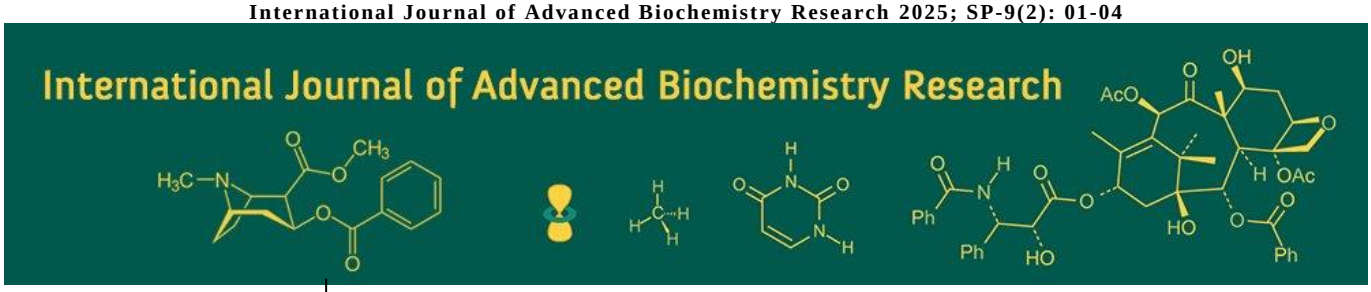

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Retention factor profiling and antibacterial potential of ethanolic extracts from selected *Sargassum* species: A short note

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Abstract
Thin Layer Chromatography (TLC) analysis of ethanolic extracts from *Sargassum polycystum*, *S. cristaefolium*, and *S. wightii* was performed using various solvent systems to determine retention factor (RF) values and zones of inhibition (ZOI) against *Pseudomonas aeruginosa*. Among the extracts, *S. polycystum* exhibited the highest antibacterial activity with a maximum ZOI of 22 mm at an RF value of 0.65, using a benzene: ethanol (3:7) solvent system. For *S. cristaefolium*, a maximum ZOI of 6 mm was observed at an RF value of 0.8 in ethanol: water (4:6). Likewise, *S. wightii* achieved a ZOI of 10 mm at an RF value of 0.69 with ethyl acetate: ethanol (3:7). Comparative solvent analysis showed that benzene: ethanol combinations consistently enhanced antibacterial activity for *S. polycystum*, with ZOIs ranging from 5 mm to 22 mm. In contrast, ethanol: water mixtures yielded modest inhibition for *S. cristaefolium* (6 mm) and *S. wightii* (5 mm). TLC revealed distinct RF values for the various solvent systems, with the benzene: hexane combination showing no inhibition for any species. These findings highlight *S. polycystum* as a promising candidate for bioactive compound isolation and underscore the influence of solvent systems on extract potency.

Keywords: Thin layer chromatography, ethanolic extracts, *Sargassum polycystum*, retention factor (RF), zones of inhibition (ZOI), *Pseudomonas aeruginosa*

Introduction
The genus *Sargassum*, a prominent member of the Phaeophyceae (brown algae), has garnered significant scientific interest due to its ecological importance and vast array of bioactive compounds (Silva *et al*., 2015, Duarte *et al*., 2022) [17, 4]. These seaweeds, predominantly found in temperate and tropical marine environments, play crucial roles in coastal ecosystems, providing habitat and nutrients to marine organisms. Beyond their ecological significance, *Sargassum* species are rich sources of secondary metabolites, such as polyphenols, phlorotannins, terpenoids, and polysaccharides, many of which exhibit antioxidant, antimicrobial, and anti-inflammatory properties (Calheiros *et al*., 2021) [3]. These bioactive compounds hold immense potential for applications in pharmaceuticals, nutraceuticals, and other industries (Santos *et al*., 2023) [12]. Thin Layer Chromatography (TLC) is a simple, cost-effective, and versatile analytical technique widely employed for the preliminary identification, separation, and characterization of chemical constituents in complex mixtures. In the study of *Sargassum*, TLC offers a rapid method for profiling secondary metabolites, enabling researchers to screen bioactive compounds efficiently. TLC not only facilitates the detection of specific compounds but also serves as a foundation for further detailed analysis using advanced chromatographic and spectroscopic methods (Gerhart, 1984, Prasad *et al*., 2010; Harvey, 2015; and Verma *et al*., 2022) [5, 9, 6, 19].
This study focuses on the application of TLC for the chemical analysis of *Sargassum* species, emphasizing its role in identifying bioactive compounds. By optimizing TLC methodologies, the research aims to enhance our understanding of the phytochemical diversity within *Sargassum* and provide insights into its potential industrial and biomedical applications.

