

ASSESSMENT OF METABOLITES AND METABOLIC PATHWAYS OF TROPICAL SEAGRASS FOR ANTI-MICROBIAL LEADS

*Submitted in partial fulfilment of the requirements for the award of
Bachelor of Science in Biotechnology*

By

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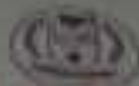
**DEPARTMENT OF BIOTECHNOLOGY
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SATHYABAMA INSTITUTE OF SCIENCE AND TECHNOLOGY**

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This is to certify that this Project Report is the bonafide work of
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DECLARATION

We **SHRUTHI LAYA S (41770031)** and **Dharun kumar . M (41770008)** hereby declare that the Project Report entitled **Assessment of metabolites and metabolic pathways of tropical seagrass for anti-microbial leads** done by us under the guidance of Dr.Thiyagarajan (Internal), Dr. Prabhakaran(Internal) and Dr. D Jeyapragash (External) at Centre for Ocean Research (Sathyabama Research Park) is submitted in partial fulfillment of the requirements for the award of Bachelor of Science degree in Biotechnology .

SIGNATURE OF THE CANDIDATE/S

DATE: 27 APRIL 2024

PLACE:CHENNA

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ABSTRACT

The emergence of drugs from marine sources, particularly marine plants, has increased in recent years. Hence, the purpose of this study was to assess, evaluate the phytochemical profile, antifungal activity of *Halodule pinifolia* extract. Furthermore, GC-MS analysis showed predominant components present in the extract which showed considerable anticancer activities. The GC-MS analysis results revealed that the methanol extract contained more number of pharmaceutical valuable photochemical in extracts. The methanol extract showed excellent antifungal activity against *Candida albicans* (20 mm) of 50 mg ml⁻¹. Furthermore, the GC-MS analysis results revealed that the methanol extract contained more than 25 compounds, the majority of which had previously been identified as pharmaceutically valuable components. These findings concluded that the components present in the methanol extract of *H.pinifolia* have pharmaceutical value and may be useful to the humanity.

Keywords: GC-MS, *Halodule pinifolia*, Anti fungal, Molecular docking.

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AMPs	Antimicrobial peptides
AMR	Antimicrobial Resistance
ACPs	Anticancer Peptides
PS	Phospholipid phosphatidylserines
H. Pinifolia	Halodule Pinifolia
BSA	Bovine Serum Albumin
YEPD	Yeast Extract Peptone Dextrose
MIC	Minimum Inhibitory Concentration
LB	Luria-Bertani
OP	optical density
C.albicans	Candida albicans
P.aeruginosa	Pseudomonas aeruginosa
FBS	Fetal Bovine Serum
MTT	Methyl Thiazolyl diphenyl- Tetrazolium bromide
DMSO	Dimethyl sulfoxide

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CHAPTER-1

INTRODUCTION

Seagrasses are marine hydrophilous angiosperms that live entirely in an estuarine or in the marine environment. Seagrass ecosystems act as a breeding and nursery ground for numerous organisms and also help in promoting the commercial fisheries. .

Seagrasses descended from terrestrial plants that reentered the ocean between 100 and 65 million years ago. Although they are all monocotyledons, they do not have a single evolutionary origin, but are a polyphyletic group. Seagrasses are important as habitats supporting high biodiversity, yet they occur at the land–sea interface and are particularly vulnerable to anthropogenic impact. It is considered to be one of the most productive ecosystems that retain the structural complexity and biodiversity shed light to some researchers to describe seagrass community as marine representation of the tropical rainforests (Danaraj Jeyapragash et al., 2021). Seagrasses do not represent the link between marine algae and terrestrial higher plants. This unique ecological group represents the “whales” of the plant kingdom (M. Kwaaitaal et al., 2021). Seagrasses cover about 0.1–0.2% of the global ocean, and develop highly productive ecosystems which fulfil a key role in the coastal ecosystem (Carlos M et al., 2002). Seagrasses have developed unique ecological, physiological, and morphological adaptations to a completely submersed existence, including internal gas transport, epidermal chloroplasts, submarine pollination, and marine dispersal. Seagrasses have relatively low biomass compared with terrestrial ecosystems, but have very high biomass compared to plankton-based oceanic communities. Highly productive seagrass ecosystems have a relatively complex physical structure, providing a combination of food and shelter that enables high biomass and productivity of commercially important fish species (F. Short et al., 2007). Seagrasses themselves are a critically important food source for dugong, manatee, sea turtles, and waterfowl. Many other species of fish and invertebrates, including sea horses, shrimps, and scallops, utilize seagrass for part of their life cycles, often for breeding or as juveniles (F.T Short 2016). Den Hartog recorded the occurrence of *Halodule uninervis* from Indian Ocean to Western Pacific Ocean and their marginal seas. Seagrass beds are a clear example of ecosystems dominated by foundation 37

