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Antibacterial Potential of Seagrass *Syringodium isoetifolium* (Ascherson) Dandy against Human Pathogens

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A B S T R A C T

To assess the antibacterial activity of *Syringodium isoetifolium* against human bacterial pathogens. The antibacterial activities of the extracts on the different test organisms using agar well diffusion method. The current investigation the antibacterial activity of different organic solvent extracts of *S. isoetifolium* showed that, methanol extract had wide spectral growth inhibitory activity than chloroform and acetone extracts. The antibiotic tetracycline exhibited markedly pronounced inhibitory zones than the crude extracts. The useful groups in seagrass extracts which expected to act antimicrobial activities were phenol and anthraglycoside. The antimicrobial activities of seagrass extracts showed the likelihood to use seagrass for biomedicine purpose.

***Key words*:** *Syringodium isoetifolium,* Antibacterial activity, Solvent extracts

Sea grasses are flowering plants belonging to monocotyledons [1] restricted to shallow marine waters with sufficient sunlight penetration. Sea grass beds are among the most productive ecosystems in the world [2] and serve as nurseries for many species of fish and shellfish [3].

Seagrasses were also used as medicine, food and livestock feed [4]. Sea grasses are well documented for the presence of diverse secondary metabolites [5]. It has been realized documented that these metabolites are biologically active and of biomedical importance and could be used as potential drugs. There is a growing concern about the use and particularly the abuse of antimicrobial drugs not only in human medicine and agriculture but also in aquaculture. Hence the need of the hour is to search for antibiotics with unique potential for which the pathogens may not have developed resistance [6] and so the search for new antibiotics is a continuous process. Marine plants and animals are reported to possess a wide spectrum of bioactive substances, which are structurally novel and biologically active. Research in the areas of marine natural products has grown geometrically in the recent past [7]. The antibacterial activity of different solvent extracts of *Halodule uninervis* against different bacterial pathogens [8].

The antifouling potential of some marine organisms against *Bacillus* and *Pseudomonas* species and reported that the seagrass *Cymodocea rotundata* exhibited mild activity against all the bacterial strains [9]. The antibacterial properties of three seagrasses namely *C. serrulata, H. ovalis* and *Zostera capensis* were tested against human pathogens such as *S. aureus*, *B. cereus*, *B. subtilis*, *E. coli*, *S. paratyphi*, *S. typhimurium* and *M. luteus*, using six different solvent system for extraction namely petroleum ether, chloroform, ethyl acetate, acetone, methanol and water [10]. The present study was undertaken to investigate the qualitative test for phytoconstituents of the sea grass *Syringodium isoetifolium* and its antibacterial activity against human pathogens.

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MATERIALS AND METHODS

Collection of Seagrass

In the present study, fresh leaves of seagrass *Syringodium isoetifolium*, (Ascherson) Dandy was collected at a depth of 1m from Tuticorin coast, Tamil Nadu, India.

Preparation of extracts

Collected seagrass *S. isoetifolium* was brought to laboratory in a polythene bag. Then the seagrass sample was washed thoroughly with running tap water and surface sterilized with 3% ethanol to remove the dirt and epiphytes. Then seagrass was dried at room temperature and then pulverized into fine powder with electrical grinder. In the present study, 100 g of seagrass powder sample was taken and subjected to percolation individually in 300 ml of solvents (methanol, chloroform and acetone) of varying polarity at room temperature. The process was repeated twice; extracts were pooled together and filtered through Whatman No:1 filter paper. Each filtrate was concentrated to dryness under reduced pressure using a rotary vacuum evaporator. Dried extracts were weighted and stored in screw cap bottles under refrigerated condition for further study.