Materials and Methods

Sample collection

Samples of three *Sargassum* species namely *S. cristaefolium*, *S. polycystum*, and *S. wightii* were collected from the Mandapam coast of Tamil Nadu, India. This region, located along the southeastern coast of the Indian peninsula, is characterized by its diverse marine biodiversity and favorable environmental conditions for seaweed growth. The collection was conducted during July 2021, ensuring optimal growth conditions to obtain healthy and mature thalli. The samples were carefully harvested by hand from shallow waters at depths ranging from "0.5 to 2 meters", ensuring minimal damage to the surrounding marine ecosystem. Each species was identified in the field based on morphological characteristics, following standard taxonomic keys, and further confirmed using reference literature. After collection, the samples were rinsed thoroughly with seawater on-site to remove any adhering sand, epiphytes, or debris. They were then transported to the laboratory in sterile, airtight containers to preserve their integrity. In the laboratory, the seaweed samples were further cleaned using distilled water, air-dried under shade at room temperature, and stored in airtight containers for subsequent analysis.

Phytochemical extraction

The phytochemicals from *S. cristaefolium*, *S. wightii*, and *S. polycystum* were extracted using a cold extraction method, where dried and powdered seaweed samples were macerated in ethanol (95%) at room temperature for 7 days, followed by filtration and concentration under reduced pressure.

Partial Thin-Layer Chromatography (Partial TLC)

Analytical TLC was carried out on TLC plates (GF 254, Merck). An aliquot of crude extract was spotted on to the silica gel plate and allowed to dry. The plates were developed with chloroform, diethyl ether, n-hexane, acetic acid (10:3:1:1, v/v/v/v) as the mobile phase in a previously saturated glass chamber with eluting solvents for 30 min at room temperature. The developed plate was dried under normal air and the spots were visualized under visible light. The retention factor values of isolated compounds and standard were calculated and compared.

Preparative TLC for purification

As the streak of crude extract was applied manually on a preparative TLC glass plate (20 cm × 20 cm; 1500 μm thickness) with inorganic fluorescent indicator binder (Analtech, Sigma-Aldrich). After air drying, the plate was developed in a saturated glass chamber. In each experiment, two plates were used in parallel. One plate from each set of experiment was sprayed with DPPH radical (for antioxidants) and TTC solution (for antimicrobials) and the bands showing antioxidant and antimicrobial activity were scraped off from the second plate of each set. The scratched samples were dissolved in HPLC grade methanol and centrifuged at 12000 g for 15 minutes. The supernatant was collected, filtered (0.22 μm filter) and dried under reduced pressure. All the dried samples were passed under nitrogen gas for 5min and then dissolved in methanol for further characterization and bioactivity analysis. Then the entire purification process was carried out under dark conditions.

Results

Thin Layer Chromatography (TLC) autography on ethanolic extract of the selected *Sargassum* species

Thin layer chromatography analyzed with different solvent systems showed maximum number of RF value in each ratio system with benzene and N-hexane extracts of *S. polycystum* with no zone of inhibition against *Pseudomonas aeruginosa* and in case of benzene and ethanol (3:7) solvent system the result showed that there was a maximum zone of inhibition (22mm) at the 0.65 RF value against the *P. aeruginosa*. In the case of *S. cristaefolium*, maximum zone of inhibition (6mm) was observed with the ethanol and water (4:6) solvent system at the range of 0.8 RF value against the *P. aeruginosa*. Meanwhile, *S. wightii* showed maximum zone of inhibition (10mm) against *P. aeruginosa* with the Ethyl acetate and ethanol solvent system at the ratio of 3:7 at 0.69 RF value (Table 1, 2 and 3).

