VERMICOMPOSTING OF SPENT TEA LEAVES BY EARTHWORM *EISENIA FETIDA* AND ITS EFFICACY ON THE GROWTH OF LADY'S FINGER (*ABELMOSCHUS ESCULENTUS*)

Dissertation submitted to

ST.MARY'S COLLEGE (Autonomous), Thoothukudi

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

in partial fulfilment for the award of the degree of

MASTER OF SCIENCE IN ZOOLOGY

By

M.AARTHI

Reg. No. 17APZO01

DEPARTMENT OF ZOOLOGY

ST.MARY'S COLLEGE (AUTONOMOUS)

(Re-accredited with 'A' Grade by NAAC)

THOOTHUKUDI-628001

2018-19

CERTIFICATE

This is to certify that this dissertation entitled, **"VERMICOMPOSTING OF SPENT TEA LEAVES BY EARTHWORM *EISENIA FETIDA* AND ITS EFFICACY ON THE GROWTH OF LADY'S FINGER *(ABELMOSCHUS ESCULENTUS)*"** is a record of original research work done by **M. AARTHI,** under my supervision, and submitted in. partial fulfilment for the degree of Master of Science in Zoology. This dissertation has not formed the basis for any other degree.

GUIDE

23/10/18
HEAD OF THE DEPARTMENT
HOD
PG & Research Department of Zoology
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

PRINCIPAL
Principal
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

30/10/18
EXAMINER

DR.J.SUMITHA M.Sc.,M.Phil.,Ph.D.,
ASSOCIATE PROFESSOR,
Department of Zoology.
RANI ANNA GOVT. COLLEGE FOR WOMEN,
GANDHI NAGAR, TIRUNELVELI - 627 008.

DECLARATION

I hereby declare that this dissertation entitled, **"VERMICOMPOSTING OF SPENT TEA LEAVES BY ERATHWORM *EISENIA FETIDA* AND ITS EFFICACY ON THE GROWTH OF LADY'S FINGER *(ABELMOCHUS ESCULENTUS)*"** submitted by me for the award of the degree of Master of Science in Zoology is the result of my original independent research work under the guidance of **Dr. A. Elezabeth Mary, M.Sc., B.Ed., Phil., Ph.D** Associate Professor, Department of Zoology, St. Mary's College (Autonomous), Thoothukudi, and it has not been submitted for the award of any degree, diploma, associateship/ fellowship of any university or institution.

Place : Thoothukudi
Date : 23.10.2018

M. Aarthi
Signature of the Candidate

PHARMACOLOGICAL EVALUATION AND BIOSYNTHESIS OF SILVER NANOPARTICLES FROM *MUREX TRIBULUS* OF THOOTHUKUDI COAST.

A dissertation submitted to

ST.MARY'S COLLEGE (Autonomous), Thoothukudi

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

in partial fulfilment for the award of the degree of

MASTER OF SCIENCE IN ZOOLOGY

By

J. ESTHER MEREEN

Reg. No.17APZO02

DEPARTMENT OF ZOOLOGY

ST.MARY'S COLLEGE (AUTONOMOUS)

(Re-accredited with 'A' Grade by NAAC)

THOOTHUKUDI-628 001

OCTOBER-2018

CERTIFICATE

This is to certify that this dissertation entitled **"PHARMACOLOGICAL EVALUATION AND BIOSYNTHESIS OF SILVER NANOPARTICLES FROM *MUREX TRIBULUS* OF THOOTHUKUDI COAST"**, submitted by **J.ESTHER MEREEN Reg.No. 17APZO02 to ST. MARY'S COLLEGE (Autonomous), THOOTHUKUDI** affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY** in partial fulfilment for the award of the degree of Master of Science in Zoology is done by her during the period of 2018-19 under my guidance and supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

Jesy Diaz

GUIDE

23/10/18

HOD

HOD
PG & Research Department of Zoology
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

Lucia Rose

PRINCIPAL

Principal
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

30/10/18

EXAMINER

DR.J.SUMITHA M.Sc.,M.Phil.,Ph.D.,
ASSOCIATE PROFESSOR,
Department of Zoology
RANI ANNA GOVT. COLLEGE FOR WOMEN,
GANDHI NAGAR, TIRUNELVELI - 627 008.

DECLARATION

I hereby declare that the dissertation entitled "**PHARMACOLOGICAL EVALUATION AND BIOSYNTHESIS OF SILVER NANOPARTICLES FROM *MUREX TRIBULUS* OF THOOTHUKUDI COAST.**" submitted by me for the award of the degree of Master of Science in Zoology, is the result of my original and independent work carried out under the guidance of **Dr. JEMMA HERMELIN JESY DIAZ M.Sc., B.Ed., M.Phil., Ph.D.,** Associate Professor, Department of Zoology, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted elsewhere for the award of any other degree.

STATION : THOOTHUKUDI

DATE : 23.10.2018

J. Esther Mereen

(J. ESTHER MEREEN)

HEAVYMETAL ACCMULATION IN SELECTED COMMERCIALLY IMPORTANT MARINE ORGANISMS FROM THE GULF OF MANNAR COAST OF THOOTHUKUDI.

Dissertation submitted to

ST. MARY'S COLLEGE (Autonomous), Thoothukudi

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

in partial fulfilment for the award of the degree of

MASTER OF SCIENCE IN ZOOLOGY

By

J. HEARTLIN

Reg. No. 17APZO03

DEPARTMENT OF ZOOLOGY

ST. MARY'SCOLLEGE (AUTONOMOUS)

(Re-accredited with 'A' Grade by NAAC -3rd cycle)

THOOTHUKUDI-628001

2018- 2019

CERTIFICATE

This is to certify that this dissertation entitled, **"HEAVY METAL ACCUMULATION IN SELECTED COMMERCIALLY IMPORTANT MARINE ORGANISMS FROM THE GULF OF MANNAR COAST OF THOOTHUKUDI"** is a record of original research work done by **J.HEARTLIN,** under my supervision and submitted in partial fulfilment for the award of degree of Master of Science in Zoology. This dissertation has not formed the basis for any other degree.

Place: Thoothukudi

Date: 23.10.18

Hermin Pasangka

Signature of the Supervisor

Lucia Rose

Head of the Institution

Principal
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

23/10/18

Head of the Department

HOD
PG & Research Department of Zoology
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

J. Sumithra 30/10/18

Examiner

DR.J.SUMITHRA M.Sc., M.Phil., Ph.D.,
ASSOCIATE PROFESSOR
Department of Zoology,
RANI ANNA GOVT. COLLEGE FOR WOMEN,
GANDHI NAGAR, TIRUNELVELI - 627 008.

DECLARATION

I hereby declare that this dissertation entitled, **"HEAVY METAL ACCUMUATION IN SELECTED COMMERCIALLY IMPORTANT MARINE ORGANISMS FROM THE GULF OF MANNAR COAST OF THOOTHUKUDI"** submitted by me for the award of the degree of Master of Science in Zoology is the result of my original independent research work under the guidance of **Dr. Mrs. HERMIN PASANGHA, M.Sc., B.Ed., Ph.D.,** Associate Professor, Department of Zoology, St. Mary's College (Autonomous), Thoothukudi, and it has not been submitted for the award of any degree, diploma, associateship/fellowship of any university or institution.

J. Heartlin

Signature of the Candidate

(J. HEARTLIN)

Place: Thoothukudi

Date: 23.10.18

A STUDY ON MACROBENTHOS WITH REFERENCE TO WATER AND SEDIMENT QUALITIES TO ASSESS THE INTENSITY OF POLLUTION ALONG THE THOOTHUKUDI COAST

Dissertation submitted to

ST.MARY'S COLLEGE (Autonomous), Thoothukudi

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

in partial fulfilment for the award of the degree of

MASTER OF SCIENCE IN ZOOLOGY

By

S. JANANI

Reg. No.17APZO04

DEPARTMENT OF ZOOLOGY

ST.MARY'S COLLEGE (AUTONOMOUS)

(Re-accredited with 'A' grade by NAAC)

THOOTHUKUDI-628001

2018-19

CERTIFICATE

This is to certify that this dissertation entitled **"A STUDY ON MACROBENTHOS WITH REFERENCE TO WATER AND SEDIMENT QUALITIES TO ASSESS THE INTENSITY OF POLLUTION ALONG THE THOOTHUKUDI COAST"** submitted by **S.JANANI, Reg.No.17APZO04** to ST.MARY'S COLLEGE (Autonomous), THOOTHUKUDI affiliated to Manonmaniam Sundaranar University in partial fulfilment for the award of the degree of Master of science in Zoology is done by her during the period of 2018-2019 under my guidance and supervision. It is further certified that the dissertation or any part of this has not been submitted elsewhere for any other degree.

Dr. S. MARY BAPTISTA JANET
M.Sc., M.Phil., B.Ed., Ph.D.,
GUIDE
Associate Professor
Department of Zoology
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

23/10/18
HEAD OF THE DEPARTMENT
HOD
PG & Research Department of Zoology
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

PRINCIPAL
Principal
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

30/10/18
EXAMINER
DR.J.SUMITHA M.Sc.,M.Phil.,Ph.D.,
ASSOCIATE PROFESSOR,
Department of Zoology,
RANI ANNA GOVT. COLLEGE FOR WOMEN,
GANDHI NAGAR, TIRUNELVELI - 627 008.

DECLARATION

I hereby declare that the thesis entitled **"A STUDY ON MACROBENTHOS WITH REFERENCE TO WATER AND SEDIMENT QUALITIES TO ASSESS THE INDENSITY OF POLLUTION ALONG THE THOOTHUKUDI COAST"** submitted by me for the Degree of Master of Science in Zoology is the result of my original and independent research work carried out under the guidance of **Dr. Mrs. S. Mary Baptista Janet, M.Sc., B.Ed., M.Phil., Ph.D.,** Associate Professor, Department of Zoology, St.Mary's College, Thoothukudi, and it has not been submitted elsewhere for of any other degree.

Place : Thoothukudi

Date : 23.10.2018

S. [signature]

Signature of the Candidate

ISOLATION AND IDENTIFICATION OF BACTERIA ASSOCIATED WITH MARINE SEDIMENTS AND MOLECULAR CHARACTERIZATION OF DESULFOVIBRIO VULGARIS

Dissertation submitted to

ST. MARY'S COLLEGE (AUTONOMOUS), THOOTHUKUDI

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

in partial fulfilment for the award of the degree of

MASTER OF SCIENCE IN ZOOLOGY

By

C. KARTHIGAIKANNI

Reg. No. 17APZO06

DEPARTMENT OF ZOOLOGY

ST.MARY'S COLLEGE (AUTONOMOUS)

(Re-accredited with 'A' Grade by NAAC)

THOOTHUKUDI-628001

2018-19

CERTIFICATE

This is to certify that this dissertation entitled, **"ISOLATION AND IDENTIFICATION OF BACTERIA ASSOCIATED WITH MARINE SEDIMENTS AND MOLECULAR CHARACTERIZATION OF DESULFOVIBRIO VULGARIS"** submitted by **C. KARTHIGAIKANNI, Reg. No. 17APZO06** to St. Mary's College (Autonomous), Thoothukudi, affiliated to Manonmaniam Sundaranar University in partial fulfilment for the award of the degree of Master of Science in Zoology is done by her during the period of 2018 – 2019 under my guidance and supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

P. J. Joslin
GUIDE

23/10/18
HEAD OF THE DEPARTMENT
HOD
PG & Research Department of Zoology
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

PRINCIPAL
Principal
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

J. Sumitha 30/10/18
EXAMINER
DR.J.SUMITHA M.Sc.,M.Phil.,Ph.D.,
ASSOCIATE PROFESSOR,
Department of Zoology,
RANI ANNA GOVT. COLLEGE FOR WOMEN,
GANDHI NAGAR, TIRUNELVELI - 627 008.

DECLARATION

I do hereby declare that this thesis entitled, **"ISOLATION AND IDENTIFICATION OF BACTERIA ASSOCIATED WITH MARINE SEDIMENTS AND MOLECULAR CHARACTERIZATION OF DESULFOVIBRIO VULGARIS"** submitted by me for the award of the degree of Master of Science in Zoology is the result of my original independent research work carried out under the guidance of **Dr. Mrs. P.J. Joslin, M.Sc., M.Phil., Ph.D.,** Associate Professor, Department of Zoology, St. Mary's College (Autonomous), Thoothukudi, and it has not been submitted elsewhere for the award of any other degree.

Place : Thoothukudi

Date : 23.10.2018

C. Karthigaikanni.

Signature of the Candidate

PROSPECTIVE ANTIOXIDANT AND ANTICANCER EFFICACY OF BIOACTIVE COMPOUNDS FROM *CHICOREUS RAMOSUS* (LINNAEUS,1758)

Dissertation submitted to
ST.MARY'S COLLEGE (Autonomaus), Thoothukudi
affiliated to
MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI
in partial fulfilment for the award of the degree of

MASTER OF SCIENCE IN ZOOLOGY

By

P.KAVIYA PRIYA

Reg. No. 17APZO07

DEPARTMENT OF ZOOLOGY
ST.MARY'S COLLEGE (AUTONOMOUS)
(Re-accredited with 'A' Grade by NAAC)
THOOTHUKUDI-628001

2018-19

CERTIFICATE

This is to certify that this dissertation entitled

"PROSPECTIVE ANTIOXIDANT AND ANTICANCER EFFICACY OF BIOACTIVE COMPOUNDS FROM *CHICOREUS RAMOSUS* (LINNAEUS, 1758)" submitted by **P.KAVIYA PRIYA** **Reg. No.17APZO07** to **St. Mary's college (Autonomous), Thoothukudi** in partial fulfilment for the award of the degree of **Master of Science in Zoology** is done by her under my supervision. It is further certified that this dissertation or any part of this has not been submitted elsewhere for any other degree.

P. Subavathy
GUIDE

23/10/18
HEAD OF THE DEPARTMENT

HOD
PG & Research Department of Zoology
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

PRINCIPAL

Principal
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

J. Sumitha 30/10/18.
EXAMINER

DR.J.SUMITHA M.Sc.,M.Phil.,Ph.D.,
ASSOCIATE PROFESSOR,
Department of Zoology,
RANI ANNA GOVT. COLLEGE FOR WOMEN,
GANDHI NAGAR, TIRUNELVELI - 627 008.

DECLARATION

I hereby declare that the thesis entitled **"PROSPECTIVE ANTIOXIDANT AND ANTICANCER EFFICACY OF BIOACTIVE COMPOUNDS FROM *CHICOREUS RAMOSUS* (LINNAEUS, 1758)"** submitted by me for the award of the degree of Master of Science in Zoology is the result of my original independent research work under the guidance of **Dr.P.Subavathy M.Sc.,M.Phil.,SET.,Ph.D.,** Assistant Professor, Department of Zoology, St. Mary's College (Autonomous), Thoothukudi, and it has not been submitted for the award of any degree, diploma, associateship, fellowship of any university or institution.

Place : Thoothukudi

Date : 22.10.2018

P. Baviga Priya

Signature of the Candidate

ANTIBACTERIAL ACTIVITY AND GC-MS ANALYSIS OF MARINE PUFFER FISH *LAGOCEPHALUS LUNARIS* FROM THOOTHUKUDI COAST

Dissertation submitted to

ST. MARY'S COLLEGE (Autonomous), THOOTHUKUDI

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

in partial fulfilment for the award of the degree of

MASTER OF SCIENCE IN ZOOLOGY

By

S.MERLIN CHRISTINA

Reg. No.17APZO08

DEPARTMENT OF ZOOLOGY

ST.MARY'S COLLEGE (autonomous)

(Re-accredited with 'A' Grade by NAAC -3rd Cycle)

THOOTHUKUDI-628001

2018-19

CERTIFICATE

This is to certify that this dissertation entitled, **"ANTIBACTERIAL ACTIVITY AND GC-MS ANALYSIS OF MARINE PUFFER FISH *LAGOCEPHALUS LUNARIS* FROM THOOTHUKUDI COAST"** is a record of original research work done by **S. MERLIN CHRISTINA,** under my supervision, and submitted in partial fulfilment for the award of the degree of **Master of Science in Zoology**. This dissertation has not formed the basis for any other degree.

S. Selvi.
GUIDE

23/10/18
HEAD OF THE DEPARTMENT
HOD
PG & Research Department of Zoology
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

PRINCIPAL
Principal
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

J. Sumitha 30/10/18
EXAMINER
DR.J.SUMITHA M.Sc.,M.Phil.,Ph.D.,
ASSOCIATE PROFESSOR,
Department of Zoology,
RANI ANNA GOVT. COLLEGE FOR WOMEN,
GANDHI NAGAR, TIRUNELVELI - 627 008.

DECLARATION

I hereby declare that the thesis entitled **"ANTIBACTERIAL ACTIVITY AND GC-MS ANALYSIS OF MARINE PUFFER FISH *LAGOCEPHALUS LUNARIS* FROM THOOTHUKUDI COAST"** submitted by me for the award of the Degree of Master of Science in Zoology is the result of my original and independent research work carried out under the guidance of **Mrs. S. Selvi, M.Sc., B.Ed., M.Phil.,** Assistant Professor, Department of Zoology, St.Mary's College, Thoothukudi, and it has not been submitted for the award of any degree, diploma, associate ship, fellowship of any University or Institution.

