

**Preliminary phytochemical analysis and antibiogram of the seagrass
Syringodium isoetifolium (Asch.) Dandy collected from
Kooduthalai Coast, Tirunelveli.**

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ABSTRACT

*Seagrasses are marine flowering plants and are distributed all over the world. They are well documented for the presence of potent, diverse secondary metabolites that are biologically active and could be used as potential drugs. The present study is about the investigation of the phytochemicals and antibacterial activity of the seagrass *Syringodium isoetifolium*. The dried seagrass powder was extracted with chloroform, acetone and ethanol separately in a ratio of 1:4 using a cold extraction method. The preliminary phytochemical analysis of the extracts showed the presence of alkaloids, steroids, saponins, flavonoids, terpenoids, glycosides and phenolic compounds. Antibacterial activity was done using a well diffusion method against one Gram-positive and one Gram-negative pathogens, such as *Bacillus subtilis* and *Pseudomonas aeruginosa*. Among the three solvent extracts, the ethanol extract showed the maximum activity of 19.66 ± 0.57 mm against *B. subtilis*. This study revealed that the seagrass *Syringodium isoetifolium* has very good antimicrobial activity due to its secondary metabolites.*

Keywords: *Seagrass, Antimicrobial activity, *Syringodium isoetifolium**

1. Introduction

Seagrass is one of the true marine flowering plants that belongs to the group angiosperms [1]. They are known to produce a wide variety of secondary metabolites that aid as defense mechanisms under stress conditions and these compounds are found to be anti-oxidative in nature [2]. These chemical compounds synthesized by secondary metabolic pathways are not involved in normal growth, development or reproduction but usually have roles in adaptation processes under stress conditions [3]. These biogenic compounds or active metabolites like polyphenols, terpenoids and halogenated compounds produced by several species of seagrass have anticancer (antitumor), antifungal or anti-inflammatory [4], antimicrobial [5], antiviral, antidiabetic activities [6], antimalarial, antioxidant [7], antiprotozoal and cytotoxic properties [8] which are effective in the prevention of different diseases and also other novel pharmacological activities. It has been realised that many of these metabolites, being biologically active, could be used as a potential drug. As antibiotic resistance has developed,

it demands a continued search for antibacterial drugs that combat harmful microorganisms [9]. Thus, we continue to require more effective, affordable, and safe medications. Therefore, the purpose of this study was to assess the antibacterial activity of the seagrass *Syringodium isoetifolium* (Asch.) Dandy against human infectious disease pathogens such as *Pseudomonas aeruginosa* and *Bacillus subtilis*, as well as examine its phytoconstituents.

Materials and methods

Sample collection and Extraction

The fresh leaves of *Syringodium isoetifolium* (Asch.) Dandy were collected from Kooduthalai coast, Tirunelveli, India and identified as *Syringodium isoetifolium* (Asch.) Dandy by morphological features according to the standard seagrass manual [10]. The collected seagrass was cleaned with tap water and shade dried. The dried materials were powdered using a mixer grinder. The powdered sample was extracted with organic solvents of increasing polarity such as chloroform, acetone and ethanol, individually in a ratio of 1:4. The extracts were filtered using Whatman No.1 filter paper. The resultant filtrate was then concentrated and used for further analysis.



Fig 1. Image of the seagrass *Syringodium isoetifolium*

Phytochemical Analysis

The qualitative test for the identification of phytochemical constituents was carried out according to standard procedure [11].

Microorganisms used

The antimicrobial activity of the seagrass extract was screened against one Gram-positive bacteria, *Bacillus subtilis* and one Gram-negative bacteria *Pseudomonas aeruginosa*, which were obtained from Scudder Laboratory, Nagercoil.

Antibacterial assay

Antibacterial activity was assayed using the agar well diffusion technique using Nutrient Agar Medium. A sterile cotton swab was used for spreading the test microorganisms evenly from the 24-hour incubated broth on the NA plates. In each of these plates, wells of

6mm diameter were made using a sterilized cork borer and 50 μ l of each extract was added to those wells separately. 25 μ l of streptomycin (10mg/ml) was used as a positive control and 25 μ l of the respective solvent used as a negative control. The extract-loaded plates were kept for incubation at 37°C for 24 hours. After incubation, a clear zone around the well was formed which was the diameter of the zone of inhibition measured in millimetres. The experiment was carried out in duplicate and the mean zone of inhibition \pm standard error was calculated using excel.

2. Results

Table 1. Phytochemical analysis of *Syringodium isoetifolium*

S. No	Phytochemicals	Acetone	Chloroform	Ethanol
1	Alkaloids	-	+	+
2	Steroids	+	-	-
3	Tannins	-	-	-
4	Phlobatanins	-	-	-
5	Saponins	+	+	+
6	Flavonoids	+	-	+
7	Terpenoids	+	+	-
8	Glycosides	+	+	+
9	Phenolic compounds	+	+	+
10	Aromatic acids	-	-	-
11	Xanthoproteins	-	-	-

Phytochemical analysis was done separately for acetone, chloroform and ethanol extracts of *S. isoetifolium*. As Table 1. Shows, the acetone extract of the seagrass shows the presence of phytochemicals such as steroids, saponins, flavonoids, terpenoids, glycosides and phenolic contents. The chloroform extract shows the presence of alkaloids, saponins,

terpenoids, glycosides and phenolic compounds. The ethanol extract shows the presence of alkaloids, saponins, flavonoids, glycosides and phenolic compounds.

