

Phytochemical composition and antimicrobial properties of *Syringodium isoetifolium* (Ascherson) Dandy ethanolic extracts

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Abstract:

The seagrass, *Syringodium isoetifolium* was obtained from the Gulf of Mannar and the ethanolic extract was prepared using the shade-dried plants. TLC results revealed the presence of phenols, steroids and tannins with ethanol extract. The GC-MS analysis of the ethanolic extracts of *Syringodium isoetifolium* detected 40 peaks, which included the fatty acids, phenolic compounds, and phytosterols. Antimicrobial activities were analysed using the mean value of zone of inhibition against the selected microorganisms.

Keywords: Antimicrobial, phytosterol, microorganism and Ethanol

Introduction

In recent times, people have forgotten traditional foods with medicinal value, which has led to the emergence of various new diseases that are epidemic to humankind. The most complex ecosystem on earth is the marine ecosystem, which is vast to be explored. In aquatic habitats, marine angiosperms are unique and they occupy the bottom line of the food chain and serve as the main nutrients for the ocean's higher organisms. Seagrasses are marine angiosperms belonging to the order Alismatales and there are four different families, such as Posidoniaceae, Zosteraceae, Hydrocharitaceae, and Cymodoceaceae, which have a unique nature that grow and complete their life cycle under the ocean. *Syringodium isoetifolium* is a herbaceous plant that grows well not only on coral flats but also on sandy to muddy bottoms up to 15 m depth. It is not seen in backwaters and estuaries. Phytochemical examinations of seagrass species have shown that they are likely sources of cell reinforcements (Ragupathi *et al.*, 2010; Rengasamy *et al.*, 2011). The main aim of a medicinal chemist is to get active extracts, fractions or compounds against a particular target. In recent times, computational chemistry has become an economically cheaper solution for drug discovery and the identification of lead molecules. This, when coupled with natural products, enables a medicinal chemist to explore more efficiently with less work (Mukhtar *et al.*, 2008). Qualitative and quantitative analysis using chromatographic methods are extensively employed in drug discovery, pharmacokinetics, drug metabolism, process and formulation development, metabolic process for research, standardization and quality control (Kazakevich and LoBrutto, 2007; Hostettmann *et al.*, 2001). Only very few studies are available on the antifungal, antibacterial and antiviral activities of crude solvent extracts of the seagrasses. In this study, the ethanolic extract of *Syringodium isoetifolium* was subjected to phytochemical analysis and antimicrobial activities.

Materials and Methods

Syringodium isoetifolium was obtained from the Gulf of Mannar. Live and healthy seagrass samples were collected during the low tide period. The samples were washed in seawater to remove epiphytes and extraneous matter. They were cleaned with fresh water to remove sand and salt to avoid the pumping of solvent during the extraction process. After draining off the water a known amount was taken for the preparation of the extract and the rest was pressed with blotting paper and shade dried at room temperature and used for dry extract preparation. The plant material was identified and authenticated.

Thin Layer Chromatography

The TLC profile of the ethanolic extract was prepared using the standard procedure explained by Talukdar *et al.* (2014).

Chromatography Analysis – GC-MS

GC ANALYSIS: The crude plant extract was subjected to centrifugation at about 10,000 rpm for about 30 minutes to remove the particulates. The clear supernatant was aspirated using a pipette and transferred into the clean vial and labelled. Then the supernatant was subjected to gas chromatography analysis using equipment THERMO GC-TRACE ULTRA version 5.0 and equipped with DB 35 Non-polar capillary column dimensions 30 mts, 0.25mm, film 0.25µm. The operation condition as follows:

Carrier gas: He flow: 1.0 ML/Min

Pressure: 17 psi for Cp ware column and 16 psi for the Cp5 column

Oven temperature: 70°C Raised to 260°C at 6°C/ min

Injection volume: 1 microliter

The peak area calculation was done by the star workstation and peak identification by comparison with authentic, wherever available. Calculation of the Kovats Retention Index was done. The Kovats Retention Index, (I) is defined and calculated by the following equation.

$$I = 100N + 100 n \log t' (N + n) - \log t' (N)$$

MS Analysis: Mass spectroscopy analysis was performed on a Shimadzu GC 17 AQP 5,000 MS coupled with a mass detector fitted with non-polar DBS (Di-phenyl di benzyl siloxane) capillary volume of length 25m 0.25 mm id GC MS operation conditions at initial temperature 60°C - 300°C. The injection volume was 0.1 µl with a helium gas carrier at the flow rate of 0.6 ml/minute. Relative Retention time (RRts) of constituents were determined using C5-C6 straight-chain alkanes as standards. Individual constituents of the extract were identified by WILEY and NIST database matching by comparison of mass spectra with published data and by comparison of their RRts