Antibacterial activity

Antibacterial activity of the crude extracts of seagrass *S. isoetifolium* was tested through standard agar well diffusion method. Muller hinton agar plates (MHA) were prepared separately and overnight culture of test bacterial pathogens were seeded individually over the surface of MHA plates using sterile cotton swabs. Thereafter wells of 6 mm diameter were made over MHA plates using sterile cork borer. The wells were then loaded with 100 µl of sample containing 250 µg of crude extracts which was prepared in dimethyl sulphoxide. Tetracycline was used as positive control; while DMSO was used as negative control. The plates were then incubated at 37°C for 24 h and growth inhibitory activity in terms of zone of inhibition (mm) formed around each well was measured and recorded. The assay was carried out in triplicates.

Statistical analysis

The data obtained in the present study were subjected to the following statistical analysis using SPSS 17.0.

RESULTS AND DISCUSSION

Percentage yield of crude extracts of S. isoetifolium

In the present investigation, the seagrass *S. isoetifolium* was extracted with solvents of varying polarity. The (Table 1) shows the results of yield (%) of crude extracts obtained using different solvent system. Amongst the extracts, methanol extract had higher yield of 23.27 ± 1.18%. Whereas, the plant extracted with chloroform and acetone showed a marked reduction in yield and accordingly it was found to be 19.66 ± 2.16 and 15.58 ± 2.18%, respectively.

Table 1 Percentage yield crude extracts of *S. isoetifolium* using various organic solvents

Organic solvent extracts	Extract yield (%)
Methanol	23.27 ± 1.18
Chloroform	19.66 ± 2.16
Acetone	15.58 ± 2.18

Each value is the Mean ± SD of three replicates

Antibacterial activity

Antibacterial activity of methanol extract of S. isoetifolium

The (Fig 1) shows the antibacterial activity of methanol extract of *S. isoetifolium* against test bacterial pathogens showed 100% growth inhibition of bacterial pathogens by forming zone of inhibition ranged between 7 ± 0.22 to 14 ± 0.92 mm. It showed higher growth inhibitory activity of 14 mm against *P. aeruginosa* and minimum of 7 mm against *E. coli* (Fig 1). The same extract tested against other pathogenic bacterial strains viz., *E. faecalis, S. pneumoniae, S. mutans, S. aureus, S. typhi, K. pneumonia* and *P. vulgaris* displayed moderate growth inhibitory activity ranged between 9 and 13 mm, respectively.

Antibacterial activity of chloroform extract of *S. isoetifolium* against test bacterial pathogens showed 66.6% growth inhibitory activity with the inhibitory zones ranged from 4 ± 0.18 to 11 ± 0.67 mm. Here, maximum growth inhibitory activity of 11 ± 0.67 mm was recorded against *S. pneumoniae* and minimum of 4 mm was noticed against *S. mutans* and *E. coli* (Fig 1).

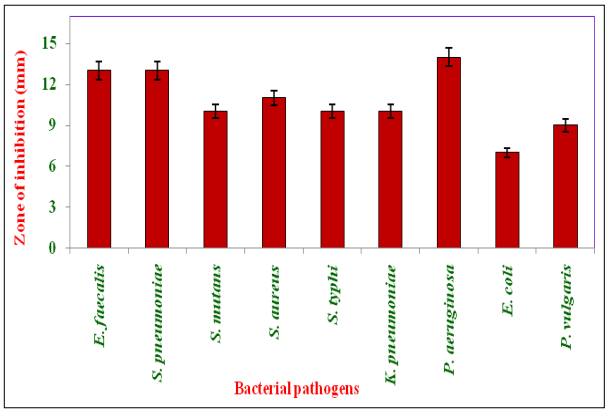

Fig 1 Antibacterial activity of methanol extract of *S. isoetifolium* against bacterial pathogens

