Molecular Analysis of Bioactive Compounds from Macroalgae *Sargassum ilicifolium*

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ABSTRACT

Macro algae metabolites are attracting the enormous attention, due to known for their pharmacological properties. In addition, seaweeds have different biological activities such as antibacterial, antioxidant, antitumor, antiviral and anti-inflammatory characteristics. Free radicals have been reported to play a role in affecting human health by causing many diseases. Thin layer Chromatography analysis of bioactive compounds, the solvent extracts from Sargassum ilicifolium have the RF value of 0.71 cm (pheophytin a), 0.88 cm in (carotene). Column chromatography reveals the Elution 1, 0.84 cm (carotene) Elution 2, 0.74 cm (pheophytin b) Elution 3, 0.49 cm (xanthophylls) Elution 4 respectively. Rf values of separated pigments were calculated and correlate to standard Rf values. The antimicrobial activity of Elution 1 and Elution 2 were used to envisage the antibacterial activity against Gram positive and negative bacteria viz., Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis and Staphylococcus. The Elution 2 and Elution 1 extract of Sargassum species showed highest potential activity against the selected human pathogens.

Keywords: *Brown algae, TLC, Column chromatography, Human pathogens*

1. Introduction

Macroalgae or commonly known as seaweed can be divided into three divisions which are green algae (Chlorophyta), red algae (Rhodophyta) and brown algae (Phaeophyta). This is due to the compounds that are responsible for the antimicrobial properties such as acrylic acid and diterpenoids in the green algae. Emergence of resistance pathogenic microorganisms to majority of antibiotics enhanced morbidity and mortality rate. Clinical and health problems that arise from antibiotic resistance and multi-resistant bacteria are becoming harder and eventually impossible to treat. Pharmaceutical company had done their best in overcoming this problem by producing better and enhanced drugs over the time. The usage of seaweeds as pharmaceutical products provides a more cost effective and proactive solution.

Macroalgae produce a diverse array of antimicrobial compounds, including terpinols, sterols, polysaccharides, dibutanolides, peptides, and protein metabolites. Antibacterial compounds have been identified in green, brown, and red algae. These marine algae are known for generating a wide range of bioactive secondary metabolites with antimicrobial, antifeedant,

antihelminthic, and cytotoxic properties [1]. These bioactive substances encompass alkaloids, polyketides, cyclic peptides, polysaccharides, phlorotannins, diterpenoids, sterols, quinones, lipids, and glycerols. Seaweeds, in particular, are recognized as a rich source of bioactive compounds due to their ability to produce a diverse range of secondary metabolites with broad biological activities.

Brown algae are widely utilized in various fields, including food, feed, pharmaceuticals, and industry. Chemical analysis of *Sargassum ilicifolium* has revealed the presence of terpenoid, acetogenic, and aromatic compounds, which exhibit a wide range of biological activities, such as antimicrobial, anti-inflammatory, and antiviral effects. The objectives of this research were to evaluate the impact of extraction solvent and time on the antibacterial activity of brown algae, as well as to explore its bioactive compounds.

Chromatographic techniques play a role in natural products chemistry and have significantly contributed to the discovery of novel and innovative compounds of pharmaceutical and biomedical importance, as studied by [2]. This study focused on a step-by-step visual demonstration of the fractionation and isolation of biologically active plant secondary metabolites using column chromatography techniques.

The present analysis deals with different crude extraction of *Sargassum ilicifolium* screening using TLC, Column chromatography and antimicrobial activity, indicates that the presence of active constituents in the seaweed, which can be exploited for the production of innovation drugs for the benefits of the humanity.

2. Materials and Methods

2.1. Sampling and Collection site

The seaweed collected from open field of sea by diving and hand-picking from the rocky substratum at depth of 2.7 m along the subtidal areas at Kurumbanai, Kanyakumari (district) during the month of December (2021).

2.2 Preparation of Algal Powder

The collected algae were shade dried and pulverized to powder in a mixer grinder. About 200 grams of the algal powder were weighed, transferred to flask and continuously extracted with methanol in Soxhlet apparatus for 24 hours. After one day of extraction, the crude mass was taken and filtered in Whatman No.1 filter paper. The filtrate was then concentrated using rotor vac evaporator and the concentrated materials were weighed to get the crude material. The crude material was diluted and subjected to chromatographic techniques. The extracts contain all the non-polar, mid polar and high polar components.