Place : Thoothukudi

Date : 23.10.18

S. Merlin Christina.

Signature of the Candidate

(S. MERLIN CHRISTINA)

SCREENING AND CHARACTERIZATION OF *P. VANNAMEI* SHELL EXTRACT FOR THERAPEUTIC ACTIVITY.

A dissertation submitted to

ST.MARY'S COLLEGE (Autonomous), Thoothukudi

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

in partial fulfilment for the award of the degree of

MASTER OF SCIENCE IN ZOOLOGY

By

M. MUTHUMARIYAMMAL

Reg. No.17APZO09

DEPARTMENT OF ZOOLOGY

ST.MARY'S COLLEGE (AUTONOMOUS)

(Re-accredited with 'A' Grade by NAAC)

THOOTHUKUDI-628001

OCTOBER-2018

CERTIFICATE

This is to certify that this dissertation entitled **"SCREENING AND HARACTERIZATION OF *P.VANNAMEI* SHELL EXTRACT FOR HERAPEUTIC ACTIVITY"** submitted by **M. MUTHUMARIYAMMAL** eg.No. **17APZO09to ST. MARY'S COLLEGE (Autonomous), HOOTHUKUDI** affiliated to **MANONMANIAM SUNDARANAR NIVERSITY** in partial fulfilment for the award of the degree of Master of cience in Zoology is done by her during the period of 2018-19 under my idance and supervision. It is further certified that this dissertation or any part of is has not been submitted elsewhere for any other degree.

Tesy Diaz
GUIDE

HOD 23/10/18

HOD
& Research Department of Zoology
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

Lucia Rose
PRINCIPAL
Principal
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

J. Sunitha 30/10/18
EXAMINER
DR.J.SUMITHA M.Sc.,M.Phil.,Ph.D.,
ASSOCIATE PROFESSOR,
Department of Zoology,
RANI ANNA GOVT. COLLEGE FOR WOMEN,
GANDHI NAGAR, TIRUNELVELI - 627 008.

DECLARATION

I hereby declare that the dissertation entitled "**SCREENING AND HARACTERIZATION OF *P. VANNAMEI* SHELL EXTRACT FOR HERAPEUTIC ACTIVITY**". submitted by me for the award of the degree of laster of Science in Zoology, is the result of my original and independent work arried out under the guidance of **Dr. JEMMA HERMELIN JESY DIAZ M.Sc., B.Ed., M.Phil.,Ph.D** Assistant Professor, Department of Zoology, St. Mary's College (Autonomous), Thoothukudi and it has not been submitted lsewhere for the award of any other degree.

STATION : THOOTHUKUDI

DATE: 23.10.2018

M. Muthumariyammal

(M. MUTHUMARIYAMMAL)

HEAVY METAL CONCENTRATION IN WATER, SEDIMENT AND THE FISH *HEMIRAMPHUS MARGINATUS* FROM TUTICORIN COAST

Dissertation submitted to

ST. MARY'S COLLEGE (Autonomous), Thoothukudi

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

in partial fulfilment for the award of the degree of

MASTER OF SCIENCE IN ZOOLOGY

By

M. MUTHUSELVI

Reg. No. 17APZO10

DEPARTMENT OF ZOOLOGY

ST.MARY'S COLLEGE (AUTONOMOUS)

(Re-accredited with 'A' Grade by NAAC -3rd cycle)

THOOTHUKUDI-628001

2018 - 2019

CERTIFICATE

This is to certify that this dissertation entitled,

"HEAVY METAL CONCENTRATION IN WATER, SEDIMENT AND THE FISH *HEMIRAMPHUS MARGINATUS* FROM TUTICORIN COAST" is a record of original research work done by **M. MUTHUSELVI,** under my supervision, and submitted in partial fulfilment for the award of degree of Master of Science in Zoology. This dissertation has not formed the basis for any other degree.

Place: Thoothukudi

Date: 23.10.2018

Signature of the Supervisor

Head of the Institution

Principal
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

Head of the Department

23/10/18

HOD
PG & Research Department of Zoology
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

Examiner

30/10/18

DR.J.SUMITHA M.Sc.,M.Phil.,Ph.D.,
ASSOCIATE PROFESSOR,
Department of Zoology,
RANI ANNA GOVT. COLLEGE FOR WOMEN,
GANDHI NAGAR, TIRUNELVELI - 627 008.

DECLARATION

I hereby declare that this dissertation entitled,

"HEAVY METAL CONCENTRATION IN WATER, SEDIMENT AND THE FISH *HEMIRAMPHUS MARGINATUS* FROM TUTICORIN COAST" submitted by me for the award of the degree of Master of Science in Zoology is the result of my original independent research work under the guidance of **Mrs. S. JESILY, M.Sc., B.Ed., M.Phil.**, Associate Professor, Department of Zoology, St. Mary's College (Autonomous), Thoothukudi, and it has not been submitted for the award of any degree, diploma, associate ship/ fellowship of any university or institution.

Place: Thoothukudi

Date: 23.10.2018

Signature of the candidate

M. Muthuselvi.

(M.MUTHUSELVI)

IDENTIFICATION OF BIOACTIVE COMPOUNDS AND ANTIMICROBIAL ACTIVITY OF MARINE ECHINODERMS *PROTOREASTER LINCKII* (BLAINVILLE, 1834) FROM THOOTHUKUDI COAST

Dissertation submitted to

ST.MARY'S COLLEGE (Autonomous), Thoothukudi

Affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

in partial fulfilment for the award of the degree of

MASTER OF SCIENCE IN ZOOLOGY

By

D. NIVETHA

Reg. No.17APZO12

DEPARTMENT OF ZOOLOGY

ST.MARY'S COLLEGE (AUTONOMOUS)

(Re-accredited with 'A' Grade by NAAC -3rd Cycle)

THOOTHUKUDI-628001

2018-2019

SCANNING ELECTRON MICROSCOPICAL STUDY OF RADULA, INVESTIGATION OF DIGESTIVE ENZYMES AND GUT MICROBIOME OF *CHICOREUS VIRGINEUS* (R.1798) AND *TURBINELLA PYRUM* (L.1758) FROM THOOTHUKUDI COAST

Dissertation submitted to

ST.MARY'S COLLEGE (Autonomous), Thoothukudi

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

in partial fulfilment for the award of the degree of

MASTER OF SCIENCE IN ZOOLOGY

By

A.SHINY THRESA

Reg. No.17APZO13

DEPARTMENT OF ZOOLOGY

ST.MARY'S COLLEGE (AUTONOMOUS)

(Re-accredited with 'A' Grade by NAAC -3rd Cycle)

THOOTHUKUDI-628001

2018-19

CERTIFICATE

This is to certify that this dissertation entitled, "**SCANNING ELECTRON MICROSCOPICAL STUDY OF RADULA, INVESTIGATION OF DIGESTIVE ENZYMES AND GUT MICROBIOME OF *CHICOREUS VIRGINEUS*(R.1798) AND *TURBINELLA PYRUM* (L.1758) FROM THOOTHUKUDI COAST**" is a record of original research work done by **A.SHINY THRESA,** under my supervision, and submitted in partial fulfilment for the degree of Master of Science in Zoology. This dissertation has not formed the basis for any other degree.

GUIDE

23/10/18

HEAD OF THE DEPARTMENT

HOD
PG & Research Department of
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

PRINCIPAL

Principal
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

J. Sumitha 30/10/18

EXAMINER

DR.J.SUMITHA M.Sc.,M.Phil.,Ph.D.,
ASSOCIATE PROFESSOR,
Department of Zoology,
RANI ANNA GOVT. COLLEGE FOR WOMEN,
GANDHI NAGAR, TIRUNELVELI - 627 008.

DECLARATION

I hereby declare that the thesis entitled **"SCANNING ELECTRON ICROSCOPICAL STUDY OF RADULA, INVESTIGATION OF GESTIVE ENZYMES AND GUT MICROBIOME OF *CHICOREUS RGINEUS* (R.1798) AND *TURBINELLA PYRUM* (L.1758) FROM HOOTHUKUDI COAST"** submitted by me for the Degree of Master of ience in Zoology is the result of my original and independent researchwork rriedout under the guidance of Dr.R.D.Thilaga,M.Sc.,M.Phil.,M.Ed.,Ph.D., sociate Professor and Head, Department of Zoology, St.Mary's College, oothukudi,and it has not been submitted for the award of any degree, diploma, sociate ship, fellowship of any University or Institution.

ace: Thoothukudi.

te: 23.10.2018

A. Shiny Thresa

(Shiny Thresa. A)

IDENTIFICATION OF BIOACTIVE COMPOUNDS AND ANTIMICROBIAL ACTIVITY OF MARINE ECHINODERM *SALMACIS VIRGULATA* (L. AGASSIZ AND DESOR, 1846) FROM GULF OF MANNAR

Dissertation submitted to

ST.MARY'S COLLEGE (Autonomous), Thoothukudi

affiliated to

MANONMANIAM SUNDARANAR UNIVERSITY, TIRUNELVELI

in partial fulfilment for the award of the degree of

MASTER OF SCIENCE IN ZOOLOGY

By

M.VELANKANNY

Reg. No.17APZO14

DEPARTMENT OF ZOOLOGY

ST.MARY'S COLLEGE (AUTONOMOUS)

(Re-accredited with 'A' Grade by NAAC -3rd Cycle)

THOOTHUKUDI-628001

2018-2019

CERTIFICATE

This is to certify that this dissertation entitled, **"IDENTIFICATION OF BIOACTIVE COMPOUNDS AND ANTIMICROBIAL ACTIVITY OF MARINE ECHINODERM *SALMACIS VIRGULATA* (L.AGASSIZ AND DESOR,1846) FROM GULF OF MANNAR"** Submitted by **M.VELANKANNY, Reg.No.17APZO14** in partial fulfilment for the award of the degree of Master of science in zoology to St.Mary's College (Autonomous), Thoothukudi affiliated to **MANONMANIAM SUNDARANAR UNIVERSITY** is done by her during the period of 2018-2019 under my guidance and supervision. It is further certified that this dissertation or any other part of this has not been submitted elsewhere for any degree.

Place: Thoothukudi

Guide

Date: 23-10-2018

Head of the institution

Principal
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

23/10/18

Head of the Department

HOD
PG & Research Department of Zoology
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

30/10/18.

EXAMINER

DR.J.SUMITHA M.Sc.,M.Phil.,Ph.D.,
ASSOCIATE PROFESSOR,
Department of Zoology,
RANI ANNA GOVT. COLLEGE FOR WOMEN,
GANDHI NAGAR, TIRUNELVELI - 627 008.

DECLARATION

I hereby declare that the thesis entitled **"IDENTIFICATION OF BIOACTIVE COMPOUNDS AND ANTIMICROBIAL ACTIVITY OF MARINE ECHINODERM *SALMACIS VIRGULATA* (L.AGASSIZ AND DESOR,1846) FROM GULF OF MANNAR"** submitted by me for award of the Degree of Master of Science in Zoology is the result of my original and independent research work under the guidance of Dr.S.R.T.Sherly Cross., M.sc., M.Phil., Ph.D., Assistant professor, Department of Zoology,St.Mary's College,(Autonomous), Thoothukudi, and it has not been submitted for the award of any degree or diploma.

Place: Thoothukudi.

Date: 23.10.2018

M. Velankanny

Signature of the candidate

(VELANKANNY.M)

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1. INTRODUCTION

The world's oceans, which cover more than 70% of the earth's surface, has been considered as a rich source of compounds possessing novel structures with rich biological activity. The increasing incidence of antibiotic resistance among bacterial pathogens and emerging new disease are posing great challenges to humans. Given the widespread misuse and over prescription of antibiotics by the medical community. The antibiotics available today are rapidly becoming less and less effective in the face of emerging multi-drug resistant pathogens of clinical concern.

Microorganisms display interesting competitive mechanisms of which antagonism has been commonly referenced. Microbial antagonism is a biological phenomenon in which certain microorganisms of the normal micro biota that suppresses the growth of other microorganisms through competition for nutrients and the secretion of inhibitory substances. Some microorganisms can inhibit other microorganisms or reduce their growth in medium thanks to their metabolites, indirect (by changing pH, osmotic pressure and surface tension) or direct (by producing toxic component, antimicrobial components, bacteriocin, antibiotics etc.,) this situation is called as antagonistic relation. Microorganisms are not only the cause of infections; they can also produce organic substances that can cure infections. The first marine bacterium based antibiotic was characterized in 1966. As the primary role of antimicrobial activity can be to antagonize competitors, bacteria may also produce

antimicrobial compounds when they sense the presence of competing organisms. Many microorganisms contain substances that have antimicrobial, antiviral, anticoagulant and cardio active properties few of these substances have unique chemical structures that are unlike any other compounds, may serve as leads to the discovery of new drugs.

The marine environment supports a wide range of microorganisms. Surfaces placed in the sea rapidly absorb bacteria, algae and protozoa. The surfaces appear to provide nutrient sinks which enable diverse microbial communities, to develop and maintain themselves at high population densities (Marshall, 1976). (Baier 1972) and (Leob and Neihof 1975) showed that the first chemical event that takes place when a solid surface is submerged in seawater is the accumulation of organic "Conditioning" film making the surface wet table (Dexter *et al*., 1975).

(Dexter 1978) The subsequent adsorption of bacteria according to (Marshall 1976) and (Marshall *et al*., 1971) involves two distinct phases. The first reversible adsorption is an instantaneous attraction, holding bacteria near the surface so that they will still exhibit Brownian motion. The phenomenon is termed reversible because the organisms can be removed easily before substantial contact of all solid surfaces has been made.

Marine invertebrate living in coastal areas may experience substantial fluctuation in environment condition. The effect of biotic and antibiotic

factors such as predation, completion, temperature, salinity, food and pollution on the survival, growth, and reproduction of marine invertebrates are well documented by various authors (Thorson 1946, 1966, Kinne 1964, 1971, Foster 1987, Pechenik 1987, Barnes 1989, Boidron - Mentairon 1995, Morgan 1995). However the effect of environmental stress experienced in one developmental stage of the life cycle on the performance of later stages has been examined only in few marine invertebrate species (Bacon 1971, Bayne 1972, Helm et al.1973, Roller & Stickle 1993, 1994, Hintz & Lawrence 1994, Pechanik et al.1996a, b, 1998).

A large proportion of natural compounds have been extracted from marine organisms, especially sponges, ascidians, bryozoans and Echinoderms and some of them are currently in clinical trials (Proksch *et al.,* 2002) To date, almost all of the drugs derived from natural sources come from terrestrial organisms. But recently, systematic searches for new drugs have shown that marine animals produce more antibiotic, anti – cancer and anti – inflammatory substances than any group of terrestrial organisms. Particularly promising groups include sponges, tunicates, ascidians, bryozoans, octocorals, annelids, some molluscs, and Echinoderms (Faulkner 2000 and Di Bella *et al*., 2008).

Most of the pathogens are increasingly resistant to the major classes of the routinely used antibiotic. Many diseases were initially controlled exclusively by the use of antimicrobial drugs. The massive use of

antimicrobial for diseases control and growth promotion in animals increases the selective pressure exerted on the natural emergence of bacterial resistance (Riguera, 1997). So there is an urgent need for the discovery of the new and novel antimicrobial drugs to effectively combat not only the drugs resistance but also the new disease producers, hence the search for active drugs from alternative sources including marine environment, obviously becomes imperative. The rich diversity of marine organism assumes a great diversity of the discovery of new bioactive substances. The ocean remains as an untapped source for many drugs and contemporary experimental studies which indicate that, pharmacologically active substances could be isolated from marine organism (Baslow, 1969).

Natural products isolated from marine organisms have increased rapidly and hundreds of new compounds being discovered every year (Faulkner, 2000; Proksch and Muller, 2006). Marine invertebrates offer good source of potential antimicrobial drugs (Bansemir et al., 2006; Mayer *et al*., 2007; Jayaraj *et al*., 2008). Studies on antimicrobial mechanisms and compounds of marine invertebrates may provide valuable information for new antibiotic discoveries and give new insight into bioactive compounds in Echinoderms.

The phylum Echinodermata is the most charismatic marine invertebrate and has become a symbol of marine life. Echinoderms contain huge potential of untapped some of bioactive molecules for therapeutic applications in

selected fields of cancer research, in the control of bacterial growth as substance with new antibiotic properties, and is also used as antifouling substances. The Echinoderms are of great interest in the field of biotechnology. In the 19^{th} and 2^{th} centuries, great cell biologists used Echinoderms (Sea Urchins) as the model systems to study basic phenomena such as mitosis cell divisions, differentia and organ formation. Today Echinoderms continue to be the model of choice for many cell and molecular biologists offering exciting overtures on the way from molecular to cell biology (Arnone *et al*., 1997).

Echinoderms are a renewable resource with an economic value to their increasing demand as food and/or source of bioactive molecules exerting antitumor, antiviral anticoagulant, antioxidant, and antimicrobial activities (Loredana *et al*., 2017). It containing approximately 7,000 living species and is a remarkable economic activity especially in Asia. With the increasing demand for sea urchin roe and trepan (a generic name for sea cucumbers) commercial culture venues have grown in order to maintain the demands for these organisms (Conand 2009 and Furesi *et al*., 2016).