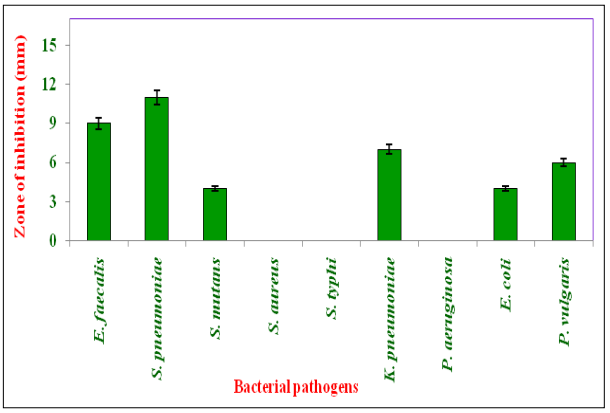

Fig 2 Antibacterial activity of chloroform extract of *S. isoetifolium* against bacterial pathogens

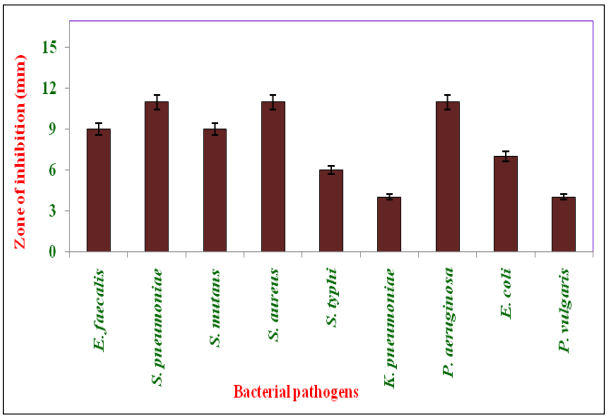

Fig 3 Antibacterial activity of acetone extract of *S. isoetifolium* against bacterial pathogens

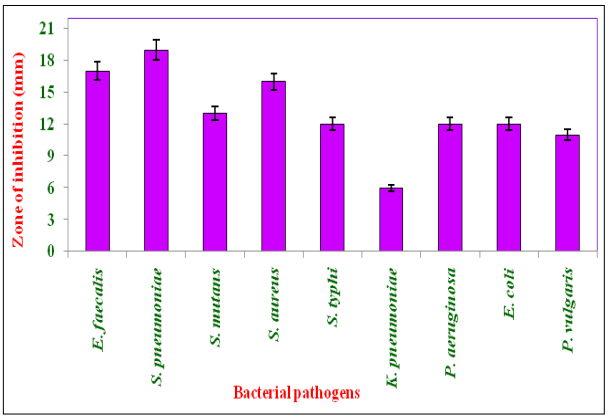

Fig 4 Antibacterial activity of antibiotic tetracycline against bacterial pathogens

The antibacterial activity of acetone extract of *S. isoetifolium* against the bacterial pathogens displayed markedly pronounced growth inhibitory activity than the chloroform extract tested with the inhibitory zones ranged between 4 ± 0.12 to 11 ± 0.72 mm (Fig 3). It showed maximum antagonistic activity of 11 mm against *S. pneumoniae*, *S. aurues* and *P. aeruginosa*; whereas, the least bioactivity of 4 mm against *P. vulgaris* (Fig 1).

Whereas, the antibacterial activity of antibiotic tetracycline (positive control) tested against pathogenic bacterial strains displayed pronounced inhibitory zones ranged between 6 ± 0.33 and 19 ± 1.24 mm. It recorded broad spectrum antagonistic activity against *S. pneumoniae* (19 mm), *E. faecalis* (17 mm) and *S. aurues* (16 mm). On the same instance it displayed minimal growth inhibitory of 6 mm against *K. pneumoniae* (Fig 1). DMSO was used as negative control which always showed no growth inhibitory activity against the bacterial pathogens. The antibacterial activity of crude methanol, chloroform, acetone and antibiotic tetracycline showed as a function of variation between pathogenic bacterial strains was statistically significant (P < 0.00) (Table 2-5).