2.4 Separation and identification of the compounds

2.4.1 Thin Layer Chromatography

Thin layer chromatography was used to calculate the Rf value of the active molecules present in the extracted samples. In TLC, the molecules in the mixture were separated on the basis of their differences in solubility and partition co efficient in a binary solvent system. Silica gel coated plates (Merck -10×6cm) and the developing solvent (n-hexane: ethyl acetate) in a ratio of 7:3 was used for TLC. Initially the chromatography sheets were pre-saturated with the solvent. 5µl of the sample was then carefully applied on the plates and the samples were allowed to dry. The loaded plates were then placed in a pre-saturated tank with caution such that the applied sample does not dip in the solvent system. The set up was left undisturbed and the solvent was allowed to move up till it reached 9cm.The plates were then removed from the tank and the spots were marked immediately. The RF values were noted in the TLC plates and calculated by the standard formula given below,

[Rf = Distance moved by the pigment / Distance moved by the solvent]

2.4.2 Isolation of active molecules using Column chromatography

Column chromatography is a method of separating the compounds according to their density. A glass column cleaned with acetone and initially packed with glass wool or cotton at the bottom end for separation and purification. Silica gel (230-400 mesh) mixed with the solvent n-hexane was poured immediately in to the column after continuous stirring without any breakage or bubbles. The column was left undisturbed for one day for proper binding of silica and later 5ml of the crude extract was loaded in the column. After the binding of extracts in the silica column, the eluting solvent n-hexane and ethyl acetate in the ratio 7:3 was added frequently for separation and purification of active molecules from crude extract. The fractions obtained were collected and stored in the bottles under 4-10 ℃ for further use.

2.5 Antimicrobial assay

2.5.1 Agar diffusion disc – Variant

The bacteria were sub cultured to Muller Hinton Agar for 24 h prior to use. One loop of each test organism was suspended in 5 ml Trypticase Nutrient Broth solution separately. Muller – Hinton Agar (MHA) was surface inoculated with the suspension of the respective organism. The disks impregnated with the crude extracts of the seaweeds were placed on the MHA medium with suitable apace and the plates were incubated at 32° C for 24 hours. Ampicillin was used as a positive and respective solvents were used as a negative control.

2.5.2 Agar diffusion well – Variant

The well diffusion assay was performed a sterilized Muller Hinton Agar medium was poured into sterilized petri dishes. Nutrient Broth containing 0.1 ml of 48 hours incubated cultures of the respective bacterial strains was spread separately on the agar medium. Wells were made using stainless steel sterilized cork borer aseptic conditions. Subsequently, 250 μl of crude extracts were loaded into corresponding wells. The standard antibiotic Ampicillin was used in order to compare the result. The plates were incubated for 24 hrs at 32° C and the diameter of the zone of complete inhibition of the bacteria was measured around the each well and reading were recorded in millimetres.

3. Results and Discussion

3.1 Active molecules from algal extract using Colum Chromatography

A 5-gram crude extract of *Sargassum ilicifolium* was loaded onto a column packed with 30 grams of silica gel (60-120 mesh) and with a diameter of 2.4 cm. The column was developed with a gradient of increasing solvent polarity, starting from hexane and progressing to ethyl acetate. Five fractions were collected from the extract, each corresponding to different polarity levels. The height of the column bed was 20 cm.

3.2 Bioactive compounds by TLC

Thin layer chromatography of different solvent elution extracts reveals various bioactive compounds. *Sargassum ilicifolium* have the RF value of 0. 71cm (pheophytin) in algae extract, 0.88 cm in (carotene) Elution 1,0.84 cm (carotene) Elution 2, 0. 74 cm (pheophytin b) Elution 3, 0.49 cm (xanthophylls) Elution 4 respectively. Thin layer Chromatography (TLC) is the most popular and widely used separation techniques because of its ease of used, cost effectiveness, high sensitivity, peed of separation as well as its capacity to analysis simultaneously the result is so suggested with [3- 6], the extract of *S. ilicifolium* has some bioactive compounds and pigments such as Pheophytin a, Pheophytin b, Carotene and Xanthophylls.

3.3 Antimicrobial Assay

3.3.1 Agar Diffusion Disc Variant

Four elutions from *Sargassum ilicifolium* are tested for antibacterial activity against four pathogens. The inhibition zone is seen around sterile disc impregnated with organic solvent extracts of *Sargassum* sps. Ampicillin used as positive control, and the pure organic solvent was used as a negative control.