More ever, recently Echinoderms have received great attention as an un exploited source of new bioactive molecules with important antimicrobial, antiviral, antiprotozoal, antifungal, and antihelminthic anticancer activities suggesting their potential applicability for drug discovery (Layson *et al*., 2014). The peculiarities of these molecules are stability, activity at low

temperature and specificity of action. As invertebrates lacking adaptive immunity, Echinoderms are an excellent model for studying innate immunity. Their defence mechanisms are mediated by cellular and humeral responses (Ramirez and Garcia 2010).

The sea is a source of novel organic bioactive molecules that have much importance in medicine, Physiology, pharmacology and biochemistry. The marine environment may contain over 80% of world's plant and animal species although marine compounds are under estimated in current pharmacopeias, it is anticipated that aquatic environment will become an invaluable source of novel compounds in future. Bioactive chemical compounds can be classified as primary metabolites and secondary metabolites from these organisms have recently gained importance as a potential bioactive compound. Like many other marine organisms, Echinoderms have been and continue to be examined as a some of biologically active compound with biomedical applications. (Kelly, 2005).

Sea urchins are small, spiny, globular animal which, with their close skin, such as sand dollars, constitute the class Echinoidea of the Echinoderm phylum. The shells are known to contain various polyhydroxylated naphtoquinone pigments. (Anderson *et.al*.1969).

Sea urchins are classic objects of research in different fields of biology, ecology, biodiversity and evolution. At the same time, they are used as raw

material to produce foodstuff, in particular, the product of processing gonads, known as “Sea Urchin Roe” (Kaneniwa and Takagi.1986) The Reo of sea urchin is considered to be a prized delicacy due to its tasty qualities in Asian and Mediterranean countries such as Barbados and Chile (Lawrence *et.al*.,1997). The gonads of sea urchins either fresh or in the form of processed food have long been using as luxury foods in Japan (Shinoabokuro.1991).

The sea urchin shells are containing various polyhydroxylated nephtoquinone pigments, spinchromes (Anderson *et al*.,1969) as well as their analogous compound, Echinochrome of which was shows bactericidal effect was reported by(service *et al*., 1984).

Japanese demand for sea urchin has risen concerned about overfishing, this making it one of the most valuable sea foods in the world. The population of the Asian pacific region (Hagen, 1996) has also been using it for long time as a remedy for improving general living tone, treatment for a number of diseases and strengthening of the sexual potency of men, especially the middle aged (Ankodinora *et.al*., 1995) Sea urchin fisheries have expanded so greatly in recent years that the natural population of sea urchins in Japan, France, China the north-eastern united states. The Canadian Maritime Provinces and the west coast of North America from California to British Colombia have been overfished to meet the great demand. (Lawrence *et.al*. 2001).

The marine environment is an exceptional reservoir of bioactive natural products, many of which exhibit structural and chemical features not found in terrestrial natural products. The richness of diversity offers a great opportunity for the discovery of new biotic compounds. Modern technologies have opened vast areas of research for the extraction of biomedical compounds from ocean and seas to the treat the deadly disease. (Proksch and Muller, 2006) The secondary metabolites have various functions. It is likely that some of them may be pharmacologically active on humans and useful medicines. (Briskin, 2000).

The bacterial diseases caused series health problem in human, all over the world. More over the bacteria diseases was different species, an instance, *Bacillus subtiles* is accountable for causing food borne gastroenteritis. *Escherichia coli, Staphyloccus aureus* and *Pseudomonas aeruginosa* caused mastitis, abortion and upper respiratory difficulties, whereas *Salmonella sp*, caused diarrhoea and typhoid fever (Jawetz *et.al*,1995). It becomes a greater problem of giving treatment against resistant pathogenic bacteria (Robert *et.al*.,1999) The emergence of antimicrobial resistance toward a number of conventional antibiotics has stimulated the search for antimicrobial agents from a variety of sources including the marine environments.

Marine organisms represent excellent source for bioactive compounds (Bickmeyer *et al*., 2005). Bioactive chemical compounds can be classified as primary metabolites, and secondary metabolites. Depending on its

biosynthetic origin, biochemical role and genual occurrence. The secondary metabolites, depending on this biopsy synthetic origin, biochemical role and general occurrence. The secondary metabolites have various functions, it is likely that some of them may be pharmacologically active on humans and useful as medicines (Briskin, 2000). A majority of pharmacologically active secondary metabolites have been isolated from Echinoderms (Arballeria *et al*., 1996).

2. REVIEW OF LITERATURE

The Phylum Echinodermata composes of approximately 7,000 living species. (Leuckart 1854) successfully established the Echinodermata as a distinct Phylum (Pawson, 2007). About 1,300 Echinoderm species of the Indo-Pacific region was recorded by (Pawson 1995). Recent taxonomic research on the Holothurians fauna has been conducted by (Thandar 1986-94). Earlier taxonomic studies were conducted by (Clark and Rowe 1971) and (Cherbonnier and Guille 1978) on Madagascar Ophiuroids; (Marshell and Rowe 1981) on Madagascar Crinoids; (Sloan *et al*., 1979) on Seychelles Echinoderms; (Cherbonnier 1988) on Madagascar Holothurians.

The Class Echinoid consists of free moving Echinoderms commonly known as sea urchins and sand dollars. The echinoid body is spherical in shape and flattened along the oral or aboral axis. The body is armed with long movable spines. Sea urchins are brown, black, purple, green, white and red in colour but some are multicoloured (Ruppert and Barnes, 1994). The anus and the madreporiteare situated at the centre of the top on regular sea urchins and the mouth containing the fine toothed chewing mechanism called Aristotle's lantern situated at the centre of the underside. Some species feed on sponges and algae and others are detritus deposit feeders. The sexes are separate and the young ones are formed indirectly by the fusion of sperm and egg, released into water. There are about 800 species of Echinoids (James, 2003.).

(Febrina *et a*l; 2000) determined the best body part of sea urchin showing antibacterial activity and the determination of proximate composition, toxicity, bioactive compound and antibacterial activity from the best body part of sea urchin.

(Uma and Parvathavarthini, 1884) elucidates that hexane extract of the sea urchin. *Temnopleurus alexandra* has an antibacterial activity of the gram-positive (*Staphylococcus areus* ATCC 25923, *Bacillus subtitles* MTCC 441, *Enterococcus faecalis* ATCC 29212) and gram negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeroginosa* ATCC 27853, *Klebsiellla pneumoniae* ATCC 15380, *Proteus volgaris* MTCC 1771 bacteria tested, hexane extract showed antibacterial activity for all the bacteria tested except *k.pneumoniae*). It shows that hexane is a potent antibacterial agent and needs further purification for the specific compound, which is responsible for the said activity.

(Parvatha Varthini and Uma 1884) elucidates and aimed at the antibacterial activity of hydro alcoholic extract of the sea urchin, *Temnopleurus alexandra* (Bell, 1984).

Bioactive Compounds from the marine habitat have been represented as the greatest under exploited source of potentially active pharmaceutical agents. They produce a variety of metabolites, some of which can be used for drug development (Chellaram and Prem Anand 2010). Echinoderms have pronounced pharmacological activities or other properties which are useful in the biomedical area.

The methanolic extract of *L.elongate* showed good antibacterial activity against the clinical pathogens. The present findings on the sea urchin demonstrate that this species may form the basis for the source of active manner of antibacterial potential in future (Maheswaran *et al;* 2016).

Bacteria have the genetic ability to transmit and acquire resistance to drugs used as therapeutic agents. Echinoderms have already been reported to contain pharmacologically active compounds with respect to antihistaminic, cytotoxicity (antiangiogenicity, and antibacterial activity. (Kelly, 2005). (Haug *et al*., 91.2002) has isolated different extracts from the sea urchin, the sea cucumber *Cucumaria frondosa* and the store fish *Asterias rubes* exhibited antibacterial activity against several strains tested. The crude extract was found to inhibit the growth of several bacteria tested. (Abubakar *et al*; 2012).

(Maheswaran et al; 2015) evaluate the antimicrobial activity of methanolic extract from *L.elongata*. The methanolic extract of *L.elongata* showed good antibacterial activity against the clinical pathogens. The present findings on the sea urchin demonstrate that this species may form the basis for the source of active manner of antibacterial potential in future.

Antibacterial activity has previously been described in a wide range of Echinoderm species. The whole body or body walls were tested for activity. Antimicrobial activity has also been reported in egg extracts of *Echinoid Paracentrotus lividus* and the asteroid *Marathasteerias glacialis* (Marimuthu *et al*; 2014).

(Laia Abubakar *et al;* 2012) evaluate extracts of the got ,gonad, spines and mouth parts of the sea urchin *Tripneustes gratilla* for antimicrobial and haemolytic activities in vitro. Antibacterial activity has previously been described in a wide range of Echinoderm species (Anderson *et al*; 1983, 1989; Bryan *et al*; 1994; Ridzwan *et al*; 1995). In most of the species studied, the whole bodies or body walls were tested for activity.

Sea urchins are common marine organisms belonging to the Phylum Echinodermata. Echinodermata is a very small phylum, which includes familiar animals such as sea urchin, sea cucumber and star fish. Sea urchins ubiquitously distributed in the world's oceans, constitute an important part of sub tidal marine communities, and are an important part of fishery resource. Sea urchins have been used as modal organisms over the past century in developmental biology. The phylogenetic position of sea urchins and their importance in studies of embryonic development motivated sequencing the genome of the purple sea urchin *Strongylo cantrotus purporatus* (Tu *et al*; 2012).

(Lola Brasseur et al; 2017) reported that biological role in sea urchin physiology, experiments are undertaken on crude extracts from four species and on four isolated spin chromes in order to test their antibacterial, antioxidant, inflammatory and cytotoxic activities first, the antibacterial arrays shows that the use of crude extracts as representation of antibacterial effects of spinochromes are in accurate.

(Bragadeeswaran *et al*; 2013) elucidates the bioactive potential of aqueous extract of sea urchin *Temnopleurus toreumaticus*. The antibacterial agents that have been active enough to complete with classical antimicrobial obtained from micro organisms (Rinehart *et al;* 1981)

Pharmacognosy is the scientific study of structural, physical, chemical and biological characteristics of crude drugs. Echinoderms have been poorly studied with reference to their biochemical potential. Chemical composition of gonad extract of sea urchin *Strongylo centrotus nudus* was assessed by (Hirano *et al.* 1978). The complete amino acid sequence of histone H2B (3) from sperm of the sea urchin *Parechinus angulosus* was proposed by (Strickland *et al.* 1978). Distribution of fatty acids in lipids of the common Atlantic sea urchin *S. droebachiensis was* studied by (Takagi *et al*. 1980). (Evans et al. 1983) discovered the protein cyclic in sea urchin embryos. The complete amino acid sequence of echinoid in, a lectin from the coelomic fluid of the sea urchin *Anthocidaris crassispina* was determined by (Giga *et al*., 1987).(Serrazanetti *et al*. 1995) studied the hydrocarbons, sterols and fatty acids in sea urchin *Paracentrotus lividus* of the Antartica Sea.

(Cook *et al*. 2000) assessed the fatty acid compositions of gonadal material and diets of the sea urchin, *Psammechinus miliari*. (Cruz- Garcia *et al*. 2000) compared the protein, amino acid and fatty acid contents in raw and canned sea urchin *P. lividus*. (Murata and Sata 2000) isolated pulcherimine, a novel bitter tasting amino acid, from the sea urchin *Hemicentrotus*

pulcherrimus ovaries. (Garcia *et al.* 2000) compared the protein, amino acid and fatty acid contents in raw and canned sea urchin (*P. lividus*) harvested in Galicia (NW Spain).

(Liyana-Pathirana *et al*. 2002) found out the effect of an artificial diet on the biochemical composition of the gonads of S. *droebachiensis*. Seasonal changes in the biochemical composition of body components of the sea urchin *P. lividus* were studied by (Montero-Torreiro and Garcia-Martinez 2003). The effect of dietary lipids on fatty acid composition and metabolism in juvenile green sea urchin *S. droebachiensis* was studied by (Castell *et al*., 2004). Variation in gonad fatty acid composition of the echinoid *P. miliaris* was examined by (Hughes *et al*., 2005).

(Amarowicz *et al*., 2012) compared the mineral, protein and pigment contents of shells from red *(S. franciscanus*) and green (*S. droebachiensis*) sea urchins. Seasonal variation in the gonad weight and biochemical composition of the sea urchin *P. lividus* was studied by (Arafa *et al*., 2012). (Diniz *et al*., 2012) compared the gross composition and nitrogen to protein conversion factors of marine invertebrates belonging to three phyla including Porifera, Mollusc and EAchinodermat.

Antimicrobial screening can be used as a rapid and simple preliminary screening for bioactive compounds during the isolation of natural products. Several drug discovery projects have screened Echinoderms for antibiotic activities. An early study by (Ryoyama 1974) reported the biological

properties of coelomic fluid preparations from three species of sea urchins *A. crassispina, Pseudocentrotus depressus* and *H. pulcherrimus*. (Wardlaw and Unkles 1978) reported that the coelomic fluid from *Echinus esculents* possess bactericidal activity against marine *Pseudomonas sp*. (Rinehart et al. 1981) reported that 43 % of 83 unidentified species of Echinoderms (collected from the west coast of Baja California and the Gulf of California) and 58 % of 36 unidentified Caribbean species displayed antimicrobial activities. Early work to document antimicrobial activities of crude extracts from Echinoderms showed a wide range of activities against bacterial and fungal isolates. (Dybas and Fankboner, 1986). (Service and Wardlaw 1984) reported that Echinochrome is a bactericidal substance in the coelomic fluid of *E. esculentus*. (Service and Wardlaw 1985) evaluated the bactericidal activity of coelomic fluid of the sea urchin, *Echinus esculentus* on different marine bacteria. (Canicatti and Roch 1989) studied the antimicrobial proteins from the Echinoderm*, H. polii*.

Antimicrobial and anti *Staphylococcal* bio film activity from the sea urchin *P. lividus* was assessed by (Schillaci et al. 2010). They also found that sub- MIC concentrations of the 5- kDa peptide fraction of the cytosol from coelomocytes of *P. lividus* were active to inhibit the formation of young and mature staphylococcal biofilm. (Uma and Parvathavarthini 2010b) elucidated that the hexane extract of the sea urchin *T. alexandri* had antibacterial activity against gram positive and gram negative bacterial strains. They also isolated

the bioactive compounds from the sea urchin by GC-MS analysis. (Uma and Parvathavarthini 2010) tested the antibacterial activity of the ethyl acetate extract of the sea urchin *T. alexandri* against bacterial strains.

(Devi *et al*., 2011) screened the red sea urchin extracts against clinical isolates of bacteria including multi- drug resistant strains and fungi. Antibacterial, antifungal and cytotoxic activities of ethyl acetate, methanol and water- methanol extracts of the cuvierian organ, coelomic fluid and body wall of sea cucumber *Bohadschia marmorata* from north coastal of Persian Gulf was evaluated by (Mokhlesi *et al*., 2011). (Park *et al*., 2011) investigated the biological activities of Red Sea cucumber *Stichopus japonicus* collected from Juju Island in South Korea and found that water-soluble fractions possesses good antibacterial effects against *Staphylococcus aurous and S. epidermidis*. (Shankarlal *et al.,* 2011) investigated the antimicrobial and antioxidant properties of *S. virgulata* methanolic extract. (Srikumaran *et al.*, 2011) elucidated the antimicrobial activity of starfishes *Protoreaster linckii and Pentaceraster regulus* against isolated human and fish pathogenic and biofilm microorganisms.

Antibacterial activities of carotenoids in flesh and coelomic fluids of three species of Holothuria (*H. scabra, H. leucospilota and H. atra*) were evaluated by (Abdullah and Ibrahim 2012). (Abubakar *et al*., 2012) evaluated the methanolic and chloroform extracts of the gut, gonad, spines and mouth parts of the sea urchin T. *gratilla* for antimicrobial and haemolytic activities

in vitro. (Bjorn *et al*., 2012) isolated antimicrobial peptide centrocin 1 from the green sea urchin.

S.droebachiensis and assessed its anti- infectious and anti-inflammatory effects. (Chamundeeswari *et al.,* 2012) evaluated the antimicrobial activity of crude tissue sample of sea star *Astropecten indicus* collected from southeast coast of India against human microbial pathogens viz., *Escherichia coli, Klebsiella pneumoniae, K. oxytoca, S. aureus, Streptococcus sp, Pseudomonas aeruginosa, Salmonella paratyphi and S. typhi* using solvent system methanol and ethyl acetate by well diffusion method. According to (Schillaci *et al.,* 2012) the smallest peptide fraction of beta thymosin detected in the coelomocytes of mediterranean Sea urchin *P. lividus* were able to inhibit biofilm formation against *Staphylococcal* strains.

The antibacterial activity of aqueous extracts from different tissues of sea Cucumber *Isostichopus badionotus* was evaluated by (Moguel-Salazar *et al.,* 2013). (Prabhu and Bragadeeswaran 2013) isolated and characterized the antibacterial compounds from starfish *Stellaster equestris*.(Schillaci *et al*. 2013) suggested that the coelomocytes and immune mediator cells in the Echinoderm *H. tubulosa* as an unusual source of antimicrobial and antibiofilm agents. The antifungal activity of the soluble matter and crude saponin extracted from the body wall of sea cucumber (*S. japonicus*) were investigated by (Husni *et al*., 2014).