Table 2 Antibacterial activity of methanol extract of *S. isoetifolium* as a function of variation due to different bacterial pathogens

Source of variations	Sum of squares	df	Mean square	F	P-value
Variation due to bacterial pathogens	118.666667	8	14.83333	36.405	P < 0.001*
Error variance	7.334	18	0.407444	-	-
Total variance	126.000667	26	-	-	-

*Statistically significant

Table 2 Antibacterial activity of methanol extract of *S. isoetifolium* as a function of variation due to different bacterial pathogens

Source of variations	Sum of squares	df	Mean square	F	P-value
Variation due to bacterial pathogens	396.6667	8	49.6	526.61	P < 0.0001*
Error variance	1.6948	18	0.09	-	-
Total variance	398.3615	26	-	-	-

*Statistically more significant

Table 3 Antibacterial activity of chloroform extract of *S. isoetifolium* as a function of variation due to different bacterial pathogens

Source of variations	Sum of squares	df	Mean square	F	P-value
Variation due to bacterial pathogens	396.6667	8	49.6	526.61	P < 0.0001*
Error variance	1.6948	18	0.09	-	-
Total variance	398.3615	26	-	-	-

*Statistically more significant

Table 4 Antibacterial activity of acetone extract of *S. isoetifolium* as a function of variation due to different bacterial pathogens

Source of variations	Sum of squares	df	Mean square	F	P-value
Variation due to bacterial pathogens	198	8	24.75	86.65629	P < 0.0001*
Error variance	5.141	18	0.286	-	-
Total variance	203.14	26	-	-	-

*Statistically more significant

Phytochemical analysis of crude extracts of seagrass S. isoetifolium

Phytochemical constituents of crude extracts of seagrass *S. isoetifolium* were shown in (Table 1). Amongst the extract tested, methanol extract showed presence of almost all phytochemical *viz.* tannins, saponins, flavonoids, alkaloids, amino acids, terpenoids and phenols. But it did not show occurrence of steroids and anthroquinones. Besides, chloroform extract revealed the presence of saponins, flavonoids, alkaloids, terpenoids and phenols. On the same instance, tannins, flavonoids, alkaloids, steroids and phenols were the phytochemical constituents recorded in the acetone extract of *S. isoetifolium*.

The natural marine compounds represent a source of new chemical structures having potent biological activities that allow us to have a deep knowledge of to utilize it for the successful development novel therapeutic agent. This diversity of chemical compounds is believed to be a consequence of competition between organisms for space and resource in most marine habitats. Due to antimicrobial actions, some of the biologically active natural products have become excellent sources of new and effective drugs such as antifungal, anti-inflammatory and anti-viral agents of the natural products isolated from marine organisms [11].

Bioactive marine natural products play an important role in chemotherapy. Marine plants are considered as a source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities. In view of the above, an endeavour has been made in the present study to investigate the antibacterial activities of seagrass *Syringodium isoetifolium.*

In recent years, there has been a marked decrease in the introduction of new antibacterial agents for clinical use. This is an alarming development, especially in light of the emergence of multidrug-resistant (MDR) bacteria, which is a challenge to the successful treatment of nosocomial as well as community acquired bacterial infections [12].

In the present study, various organic solvent extracts of seagrass *S. isoetifolium* was tested against both gram positive and gram-negative bacterial strains. However, in the present study it was observed that methanol extract of *S.*