Brown algae extract have stronger activities against gram positive bacteria (*Bacillus subtilis*). than gram negative bacteria (*Escherichia coli* and *Pseudomonas*). The elutions of

Sargassum ilicifolium exhibited promising inhibition effect against *Bacillus subtilis* (0.8), *E. Coli* (1.5) mm, *Streptococcus* (0.9), *Pseudomonas* (0.8) mm, *Enterobacter* (0.8) mm, *Staphylococcus* (0.8) mm.

The Elution 1 of *Sargassum* sps exhibited promising inhibition effect against *Bacillus subtilis* (0.9), *E. coli* (0.8), *Streptococcus* (0.8), *Pseudomonas* (1.1), *Enterobacter* (0.8), *Staphylococcus* (0.8). The Elution 2 of *Sargassum* sps exhibited promising inhibition effect against *Bacillus subtilis* (0.8), *E. Coli* (0.7), *Streptococcus* (0.8), *Pseudomonas* (0.9), *Enterobacter* (0.8), *Staphylococcus* (0.8).

The Elution 3 of *Sargassum* sps exhibited promising inhibition effect against *Bacillus subtilis* (0.8), E. Coli (0.9), *Streptococcus* (0.8), *Pseudomonas* (0.8), *Enterobacter* (0.8), *Staphylococcus* (0.9). The Elution 4 of *Sargassum* sps exhibited promising inhibition effect against *Bacillus subtilis* (1.2), *E. coli* (1.1), *Streptococcus* (0.9), *Pseudomonas* (1.1), *Enterobacter* (1.1), *Staphylococcus* (1.4) was recorded.

The Brown algae extract of *Sargassum ilicifolium* exhibited promising inhibition effect against *Bacillus* (0.8), *E. coli* (0.8), *Streptococcus* (0.8), *Enterobacter* (0.8), *Pseudomonas* (0.8) and *Staphylococcus* (0.8) was recorded.

3.3.2 Agar Diffusion Well Variant

Different elution of *Sargassum* sps tested against four pathogens bacteria by diffusion well variant method. Ampicillin used as a positive control and the pure organic solvent was used as a negative control. The study clearly showed that the Elution was effect in inhibiting the growth of the bacteria pathogens. The Elution showed the highest zone of Inhibition was 2.1 cm for *E. coli* and *Staphylococcus*. Elution 1 of *Sargassum* sps showed the highest Inhibition activity Enterobacter (1.6) > *Staphylococcus* (1.2) > *E. coli* (1.1) > *Bacillus subtilis* (1.0) >*Pseudomonas* (0.9). Elution 3 of *Sargassum ilicifolium* showed the higher Inhibition activity *Staphylococcus* (1.4) > *Enterobacter* (1.2) > *E. coli* and *Bacillus subtilis* (1.0) >*Pseudomonas* (0.9).

Elution 5 of *Sargassum* sps showed the higher zone of Inhibition activity *E. coli* and *Staphylococcus* (2.1) >*Enterobacte*r and *Pseudomonas* (2) > *Bacillus subtilis* (1.4) was recorded. The Brown algae extract of *Sargassum* sps exhibited promising inhibition effect against *Bacillus* (1.0), *E. coli* (2.0), *Streptococcus* (1.4), *Enterobacter* (1.2), *Pseudomonas* (1.0) and *Staphylococcus* (1.2).

In this study, the Chloroform extracts of *S. ilicifolium* were found more active, when compare with Petroleum ether, Ethanol, Ethyl acetate and aqueous extracts that confirms the previous finding of [3-5]. The crude extracts of tested *S. ilicifolium* showed a significant

antimicrobial activity against Gram positive and Gram negative. Antimicrobial activity of brown seaweed *Sargassum ilicifolium* showed significant activity against gram positive and Gram-negative bacteria, which supports the present investigation by [7-9].

4. Conclusion

Marine algae are crucial components of marine ecosystems and serve as valuable sources of bioactive metabolites with diverse antimicrobial properties. This study confirmed that *Sargassum ilicifolium* exhibits the highest activity against both Gram-positive and Gram-negative bacteria. The bioactive compounds in these algae may bind to microbial cell walls, inhibiting their growth. Specifically, the extracts from Elution 2 and Elution 1 of *Sargassum ilicifolium* demonstrated the strongest potential activity against human pathogens.

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