(Liu *et al.,* 2002) studied the hypolipidemic effect of glycosaminoglycans from the sea cucumber *Metriatyla sabre* in rats fed on cholesterol- supplemented diet.(Kim *et al*., 2002) studied the effects of sea urchin shell on chicken egg quality. Recent reports have shown that the use of sea urchin shells confers certain beneficial advantages, including antioxidant and pharmaceutical effects (Kim *et al*., 2002 and Shankarlal *et al.,* 2011). (Althunibat *et al*., 2009) revealed that the Malaysian sea cucumbers *H. Scare, H. leucospilota and S. chloronouts* are potential sources of natural antioxidant and anticancer agents. Antioxidant property of polyhydroxylated napthoquinone pigments from shells of purple sea urchin *A. crassispina* was examined by (Kuwahara et al. 2009).(Hu *et al*. 2010) alleviated orotic acid-induced fatty liver in rats by dietary saponins of sea cucumber *Pearsonothuria graeffei.* Angiogenic potential of hexane extract of sea urchin *T. alexandri* was determined by (Uma and Parvathavarthini 2010).

Bioactive Steroidal Glycosides from the Starfish *Anasterias Minuta* was detected by (Chludil *et al*., 2000). (Diazde-Vivar *et al.,* 2000) isolated two sulphated glycosides and a pentahydroxylated steroid from the Antarctic starfish *Labidiaster annulatus*. (Beauregard *et al*. 2001) detected and isolated a novel antimicrobial peptide from the Echinoderm, (Frondosa Bell *et al*., 2001) synthesized eicosa pentaenoic acid in the sea urchin *P. miliaris*.(Borisovets *et al*., 2002) compared the major carotenoid contents in gonads of sea urchins (S. intermedius and *S. nudus*) during maturation.(Kawatake *et*

al., 2002) determined the structure of six glucocerebrosides from the starfish *L. maculata*. (Pineiro-Sotela *et al*., 2002) determined the purine bases in sea urchin *P. lividus* gonads by HPLC.

(Shankarlal *et al*., 2011) reported that *Salmacis virgulata* has a potent antimicrobial and antioxidant property. The *Salmacis virgulata* shows maximum inhibition against the proteus species, so that *Salmacis virgulata* will may be utilized for the investigate against the urinary tract infections and the *Salmacis virgulata* shows significant activity against the *Vibrio chrolerae* and *Salmonella typhi* , *Salmacis virgulata*(77.51%) showed potent activity at the concentration of 100 µg/ml than compared to standard ascorbic acid. This study supported, methonalic extract of *Salamis virgulata* has potential antimicrobial and antioxidant activity.

(Maoka *et al*., 2014) described the comparison of methanol crude extracted obtained from eggs of farmed and wild specimens reviled a higher bioactivity in farmed individuals with a customized fodder. Several Echinoderms, including sea urchins, are valuable sources of bioactive compounds but their nutraceutical potential is largely unexplored. In fact, The gonads of some sea urchin species contain antioxidants including Carotenoids and polyhydroxylated naphthoquinones , such as Echinochrome *A. Astaxanthin* is known to have particular bioactivity for the prevention of neuro degenerative disease (Paola cirino *et al*., 2017).

Antimicrobial substance is widely produced among bacteria. Bacteriocins and bacteriocin like inhibitory substance (SLIS) are ribosomal synthesized antimicrobial peptides produced by a number of different bacteria that are often effective against closely related species (Riley and warts 2002, cherif *et al.,* 2003)

The number of natural products isolated from marine organisms increases rapidly, and now exceeds with hundreds of new compounds being discovered every (Proksch and Muller 2006) the secondary metabolites have various functions. It is likely that some of them may be pharmacologically active on humans and useful as medicines (Briskin 2000). A majority of pharmacologically active secondary metabolites have been isolated from Echinoderms (Caballero *et al.,*1996).The analogous compound Echinochrome , of which was showed bactericidal effect was reported by (Wardlaw1984).The sea urchin gonads contain Polyhydroxylated naphthoquinone, echinochrome A, which is potential in antioxidant activity.(Lebedev *et al*., 2001) sea urchin gonads are also rich In valuable bioactive fatty acids(PUFAS) and β-carotene (Dincer andcakli 2007).

3. OBJECTIVES

Importance of bioactive substance in the present scenario triggered to carry out this present work with the following objectives.

- ❖ To collect the probable bioactive extracts from the whole body tissue of *Salmacis virgulata*.
- ❖ To test the antibacterial activity against pathogenic bacteria.
- ❖ To purify the potent fraction with superb antibacterial activity.
- ❖ To find out the bioactive compounds of the extract by GC.MS analysis.

4. EXPREMENTAL DESIGNS

Collection of *Salmacis virgulata* from the

Gulf of Manner coastal region of Thoothukudi.

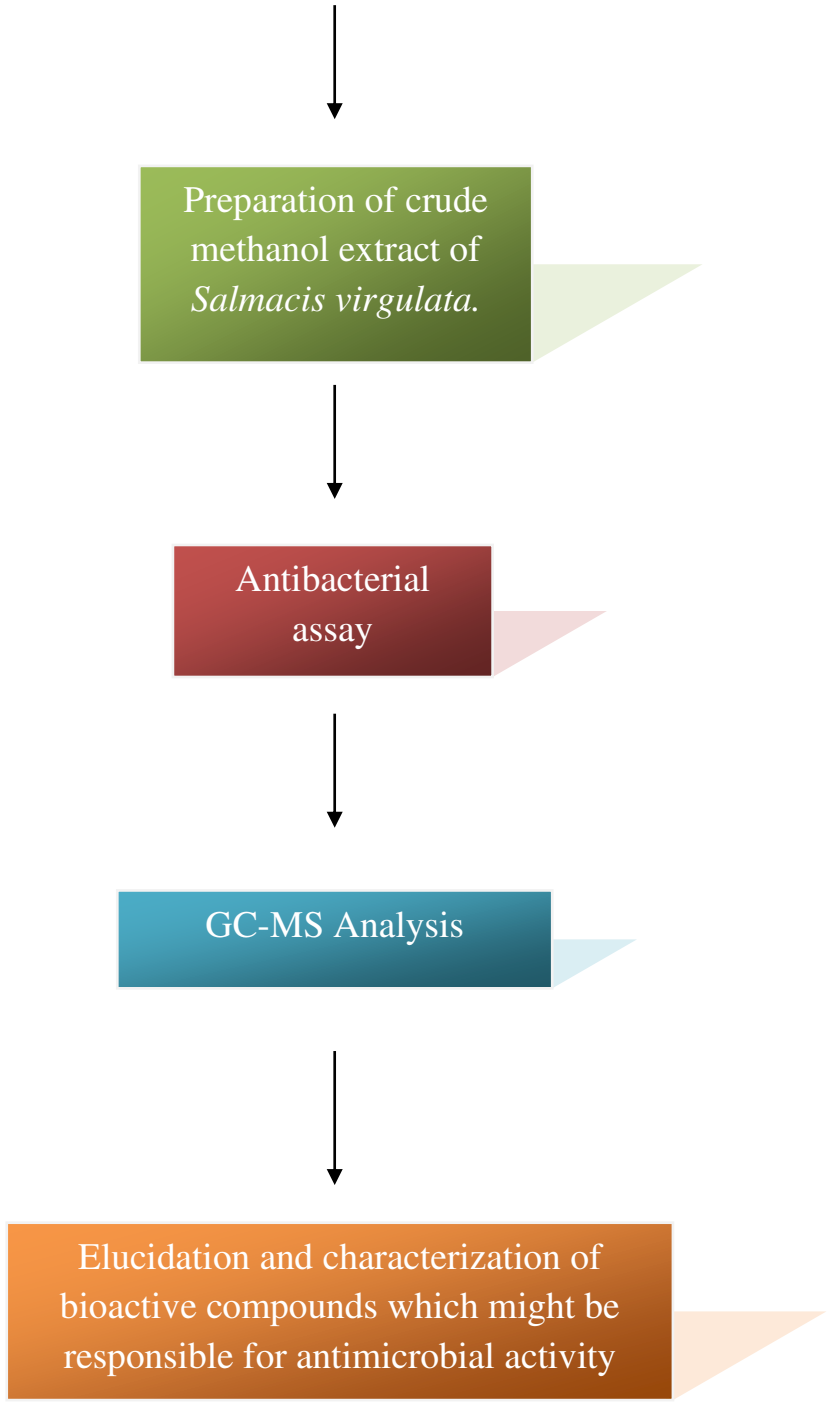
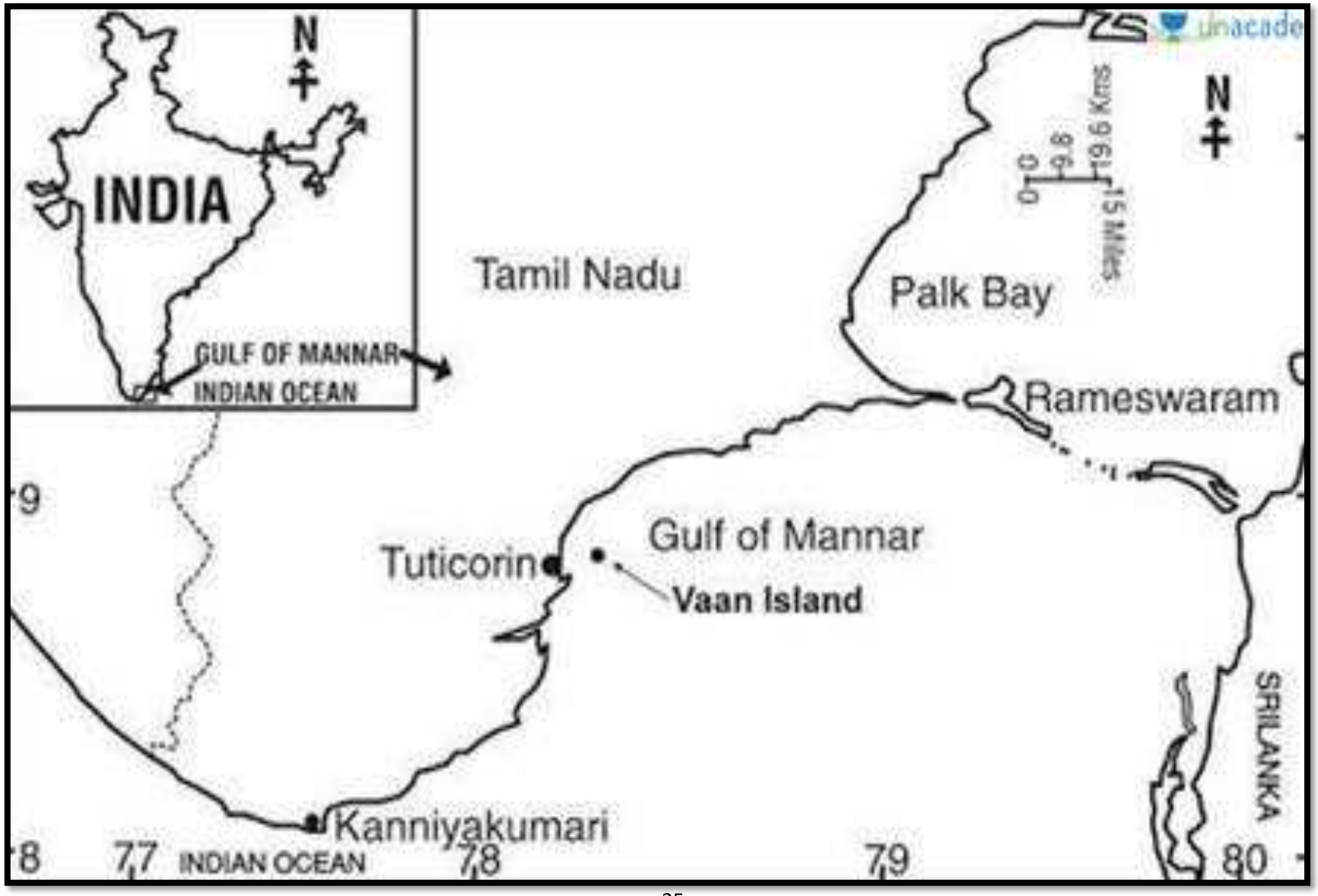


Figure: 1 MAP SHOWING THOOTHUKUDI SHORE, THEGULF OF MANNAR



5. MATERIALS AND METHODS

5.1 SYSTEMATIC POSITION OF EXPERIMENTAL ANIMAL

Salmacis virgulata

Phylum	-	Echinodermata
Class	-	Echinoidea
Order	-	Echinoida
Family	-	Echinidae
Genus	-	Salmacis
Species	-	S. *virgulata*

Salmacis virgulata, sea urchins or urchins are typically spiny, globular animals, Echinoderms in the class Echinoidea. Their tests (hard shell) are round and spiny, typically from 3 to 10 cm (1 to 4 in) across; although the largest species can reach up to 36 cm (14 in) they have a rigid, usually spherical body bearing moveable spines. Sea urchins are covered in long thin spikes where others have a hard shell that is made up of chalky plates. Sea urchin have a round shaped body and with long spines that come off it. The spines of the sea urchin are used for protection, to move about, and trap food particles that are floating around in the water. Sea urchins have five paired rows of tiny tube feet which are found amongst the spines. The feet of the sea

urchin have suckers which help the sea urchin to move about, capture food, and to hold onto the ocean floor.

The sea urchins also have little claw– like structure among their spines which the sea urchin uses for protection. These structures (known as pedicellarines) are small stinging structures that are not only used for defence and obtaining food, but are also vital in keeping the body of the sea urchin clean.

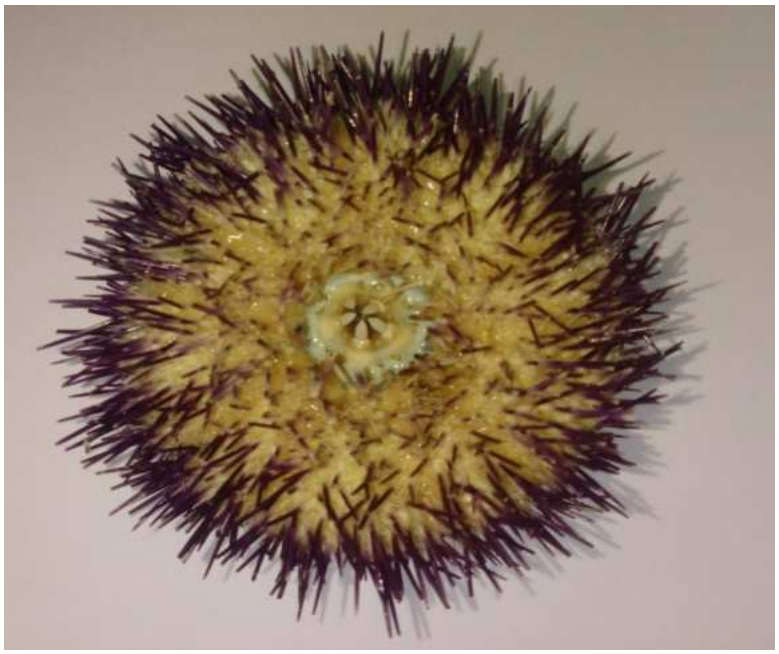
The mouth of the sea urchin (known as the Aristotle's lantern),is found in the middle on the underside of the sea urchins body and has five tooth-like plates for feeding. The anus of the sea urchin is located on the top of the body. As with other Echinoderms, sea urchins do not have a brain and instead rely on their water-vascular system which is like a circulatory system and comprises of water-filled channels that run through the body of the sea urchin.

PLATE - 1 SALMONELLA VIRGULATA

STUDY ANIMAL

DORASAL

VENTRAL



5.2 STUDY AREA

Specimens of *Salmacis virgulata* used in the present study were collected from Gulf of Mannar coastal region. Gulf of Mannar is situated along the south east coast of India. This area is remarkable for its richness and variety of fauna and the inshore sea bottom which forms an ideal habitat for growth of the shell fishes which sustains a good fishery. The Indian part of Gulf of Mannar covers approximately an area of 10,500 km^2 along lat 8°35 - 9°25 N long 78°08 - 79° 30 E. (fig .1)

It is a part of southward extension of Bay of Bengal, it meets in the Indian Ocean. This geographical area runs from Pamban island including Rameshwaram to Cape Comarin to a distance of 170 nautical miles. This coast contains a rich biological diversity of flora and fauna largely due to diversified microhabitats such as mangroves, corals, seaweed beds, sea grasses, sandy, rocky and muddy shores etc. The faunal diversity is also well pronounced with reference to different Echinoderm groups.

