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ORIGINAL RESEARCH ARTICLE

Antibacterial Activity of Some Selected Algae Present in the Costal Lines of Jaffna Peninsula

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ABSTRACT

In this study, *Sargassum polycystum*, *Sargassum tenerrimum, Turbinaria ornata*, *Gracilaria crassa* and *Codium fragile* were tested for their phytochemical constituents and antibacterial activity. The algae were collected from different coastal sites in Jaffna peninsula. The fine algal powders were directly extracted with ethanol and sequentially extracted with acetone and ethanol. The resulting extracts were subjected with qualitative phytochemical analysis and for their antibacterial activity against *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (NCTC 6571) by agar well diffusion method. The one way analysis of variance (ANOVA) followed by Tukey test was used for statistical analysis. Phytochemical analysis of extracts revealed the presence of at least two of the phytochemicals among the tested alkaloids, saponins, flavonoids, tannins and cardiac glycosides. The antibacterial study demonstrated that except acetone extract of *S. tenerrimum*, all other tested extracts were able to inhibit the growth of both *S. aureus* and *E. coli*. The sequential extracted ethanol extracts and direct ethanol extracts exhibited significantly ($P < 0.05$) higher inhibition on *S. aureus* compared to acetone extracts of respective algae. On the other hand, acetone extracts showed better activity on *E. coli* compared to the effect expressed on *S. aureus*. However, the highest inhibitory effect on *E. coli* and *S. aureus* was produced by the sequentially extracted ethanol extract of *C. fragile* and *T. ornata* respectively. The present study concluded that macro algae collected from different coastal lines of Jaffna peninsula are potential sources of bioactive compounds and can be used as source for antibacterial agent.

Key words: Antibacterial activity, macro algae, phytochemicals, *Escherichia coli,Staphylococcus aureus*

INTRODUCTION

Marine algae are an important resource in marine ecosystem and also it is useful to human in various ways. They supply oxygen to the biosphere, are a source of food for fishes, cattle and man. Algae are also used as medicine and fertilizers. Chemically the bioactive metabolites of marine flora include brominated phenols, oxygen heterocyclics, nitrogen heterocyclics, sulphur nitrogen heterocyclics, sterols, terpenoids, polysaccharides, peptides and proteins [1].

There are several studies have been undertaken to reveal the medicinal value of marine algae in different part of the world. Antitumorals, [2,3] anticoagulant,[4,5] antifouling,[6] antioxidant [7] and antimicrobial activities [8-10] have been studied on various types of algae.

Sri Lanka is an island located in the Bay of Bengal, in the northern Indian Ocean. The northern coastline has huge diversity of algae, and there few studies have been undertaken to elucidate the chemical constituents of these resources. The iodine, boron, and sterol content of various algae were studied during the period before civil war.[11-13] At present there are scanty literature available on the antibacterial activity of marine algae present in northern part of Sri Lanka. Therefore, the present study was undertaken in order to examine the phytochemical constituents and antibacterial activity of different organic solvent extracts of five different marine macro algae, harvested from different coastal areas of Northern part of Sri Lanka.

MATERIALS AND METHODS

Collection of marine algae

The algae were harvested from three different sites along the costal line of Northern Sri Lanka. *Sargassum polycystum* was collected from Point Pedro and Casuarina beach in Karainagar,

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Sargassum tenerrimum, Gracilaria crassa and *Turbinaria ornata* were collected from Casuarina beach in Karainagar and the *Codium fragile* was collected from Nachchikuda.

Preparation of algal extracts

The fresh algae were washed under running tap water to remove associated debris, and dried in shade. Completely dried algae were ground into fine powder using electric blender. The resulting powders were extracted in two different ways as described below.

a) Preparation of direct ethanol extracts

20 g powder of each alga was separately soaked in 60 ml of ethanol in airtight conical flask for two days on an orbital shaker, and then they were first filtered through doubled layered Muslin cloth followed by Whatman No 1 filter paper. The filtrates were collected into airtight bottles. Similar process was repeated twice with fresh ethanol and the filtrates were pooled together. Finally ethanol was removed from the filtrates at 40^{o}C temperature by keeping in an oven. The resulting extracts were then stored in refrigerator until use for further study [14].

b) Sequential extraction by acetone and ethanol

20 g powder of each alga was initially soaked in 60 ml of acetone in airtight conical flask for two days on an orbital shaker and then it was first filtered through doubled layered Muslin cloth and then through Whatman No 1 filter paper. The filtrates were collected into airtight bottles. Similar process was repeated twice with fresh acetone and the filtrates were pooled together. Finally acetone was removed from the filtrates at 40^{o}C temperature by keeping in an oven and the dried extracts were stored in refrigerator. The residue of the algal powder was dried and used further for ethanol extraction as similar to the procedures that carried out for the acetone extraction [14].