Table 1: TLC autography on ethanolic extract of *S. polycystum*

Solvent system	Solvent ratio	*S. polycystum* (RF value)	Autography in ZOI in mm
			P. aeruginosa
Benzene: Hexane	6:4	0.55	-
		0.58	-
		0.45	-
		0.67	-
		0.74	-
		0.76	-
	4:6	0.14	-
		0.27	-
		0.32	-
		0.44	-
		0.98	-
	3:7	0.31	-
		0.39	-
		0.47	-
		0.56	-
		0.66	-
		0.93	-
		0.96	-
Ethyl acetate: ethanol	6:4	Mixed compound	14
	4:6	Mixed compound	15
	3:7	Mixed compound	10
Benzene: ethanol	6:4	0.51	17
		0.53	14
		0.82	15
		0.8	16
	4:6	0.18	5
		0.49	5
		0.50	5
	3:7	0.65	22
Ethanol: water	6:4	Mixed compound	10
	4:6	0.76	16
	3:7	Mixed compound	-

Table 2: TLC autography on ethanolic extract of *S. wightii*

Solvent system	Solvent ratio	*S. wightii* (RF value)	Autography in ZOI in mm
			P. aeruginosa
Benzene: Hexane	6:4	0.28	-
	4:6	0.2	-
		0.32	-
	3:7	0.38	-
		0.35	-
Ethyl acetate: ethanol		Mixed compound	5
		Mixed compound	-
		Mixed compound	-
Benzene: ethanol	6:4	0.24	-
	4:6	0.28	-
		0.91	-
		0.95	-
		0.33	-
		0.29	-
Ethanol: water	6:4	Mixed compound	5
	4:6	0.8	6
	3:7	Mixed compound	-

Table 3: TLC autography on ethanolic extract of *S. cristaefolium*

Solvent system	Solvent ratio	*S. cristaefolium* (RF value)	Autography in ZOI in mm
			P. aeruginosa
Benzene: Hexane	6:4	0.29	-
		0.39	-
		0.80	-
	4:6	0.26	-
		0.28	-
		0.32	-
		0.36	-
	3:7	0.27	-
		0.36	-
Ethyl acetate: ethanol	6:4	0.39	-
	4:6	0.65	-
	3:7	0.69	10
Benzene: ethanol	6:4	0.24	3
	4:6	0.28	-
		0.91	-
	3:7	0.75	5
Ethanol: water	6:4	Mixed compound	-
	4:6	Mixed compound	6
	3:7	Mixed compound	-

Fig 1: TLC Autography

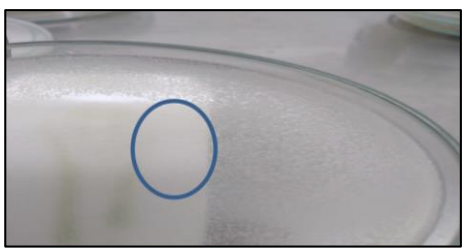

Fig 2: Zone of Inhibition

Discussion

In the present investigation, *S. polycystum* showed maximum number of phytoconstituent compound with the respect to benzene and N-hexane and it was observed that there was no zone of inhibition against *P. aeruginosa* but in case of benzene and ethanol solvent system maximum zone of inhibition was observed in comparison with *S. cristaefolium* and *S. wightii*. Premkumar *et al*. (2021) [10], Annegowda *et al*. (2013) [1] reported lipid soluble extracts from marine macroalgae as a source of substances with pharmacological properties. Sastry and Rao., (1994) [13] reported that antibacterial activity against the Gram positive and Gram negative pathogenic strains after successive extraction was found with methanol, benzene and chloroform. According to Sridhar and Vidyavathi (1991) [18], the antibacterial activity in the chloroform and acetone extract of *Falkenbergia hillebrandii* against the *S. aureus* was lowest. Madkour *et al*. (2019) [7] demonstrated that antibiotic activity from Xanthophyta was due to the presence of unsaturated fatty acids, phenolic compounds and organic acids.