species. They typically ameliorate stress, for example, by creating shelter and sediment stabilization, resulting in lower water turbidity and amelioration of wave action (Marieke M. van Katwijk et al., 2016). Seagrasses develop dense canopies that impede water movement, damping current and wave energy (Grizzle et al. 1996). The seagrasses themselves assimilate and cycle nutrients and other chemicals. Their extensive above- and below-ground biomass traps sediments, reducing coastal turbidity and erosion, as well as providing habitat for other organisms both attached and free-living (Hemminga et al., 2000). Many seagrass species are found extensively around the subcontinent of India and its islands, although little information exists on their spatial distribution (Jagtap et al. 2003). extent of seagrass meadows is conservatively estimated to be higher than mangrove, saltmarsh and kelp habitats, but marginally lower than coral reefs (Len J McKenzie et al 2020) There is a growing awareness of “seagrass blue carbon,” referring to the fact that seagrasses sequester and store carbon in their roots and sediments. Although seagrasses represent only a small area (0.2 % of the oceans’ surface), it is estimated that they store 20 % of oceanic blue carbon. (F.T. Short) . Over the past decades, seagrasses have been reported to produce biologically active compounds with wide range of biological activities of potential medicinal value . Our earlier works on nutritional and biological activity of seagrasses from this region revealed *Halodule pinifolia* has tremendous opportunity for the search of potentially active novel compounds. (Rengasamy Ragupathi Raja Kannan et al., 2013)

CHAPTER-2

LITERATURE SURVEY

Halodule pinifolia on the eastern coast of Sri Lanka, approximately 310 km along the coastline from the previously recorded location (Kapparahotta, Weligama). Further, this study describes the new locality of the nationally endangered seagrass species *Halophila beccarii* to Valaichchenai Lagoon, 26.5 km north of Batticaloa Lagoon, where it was previously recorded, along with the presence of two other species, *Halophila ovalis* and *Halodule uninervis*. While the species composition the seagrass habitat of Valaichchenai Lagoon might change in the near future due to the impact of climate change, multiple threats already exist at these newly discovered seagrass habitats, including multi day fishing vessel movements, the collection of shells on the seagrass beds, and solid waste dumping into the lagoon. Gas chromatography–mass spectrometry (GC–MS) has the potential to be a powerful tool in routine analytical laboratories by increasing sample throughput and improving laboratory efficiency. Gas chromatography/mass spectrometry (GC/MS) has long been the method of choice for identifying volatile compounds in complex mixtures. This method can fail, however, when acquired spectra are “contaminated” with extraneous mass spectral peaks, as commonly arise from co-eluting compounds, column bleed, and ion-chamber contaminants. These extraneous peaks can pose a serious problem for automated target compound identification methods where they can cause identifications to be missed by reducing the spectrum match factor below some pre-set identification threshold. In addition, the presence of spurious peaks in a spectrum adds to the risk of making false identifications. Although no single analytical system can cover the whole metabolome, GC-MS has a relatively broad coverage of compound classes, including organic and amino acids, sugars, sugar alcohols, phosphorylated intermediates and lipophilic compounds. Recovery experiments of all measurable classes of compounds have been done during method validation. For unknown compounds, recovery rates can be determined by recombination

experiments in which extracts of two plant species are evaluated both independently and after mixing (Jan Lisec et al., 2006). In chromatographic methods, such as gas or liquid chromatography coupled with mass spectrometry detectors, the goal is to identify compounds and compare their concentrations across and within samples. To achieve this goal, data processing must fulfil two criteria. It must correctly determine the mass spectrum of the individual compounds for identification and it must accurately calculate the abundance of chromatographic peaks corresponding to those compounds in each sample (Lea G. Johnsen et al., 2017). In the field of GC-MS analysis, many separation scientists occupy one of the two following categories: some analysts pay scarce attention to the GC process, and prefer to circumvent insufficient chromatographic separation by directing the column effluent to a mass-analyzing second dimension: multicomponent bands are transformed into a group of ions that are resolved and detected on a mass basis. Mass spectrometry can be very useful for the structural elucidation of overlapping GC peaks. On the other hand, many GC specialists devote themselves almost entirely to the optimization of the chromatographic process, and tend to under-exploit the great potential of MS detection. Such an approach is fine if the ion source receives totally separated solutes, identified commonly by using dedicated MS libraries (Luigi Mondello et al., 2008). GC-MS is a technique which combines the properties of gas chromatography–mass spectrometry which identifies various substances within the test sample which includes hydrocarbons, alcohols, acids, esters, alkaloids, steroids, amino and nitro compounds, and so on. GC-MS also helps to find the traces in materials. GC-MS has been commonly called as “gold standard” for forensic substance identification since it can be used to analyze a specific test (AMUDHA P et al., 2018). Seagrasses, a type of marine plant, have drawn a lot of attention lately because they produce secondary metabolites that are thought to have biological significance and may be employed as possible pharmacological substances (Faulkner et al., 2016). In the Mediterranean Sea, the seagrass *Posidonia oceanica* is an endemic plant and is crucial to the marine ecosystem ecological functioning (Duarte C M et al., 1994). Few studies have been reported this seagrass have pharmacological important metabolites having potential antitumor activity (Hale J