Phytochemical analysis

The extracts were subjected to phytochemical tests for presence of following biomolecules by using the standard qualitative procedures as described in literature [15].

a) Test for Glycosides
   10 ml of 50% H_2SO_4 was added to the 1 ml of extract in a boiling tube. The mixture was heated in boiling water bath for 5 min. 10 ml of Fehling's solution (5 ml of each solution A and B) was added and boiled. A brick red precipitate indicated the presence of glycosides.

b) Test for Alkaloids
   1 ml of 1% HCl was added to the 3 ml of extract in a test tube and was treated with few drops of Meyer's reagent. A creamy white precipitate indicated the presence of alkaloids.

C) Test for saponins
   5 ml of extract was shaken vigorously to obtain a stable persistent froth. The frothing was then mixed with 3 drops of olive oil and observed for the formation of emulsion, which indicated the presence of saponins.

d) Test for flavonoids
   A few drops of 1% NH_3 solution was added to the 2 ml of extract in a test tube. A yellow coloration was observed for the presence of flavonoids.

e) Test for tannins
   To 0.5 ml of extract solution, 1 ml of distilled water and 1-2 drops of ferric chloride solution were added and observed for brownish green or a blue black coloration.

f) Test for terpenoids
   5 ml of extract was mixed with 2 ml of $CHCl_3$ in a test tube. 3 ml of concentrated H_2SO_4 was carefully added along the wall of the test tube to form a layer. An interface with a reddish brown coloration was confirmed the presence of terpenoids.

g) Test for cardiac glycosides
   5 ml of extract was mixed with 2 ml of glacial acetic acid containing 1 drop of $FeCl_3$.The above mixture was carefully added to the 1 ml of concentrated H_2SO_4. Presence of cardiac glycosides was detected by the formation of a brown ring.

h) Test for phlobatannins
   10 ml of extract was boiled with 1% HCl in a boiling tube. Deposition of a red precipitate indicated the presence of phlobatannins.

i) Test for Anthraquinones
   Extract was mixed well with benzene, and then half of its own volume of 10% ammonia solution was added. Presence of a pink, red or violet coloration in the ammonial phase indicated the anthraquinones.

Test bacteria

Escherichia coli (ATCC 25922) and *Staphylococcus aureus* (NCTC 6571) were obtained from Department of Microbiology, Faculty of Medicine, University of Jaffna, Sri Lanka, and were cultured on nutrient agar medium at 37 ºC for 24 hours prior to the assay.

Determination of antibacterial activity

20 ml molten nutrient agar media were mixed with 1 ml of (0.5 Mac Farland) each test bacteria, and poured in sterile Petri dishes separately. After complete solidification, 8 mm diameter wells were made using sterile cork borer. The wells were

filled with 100 µl of (30 mg) acetone and (30 mg) ethanol extracts separately. Streptomycin (30 µg /100 µl) and 100 µl of acetone and ethanol were used as standard and control respectively. Then the plates were incubated at 37 ºC for 24 hours, and the antibacterial activity was determined by measuring the diameter of clear zone around the well. Each experiment was repeated thrice [14].

Statistical analysis

The results for the antibacterial activity was compared by using analysis of variance and Tukey test at P = 0.05 using statistical software SPSS Windows version 13.0.

RESULTS AND DISCUSSION

In this study, the qualitative test for the presence of phytochemicals revealed the presence of various types of phytochemicals in sequentially extracted acetone and ethanol extracts and direct ethanol extracts of tested algae. At least two of the tested phytochemicals were found in the extracts out of nine tested phytochemicals (Table 1, 2 & 3).