Thin Layer Chromatography (TLC) has proven to be an effective tool for the preliminary screening and characterization of bioactive compounds in the ethanolic extracts of *Sargassum* species. The current study employed TLC autography to analyze the phytochemical profiles of *S. cristaefolium*, *S. wightii*, and *S. polycystum*, revealing the presence of various secondary metabolites such as phenolics, flavonoids, and terpenoids. The separation patterns and RF values obtained highlight the potential diversity of bioactive compounds within these species. The distinct banding patterns observed under UV light and after visualization with specific reagents (e.g., iodine vapor or anisaldehyde-sulfuric acid) indicate that the *Sargassum* species possess unique phytochemical profiles. These differences are likely influenced by species-specific biosynthetic pathways and environmental factors such as salinity, nutrient availability, and light exposure at the Mandapam coast. The presence of bioactive compounds, particularly those with antioxidant and antimicrobial properties, aligns with previous studies on *Sargassum* species, emphasizing their therapeutic potential. TLC autography also revealed zones of bioactivity, where specific bands inhibited microbial growth in subsequent bioautography assays. This confirms the functional properties of certain compounds in the extracts, further validating the ethnopharmacological importance of these seaweeds. However, the technique's limitations, such as the inability to definitively identify compounds without complementary methods like high-performance liquid chromatography (HPLC) or mass spectrometry (MS), should be acknowledged.

Petroleum ether extracts and unsaponified fractions of red and green seaweeds and methanol extracts, lipophilic fractions and unsaponified fractions of brown seaweeds separated on TLC were tested for their efficacy against *Xanthomonas oryzae* pv. *oryzae*. Two active zones from red seaweeds, one to five from brown seaweeds and two to three from green seaweeds were isolated through TLC profiles. Among eleven seaweeds *Gracilaria edulis*, *Sargassum wightii* and *Enteromorpha flexuosa* showed high antibacterial activity. The Rf 0.30 substance obtained from unsaponified fractions of *E. flexuosa* showed the maximum

antibacterial activity against the test bacterium (Arun and Rengasamy 2000; and Rajauria and Abu, 2013) [2, 11]

TLC-guided approach (analytical, preparative, and bio-autographic) was used to screen and purify the bioactive compounds from *H. elongata* seaweed. One active compound with potential antioxidant and antimicrobial properties was identified as fucoxanthin. *H. elongata* may therefore be considered as a potential source of functional ingredients (Rajauria and Abu, 2013; and Nunes *et al*., 2020) [11, 8]. The seaweed-associated bacteria *Caulerpa racemosa* and *Halimeda opuntia* have been proved to show antibacterial ability against bacteria that cause skin diseases, namely the pathogenic bacteria *Staphylococcus aureus*, *P. aeruginosa*, and *Micrococcus luteus*. The characterization of compounds in potential isolates identified the presence of groups of alkaloid, terpenoid, and flavonoid compounds in TLC identification (Setyati *et al*., 2024) [14].

Conclusion

These findings highlight *S. polycystum* as a promising candidate for further exploration of antimicrobial compounds and suggest that solvent selection is critical for maximizing bioactive compound extraction and activity. Future studies can build on these findings by employing advanced chromatographic and spectroscopic techniques to identify and quantify the bioactive constituents of these *Sargassum* species. Additionally, exploring their activity in various biological assays can provide insights into their potential applications in pharmaceuticals and nutraceuticals. This study underscores the value of TLC as a rapid, cost-effective method for preliminary phytochemical analysis, particularly for underexplored marine resources.

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