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Research Article

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Phytochemical Screening and Antimicrobial Potentialo of Halodule Pinifolia

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Abstract: The aim of this work was to study the phytochemical and antimicrobial activity of seagrass extracts of Halodulepinifolia on clinical isolated pathogens. Seagrass species have very potential groups producing several secondary metabolites. The bioactive potential of seagrass species viz., Halodulepinifolia occurring commonly along the Thanjavur coastal area was selected. We evaluate the phytochemical and antimicrobial potential of different extracts of Halodulepinifolia. The extract of the seagrass was tested against E.coli, B.subtilis, A.nigerand C.albicans by agar diffusion method. Phytochemical screening revealed the presence of carbohydrates, reducing sugars, alkaloids, saponins, phenolic compounds and flavonoids in aqueous seagrass extract. The results of the present study conclude that the studied plant possesses broad-spectrum antimicrobial properties and may act as a potent antioxidant for biological systems susceptible to free radical-mediated reactions.

Keywords: Halodulepinifolia, Seagrass Extract, Phytochemical Activity, Antimicrobial Activity.

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1. INTRODUCTION

Seagrass modulates dissolved oxygen and reduces suspended solids and nutrients in water bodies, thereby altering the physical and chemical environment[1,2]. Seagrasses are important for the production of organic carbon in the ocean. Its root and rhizome system binds and stabilizes bottom sediments, and its leaves deflect water flow and improve water quality by filtering suspended =particles. Seagrass beds also prevent coastal erosion and provide natural coastal protection Natural products have always been an important resource for protecting life. Several life-saving drugs have been developed from these plants. The Plant Kingdom provides an endless source of medicinal plants, originally used in their raw form as herbal teas, syrups, infusions, ointments, liniments and powders. Herbal and alternative medicines are used all over the world, and in the past, herbal medicines were often the original source of most medicines. Marine species are known to produce a large number of structurally diverse secondary metabolites[3]. Seagrasses are a group of marine flowering plants that inhabit the intertidal and subtidal zones of shallow and sheltered seas, bays, backwaters, lagoons and estuaries in temperate and tropical coasts of the world [4,5]. With only about 72 species and 13 genera, seagrasses play important ecological roles in fisheries production, sediment accumulation and stabilization[6] and are of immediate value to humans as food, fodder, cover crops and medicine [7,8]. Phytochemical analysis of seagrass species shows that they are antioxidants[9,10], antibacterial, antifungal, and anti-inflammatory agents[11,12] and sources of anticancer agents [13]. Anti-microbials have saved the existences of millions of individuals and have added to the significant increases in future over the course of the past 100 years. Notwithstanding, the clinical viability of many existing anti-microbials is being compromised by the rise of multi-drug safe (MDR) pathogens[14] the new appearance of strains with decreased defenselessness as well as, unfortunate results of certain antibiotics[15]. Irresistible infections brought about by safe microorganisms are related with delayed hospitalizations, inflated cost, and more serious gamble for dreariness and mortality. The opposition issue requests that a reestablished exertion be made to evaluate different therapeutic plants for their possible antimicrobial qualities, which are because of mixtures combined in the auxiliary digestion of the plant. The most significant of these bioactive mixtures of vegetative are alkaloids, flavonoids, tannins, phenolic compounds, steroids, pitches, unsaturated fats and gums which are equipped for delivering clear physiological activity on body. One more driving element that urged researchers to look for new antimicrobial substances from different sources including therapeutic plants has been the quick pace of plant species annihilation. Therapeutic plants are depended upon by 80% of the total populace and in India there is a rich practice of involving home grown medication for the treatment of different irresistible infections, irritations, wounds and different sicknesses. A large number of the plant materials utilized in conventional medication are for the most part demonstrated more powerful and moderately less expensive than current medicine[16] against specific sicknesses while at the same time alleviating a considerable lot of the incidental effects that are frequently connected with manufactured antimicrobials[17]. This study examined the phytochemical analysis as well as the antibacterial activity of the seagrass *Halodulepinifolia*.

2. MATERIALS AND METHODS

2.1 Sample Collection

The algae were collected from the Thanjavur area in the East Coast District of Tamil Nadu and sorted using their morphology[18, 19]. Wet algal species are first washed with ocean water to eliminate debris such as sand, shells, wood chips and small stones. It was dried in the shade for 24 hours and finally dried in a tray dryer at 60°C to remove moisture. Chop the dried seaweed and grind it to a fine powder with a mortar and pestle. Microwave drying speeds up the drying process without destroying cellular components.