5.3 ANTIBACTERIAL ACTIVITY

In the present study whole body tissue extract of *Salmacis virgulata* was used for the antibacterial assay. The freshly collected samples were cleaned and washed with fresh seawater to remove all impurities. The shells were removed and the tissues were then dried in hot air oven at 56^0c for 48hours and used for further studies.

a. PREPARATION OF EXTRACT

Methanol extract of the whole body tissues was prepared by following the slightly modified technique. Dried tissue soaked in 100% A.R grade methanol for 10days at room temperature. After filtration with whatmann no1 paper, the methanol extract was reduced by vacuum evaporation. The extract reduce was resuspended in 20ml of 100% A.R crude methanol. The methanol soluble extracts were dried and solubility in deionizer water. Different concentrations of extracts were prepared and stored at 0^0c for further use.

b. ANTIBACTERIAL ASSAY:

Antibacterial activity of the extract of *Salmacis virgulata* was determined against ten bacterial strains viz, *Pseudomonas, Vibrio cholerae, Esherichia coli,* Steptococus *sps, Salmonella typhi* these pathogens were obtained from the microbiology department of Sri Paramakalyani College, Alwarkuruchi.

c. PREPARATION OF BACTERIAL CULTURE

Nutrient broth medium was prepared and sterilized in an autoclave at 151b pressure for about 30 minutes. Ten bacterial species were inoculated in the nutrient broth and incubated at 28 2^0c for 24 hours. Nutrient agar medium was also prepared, autoclaved and transfer aseptically into sterile petri dishes. On this 24 hours old bacterial broth culture were inoculated by using a sterile cotton swab.

In vitro bacterial assay was carried out by slightly modified disc diffusion whatman no.1 paper discs with 6mm diameter were impregnated with a known amount of extract of *Salmacis virgulata*.

The impregnated disc along with the control (incorporated with solvent along) was kept at the centre of agar plates, seeded with test bacterial cultures. After incubation at room temperature for 24hrs, the inhibition zones were measured with the outside of the disc to inner side of the inhibition zone. The extracts showing broad spectrum activity were examined for minimum inhibitory concentrations by testing at different concentration viz. The more potent fraction was characterized to know the functional groups through GC-MS study at Indian institute of crop processing technology, Tanjore.

5.4 GC-MS ANALYSIS:

GC-MS analysis was carried out on a GC Clara's 500 perking Elmer system comprising a AOC 200C auto sample and gas chromatography

interfaced a mass spectrophotometer (GC-MS) instrument employing for following conditions such as columnelite-5 MS fused silica capillary column (30 X 0.25mn ID X 0.25µm df, composed of 5% diphenyl 95% diphenyl poly giloxane), operating in electron impact mode at 70ev; Helium (99.999%) was used as a carrier gas at constant flow of 1ml/min and an injection volume 3µl (split ratio of 10:1) injector temperature 250^0c. The oven temperature was programmed from 1100c/min to 200^0c, the 5^0c/min to 280^0c.Mass spectra were taken at 700^0c; a scan interval of o.5s and fragments from 45to450Da.

a. IDENTIFICATION OF COMPOUNDS:

Interpretation on mass spectrum was conducted using the database of National institute of standard technology (NIST ver.21) WILEY 8 and FAME having more than 62,000 patterns. The unknown component found in the body tissues of *Salmacis virgulata*. were matched with the spectrum of the known component stored in NIST, WILEY and FAME the MS library and predicted from Duke's Ethnos Botanica.

6. RESULTS

Antibacterial activity of *Salmacis virgulata*

Antibacterial activity of methanol, benzene, hexane, chloroform and distilled water (aqueous extract) of the whole body tissue of *Salmacis vergulata* was tested against five bacterial pathogens Pseudomonas, Escherichia coli , Salmonella typhi, Vibrio cholerae, Steptococcus sps in (fig 2.1 to 2.5). The level of activity was measured by inhibition zones. The extracts developed different zones of inhibitions at different concentrations.

The methanol extract of Salmacis *virgulata* showed activity with the inhibition zones ranging from 0.2 mm to 0.5 mm. The highest activity of 0.5 mm zone was recorded against *Pseudomonas* at 100 mg/ml concentration, whereas a minimum of 0.3 mm inhibition zone was observed against *Pseudomonas* at 50 mg/ml concentration. And very negligible activity of 0.2 mm was showed at 10 mg/ml concentration.

The benzene extract of the *Salmacis virgulata* showed the activity with the inhibition zones ranging from 0. 2 mm to 0.4 mm .The highest activity of 0.4 mm was observed against E.coli at 100 mg/ml concentration. And minimum activity of 0.3 mm was recorded against *E. coli* at 50 mg/ml concentration .And lowest activity of 0.2 mm was recorded at 10 mg/ml concentration of benzene extract.

Hexane extract of *Salmacis virgulata* showed activity by developing the zones of inhibition ranging from 0.1 mm to 0.3 mm .The highest activity showed 0.3 mm at 100 mg/ml concentration. The minimum activity of 0.2 mm at 50 mg/ml concentration and lowest activity of 0.1 mm at10 mg/ml concentration noted against *Salmonella typhi.*

Chloroform extract of *Salmacis virgulata* showed activity with the inhibition zones ranging from 0.1 mm to 0.4 mm. The highest activity showed 0.2 mm at 100 mg/ml concentration. And minimum activity of 0.2 mm at 50 mg/ml concentration .And very negligible activity was showed in 0.1 mm against *Vibrio cholerae.*

Distilled water(aqueous extract) of *Salmacis virgulata* showed activity with the inhibition zones ranging from 0.2 mm to 0.4 mm. The highest activity of 0.4 mm at 100 mg/ml concentration against *Steptococcus sps*. The minimum of 0.3 against *steptococcus sps* at 50 mg/ml concentration. And very lowest activity of 0.2 mm noted against stoptococcus at 10 mg/ml concentration respectively. Of the three concentration of extracts tested in the present study, 100 mg/ml showed more potent activity than the 50 mg/ml and 10 mg/ml. The zone of inhibition increased with increased as the concentration increased.

And of all the extracts tested in the present study methanol extract showed maximum activities against all the pathogens tested. Hence the methanol extract of *Salmacis virgulata* was characterised further by GC-MS

analysis to know the type of bioactive compounds which could be responsible for antimicrobial activity in the present study.

TABLE 1

ANTIBACTERIAL ACTIVITY OF SALMACIS VIRUGULATA

S.NO	EXTRACT	PATHOGENS	10µg/ml	50µg/ml	100µg/ml
1	Methanol	Pseudomonous	0.4mm	0.3mm	0.2mm
2	Benzene	Escerichia coli	0.5mm	0.3mm	0.2mm
3	Hexane	Salmonalella typhi	0.3mm	0.2mm	0.1mm
4	Chloroform	Vibrio cholere	0.4mm	0.2mm	0.1mm
5	Distilled water	Steptococus sps	0.5mm	0.4mm	0.2mm

Table.2. COMPOUNDS IDENTIFIED IN THE METHANOL, EXTRACT OF TISSUE OF SALMACIS VIRGULATA BY GC-MS

S.NO	RT	Name of the compound	Molecular formula	Molecular weight	Peak Area %
1	3.96	Carveol	$C_{10}H_{16}O$	152	3.02
2	27.73	Dasy carpidan-1-Methanol,aoetate (ester)	$C_{20}H_{26}N_2O_2$	326	4.50
3	28.14	Calcitriol	$C_{27}H_{44}O_3$	416	13.62
4	31.14	Ursodeoxycholic acid	$C_{24}H_{40}O4$	392	71.00
5	32.34	Desoximetasone	$C_{22}H_{29}F0_4$	376	7.86

Table: 3.ACTIVITY OF COMPONENTS IDENTIFIED IN THE METHANOL, EXTRACT OF TISSUE OF *SALMACIS VIRGULATA* BY GC-MS

No.	RT	Compound Name	Molecular Formula	MW	Peak area %	Compound Nature	**Activity
1	3.96	Carveol	$C_{10}H_{16}O$	152	3.02	Monoterpenoid alcohol	Fragrance in cosmetics Flavor in foods Anti-inflammatory Antimicrobial
2	27.73	Dascarpidan-1-methanol, acetate (ester)	$C_{20}H_{26}N_2O_2$	326	4.50	Alkaloid compound	Antimicrobial Anti-inflammatory Antioxidant
3	28.14	Calcitriol	$C_{27}H_{44}O_3$	416	13.62	Vitamin D	Used for Vitamin D deficiency, Bone diseases, Pre-dialysis and dialysis patients, Postmenopausal osteoporosis.
4	31.57	Ursodeoxycholic acid	$C_{24}H_{40}O_4$	392	71.00	Bile acids	Reduce cholesterol absorption, Anticancer, Antimicrobial Anti-inflammatory Chemo preventive
5	32.34	Desoximetasone	$C_{22}H_{29}FO_4$	376	7.86	Corticosteroid	Used in skin diseases Cure psoriases Antimicrobial Anti-inflammatory