Antibacterial Activity (Agar Well Diffusion Method)

Procedure:

Petri plates containing 20 ml nutrient agar medium were seeded with 24 hr culture of bacterial strains were adjusted to 0.5 OD value according to McFarland standard, (*Pseudomonas aeruginosa*- 424 and *Propionibacterium acnes*-1951) Wells were cut and concentration of sample (500, 250, 100 and 50 µg/ml) was added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Gentamicin antibiotic was used as a positive control. The values were calculated using GraphPad Prism 6.0 software (USA).

Antifungal Activity (Agar Well Diffusion Method)

Procedure:

Petri plates containing 20 ml potato dextrose agar medium were seeded with 72 hr culture of fungal strain (*Candida albicans* and *Aspergillus flavus*). Wells were cut and different concentration of the sample was added. The plates were then incubated at 28°C for 72 hours. The anti-fungal activity was assayed by measuring the diameter of the inhibition zone formed around the wells. Amphotericin B was used as a positive control. The values were calculated using GraphPad Prism 6.0 software (USA)

Results and Discussion

The shade-dried and powdered materials of the selected plants were extracted with ethanol and was used for TLC profiling. After developing the TLC plates, the phytochemical profiling of the two selected plants is given in the Table 1. The number of phytochemicals and their retention factor (Rf) values of the plants are presented in the Table. TLC revealed the presence of phenols (Rf value of 0.75), steroids (Rf value of 0.52) and tannins (Rf value of 0.48) with ethanol extract.

Table 1: Retention Factor of Phytochemical Constituents by Thin Layer Chromatography

S.No	Rf Values		Compounds
	Standard	Test	
1.	0.73	0.8±0.1	Phenolic
2.	0.48	0.49±0.1	Tannins
3.	0.52	0.66±0.2	Steroids

GC-MS analysis

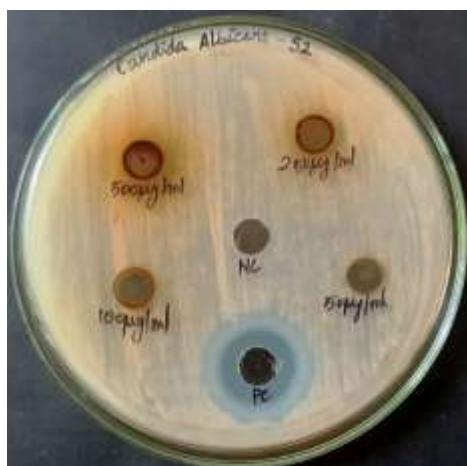
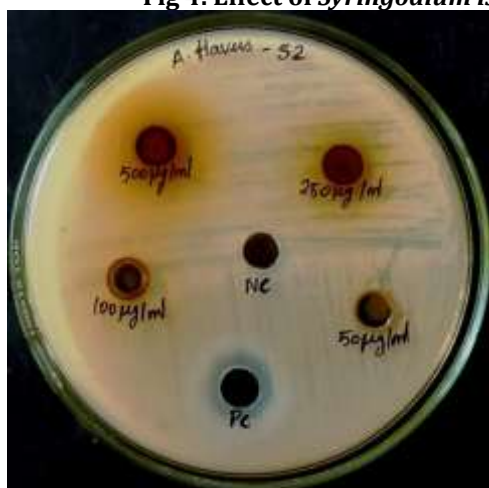
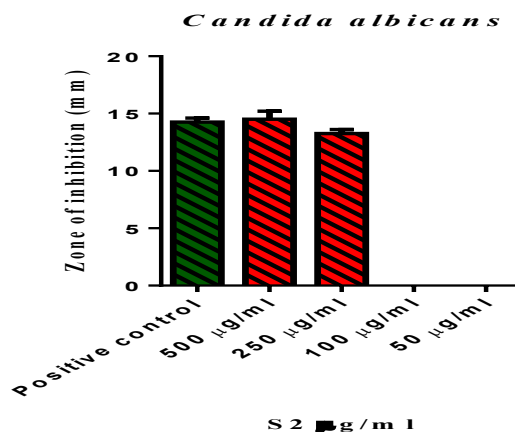
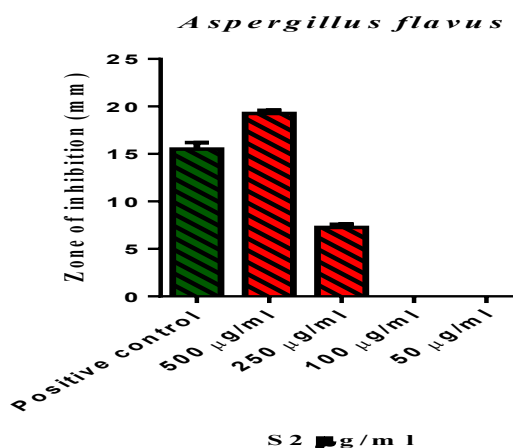
The ethanolic extracts of *Syringodium isoetifolium* were analyzed and a total number of 40 peaks were identified, which included phyto components, fatty acids, phenolic compounds and phytosterols. The composition of bioactive compounds in the GC-MS chromatogram of these extracts was analyzed and it was found that they contained a wide range of compounds, correlating the presence of certain sterols, phenolics, fatty acids and terpenes. GC-MS technique provides the identification and quantification of chemical compounds based on their characteristic fragmentation patterns at specific retention times.