2.2. Preparation of extract

Organic extract preparation:30g of biomass of algal *Halodulepinifolia (*Authenticated by Dr. P. Anantharaman, Dean and Professor, CAS in Marine Biology, Annamalai University*)* was lyophilized with a freeze dryer. 10g of the dried algal was extracted successively with hexane, acetone and ethanol (1L about each solvent) to obtain three different crude extracts of algae. Obtained crude extracts were subjected to bioactivity testing[20].

2.3. Phytochemical Screening

Qualitative phytochemical screenings were performed using standard procedures[21, 22]. The occurrence of phytochemicals in the crude extracts of *Halodulepinifolia* was determined.

2.4. Screening of Antimicrobial Activity

In-vitro antimicrobial screenings were carried out under laboratory conditions, for this various micro organisms were collected from microbiology laboratory from GOVT Medical College, ThanjavurDist, Tamilnadu, India. with bacterial strain of *E.coli*(MTCC 1302) and *B.subtilis*(MTCC-9102)and fungal strain of *Aspergillus niger*(MTCC 872) and*Candidaalbicans*(MTCC 1637). The microorganisms were cultured on recommended cultural medium and finally transferred& maintained. The inhibitory effect of each extract was compared with the commercially available standard antibiotics (amoxicillin andtrihydrate) against bacteria and fungi respectively. Initially, the stock cultures of bacteria and fungi were revived by inoculating in broth media and grown at 37°C for 24 hrs. The Nutrient agar and PDA plates were prepared and wells were made in the plate[23]. Each plate was inoculated with 24-hour-old cultures. (100 µ l, 10^{-4}cfu) and spread evenly on the plate. After 20 min, the wells were filled with methanol plant extracts (200 µl/ml). Antimicrobial activity of seagrass *Halodulepinifolia* have been carried out by using disc diffusion method[24].

2.5. Qualitative analysis of secondary metabolites by TLC: Preparation of TLC slide

TLC (Thin Layer Chromatography)plate was prepared by mixing silica gel-G in distilled water. Mixture was prepared in a colloidal form, poured and spread to the glass slide as a thin layer.

2.6. Application of sample

Silica gel coated TLC slide was taken. Starting line was drawn 15mm above the lower edge using a marking pencil. Algal

extract was applied on the starting lines as spot by making use of capillary tube. All the extracts and fractions of the algal applied in various plates. A spot was made and allowed to cool to room temperature

2.7. Development of the chromatogram

TLC slide was placed in a beaker saturated with solvent such as, Acetone and the chromatogram was allowed to run, developed at room temperature by allowing the solvent to ascend the specified distance. The TLC plate was removed from the beaker and the position of the solvent was marked. Solvent available in the plate was allowed to evaporate at room temperature.

2.8. Observation

The TLC plate was observed in daylight initially. The Iodine chamber was used as a Spraying agent. The distance of each spot to the point of allocation was recorded. RF-Value was calculated making use of the formula RF=Distance travelled by the solute/Distance travelled by the solvent.

3. RESULT AND DISCUSSION

3.1. Preliminary Phytochemical Screening

Pharmacognostic methods are used to obtain a general evaluation of the chemical composition of a plant, to give an indication of the important groups of secondary metabolites present[25]. The phytochemicals were analyzed qualitatively by using standard protocols in different solvent extracts of *Halodulepinifolia*. The protein, reducing sugar, phenol, tannins, amino acid and steroids were found in all the extracts. The flavonoids, anthraquinones and terpenoids were present in ethanol and acetone extracts. Tannins, alkaloids, amino acids, steroids and phenol were present in the hexane extract of *H. pinifolia.* The saponins, resins and glycosides were present only in the ethanol extracts of seagrass*H. pinifolia.* This is predictable with the discoveries [26] who had reported the above phytoconstituents in the methanolic concentrates of five seagrasses like *Enhalusacoroides, Thalassiahemprichii, Halodulepinifolia, Cymodoceaserrulata*and *Cymodocearotundata*from Chinnapallam bank of Tamil Nadu. Athiperumalsamie*t al*. 21 screened four seagrasses like *Halophilaovalis, S. isoetifolium, C. serrulata and H. pinifolia*and announced 15 phytochemicals from benzene and petroleum ether concentrate of *S. isoetifolium* gathered from Gulf of Mannar.The proximate content has showed excellent quantity from methanolic extract of seaweed than the hexane extract of H.pinifolia. The maximum qualitativephytochemicals of H.pinifolia with methanolic extract were analysed than the other extract. Thequantitative phytochemicals like alkaloids, anthroquinone, flavonoids, phenols, proteins, reducing sugar, saponin, steroids, terpenoids, tannin and triterpenoids was 0.69, 0.87, 0.58, 0.18, 3.67, 0.63,0.71, 0.18, 0.73, 0.23 and 0.73 mg/g recorded respectively[27]. The aftereffects of the current review is additionally in accordance with the consequences of[28] who reported the presence of ten phytoconstituents in the methanol concentrates of *H. pinifolia* gathered from the review (Table-1).