PLATE -2.1 ANTIBACTERIAL ACTIVITY OF METHANOL EXTRACT OF SALMACIS VIRGULATA

A) Pseudomonas

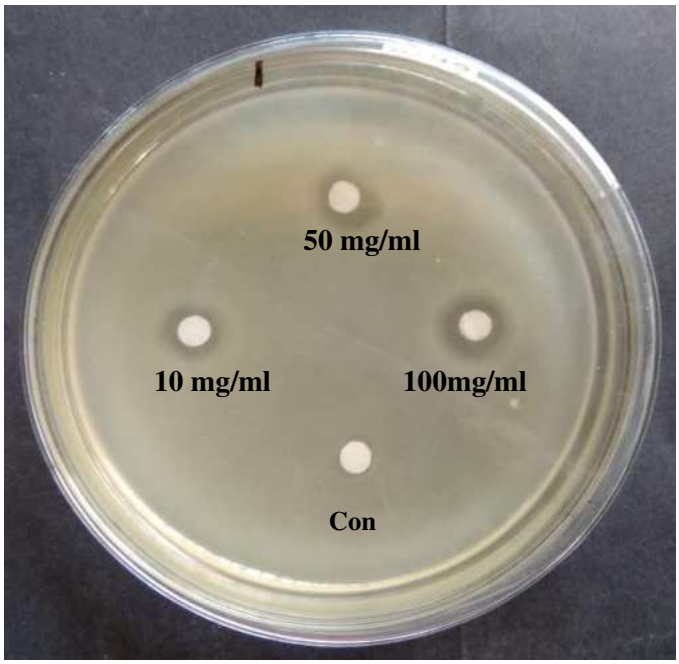


PLATE -2.2 ANTIBACTERIAL ACTIVITY OF BENZENE EXTRACT OF SALMACIS VIRGULATA

B) Escherichia coli

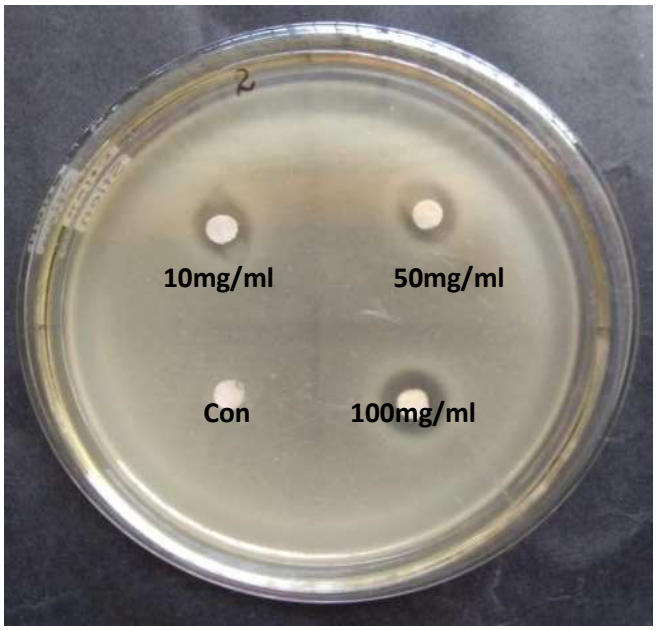


PLATE -2.3 ANTIBACTERIAL ACTIVITY OF HEXANE EXTRACT OF SALMACIS VIRGULATA

C) Salmonella typhi

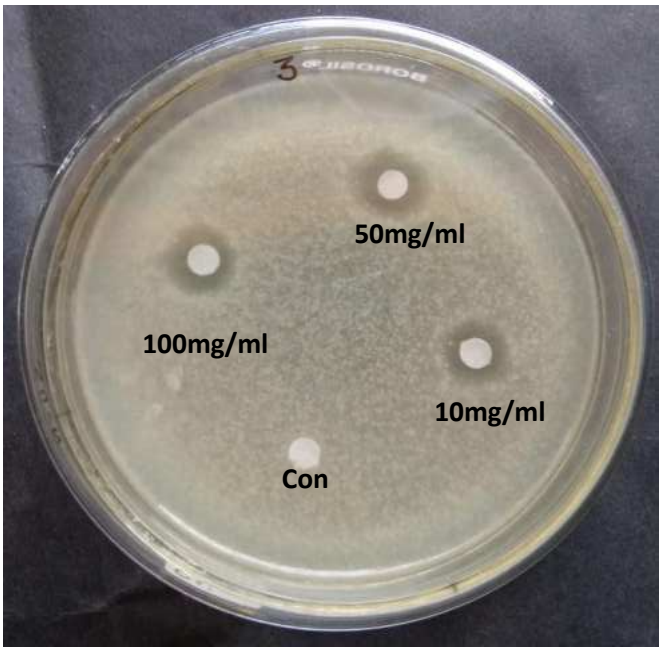


PLATE -2.4 ANTIBACTERIAL ACTIVITY OF CHLOROFORM EXTRACT OF SALMACIS VIRGULATA

D) Vibriocholera

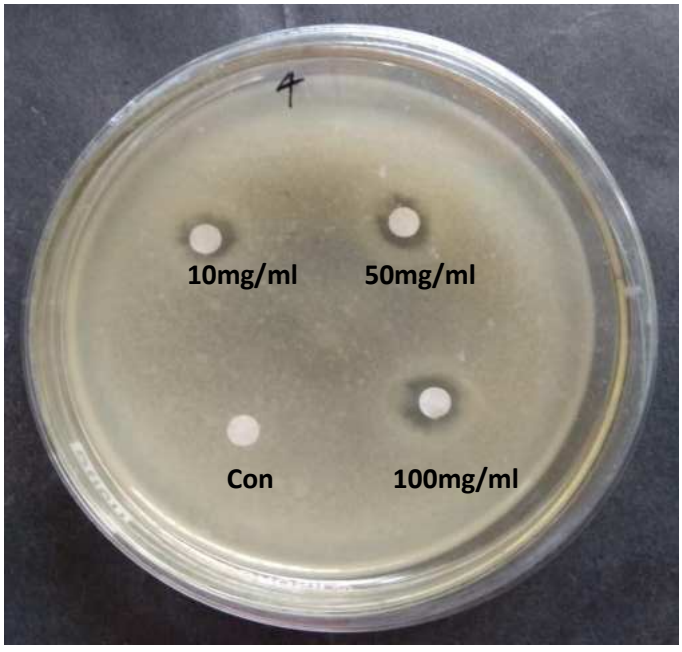


PLATE -2.5 ANTIBACTERIAL ACTIVITY OF DISTILLED WATER EXTRACT OF SALMACIS VIRGULATA

E) Streptococcus sps

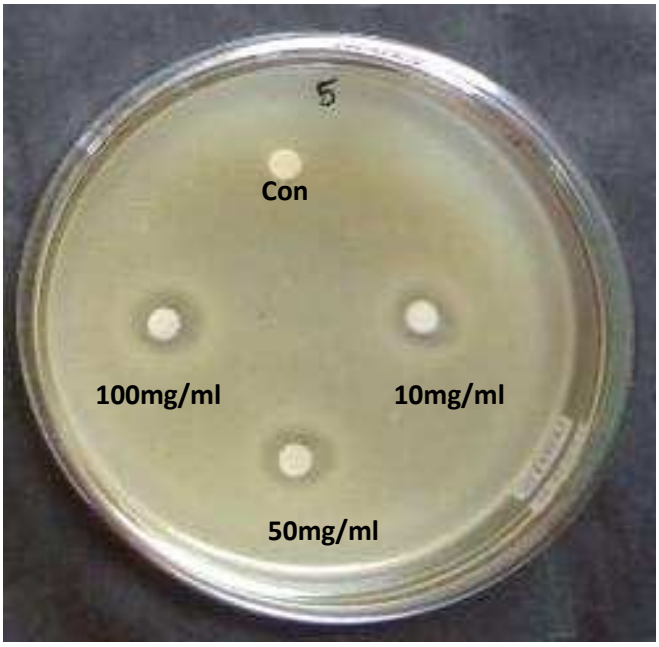


Fig 2: Antibacterial activity of methanol extract of Salmacis virgulata

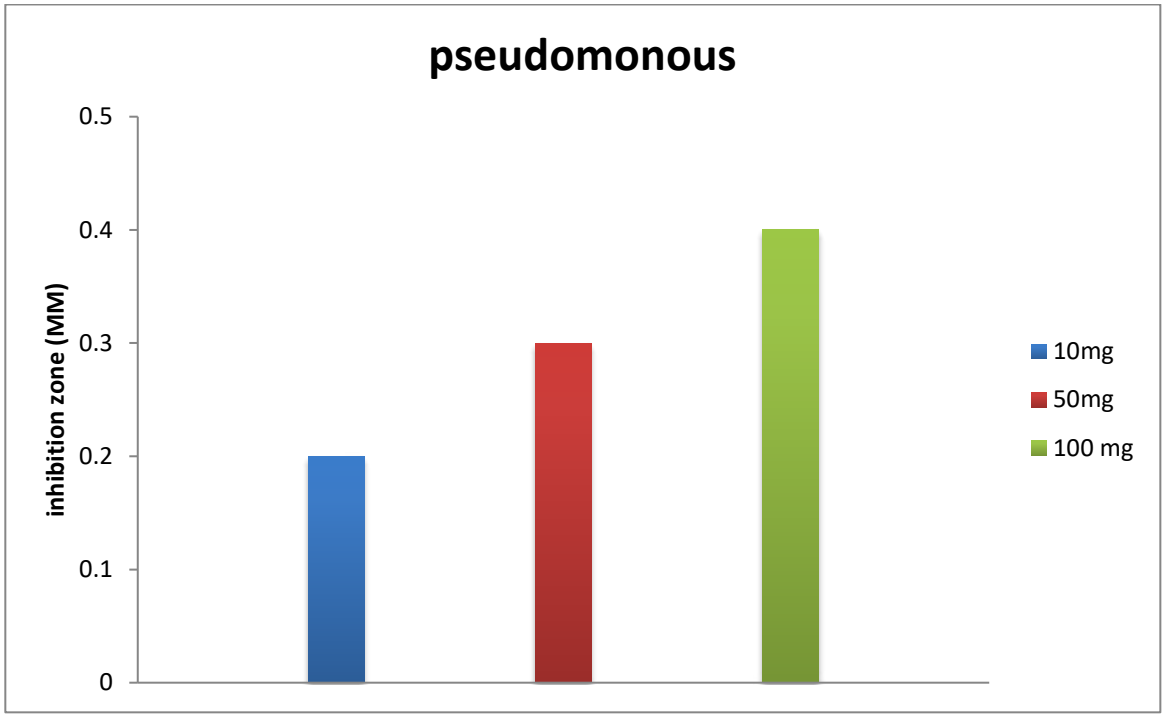


Fig 3: Antibacterial activity Benzene extract of Salmacis virgulata

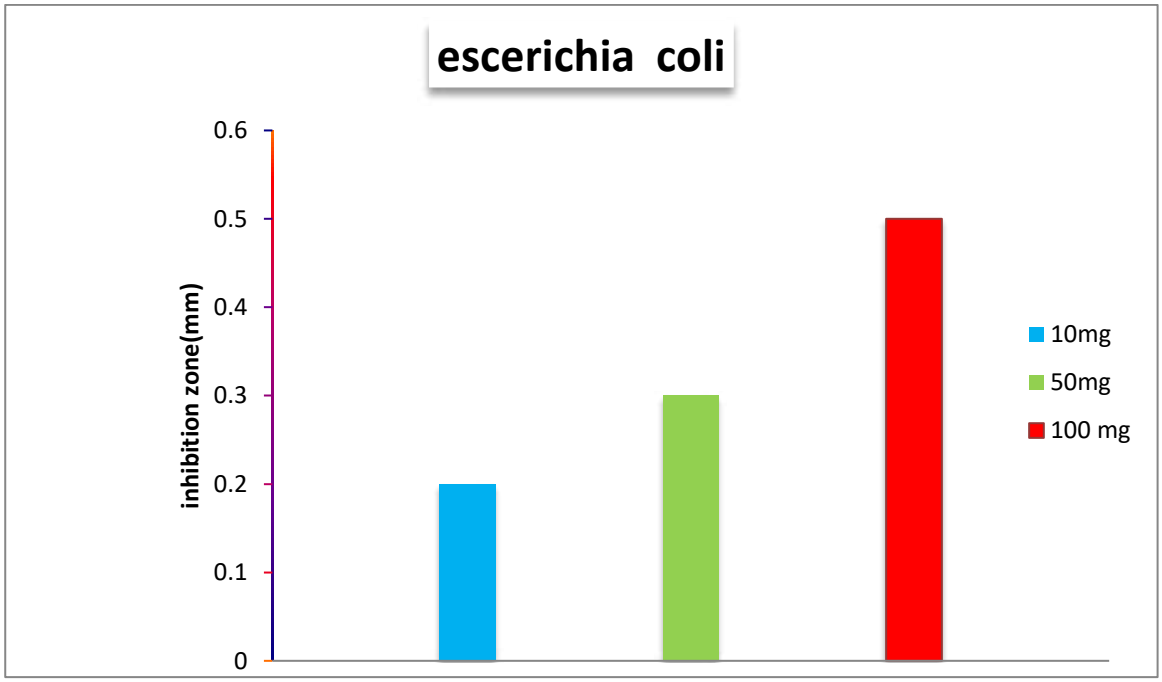


Fig 4 : Antibacterial activity of hexane extract of Salmacis virgulata

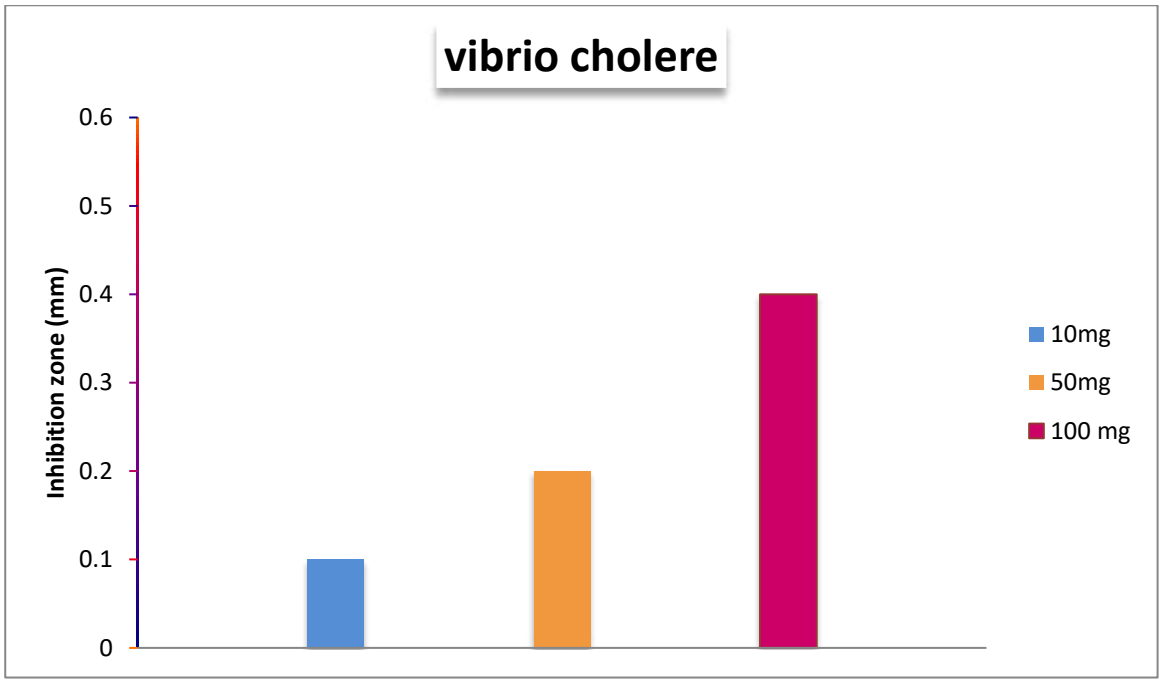


Fig 5: Antibacterial activity of chloroform extract of Salmacis virgulata

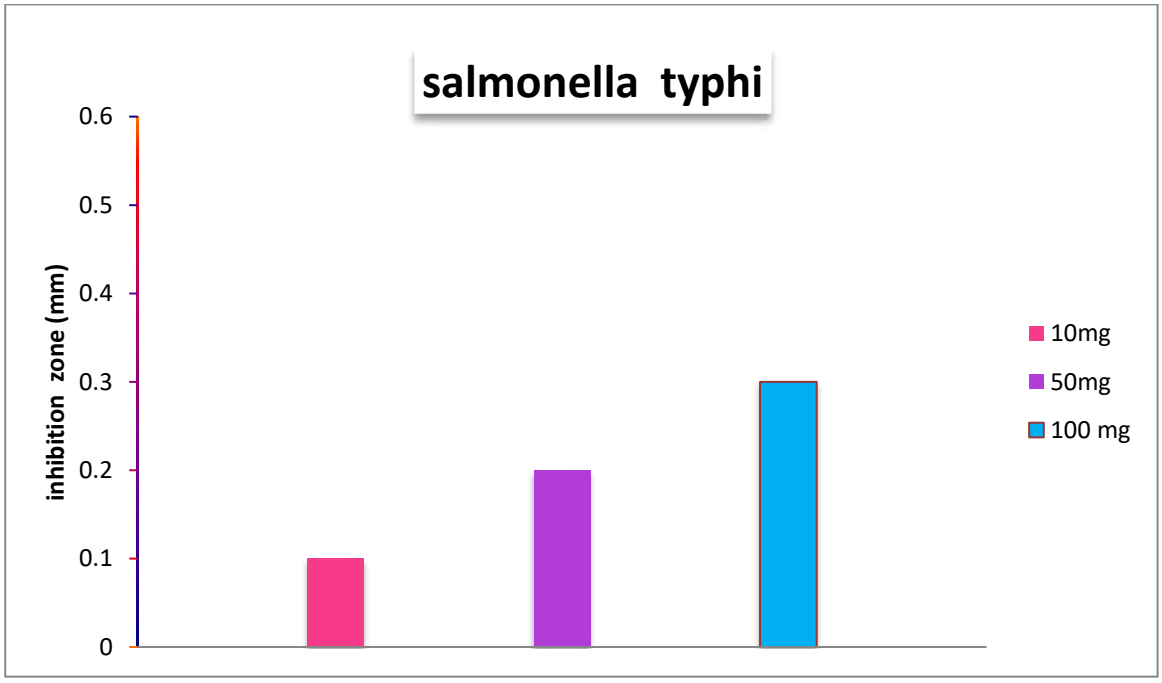


Fig 6: Antibacterial activity of distilled water extract of Salmacis virgulata

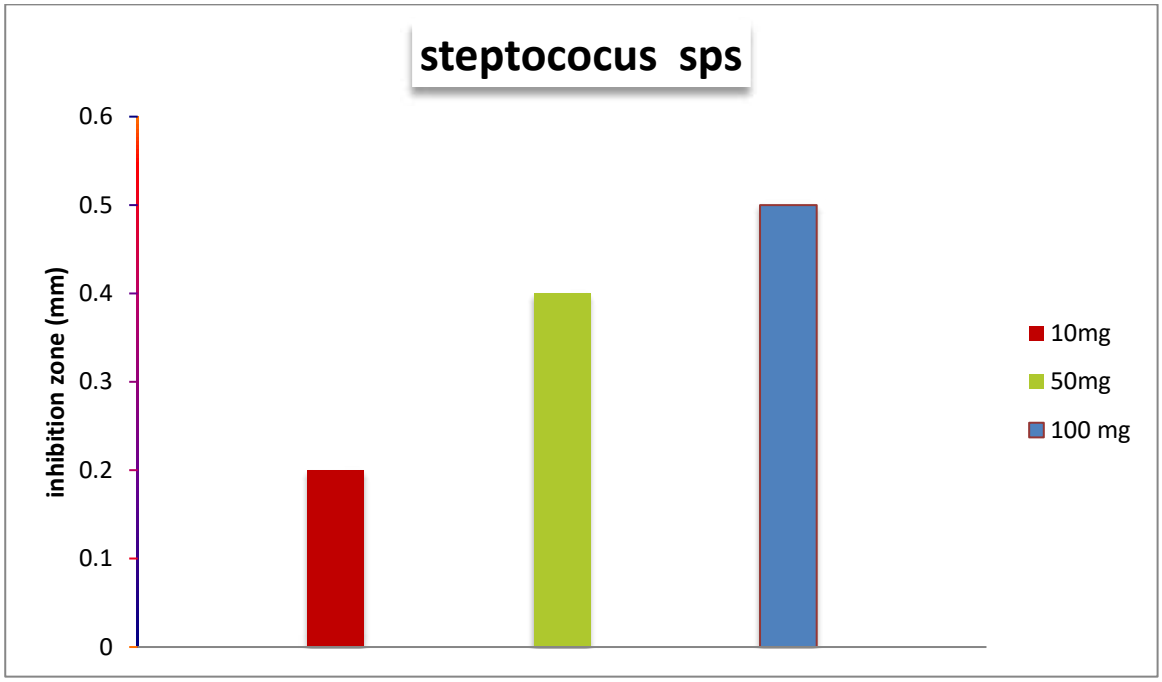
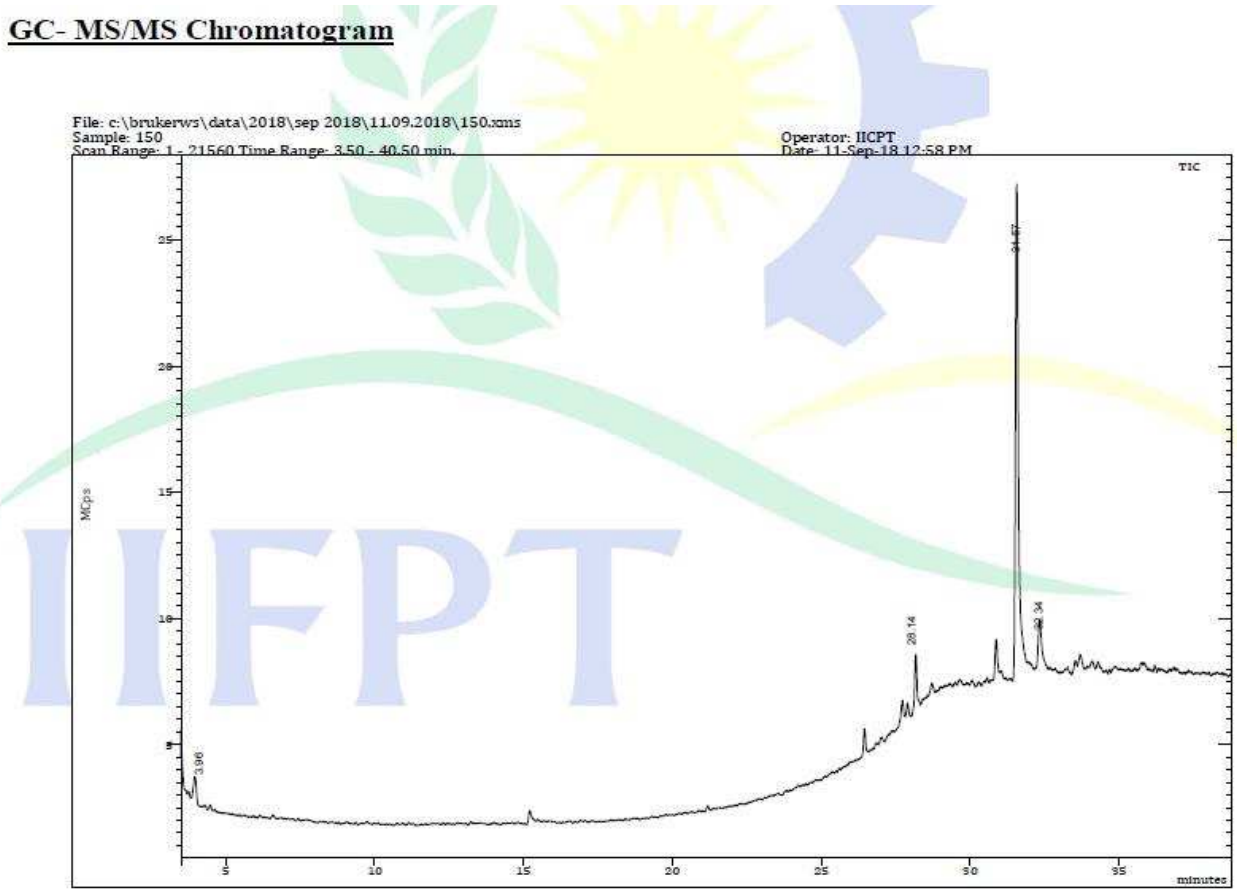


FIG: 7 CHROMATOGRAM OF COLUMN EXTRACT OF *SALMACIS VIRGULATA* BY GC-MS



GC-MS ANALYSIS

Methanol extraction of the whole body tissue of *Salmacis virgulata* showed significant antimicrobial activity. Hence this fraction was subjected to GC-MS analysis to isolate and characterise the compounds responsible for antimicrobial activities. (Table 3&4).

GC-MS analysis of body tissue of *Salmacis virgulata* exhibited five peaks, with retention times ranging from 3.96 to 32.34 min. All the five compounds were characterised as carveol, Dasycarpidan-1-methanol, acetate (ester), calcitriol, Ursodeoxycholic acid, Desoximetasone.

Among the identified compound carveol, Dasycarpidan-1-methanol, acetate (ester), Ursodeoxycholic acid, Desoximetasone was the most abundant antimicrobial compound present in the methanol tissue extract of *Salmacis virgulata* compound, have the role in fragrance in cosmetics , flavour in foods, anti-inflammatory, antimicrobial, antioxidant, used for vitamin D deficiency, bones diseases, pre-dialysis and dialysis patients, postmenopausal osteoporosis, reduce cholesterol, absorption, anticancer, chemo prevention, used in skin diseases compounds, Cure psoriases effects. These compounds constitute promising novel class of pharmaceuticals for the treatment of diseases. So it is recommended as a drug. However further studies will need to be undertaken to ascertain its bioactivity.