A et al., 2004). Antioxidant actions against bacteria and fungi that are primarily associated with their secondary metabolites(alkaloids, phenols, tannins, phenols, alkaloids, saponins, sterols, phenols, phenols, reducing polysaccharides and resins made of sugar, etc (Kandaiah V et al., 2021). According to recent studies on seagrass phytochemistry, seagrasses are a significant source of minerals, antibacterial agents (Rengasamy RRK et al.2010), antioxidants (Rengasamy RRK et al., 2010), and potentially anti-tumor agents (Folmer F et al., 2010). Molecular docking is such a structure-based drug design method that simulates the molecular interaction and predicts the binding mode and affinity between receptors and ligands. In recent years, this technology has been widely used in drug design research field. Using the compounds database to screen the potential pharmacophores is not only convenient for researchers to purchase, synthesize and complete follow-up pharmacological tests, but also greatly improves the efficiency and reduces the research cost (Jiyu Fan et al.,2019). Currently, molecular docking has become a key tool in computer-assisted drug design. The aim of docking is to accurately predict the structure of a ligand within the constraints of a receptor binding site and to correctly estimate the strength of binding.

CHAPTER-3

AIM AND SCOPE

- 1) To collect and extract metabolites from seagrass *Halodule pinifolia*
- 2) To perform antifungal activity against *Candida albicans*.
- 3) To carry out metabolites identification of *Halodule pinifolia* using GC-MS.
- 4) Bioinformatics analysis of identified metabolites against Hepatocellular carcinoma proteins.

CHAPTER-4

MATERIAL AND METHODS

4.1 *H. Pinifolia* collection and Extract Preparation

Fresh seagrass samples of *H. Pinifolia* were collected in low tide from parangipettai estuary, india, during january 2024. Seagrass species were carefully transported to halophytes conservation lab, sathyabama institute of science and technology, chennai, tamil nadu. specimen was preserved in 5% formalin solution (Danaraj J et al., 2020). Then samples washed completely with sterile sea water to remove ephiphytic components. Leaves and rhizomes separately and again washing it with freshwater .The leaves samples were dried using tissue paper and then weighed of about 5g. The green leaves were grinded using mortar& pestel.

4.2 Preparation of methanol extracts

The dried plant materials (3 g dry weight) were ground to fine powder and soaked in 25 ml methanol for 24 h and the extract was filtered through a buchner funnel with whatman no. 1 filter paper for the complete extraction of compounds from extracts.

4.3 Preliminary phytochemical screening

The phytochemicals like phenolic compounds, carbohydrate, phenol, saponins, tannin, cardiac glycosides, flavonoides, proteins , aminoacids, polysteroides and alkaloides were analysed using standard method (Sofowora, 1931) using concentrated crude extracts from solvents such as methanol, (Kebede, T et al., 2021).

4.4 Gas chromatography – Mass spectrometry analysis

After the extraction process, the each extract were analyzed using GCMS-QP2010 Ultra Gas Chromatograph Mass Spectrometer (Shimadzu Co., Kyoto, Japan) equipped with HS-20 Trap with a capillary column (Inert Cap Pure WAX, 32 m length; 0.25 mm internal diameter; 0.25 µm film thickness). For GC-MS analysis a helium (99.999%) carrier gas set at a flow rate of 1.76 mL/min was maintained and the mass spectra data were acquired and processed.. The injector and detector

temperatures were set at 350°C, while the initial column temperature was set at 80°C. A 2 µl each extract was injected into the column after 2 min, the oven temperature was raised to 150°C at a ramp rate of 10°C/min. Metabolites were identified by comparison of their mass with spiked standards (Danaraj, J et al 2021).

4.5 ANTIFUNGAL ACTIVITY

Growth inhibition of *Candida albican* by seagrass methanol extract was assessed using the well diffusion method. The study fungal strain *Candida albican* used. The microbiological assay was conducted using Yeast Extract Peptone Dextrose (YEPD) broth as the media. Initially, we prepared the YEPD media and poured it into petri plates within a laminar airflow hood. After allowing the media to dry for 10 minutes, *Candida albicans* pathogen was added to the petri plates using a pipette. Subsequently, the *Candida albicans* was streaked onto the plates using a cotton swab. Three wells were receiving 50 µL (flucanazole) and 100 µg/ml of antimicrobial peptides, respectively. The plates were then placed in an incubator for 24 to 28 hours, after which the activity was assessed. The plates were kept incubated at 37°C for 18 h. The growth inhibition was assessed as the diameter (in mm) of the zone of inhibited microbial growth (Jothi, R et al 2024).

4.6 Ligand and receptor preparations

The molecular docking analysis was carried out for all the selected phytocompounds from the GC-MS data fractions. Chemical structures of phytocompounds retrieved from pubchem database in sdf format and converted into protein data bank (PDB) format using pymol version 3.0. Software. The structural information of the identified compounds was also retrieved from the pubchem database in sdf format and converted into pdb format using an open babel server. (Gowrishankar, et al., 2021)

4.7 Purification and refinement of proteins and ligands

Biovia discovery studio was used to remove undesirable ligands, molecules of water, and other impurities from all of the proteins involved in the study. Polar

hydrogens were attached to the protein during the preparation process for improved interactions, followed by kollman charges.

