae*, *Candida albicans* and *Candida glabrata*. The methanol crude extract of *Padina gymnospora* (10 mg/ml) showed highest mean zone of inhibition (10 ± 0.6 mm) against *Aspergillus niger* followed by *Candida glabrata* (9 ± 0.7 mm), *Saccharomyces cerevisiae* (9 ± 0.6 mm), *Aspergillus flavus* (8 ± 0.6 mm), *Candida albicans* (8 ± 0.3 mm) and *Aspergillus fumigatus* (7 ± 0.6 mm). The minimum inhibitory

concentration (MIC) value of *Padina gymnospora* against fungi was ranged between 2 mg/ml to 32 mg/ml. The lowest MIC (2 mg/ml) value was recorded against *Aspergillus fumigatus*.

The extracts of different seaweeds in different solvents exhibited different antimicrobial activities. In case of *Sargassum ilicifolium, Padina tetrastromatica,* of the various solvents used for seaweed extractions, maximum inhibition was noticed with ethanol extracts and minimum with chloroform crude extracts while in case of *Gracilaria corticata*, maximum inhibition was noticed with methanol and minimum with chloroform extracts. The ethanol extract showed no activity against *Saccharomyces cerevisiae* and *Mucor racemosus.* Chloroform extract showed no activity against *Aspergillus niger, Saccharomyces cerevisiae,* mild response towards *Aspergillus niger* Subba Rangaiah *et al*. (2010).

Manivannan *et al*. (2011) evaluated the antimicrobial activity of *Turbinaria conoides*, *Padina gymnospora* and *Sargassum tenerrimum* against human fungal pathogens. The antimicrobial activity of the seaweeds extracts was determined by disc diffusion method. The methanol extract showed better results than others. In our study also, methanol extracts gave promising results.

ANALYSIS OF BIOACTIVE COMPOUNDS

In the present study, the highest antimicrobial activity was exhibited by *Gracilaria corticata*. Based on this maximum activity of seaweed, *Gracilaria corticata* was subjected to GC-MS analysis and further spectral studies, which showed the presence of components *viz*., Hexadecane, 1, 10 – Decanediol, 2 – Hepatone, 5- methyl, 2,4 – Dimethylcyclopentanol, Pentanal, 2 – methyl - , (2S, 3S) – (-) – 3 – Propyloxiranemethanol, 3,4 – Hexanediol, 2,5 – dimethyl -, Octane, 3, 4, 5, 6 – tetramethyl – and Diazoprogesterone.

Ulku Karabay *et al.* (2006) studied the methanol, dichloromethane, hexane, chloroform and volatile oil extracts of the red alga *Jania rubens* for their antimicrobial activity. GC-MS analysis of the volatile components of *Jania rubens* identified 40

compounds which constituted 77.53% of the total. The volatile components of *Jania rubens* consisted of *n*-docosane (6.35%), *n*-eicosane (5.77%) and *n*-tetratriacontane (5.58%) as major components. In this research, Hexadecane, 1, 10 – Decanediol, 2 – Hepatone, 5- methyl, 2,4 – Dimethylcyclopentanol, Pentanal, 2 – methyl - , (2S, 3S) – (-) – 3 – Propyloxiranemethanol, 3,4 – Hexanediol, 2,5 – dimethyl -, Octane, 3, 4, 5, 6 – tetramethyl – and Diazoprogesterone presence were revealed.

Aseer Manilal *et al*. (2010) assayed a total of ten seaweed species for antifouling assays against the common fouling organisms such as *Balanus* sp., *Mytilus edulis* and three biofilm forming bacteria, *Vibrio* sp., *Colwellia* sp. and *Pseudoalteromonas* sp. Of all the seaweeds tested, only one species, the red algae, *Laurencia brandenii* displayed broader spectrum of activity. The active principle of *Laurencia brandenii* was purified in Column chromatography and was identified by GC-MS. The GC-MS profile of *Laurencia brandenii* suggested the purified fraction is primarily composed of octadecadienoic acid (49.75%) followed by "n-Hexadecanoic acid" (14.24%) which could have functional role in the chemical defense against fouling organisms and it could be utilized for the development of ideal antifoulants in future. This present research showed the presence of bioactive components *viz*., Hexadecane, 1, 10 – Decanediol, 2 – Hepatone, 5- methyl, 2,4 – Dimethylcyclopentanol, Pentanal, 2 – methyl - , (2S, 3S) – (-) – 3 – Propyloxiranemethanol, 3,4 – Hexanediol, 2,5 – dimethyl -, Octane, 3, 4, 5, 6 – tetramethyl – and Diazoprogesterone.

According to the above reports and taking into an account by the results detailed in the present contribution with respect to the seaweeds from our coasts reveal significant bioactive components, and thus deserve vital place in medical microbiology, marine biotechnology, vector control programmes to examine the properties of natural products. The extracts of seaweeds showed a real potential with good yields. These results suggest the possibility of using marine algal extracts in therapy as natural alternatives to antibiotics

currently in the market and has clearly showed that seaweeds from the coast of Mandapam in Tamil Nadu are valuable source of biologically active compounds. Further, this research paves way to determine the structure and nature of these bioactive components from marine species which constitutes approximately a half of the total global biodiversity

12.7. CONTRIBUTION TO THE SOCIETY

Production of bioactive compounds from selected seaweeds against for controlling human pathogenic microorganisms. The wonderful highly available natural biomass is an excellent source to meet out the growing demand of antimicrobial agents is achieved to some extent in this project. Exploring marine sources as natural drug is a novel approach of this project. After a detailed pharmacological study the isolated marine bioactive compound diszoprogesterone, hexadecane and 2,4 dimethylcyclopentanol from the *Gracilaria corticata* may be used a effective drug against the tested bacterial and fungal pathogens.

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