Table 2. Zone of inhibition (mm±SE) of *S. isoetifolium* on human pathogens

Bacteria	Control	Standard (Streptomycin)	Acetone (25µl)	Chloroform (25µl)	Ethanol (25µl)
<i>B. subtilis</i>	-	30	13±0.8	9±0.9	19.6±0.57
<i>P. aeruginosa</i>	-	17	9±1	8±0.6	14±1.2

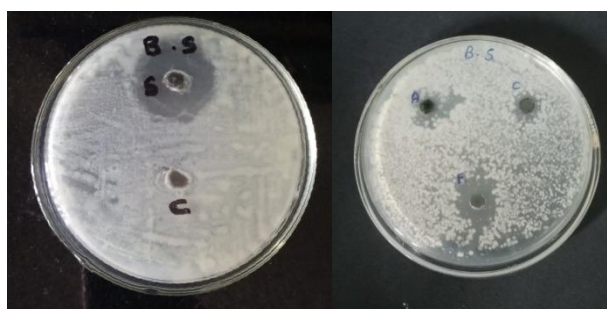
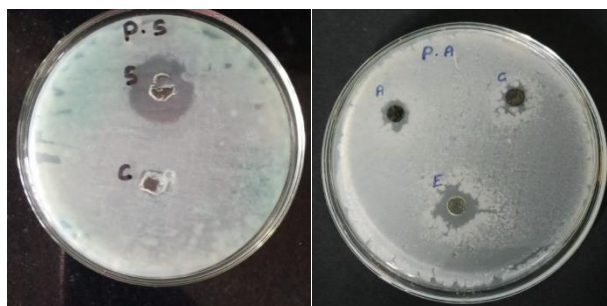


Fig. 2. Antibacterial activity of *S. isoetifolium* against *B. subtilis*



(S-streptomycin, C-control, A- acetone, C-chloroform, E-ethanol)

Fig. 3. Antibacterial activity of *S. isoetifolium* against *P. aeruginosa*

From Table 2. and Figs. 2 and 3, among the three solvent extracts, the ethanol extract showed the highest activity against both the tested pathogens, with the maximum activity of 19.66±0.57 mm against *B. subtilis*.

3. Discussion

Natural products are considered an important source of new antibacterial agents. Many chemically unique compounds of marine origin with different biological activities have been isolated and a number of them are under investigation and/or are being developed as new pharmaceuticals [12,13]. The present study revealed that the seagrass *S. isoetifolium* contains phytochemicals such as alkaloids, steroids, saponins, flavonoids, terpenoids,

glycosides and phenolic compounds. The qualitative phytochemical analysis showed that most of the phytoconstituents were extracted with acetone extraction compared to the other two.

Seagrasses have the highest value of soluble phenolic content which exhibit multiple bioactive roles against pathogenic microorganisms. In this study, the solvent extracts of seagrass *S. isoetifolium* displayed good antibacterial activity against Gram-positive as well as Gram-negative pathogens. Among the three solvents, the ethanol extract of *S. isoetifolium* effectively inhibited both *B. subtilis* (19.6 mm) and *P. aeruginosa* (14 mm). It is followed by acetone extract which inhibits *B. subtilis* (13 mm) effectively. The chloroform extract showed the lowest activity among the three extracts. Similar findings were also seen in some previous reports and also confirm that the polar solvents like methanol and ethanol extracts of seagrasses *Halophila ovalis* and *Halodule pinifolia* showed better zones of inhibition against pathogens than any other tested extracts [14]. Likewise, *Heracleum sphondylium*'s ethanol and aqueous extracts have shown antibacterial properties against both Gram-positive and Gram-negative bacteria [15]. The variation in antibacterial activity of the extracts might be due to the distribution of antimicrobial substances, which varied from species to species [16]. The present study reveals that the ethanol extract has effective inhibition of human pathogens and could be used to extract potent antimicrobial compounds. But previously, it was reported that the acetone and hexane leaf extracts were found to have the strong antimicrobial and anti-inflammatory activities [17]. The marine plant's antibacterial effect may be a result of phytochemicals present in plant extracts, which include alkaloids, tannins, flavonoids, and sugars [18].

4. Conclusion

The prospect of developing antimicrobials from plant extracts emerges satisfying, as it will lead to the development of phytomedicines to act against microorganisms. The sea grass *Syringodium isoetifolium* has a variety of biologically active molecules that can be used as a source of antibiotics. Further purification of active compounds and structural elucidation can be used for drug discovery.

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