Table 2: SD± Means of zone of inhibition obtained by *Syringodium isoetifolium* against *Pseudomonas aeruginosa* and *Propionibacterium acnes*.

S. No	Name of the test organism	Zone of inhibition (mm) SD ± Mean				
		500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	<i>Pseudomonas aeruginosa</i>	15.5±0.7	13.5±0.7	10.5±0.7	5.25±0.35	16.5±0.7
2.	<i>Propionibacterium acnes</i>	17.5±0.7	12.5±0.7	11.5±0.7	5.5±0.7	17.5±0.7

SD – Standard Deviation, *Significance - $p < 0.05$ **Antifungal activity:**

The effect of the selected sample extracts against *Candida albicans* (1 µg/ml) and *Aspergillus flavus* (1 µg/ml) were tested and the zone of inhibition (mm) were presented with different concentrations and Amphotericin B was used as a positive control. The results obtained showed that the zone of inhibition was increased with increase in extract concentration. The extracts of *Syringodium isoetifolium* showed maximum effect against *Candida albicans* (1.0 µg/ml), at 500 µg/ml when compared to the positive control and similar effect was observed against *Aspergillus flavus*. High inhibition was observed only in the concentration of 500 µg/ml which was next to the positive control and the other lower concentrations failed to show any significant inhibition. The Mean value of zone of inhibition obtained by *Syringodium isoetifolium* against *Candida albicans* and *Aspergillus flavus* ranges between 0-19.25±0.35 µg/ml

**Fig 4. Effect of *Syringodium isoetifolium* against *Candida albicans*.****Fig 4. Effect of *Syringodium isoetifolium* against *Aspergillus flavus*.****Table 3: SD± Means of zone of inhibition obtained by *Syringodium isoetifolium* against *Candida albicans* and *Aspergillus flavus*.**

S.NO	Name of the test organism	Zone of inhibition (mm) SD ± Mean				
		500 µg/ml	250 µg/ml	100 µg/ml	50 µg/ml	PC
1.	<i>Candida albicans</i>	14.5±0.7	13.25±0.35	0	0	14.25±0.35
2.	<i>Aspergillus flavus</i>	19.25±0.35	7.25±0.35	0	0	15.5±0.7

Summary and Conclusion

Syringodium isoetifolium has been used to study its potential as a medicinal plant. extract. TLC results revealed the presence of Phenols, steroids and tannins with the ethanol extract and the petroleum ether extract showed no bands. In Antibacterial studies, the Mean value of the zone of inhibition obtained by *Syringodium isoetifolium* against *Pseudomonas aeruginosa* and *Propionibacterium acnes* showed a good inhibition range. The Mean value of zone of inhibition obtained by *Syringodium isoetifolium* against *Candida albicans* and *Aspergillus flavus* also showed a good range of inhibition. The GC-MS analysis of the ethanolic extracts of the selected seagrasses detected 40 peaks in *Syringodium isoetifolium*, which included phytochemical components, fatty acids, phenolic compounds, and phytosterols. Fractionation of these components are the future and analysing their properties may be studied which would be useful in drug research.

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