isoetifolium showed pronounced antagonistic activity (7 to 14 mm) than chloroform and acetone extracts. The results of the present study consisted with some preceding studies of seagrass antibacterial activity [13]. Similarly extracts from *Cymodocea rotundata* Ehrenberg and Hemprich ex Ascherson (Cymodoceaceae) was effective against *Bacillus* species [14]. It was reported that ethanol and methanol extracts of seagrasses showed better zones of inhibition against bacterial pathogens [15]. The methanol and diethyl methyl formamide extracts of seagrass sp. was found to be active against Gram-positive pathogens [16]. Alam *et al.* [17] who found that methanol extract of *Enhalusa coroides* were effective against *S. aureus, K. pneumoniae* and *P. aeruginosa* than the hexane extract. This significant variation in antibacterial activity could be ascribed to differences in cell wall composition between gram negative and gram-positive bacteria, which make gram negative bacteria more resistant to different antibacterial substances. As suggested by Schwarz and Noble [18] reported these bacterial strains may have some kind of resistance mechanisms e.g., enzymatic inactivation, target sites modification and decrease intracellular drug accumulation or the concentration of the compound used may not be sufficient. No inhibition was observed with negative controls, which proves that the solvents could not act as antibacterial agents. Besides, there are several factors such as the age of the plant, duration of storage, temperature, preparation of the media and pH which could indirectly affect the degree of antibiotic activity [19]. Fennel *et al.* [20] reported that, the antibacterial activity exhibited by the marine plant parts could be due to the presence of phytochemical like alkaloids, tannin, flavonoids and sugars present in the plant extracts. Olvi Cristianawati *et al.* [21], reported antibacterial activity of seagrass associated bacteria from the North Java Sea, Indonesia against multidrug-resistant bacteria.

In the present study methanol extract of *S. isoetifolium* showed presence of majority of the phyto-constituents like tannins, saponins, flavonoids, alkaloids, amino acids, terpenoids and phenols. This is consistent with the findings of Kannan *et al.* [22] who had reported the qualitative analysis of the above phytoconstituents in the methanol extracts of six seagrasses like *Enhalusa coroides, Thalassia hemprichii, Halodule pinifolia, Syringodium isoetifolium, Cymodocea serrulata* and *Cymodocea rotundata* from Chinnapallam coast of Tamil Nadu. Athiperumalsami *et al.* [23] screened four seagrasses such as *Halophila ovalis, S. isoetifolium, C. serrulate, 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.* [24] who reported the presence of ten phytoconstituents in the methanol and acetone extracts of *H. pinifolia* collected from the coast of Tuticorin. Windyaswari observed phytochemical profile of sea grass extract *Enhalus acoroides*.

Seagrasses are the potential resources in marine environment which provides a great significance in drug development. Several types of secondary metabolites have been studied in seagrass, often from a chemotaxonomic view point. In the present study, alkaloids, flavonoids and phenolics were much higher in methanol extract. In accordance to our study, higher alkaloid content was also observed in seagrass *H. ovalis* collected from the Chunnamber estuary in Pondicherry [25]. Alkaloids are toxic due to their stimulatory effects, leading to excitation of cells and neurological dysfunction [26].

Besides alkaloids, in the present investigation flavonoid was also rich in methanol extract. Flavanoids are most commonly known for their antioxidant activity. Flavanoids are potent antioxidants and have aroused considerable interest recently because of their potential beneficial effects on human health in fighting disease. They show antiallergic, anti-inflammatory, antimicrobial and anticancer activity [27]. Several authors already reported on flavonoids groups exhibited a wide range of biological activities such as antioxidant, anti-inflammatory, antimicrobial, anti-angionic, anticancer and anti-alergic [28-29]. In the present study analysis of phenolic content in the crude extracts of *S. isoetifolium* inferred that, incidence of phenolics were much high in methanol extract rather than chloroform and acetone extract. This could be attributed to polar nature of the methanol solvent. Several authors reported that high solubility of phenols in polar solvents provide high concentration of these compounds in the extracts obtained using polar solvents for the extraction [30].

CONCLUSION

The present study concluded that the antibacterial activity of various organic solvent extracts of *S. isoetifolium* indicated that, methanol extract had wide spectral growth inhibitory activity than chloroform and acetone extracts. The antibiotic tetracycline exhibited markedly pronounced inhibitory zones than the crude extracts. However, through purification and characterization of the acrtive components will reveal the exact nature of the active principles involved in the antibacterial assay. Phytochemical analysis of different solvent extracts of *S. isoetifolium* revealed that methanol extract had higher number of phytochemicals, followed by acetone and chloroform extract. Results on total alkaloid, flavonoid and phenolic content of various organic solvent extracts of *S. isoetifolium* depicted that they were in the following order: Methanol > Chloroform > Acetone.

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