4.8 Molecular docking studies

The docking calculation and algorithms to create the pocket binding residues using the lamarckian genetic algorithm in autodock vina 4.2. The docking analysis with the standard parameters. The molecular docking studies were performed on all selected phytocompounds from the gc–ms data sections. Chemical structures of phytocompounds were obtained in sdf format from the pubchem database and were translated to pdb format with pymol version 3.0. The targets used in the investigation were chosen based on the evidence from past research, and all 3d crystal structures were collected in .pdb format from the protein data bank.

CHAPTER- 5

RESULTS AND DISCUSSION



Figure 5.1 : Collection and extraction of bioactives from *Halodule pinifolia* leaves.

5.1 Preliminary phytochemical screening

The preliminary analysis of phytochemicals from methanol, chloroform and ethyl acetate extracts of seagrasses *halodule pinifolia* is presented in table 1. The methanol and ethyl acetate extracts of *h. Pinifolia* showed the presence of carbohydrate, cardiac glycosides, coumarins, flavonoides and sugar. In the case of chloroform extract from *h. Pinifolia* showed the presence of alkaloids, phytosteroides, protein and amino acids.

Table 5.1 Phytochemical prescreening of *Halodule pinifolia*

S.no	Phytochemicals	Methanol solvent
1.	Carbohydrate	+
2.	Phenol	+
3.	Saponins	+
4.	Tannin	+

5.	Cardiac glycosides	+
6.	Flavonoides	+
7.	Proteins & aminoacids	+
8.	Polysteroides	—
9.	Alkaloides	—

The marks '+' and '-' indicate 'present' and 'absent', respectively.

5.2 Antifungal activity

Upon incubation for 24 to 28 hours, the assay revealed distinct zones of inhibition around the wells containing flucanazole and antimicrobial peptides. Specifically, the zone of inhibition observed for not observed for positive control and negative control. But ZOI was observed for methanol extract and it was found to be 6 mm for 50 µg/ml and 11m for 100µg/ml with potential inhibitory effect.

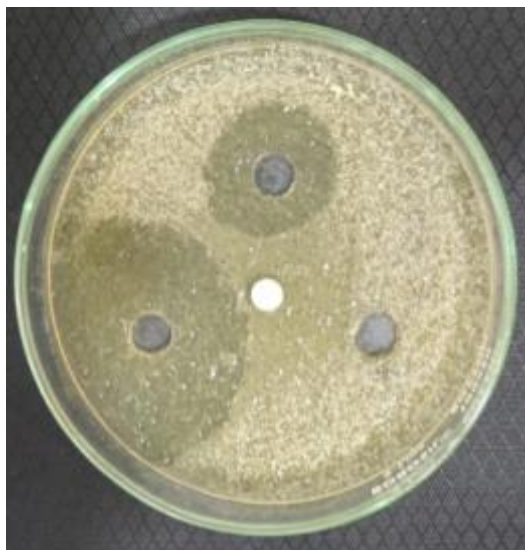


Figure 5.2 : Zone of inhibition of Methanol extract against *C. albicans*

These results highlight the potential efficacy of methanol extract as an alternative or adjunct therapy for combating fungal infections caused by *Candida albicans*.

5.3 GC-MS analysis

Methanolic extract shows hexadecanoic acid,9,12-octadecadienoic acid (z,z)-,

9-octadecenoic acid (z)-, cis-9-hexadecenal(z)-4-decen-1-ol, 6-octen-1-ol, 3,7-dimethyl, 11-tridecen-1-ol, 9,12-octadecadien-1-ol, pentadecanal-, z-7-pentadecenol, 9-octadecen-1-ol,(z), undecanenitrile, cyclohexanemethanol, 2-hydro 5-cyclohexyl-1-pentene, z-8-tetradecen-1-ylacetate, 2-hydroxy-1,, decane,1-iodo-, 9-octadecenamide, 9,12-octadecadienoicacid(z,z)-,2, oleoyl chloride, ethyl (9z,12z)-9,12-octadecadieno, 9-octadecenoicacid,1,2,3-propanetriylester, glycidol stearate, 9-tetradecenal, methyl18-fluoro-octadec-9-enoate,flavone4'-oh,5-oh,7-di-triarachine,1,2, bis(trimethylsilyl)benzene and z,z-2,13-octadecadien-1-ol compounds were

identified. more than 25 metabolites were identified in the saponifiable part of the fraction methanol extract of *halodule pinifolia*. The metabolite 9 octadecenoic acid, 1,2,3-propanetriylester with area 32.98% in major and minor 9-octadecenamide with the area of 0.28% according to gcms 17.62% of cis-9-hexadecenal, 14.54 % area of ethyl(9z,12z)-9,12octadecadieno, 4.34% glycidol stearate and lowest area of 0.29% 1,2-bis(trimethylsilyl)benzene, 0.39% of hexadecanoic acid, methyl ester and area of 0.51% 9-tetradecanal,(z) shows the activity of antibacterial, antimicrobial and antioxidant activity