Table.1 Qualitative phytochemical analysis for the extracts of *H. pinifolia*

Sl.No	Phytochemicals	Solvents		
		Ethanol	Acetone	Hexane
1	Alkaloids	+	+	+
2	Amino acids	+	+	+
3	Anthraquinones	+	-	+
4	Flavonoids	+	+	+
5	Glycosides	+	+	+
6	Phenol	+	+	+
7	Proteins	+	+	+
8	Reducing sugar	+	+	+
9	Resins	+	-	-
10	Saponins	+	+	+
11	Steroids	+	+	+
12	Tannins	+	+	+
13	Terpenoids	+	+	+

+, present ***-, absent***

3.2. Antimicrobial Analysis

The antimicrobial analysis (Table 2) showed a remarkable activity against the bacterial and fungal pathogens with different solvents extracts of *H. pinifolia* (200 µl/ml). The zone of inhibition measured for *B. subtilis*(MTCC-9102) and *E.coli* (MTCC 1302) using well diffusion method were 16 mm and of 10 mm. The maximum activity compared to the control shows the potential of the seagrass and is an indicator for determining the significance of the activity against the pathogens. The overall antimicrobial analysis reveals maximum against the *B. subtilis* and minimum activity was noted against the *E.coli*. Against fungal pathogens activity was maximum towards *Aspergillus niger*(MTCC 872) and minimum activity was seen against *C.albicans*(MTCC 1637). Overall observation reveals that the plant has inhibitory activity against all the pathogens studied. *H. pinifolia* is a potential source of broad-spectrum antimicrobial agents due to the presence of phenolic compounds (Table-2 and Fig-1), which have been accounted for to be engaged with hindrance of nucleic corrosive biosynthesis and other metabolic cycles [29]. Some of the seagrasses have been used in traditional medicine for example in India for malaria, skin diseases and the early stage of leprosy. Some extracts also have antibacterial activity [30,31,3]. During the long period of coevolution, a cooperative relationship has been formed

between each endophyte and its host plant. Some endophytes have the ability to produce similar bioactive compounds to those that originate from their terrestrial host plants[32] Devarajanet *al.*[33]isolated many endophytic fungi from three seagrass species commonly found in the south of Thailand and screened them for their ability to produce antimicrobial metabolites. Although low colonization densities of endophytic fungi have been reported in seagrasses, the percentage of active isolates derived from seagrasses (69%) was in the same range as those derived from mangrove plants (61%)[34] or even higher than those isolated from other terrestrial plants such as Garcinia species[35] . The number of active extracts and active isolates among the three studied seagrasses was similar. This indicated that these seagrasses are a good source of antimicrobial-producing endophytic fungi.

Table 2. Antimicrobial activity of *H. pinifolia*extract against pathogens

Pathogens	Crude extracts (Zone of inhibition-mm)			Standard
	Ethanol	Acetone	Hexane	
E.coli	14.6 ± 0.15	12.9 ± 0.11	11.7 ± 0.25	17.7 ± 0.65
B. subtilis	17.2 ± 0.28	15.8 ± 0.17	14.5 ± 0.18	21.5 ± 0.16
A. niger	10.8 ± 0.14	9.4 ± 0.22	8.9 ± 0.31	11.8 ± 0.28
C.albicans	9.6 ± 0.25	8.7 ± 0.12	8.1 ± 0.16	10.2 ± 0.17

Each value is the Mean ± SD of three replicates

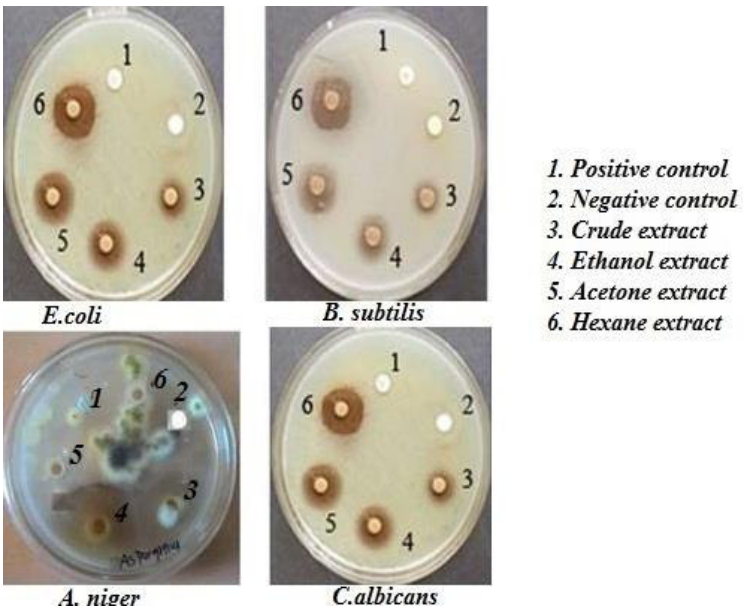

Fig- 1. Antimicrobial potential of Seagrass different solvents extract.