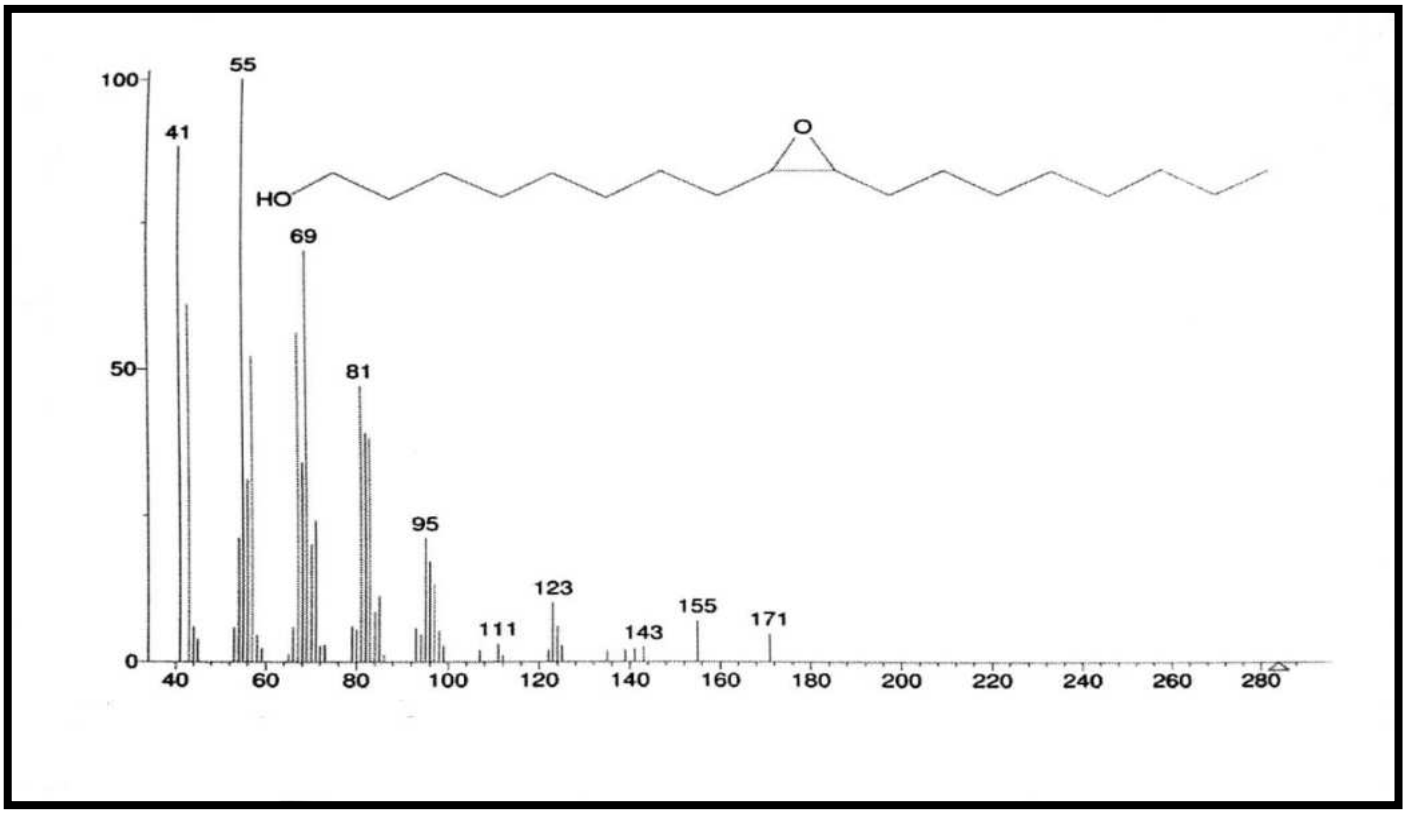
Chromatogram

Fig: 8

Name: Carveol

Formula: $C_{10}H_{16}O$

MW: 152



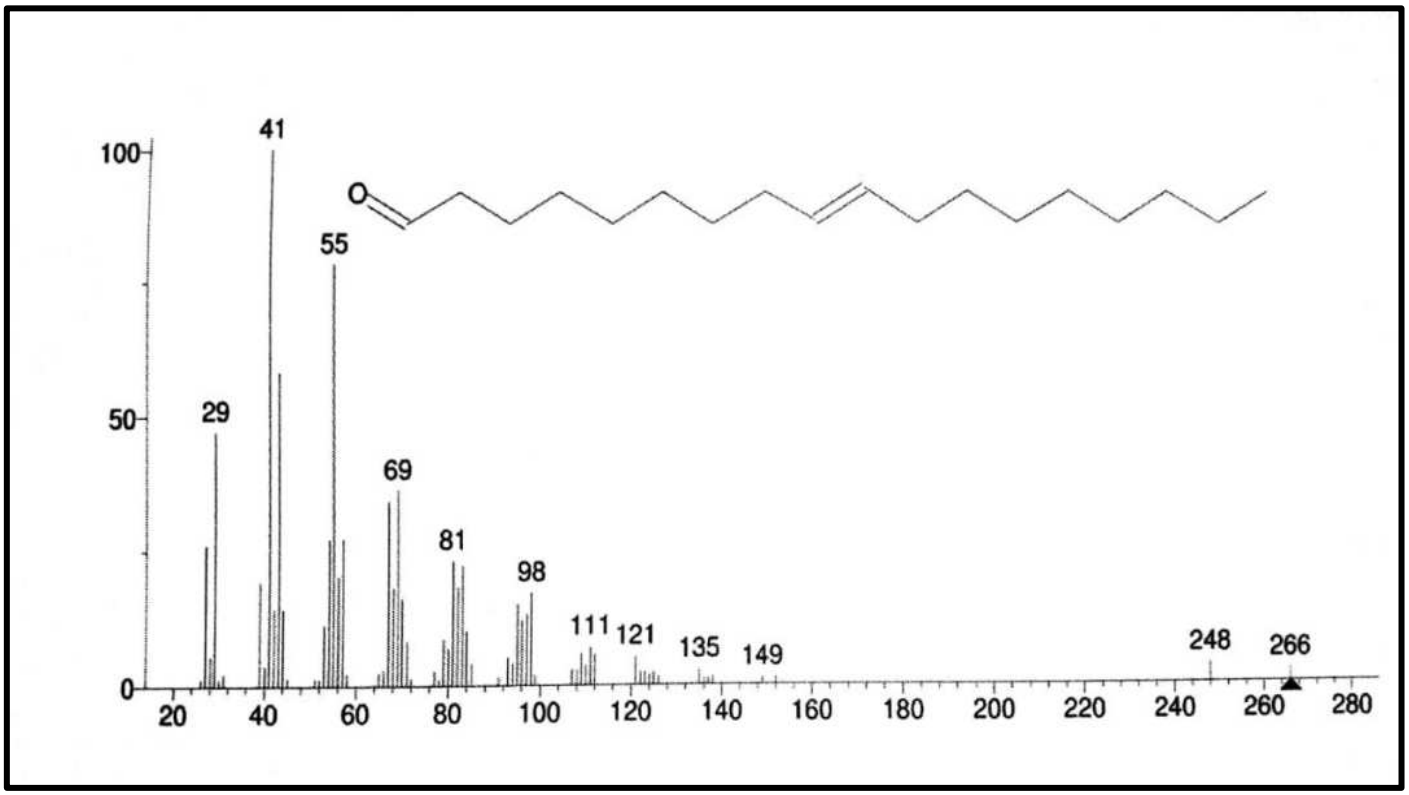
Chromatogram

Fig: 9

Name: Dasycarpdian-1 –methanol, acitate (ester)