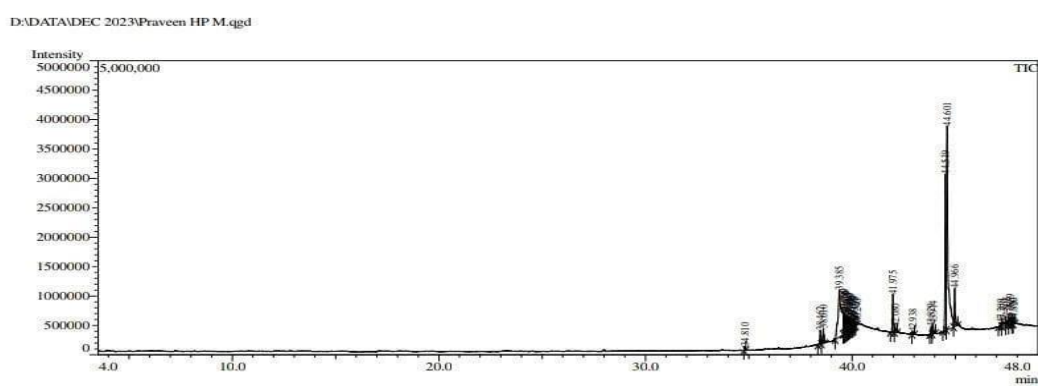
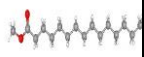
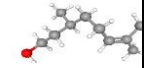
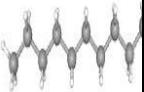


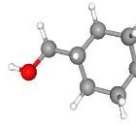
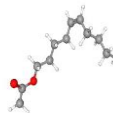
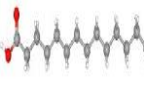
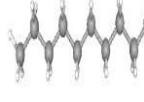
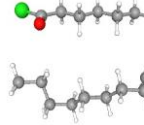
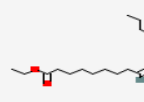
Figure 5.3: GC-MS peaks of methanol extract

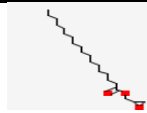
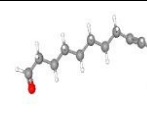
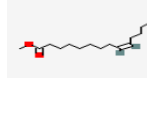
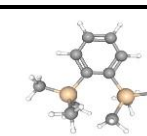
Peak Report TIC				
Peak#	R.Time	Area	Area%	Name
1	34.810	170369	0.33	HEXADECANOIC ACID, METHYL ESTER
2	38.462	748339	1.43	9,12-OCTADECADIENOIC ACID (Z,Z)-, M
3	38.610	754315	1.44	9-OCTADECENOIC ACID (Z)-, METHYL
4	39.385	9232868	17.62	cis-9-Hexadecenal
5	39.590	1178578	2.25	(Z)-4-Decen-1-ol, pentafluoropropionate
6	39.635	486208	0.93	6-OCTEN-1-OL, 3,7-DIMETHYL-
7	39.670	553281	1.06	11-Tridecen-1-ol
8	39.715	1275529	2.43	9,12-Octadecadien-1-ol, (Z,Z)-
9	39.800	974168	1.86	Pentadecanal-
10	39.835	855451	1.63	Z-7-Pentadecenol
11	39.928	846274	1.62	9-Octadecen-1-ol, (Z)-
12	39.990	432302	0.83	Undecanenitrile
13	40.025	416030	0.79	CYCLOHEXANEMETHANOL, 2-HYDROXY-
14	40.075	366897	0.70	5-Cyclohexyl-1-pentene
15	40.135	313631	0.60	5-Cyclohexyl-1-pentene
16	40.240	283103	0.54	Z-8-Tetradecen-1-yl acetate
17	41.975	2132168	4.07	HEXADECANOIC ACID, 2-HYDROXY-1,
18	42.080	536802	1.02	Decane, 1-iodo-
19	42.938	145186	0.28	9-OCTADECENAMIDE
20	43.829	351599	0.67	9,12-OCTADECADIENOIC ACID (Z,Z)-, 2
21	43.914	551188	1.05	Oleoyl chloride
22	44.519	7616322	14.54	ETHYL (9Z,12Z)-9,12-OCTADECADIENO
23	44.601	17280109	32.98	9-Octadecenoic acid, 1,2,3-propanetriyl ester
24	44.966	2275645	4.34	Glycidol stearate
25	47.209	268842	0.51	9-Tetradecenal, (Z)-
26	47.320	578613	1.10	Methyl 18-fluoro-octadec-9-enoate
27	47.505	604218	1.15	03027205002 FLAVONE 4'-OH,5-OH,7-DI
28	47.659	811457	1.55	Triarachine
29	47.760	149505	0.29	1,2-Bis(trimethylsilyl)benzene
30	47.820	204176	0.39	Z,Z-2,13-Octadecadien-1-ol
		52393173	100.00	

Figure 5.4 : GC-MS peaks of methanol extract with metabolites

Table 5.2 : GC-MS identified metabolites with biological activities.