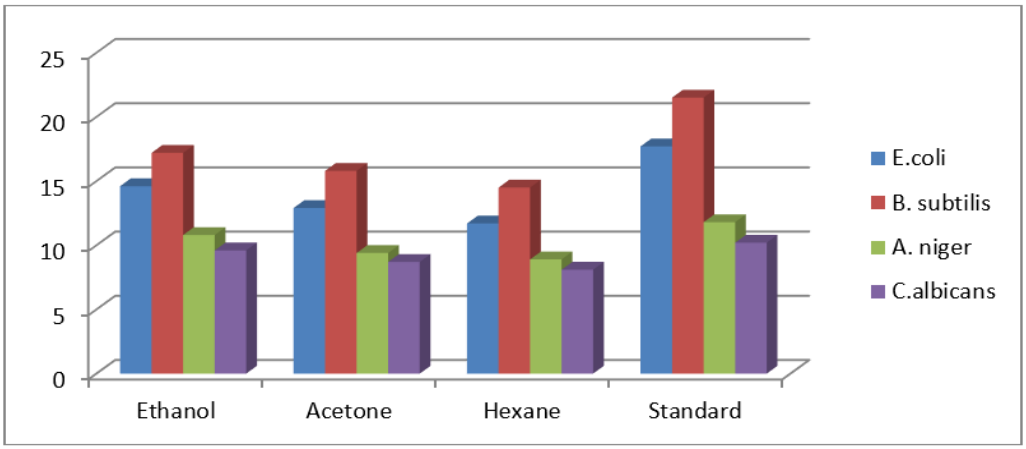

Fig-2: Graph showed the antimicrobial activity of *H. pinifolia* extract against Clinical isolated pathogens

Different Solvent extracts of marine algae which showed maximum antibacterial activity (9th day) were purified. The eluents with different colors were subjected to Thin Layer Chromatography (TLC). Spots with Rf value 0.66, 0.51, 0.79 and 0.68 indicated the presence of marine algae compounds. This showed the presence of the bioactive compound flavonoid. The identity of the quercetin bands in sample chromatograms was confirmed by the chromatogram

obtained from the sample with that obtained from the reference standard solution[34] (Table-3).

Table-3 Thin layer chromatography (TLC) profile of seagrass crude extracts in different solvent systems.

Sample	Flavonoids R_fValues	Alkaloids R_fValues	Steroids R_fValues	Anthraquinones R_fValues
H. pinifolia Ethanol extract	0.66	0.51	0.79	0.68
Acetone extract	0.64	0.48	0.65	0.65
Hexane extract	0.61	0.49	0.68	0.66

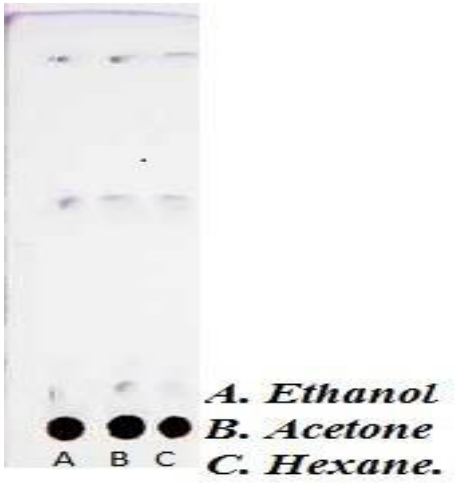

Fig 3. Images of thin layer chromatography (TLC) profile of seagrass crude extracts in different solvent systems.

4. CONCLUSION

On the basis of the results obtained in the present study, it is concluded that ethanol extract of H. pinifolia has potent anti microbial activities. Thus the H. pinifolia extract may be attributed to the presence of phenolic compounds and flavonoids etc., therefore, further investigation is needed to isolate and identify the active compounds present in the seagrass extract and its efficacy. Thus, the search for new antimicrobial substances from H. pinifolia with low residual effects on the environment could be of importance for clinical sides.

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6. CONFLICT OF INTEREST

Conflict of interest declared none.

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