Formula: $C_{20}H_{26}N_2O_2$

MW: 326



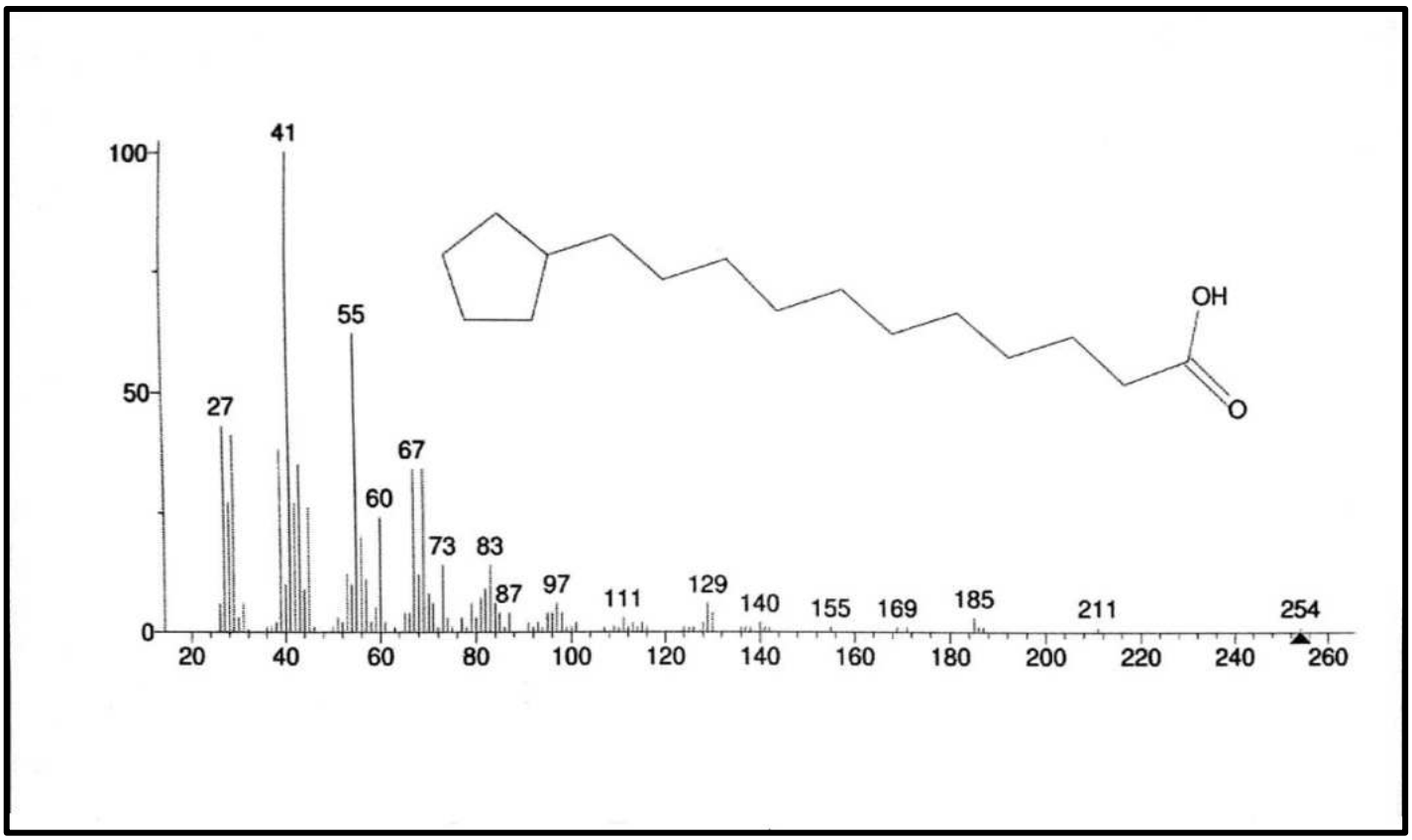
Chromatogram

Fig: 10

Name: Calalcitrion

Formula: $C_{27}H_{44}O_3$

MW: 416



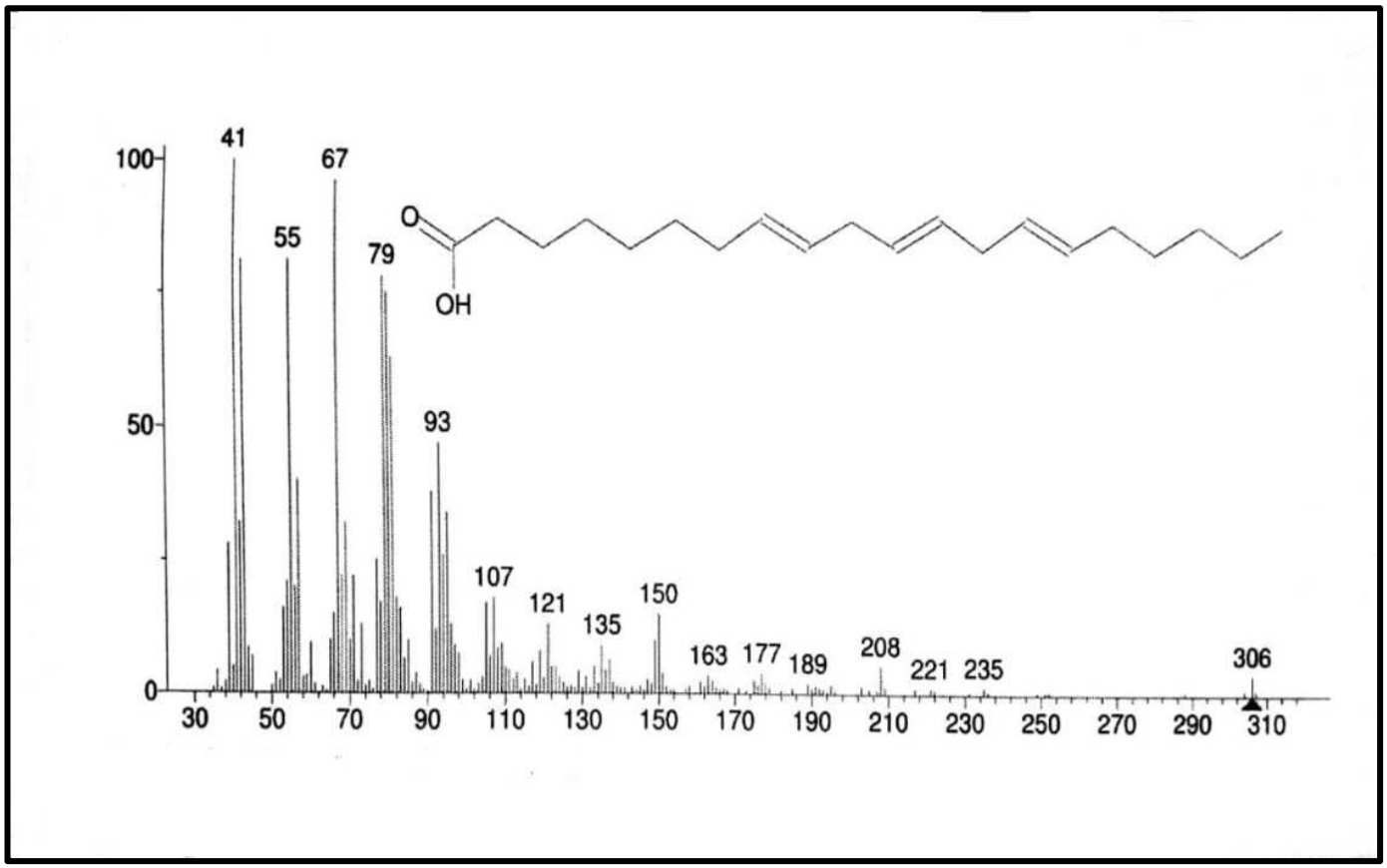
Chromatogram

Fig: 11

Name: Ursodeoxycholic acid

Formula: $C_{24}H_{40}O_4$

MW: 392



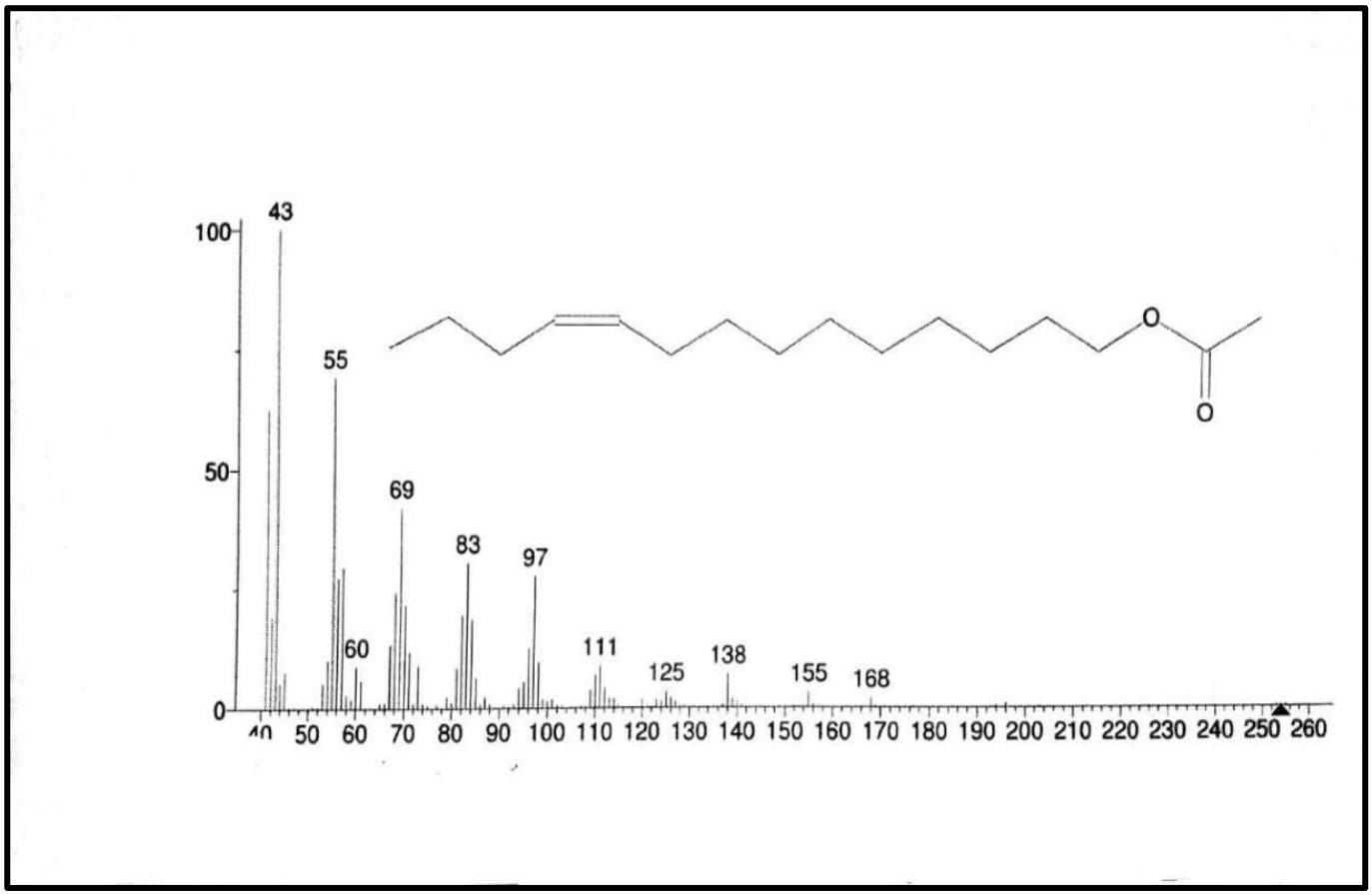
Chromatogram

Fig: 12

Name: Desoximetasonia

Formula: $C_{22}H_{29}O_4$

MW: 376



7. DISSCUSSION

The marine environment harbours a wide range of capable of microbes exhibiting bacteriolytic and antibacterial activity. It plays a primary role of the antibiotic substance which could be attributed to ecological competition. The beneficial associations between associated bacteria and their coast have widely reported to produce novel chemical substances, and hence may form the basis of new drugs. The discovery of new classes of antibiotics is necessary due to the increased incidence of multiple resistances, human pathogenic micro organisms to conventional drugs. So, the bacteria associated with barnacle may play a similar role and antibacterial activity could be explained. Similarly, bio film forming marine bacterium D_2 (*Pseudomonas tunicate)* isolated from the surface of the tunicate coined industinalysis was found to produce a novel protein with bacterial activity against wide variety of marine and medical bacterial isolates as in (zheng *et al.,* 2005).

The antimicrobial activity of sea urchin T.*gratilla* appears to be concentrated mainly in the gut and gonads extracts- little or no activity was observed in the spine and mouth extracts. Further study is required to establish if this observed activity is attributing to proteinaceous (including lysosme-like or non – proteinaceous factors. (Laila *et al*., 2002).

Similarly antifungal activities of various marine organisms and plants have been reported by many workers. (Shanmugam *et al*., 2008) noticed in the methanol extract of *Sepia aculeate* which exhibited the maximum activity

against *A.flavus*. (Bhosale *et al*., 1999) in their study reported that the methanol extract of polychaete *Sabellaria cementifera* showed maximum activity against *A.flavus* and minimum against *A.niger* also support the present study. Similarly antifungal activities of various marine organisms and plants have been reported by many workers. (Shanmugam *et al*., 2008) noticed in the methanol extract of *Sepia aculeate* which exhibited the maximum activity against *A.flavus*. (Bhosale *et al.,* 1999).

Antimicrobial peptides (AMP_S) considered as important immune defence molecules of Echinoderms lacking vertebrate-type adaptive immune system (smith *et al*., 2010) of Echinoderms has been reviewed earlier(Li *et al*., 2010).AMP_S and lysosome like compounds were recorded in various star fishes *viz.,Asterias forbesi, A.rubens* and *Marthasterias glacialis* (Haug *et al*., 2002),(Leonard *et al*., 1990),*Canicatti* and (Ancona 1990).

Marine organisms has been a rehabilitated to much natural products especially in therapeutic possible to cure many infectious diseases. (Sreedevi 2014, minh *et al.,* 2005). Several drug discovery projects have screened Echinoderms for antibiotic activities. The 83 unidentified species of Echinoderms from the Gulf of California showed 43% of antimicrobial activity. (Rinehart *et al*., 1981)In addition, the 36 unidentified species of Echinoderms from Caribbean showed 58% of antimicrobial activity; wearers the 22 species of Echinoderms are collected from the northern Gulf of Mexico showed 80% in antimicrobial activity. (Bryan *et al*., 1994).

Antibacterial activity was detected in extracts of several tissues from the green sea urchin S.*Droebachiensis*, the common sea star *Asterias rubens,* and the sea cucumber *Cucumaria frondosa* (haug *et al.,* 2002).It is an interesting finding that sea urchin being marine animal has the ability to dispose the bacterial boon infection. As the bacterium is a human pathogen, it is important that sea water should not be in the water and the peptides can kill more efficient that the conventional antibiotics. Antibacterial activity from the body wall of several Echinoderm species has been study by (Billasin and Pomory, 2000). (Stabili *et al.,* 1996) have been studied the antibacterial activity in the coelomocytes of the sea urchin *Paracentrotus lividus*. Antimicrobial activity has been found from the eggs of other marine invertebrates as well (Benkendorff *et al*., 2001; Haug *et al.,*2002) and both of these studies showed that at least some of the antibacterial compounds are not proteinaceous.

The composition of valuable components, However, varies greatly among different urchin species and is influence by their natural diet as well as physiological process i.e. reproductive stage (Lawrence 2007, Fernandez, 1998).On the other hand, the high levels of AA and EPA recently dedicated in *Diadema setosum* and *Salanacis sphaeroides* supported the development of aquaculture of sea urchins (Chen *et al*., 2010), since PUF as are important for human nutrition (Lawrance 2007).

(Anand and Edward 2001) noted that the crude methanol extracts of *Cypraea errones* exhibited promising results for antibacterial activity. Antibacterial activity of opercular extracts of *Chicoreus ramosus and Pleuroplaca trapezium* against six bacterial pathogens was reported by (Murugan and Ayyakkannu., 1997). The maximum antibacterial activity against *S.Aureus and E.coli of Trochus radiates* was reported by (Mary Elizabeth, 2003). (Santhana Ramasamy and Murugan., 2003) have reported that the crude methanol extract of *Didemnum psammathodes* inhibited the growth of bacteria. (Mohammed Hussain and Anandhan., 2009) reported that the methanol extract of *D.Candidum* exhibited maximum antibacterial activity against *S.typhi, P.Aerogenosa and V.Cholerae*.

Sea urchins are prone to infestation by the gastropod *Vexillaa Vexillum* that can lead to lethal bacterial infections. Out of the four studied sea urchins, only *T.Gratilla and E.Mathaei* can be infested and develop the disease, the first being 5 times more affected .*D.Savignyi and T.Pileolus* are probably better production than the two other species thanks to their efficient spines or their pedicellariae toxins .

The Echinoderms have stronger antibacterial effects then porifera, molluscs, Bry-zoar annelids (Ridzwan *et al*.,1995).Antibacterial activity has previously been described in some species of Echinoderms (Haug *et al*., 2002-kiani et al 2014- Ridzwan *et al*.,1995).(Haug *et al*.,2002) studied the star fish *Asterias rubens*,and the sea cucucmber *Cucumaria frondosa* against

gram- positive and negative bacteria. They showed antibacterial activities the extract of several tissues from *A.Rubens and C.Frondosa*.the coelomocytes of the sea urchin *Paracentrotus lividus* showed antibacterial activity against *Vibrio alginolyticus* (stabili *et al.*,1996). (Rinehart *et al*., 1981) examine 83 unidentified species of Echinoderms from the west coast of Baja California and the Gulf of California and found 43% of them had antimicrobial activity. In the same study, 58% out of 36 unidentified Caribbean species showed antimicrobial activity. Out of 22 species of Echinoderms collected from the northern Gulf of Mexico, 80% had microbial activity (Bryan *et al*., 1994).This study demonstrated the presence of antibacterial factors in several tissues such as gonads and test of *E.Mathaei*. Whether the same antibacterial factors are responsible for the activity in all organs, is unknown. However, it seen that the antibacterial factors have an important functions as a first line of defines against pathogenic microorganisms.

Antimicrobial activity was observed in both the methanol and the chloroform extracts of the ovary; however the higher inhibition was exhibited by the methanol extracts. This suggested that the antimicrobial components might be present in the sea urchin ovary. The antimicrobial susceptibility showed that, sea urchin ovary extract has the higher zone of inhibition against a few bacteria compared to the conventional antibiotics such as streptomycin, ampicillin, cephalexin and gentamicin. For example, ampicillin showed a very high antibacterial activity against *B.Subtilis* and *S.Typhi*. However, the

methanol extract of sea urchin showed better zone of inhibition against *S.Flexneri, S.Typhimurium*, *A.Hydrophila, K.Pneumoniae, C.Freundii, and S.Aureus. Citobacter freundii* was not inhibited by ampicillin, cephalexin and gentamicin. The methanol extract of sea urchin ovary showed inhibition against these bacteria.

Antibacterial activity by the hexane extract of T.Alexndri. Highest activity was observed with the maximum dose of hexane extract and the zone of inhibition was increasing with respect to increasing dose. Echinoderms have ready been reported to contain pharmacologically active compounds with respects to antihistaminic, cytotoxicity and antiangiogenicity. The *Ophoplocas januarii* from Argentina contained one new antiviral steroidal sulphate. Similarly, Neothyoside is an antifungal triterpene diglycoside from the Gulf of California Holothurians Neothyone *gibbons*. The major components in the present hexane extract could have been responsible for the antibacterial activity. The biological activities of these major bioactive components in relation to parasitism (Paul *et al*, 2002), apoptosis (Jae *et al*., 2008) and antimicrobial activity (Liu *et al*., 2010) have already been established, supporting the fact they might have had the antibacterial activity as well. Since antibacterial agents that poses antibacterial activity area of interest in the field of pharmacology, further fractionation, purification, and identification of the exact bioactive compound present in the present hexane extract is of much importance.

In the present study the activity of *Salmacis virgulata* was found to be high which may be due to species specific characteristics more ever the antibacterial activities can be depend upon the nature of solvent and the compounds extracted (sugesh *et al.,* 2013). The different solvent system extracts of *Salmacis virgulata* are showing antibacterial properties against the bacterial species tested here. Thus the current studies revealed the presence of potent antimicrobial compounds from Echinoderms *Salmacis virgulata* of Tuticorin coast.

In the present study GC-MS analysis of *Salmacis virgulata* showed 100 percentage successes in the identification of bioactive compounds responsible for antimicrobial activities. The magnitude of crude extracts of *Salmacis virgulata* possibly reveals the presence of five antimicrobial compounds. Carveol, Dasycarpidan-1-methanol, acetate (ester), calcitriol, Ursodeoxycholicacid, Desoximetasone. (Table: 3&2), (Figures: 7to14), identified from GC-MS analysis might be responsible for antimicrobial activity. As the Echinoderms resources are rich and varied Indian cost, there exist a great potential for the extraction of bio active compounds of medicinal importance at a lower cost.

8. SUMMARY

The present investigation has been undertaken to find out the antibacterial activities of the marine Echinoderm *Salmacis virgulata.* Antibacterial activity was tested against five bacterial pathogens *Pseudomonas, Escherichia coli, Salmonella typhi, Vibrio cholerae, and Streptococcus sps*. The growth of all tested bacterial inhibited by the crude extract of *Salmacis virgulata* and the inhibitory zones varied from 0.1 mm to 0.5mm. The maximum inhibition zone (0.5mm) was developed against *Pseudomonous* at 100µg/ml concentration and lowest inhibition zone (0.1mm) was recorded against *Salmonella typhi* and *Vibrio cholerae* at 10µg/ml concentration. And methanol is considered to be the most potent fraction.

The methanolic extractions of tissue were analyzed by GC-MS to characterize the compound responsible for antimicrobial activities. GC-MS analysis of tissue of *Salmacis virgulata* exhibited five peaks; with the retention times ranging from 3.96 to 32.34 min. and GC-MS study revealed the presence of five compounds from the fraction of the five compounds the following as Carveol, Dasycarpidan-1-methanol, acetate (ester), calcitriol, Ursodeoxycholic acid, Desoximetasone. Among the compounds identified Ursodeoxycholic acid was the most abundant antimicrobial compounds (71.00%) present in the methanol tissue extract of *Salmacis virgulata.*

The result of the present study showed that the whole body tissues extracts showed potential antimicrobial activity against pathogenic bacterial strains, which indicates the presence of potent bioactive substance in them, and correct understanding and utilization may lead to its use as antibiotic drugs.

9. CONCLUSION

Echinoderms are considerably important as a medical source as well in scientific investigation. There is an ever continuous and urgent need to discover new antimicrobial compounds with diverse chemical structures and novel mechanisms of action due to the alarming increase that has been witnessed in the incidence of both new and emerging infectious diseases. A further big concern is the development of resistance to the antibiotics in current clinical use.

The present study recommends that the natural bioactive substance have the least quantum of side effects when compared to synthetic products. Although most antibiotic have been derived from the terrestrial products it is the marine world that provide the pharmaceutical industries the next generation of medicines.

GC-MS analysis has also aided the evaluation of the major and minor compounds present in methanol extracts of tissues. A novel therapeutic compound from this marine source would be of much use in eradicating the microbial pathogens and it would definitely aid in the control and emergence of drug resistance strain.

10. SUGGESTION

Commercial antibiotics are highly effective to kill the bacterial pathogens involved in common infection. Today people prefer to use pharmaceutical products from natural origin because of their less side effects and nutritive value. So for very few studies have been carried out to show the presence of antimicrobial compounds. This study revealed the presence of bioactive compounds that would inhibit the microbial contaminants in marine or aquaculture field. The results of the presence study suggest that the Echinoderm of Indian origin with rich bioactive compounds would be used as antimicrobial agents for alternative therapy. New Echinoderm species of Indian waters need to be exposed out with strong antimicrobial agents. The strong antimicrobial activity of *Salmascis virgulata* of Tuticorin coast can be used for future pharmacological research to solve the problems of multi drug resistance in all fields. Studies on the Echinoderm species *Salmascis virgulata* and identification of antimicrobial agents are further needed in the future to solve the problems of multidrug resistance in the microorganisms. Although substantial progress has been made in identifying novel drugs from marine sources, great endeavours are still needed to explore these molecules for clinical applications without altering or disturbing the biodiversity on marine organism. If any animal is found to be suitable candidate species for the exploitation of drugs. The animal can be cultured by suitable aquaculture

practice and thereby we can conserve the fauna as well as not modifying the diversity for the sake of mankind.

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EXPLORATION OF BIOMEDICAL POTENTIALS OF *PORTUNUS SANGUINOLENTUS* (HERBST, 1783) FROM GULF OF MANNAR

Dissertation submitted to

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By

Y.YAKINSHA

Reg. No. 17APZO15

DEPARTMENT OF ZOOLOGY

ST.MARY'S COLLEGE (AUTONOMOUS)

THOOTHUKUDI-628001

OCTOBER 2018

Sr.C.Shibana M.Sc., B.Ed .,M.Phil.,
Assisstant Professor
Department of Zoology,
St. Mary's College (Autonomous),
Thoothukudi – 628 001.

CERTIFICATE

This is to certify that this dissertation entitled **"EXPLORATION OF BIOMEDICAL POTENTIALS OF *PORTUNUS SANGUINOLENTUS* (HERBST,1783)FROM GULF OF MANNAR"**isa record of original research work done by **Y.YAKINSHA**under my supervision, and submitted in partial fulfilment for the degree of Master of Science in Zoology. This dissertation has not formed the basis for any other degree.

Place: Thoothukudi

Date: 23.10.2018

Sr. C. Shibana
Signature of the Supervisor

Head of the Institution
Principal
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

23/10/18
Head of the Department
HOD
PG & Research Department of Zoology
St. Mary's College (Autonomous)
Thoothukudi - 628 001.

20/10/18
EXAMINER
DR.J.SUMITHA M.Sc.,M.Phil.,Ph.D.,
ASSOCIATE PROFESSOR,
Department of Zoology,
RANI ANNA GOVT. COLLEGE FOR WOMEN,
GANDHI NAGAR, TIRUNELVELI - 627 008.

Y.YAKINSHA
Department of Zoology,
St.Mary's College (Autonomous),
Thoothukudi – 628 001.

DECLARATION

I hereby declare that the thesis entitled **"EXPLORATION OF BIOMEDICAL POTENTIALS OF *PORTUNUS SANGUINOLENTUS* (HERBST,1783) FROM GULF OF MANNAR"** submitted by me for the Degree of Master of Science in Zoology is the result of my original and independent research work carried out under the guidance of Sr.Shibana M.Sc., B.Ed., M.Phil., Assisstant Professor of Zoology, St.Mary's College, Thoothukudi, and it has not been submitted for the award of any degree, diploma, associate ship, fellowship of any University or Institution.

Place: Thoothukudi.

Date: 23.10.2018

Y.Yakinsha

(Y.YAKINSHA)