S.No	Retention time	Compound name	Molecular formula	Molecular Weight g/mol	Structure	Biological activity
1	34.810	HEXADECANOIC ACID, METHYL ESTER	C17H34O2	270.45		anti-inflammatory activities[38]
2	38.462	9,12-OCTADECADIENOIC ACID (Z,Z)-, M	C18H32O2	280.4455		antimicrobial activity[39]
3	38.610	9-Octadecenoic acid (Z)-, methyl ester	C19H36O2	296.4879		Neurotoxicity[40]
4	39.385	cis-9-Hexadecenal	C16H30O	238.4088		Cytotoxicity[41]
5	39.590	(Z)-4-Decen-1-ol, pentafluoropropionate	C13H19F5O2	302.2808		adulticidal activities[42]
6	39.635	6-Octen-1-ol, 3,7-dimethyl-	C8H16O	128.21		anticonvulsant activity[43]
7	39.715	9,12-Octadecadien-1-ol	C18H34O	266.47		antimicrobial activity[39]
8	39.800	Pentadecanal-	C15H32O	212.4146		antibiofilm activity[44]
9	39.928	9-Octadecen-1-ol, (Z)-	C18H36O	268.5		anti-inflammatory[45]
10	39.99	Undecanenitrile	C10H19N	153.2646		mutagenic activity[46]

11	40.025	CYCLOHEXANE METHANOL,2-H YDRO	C ₈ H ₁₆ O	144.2114		frothing agen[47]
12	40.075	5-Cyclohexyl-1-p entene	C ₁₁ H ₂₀	152.28		safety/hazards/ toxicity information
13	40.24	Z-8-Tetradecen- 1-ylacetate	C ₁₆ H ₃₀ O	254.4082		Antibacterial Activity[48]
14	41.975	HEXADECANOI CACID,2-HYDR OXY-1,	C ₁₉ H ₃₈ O ₄	330.5026		ANTIBACTERI AL AND ANTIFUNGAL ACTIVITIES [49]
15	42.08	Decane,1-iodo-	C ₁₀ H ₂₁ I	268.1782		antibacterial agent[50]
16	42.938	9-OCTADECEN AMIDE	C ₁₈ H ₃₅ N O	281.48		antioxidative and hypolipidemic[51]
17	43.829	9,12-OCTADEC ADIENOICACID(Z,Z)-,2	C ₁₈ H ₃₂ O	280.4455		antimicrobial activity[39]
18	43.914	Oleoyl chloride	C ₁₈ H ₃₃ C IO	300.91		Antibacterial activity[52]
19	44.519	ETHYL (9Z,12Z)-9,12-O CTADECADIEN O	C ₂₀ H ₃₆ O	308.4986		antibacterial and anti-inflammat ory[53]

20	44.601	9-Octadecenoic acid, 1,2,3-propanetriylester	C ₅₇ H ₁₀₄ O ₆	885.4321		
21	44.966	Glycidol stearate	C ₂₁ H ₄₀ O ₃	340.54		induces the formation of subcutaneous sarcomas[54]
22	47.209	9-Tetradecenal	C ₁₄ H ₂₆ O	210.36		Antimicrobial[55]
23	47.32	Methyl 18-fluorooctadec-9-enoate	C ₁₉ H ₃₆ O	312.5		Biodegradation of textile dye[56]
24	47.505	03027205002FL AVONE 4'-OH, 5-OH, 7-DI-	C ₁₅ H ₁₀ O ₂	222.239		Antioxidant and allelopathic Properties
25	47.659	Triarachine	C ₆₃ H ₁₂₂ O ₆	975.6392		potential therapeutic effects in various diseases
26	47.76	1,2-Bis(trimethylsilyl)benzene	C ₁₂ H ₂₂ Si ₂	222.478		antioxidant

5.4 MOLECULAR DOCKING against HEPATIC LUNG CARCINOMA

Any compound having more than one violation of Lipinski's rule and showing any positive result for selected toxicological parameters was excluded from the in-silico study. Out of thirty compounds identified by GCMS analysis we select 6 octa

decanoic acid compounds were filtered on this criteria and subjected to molecular docking for evaluation of anticancer potential. Molecular Docking analysis for hepatocellular carcinoma inhibitors here we take the 3D structure of target proteins and the 2D structure of selected phytocompounds. It showed better docking scores against target proteins and had the potential to develop into an anticancer drug

