Original Research Article

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL POTENTIAL OF *HALODULE PINIFOLIA*

ABSTRACT

Seagrass species have a very potential groups were producing several secondary metabolites. The bioactive potential of seagrass species viz., *Halodule pinifolia* occurring commonly along the Thanjavur coastal area was selected. We evaluate the phytochemical and antimicrobial potential of different extract of *Halodule pinifolia*. The extract of the seagrass were tested against *E.coli, B.subtilis, A.niger* and *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: *Halodule pinifolia*, seagrass extract, phytochemical activity, Antimicrobial activity**.**

UNDER PEER REVIEW

INTRODUCTION

Seagrass regulate dissolved oxygen, reduce suspended sediments and nutrients in the water column and there by modify physical and chemical environments. Seagrass are important in the production of organic carbon in the oceans. Its root and rhizome systems bind and stabilize bottom sediments and its leaves baffle currents and improve water quality by filtering suspended matter. Seagrass beds also prevent coastal erosion thereby offering natural shoreline protection.

Natural products have been an important resource for the maintenance of life for ages. Several life-saving drugs have been developed from the plants. The plant kingdom has provided an endless source of medicinal plants first used in their crude forms as herbal teas, syrups, infusions, ointments, liniments and powders. Herbal remedies and alternative medicines are used throughout the world and in the past herbs often represented the original

sources of most drugs. Marine species are known to produce a large number of structurally diverse secondary metabolites (Sundaram Ravikumar *et al.*, 2011).

Seagrasses, a group of marine flowering plants, inhabit the tidal and sub-tidal zones of shallow and sheltered localities of seas, gulfs, bays, backwaters, lagoons, and estuaries along temperate and tropical coastlines of the world (Green and Short, 2003; Short *et al.*, 2001). With only about 72 species and 13 genera, seagrasses play key ecological roles in fisheries production, sediment accumulation, and stabilization (Ronnback *et al.*, 2007) and have direct value to humanity as food, feed, green manure, and medicine (Newmaster *et al.*, 2011; Ragupathi *et al.*, 2013). Phytochemical analyses of seagrass species have shown that they are potential sources of antioxidants (Ragupathi *et al.*, 2010; Rengasamy *et al.*, 2011), antibacterial, antifungal and anti-inflammatory agents (Puglisi *et al.*, 2007 and Yuvaraj *et al.*, 2012), and source of anticancer compounds (Folmer *et al.*, 2010). The present study the phytochemical analysis of seagrasses *Halodule pinifolia* along with an antimicrobial activity.

MATERIALS AND METHODS

Sample Collection

Algal samples will be collected from Thanjavur district, East costal region, Tamil Nadu. The w*et al*gal species were identified by standard according to their morphologies (Menez *et al.*, 1983 and Coles *et al.*, 2004).W*et al*gal species will be first washed with sea water to remove the debris like sand, sea shells, pieces of wood and tiny stones. It will be shade dried for 24 hours and then finally dried in a tray drier at 60°C to remove the water content. Dry algae obtained will be finely chopped into pieces and then ground into fine powder using mortar and pestle. Microwave drying makes the drying process faster without any degradation of cell components.

Preparation of extract

For extraction, different solvents such as ethanol, n-hexane and acetone were added to 100 g of powdered leaves separately and placed in Soxhlet apparatus for 24 h. The extracts were filtered with Whatman 40 filter paper and then concentrated using a rotary evaporator to give rise to a semi-solid mass. Each solvent extraction method was repeated thrice for the purpose of accuracy. The residues obtained were stored in refrigerator for further analysis.

Phytochemical Screening

UNDER PEER REVIEW

Qualitative phytochemical screenings were performed using standard procedures (Sofowora, 1993; Trease and Evans, 1989). The occurrence of phytochemicals in the crude extracts of *Halodule pinifolia* was determined.

Screening of Antimicrobial Activity

In-vitro antimicrobial screenings were carried out under laboratory conditions, for this various micro organism were collected from microbiology laboratory, with bacterial strain of *E.coli* and *B.subtilis* and fungal strain of *Aspergillus niger* and *candidas albicans*. All the stains suggested microorganisms were cultured on recommended cultural medium and finally transfer & maintained on agar broth for O/N. Antimicrobial activity of seagrass *Halodule pinifolia* have been carried out by using disc diffusion method (Kavanagh, 1972). The inhibitory effect of each extracts was compared with the standard antibiotics penicillin and mycostatin against bacteria and fungi respectively.

RESULT AND DISCUSSION

Preliminary Phytochemical Screening

The phytochemicals were analysed qualitatively by using standard protocols in different solvent extract of *Halodule pinifolia.* 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. Tanins, 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 sea grass *H. pinifolia*.

This is consistent with the findings of Ragupathi *et al.* (2013a) who had reported the qualitative analysis of the above phytoconstituents in the methanolic extracts of five seagrasses like *Enhalus acoroides, Thalassia hemprichii, Halodule pinifolia, Cymodocea serrulata* and *Cymodocea rotundata* from Chinnapallam coast of Tamil Nadu. Athiperumalsami *et al*. (2008) screened four seagrasses such as *Halophila ovalis, S. isoetifolium, C. serrulata and H. pinifolia* and reported 15 phytochemicals from benzene and petroleum ether extract of *S. isoetifolium* collected from Gulf of Mannar. The results of the present study is also in line with the results of Girija *et al*. (2013a) who reported the presence of ten phytoconstituents in the methanol extracts of *H. pinifolia* collected from the study site.

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

UNDER PEER REVIEW

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

+, present -, absent

Antimicrobial analysis

The zone of inhibition measured for *B. subtilis* and *E.coli* using well diffusion method were 16 mm and of 10 mm. The antimicrobial analysis (Table 2) showed a remarkable activity against the bacterial and fungal pathogens with different extract of *H. pinifolia*. 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* and minimum activity was seen against *C.albicans*. 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, which have been reported to be involved in inhibition of nucleic acid biosynthesis and other metabolic processes (Cushnie & Lamb, 2005).

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 (Engel *et al*., 2006; Ross *et al*., 2008 and Ravikumar *et al*., 2011). During the long period of co-evolution, 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 (Zhao *et al*., 2011). Devarajan *et al*. (2002) studied isolated many endophytic fungi from three seagrass species commonly found

UNDER PEER REVIEW

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%) (Buatong *et al*., 2011) or even higher than those isolated from other terrestrial plants such as Garcinia species (Phongpaichit *et al*., 2006). 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

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 plant extract and its efficacy.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

UNDER PEER REVIEW

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UNDER PEER REVIEW

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