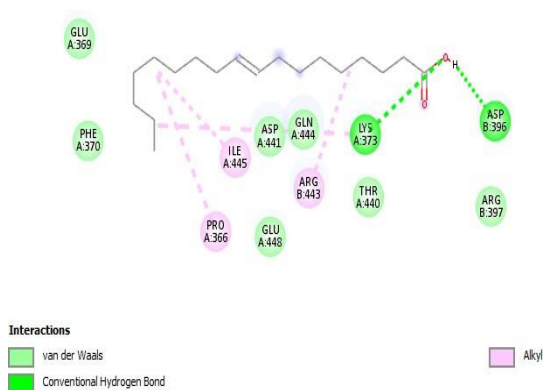
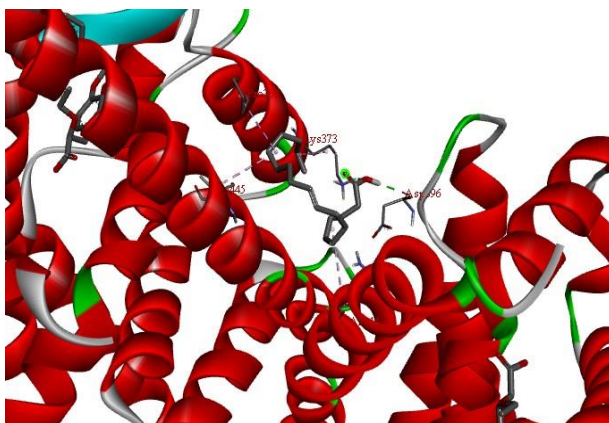


Figure 5.5 : Molecular interaction and binding energies of selected drug compounds taken from methanol extract. (**1i7i** with ligand shown Binding affinity -3.4)

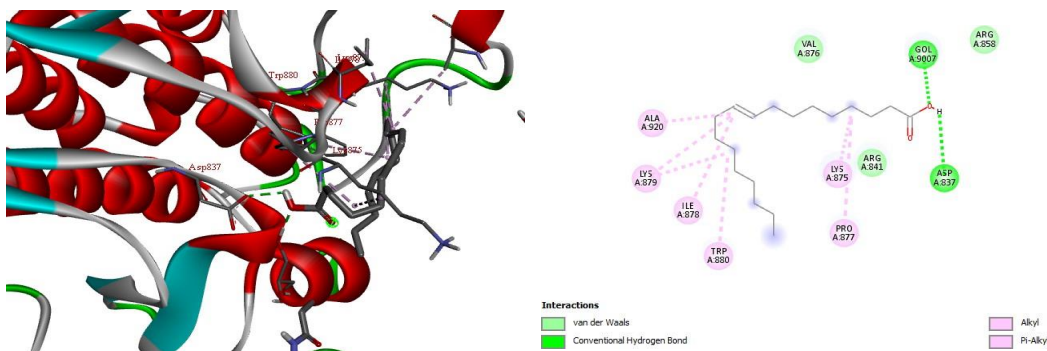


Figure 5.6 : Molecular interaction and binding energies of selected drug compounds taken from methanol extract. (5ug8 with ligand shown Binding affinity -4.75)

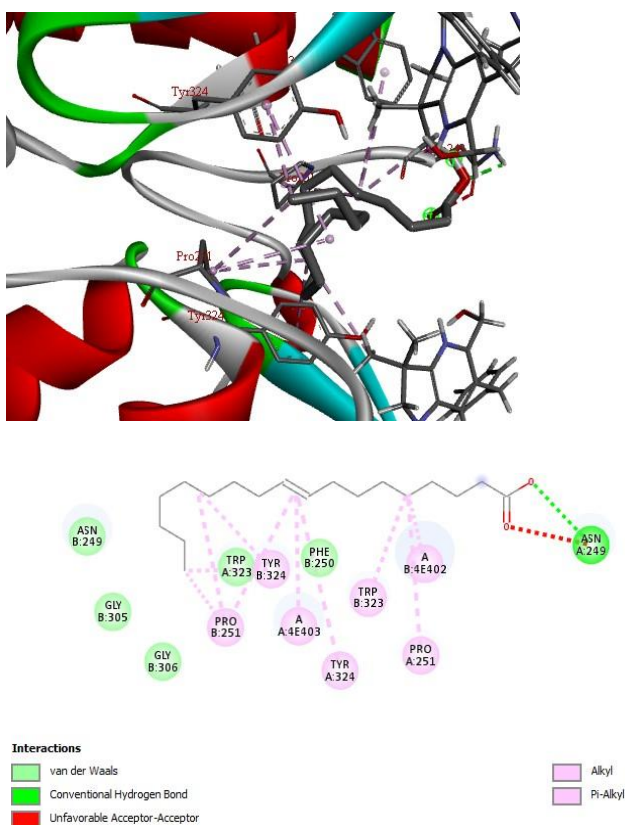


Figure 5.7: Molecular interaction and binding energies of selected drug compounds taken from methanol extract. (5oqw with ligand shown Binding affinity -5.71).

5.5 LIGHT MICROSCOPIC VISUALIZATION

To comprehensively inspect the *Halodule pinifolia* methanol extract mediated biofilm destruction on glass surfaces, light and fluorescence microscopic analyses were performed (Fig. 8). The light microscopic image of untreated control unveiled a robust crisscross architecture of biofilm. In contrast, this characteristic of biofilm architecture was immensely collapsed in a concentration-dependent manner upon 24 h of treatment with extract. Significant inhibition of biofilm was found in *C. albicans*, *P.aeruginosa* and *K.pneumoniae* treated with extract at 128 µg/mL. Likewise, a notable biofilm inhibition was observed at the concentrations of 64 and 32 µg/mL. Microscopic examinations depicted that extract attenuates planktonic cells towards forming biofilm architecture in a dose-dependent fashion.

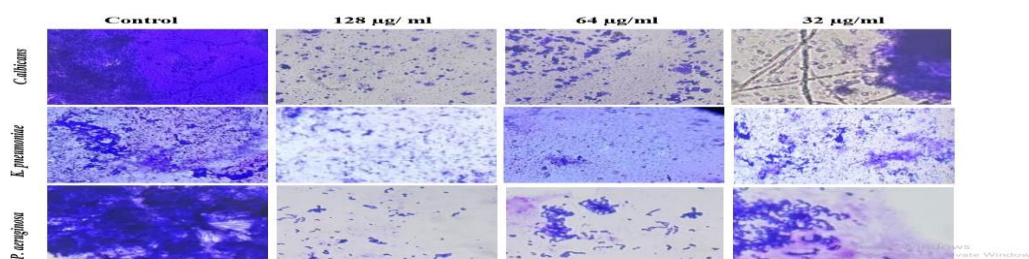


Figure 5.8 : Effect of methanol extract on biofilm cells and planktonic growth. After 24 h incubation, control and treated *C. albicans* were photographed under light microscope(40X Magnification).Extract treated groups exposed a reduction in biofilm formation.

CHAPTER- 6

Summary and Conclusion

Candida albicans are opportunistic microorganisms. The aim of this study was to evaluate the antifungal effect of *H. pinifolia* against *C. albicans*. It was found that the extract has an effective antifungal effect against *C. albicans*. Medicinal plants are an important source of new chemical substances with potential antimicrobial and therapeutic effects. Literature data have shown that the extract are the most popular source of active compounds. Our investigations showed that crude methanol extract possesses effective antifungal activity. It reduces *C. albicans* growth 100% at a concentration of 100 µg/ ml. The changes observed

in *C. albicans* biofilms after treatment with different concentration of extract. The results obtained leads to the conclusion that the *C. albicans* cell wall is a potential target for the antifungal activity of the active components of the *H. pinifolia* extract.

About 60% of marketed drugs are plant-based with effective pharmacological properties. In recent years, many novel anticancer drugs have been commercially derived from different plants, including vinca alkaloids, podophyllotoxin, taxanes, vincristine, and their derivatives. So, natural anticancer agents are safer than synthetic medicines that affect even normal cells. More than 25 metabolites were identified in the saponifiable part of the fraction methanol extract of *halodule pinifolia*. The metabolite 9 octadecenoic acid, 1,2,3-propanetriylester with area 32.98% in major and minor 9-octadecenamide with the area of 0.28% according to gcms 17.62% of cis-9-hexadecenal, 14.54 % area of ethyl(9z,12z)-9,12octadecadieno, 4.34% glycidol stearate and lowest area of 0.29% 1,2-bis(trimethylsilyl)benzene, 0.39% of hexadecanoic acid, methyl ester and area of 0.51% 9-tetradecanal,(z) shows the activity of antibacterial, antimicrobial and antioxidant activity

GCMS analysis to profile pharmacologically active phytochemicals. 4 octadecanoic acid were selected for molecular docking based on toxicological and drug-like parameters. This compounds showed better results against hepatic lung carcinoma and showed the best hits against most of the target macromolecules to inhibit hepatocarcinogenesis via inhibiting inflammation, promoting apoptosis, and reducing angiogenesis in malignant cells. According to our knowledge, most of the biological activities of *H. pinifolia* are not well-documented previously. Thus, this plant, especially its methanol extract, has great potential to develop into a safe, specific, and effective medication for preventing and managing hepato lung cellular carcinoma. For new medication formulations, additional in-vivo studies are required to authenticate its anticancer role via exploring various underlying mechanisms involved in inflammation-induced liver carcinogenesis. Molecular docking of best-hit biomolecules derived from the methanol extract of *H. pinifolia* showed better modulation of anticancer, and apoptotic potential to define their promising role in developing the multiple-targeted drugs. Out of thirty compounds identified by GCMS analysis we select 6 octa decanoic acid

compounds were filtered on this criteria and subjected to molecular docking for evaluation of anticancer potential. Molecular Docking analysis for hepatocellular carcinoma inhibitors here we take the 3D structure of target proteins and the 2D structure of selected phytochemicals. It showed better docking scores against target proteins and had the potential to develop into an anticancer drug. So, these compounds are proven good candidates to be a part of different anticancer therapeutic regimens against Hepatic lung carcinoma but should be further explored using different in-vivo studies.

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