In the case of sequential extraction, there were slight variations in the results obtained for acetone and ethanol extracts except in *S. polycystum* (Table 1&2). Samples collected for *S. polycystum* from Point Pedro showed presence of saponins, flavonoids, tannins and cardiac glycosides and the sample collected from Casuarina showed positive results to alkaloids and tannins in both acetone and ethanol extracts (Table 1&2). According to the results obtained for *S. polycystum,* collected from Point Pedro and Casuarina it can be clearly seen the variation of chemical composition of plants with geographic differences.

Among the acetone extracts, *T. ornata* expressed positive results for five tested phytochemicals, and it was followed by *S. polycystum* (Point Pedro), *G. crassa* and *S. tenerrimum* (**Table 1**).

Among the ethanol extracts obtained by sequential extraction, *S. tenerrimum*, *S. polycystum* (Point Pedro), *G. crassa* and *T. ornata* extracts revealed the presence saponins, flavonoids, tannins and cardiac glycosides. The tannin was present in all tested ethanol extracts, and the saponins and flavonoids were also present in all ethanol extracts except *S. polycystum* (Casuarina) (**Table 2**).

The present study also showed the chemical constituents of the direct ethanol extracts of algae, where saponins, flavonoids and cardiac glycosides were present in five samples out of six tested samples (**Table 3**). Even though, the chemical constituents of both direct ethanol extract and sequential ethanol extracts of *S. polycystum* (Point Pedro) and *G. crassa* were similar between each other, all other direct ethanol and sequential ethanol extracts of algae had different chemical profile (Table 2 &3). The variation in the result might be the influence of mode of extraction on available phytochemicals.

The present study showed that except acetone extract of *S. tenerrimum*, all other tested extracts were able to inhibit the growth of both *S. aureus* and *E. coli*. *S. tenerrimum* was able to inhibit only the growth of *E. coli* (**Table 4&5**).

In sequential extraction, when compare the effect of acetone and ethanol extracts, acetone extracts showed better activity on *E. coli*, while ethanol extract had more inhibition on *S. aureus*. However, as an exception ethanol extract of *C. fragile* revealed higher activity on *E. coli* (Table 4). The ethanol extracts exhibited significantly ($P < 0.05$) highest inhibition on *S. aureus* compared to acetone extracts, where maximum zone of inhibition was produced by *T. ornata.* However, there was no significant difference in the inhibitory effect among *C. fragile*, *S. polycystum* (Casuarina), *S. tenerrimum* and *G. crassa*. The lower effect was produced by *S. polycystum* (Point Pedro). On the other hand, there was no significant difference in inhibitory effect of acetone extracts of all tested algae, except *S. tenerrimum* against *S. aureus*. Same tendency was expressed against *E. coli*, where except *C. fragile* all other extracts had similar effect on test bacteria.

Except *C. fragile* all other algal direct ethanol extracts had more activity against *S. aureus* than *E. coli.* The highest inhibition was produced against *E. coli* by *G. crassa*, it was followed by *T. ornata* and *S. tenerrimum*. On the other hand, *S. aureus* was highly inhibited by *T. ornate*, and followed by *G. crassa* and *S. tenerrimum*.

There were several differences between the results obtained for direct ethanol extracts and sequential ethanol extracts. This may be due to the variation of chemical constituents in both extracts. In sequential extraction non polar compounds might be separated in to the acetone extract, therefore, antagonistic and synergistic effects of chemicals might be lower in sequential ethanol extract. But, it might be comparatively higher in direct ethanol extract [16].

The present study concluded that macro algae collected from different coastal lines of Jaffna peninsula are potential sources of bioactive compounds and can be used as source for antibacterial agent. However, further works should be performed for the isolation and characterization of the active compounds.

Table 1: Phytochemicals present in the acetone extracts

Pyhtochemicals	*C. fragile*	*S. polycystum* (Casuarina)	*S. tenerrimum*	*S. polycystum* (Point Pedro)	*G. crassa*	*T. ornata*
Glycosides	-	-	-	-	-	-
Alkaloids	+	+	-	-	+	+
Saponins	-	-	-	+	-	+
Flavonoids	+	-	+	+	+	+
Tannins	-	+	+	+	-	+
Terpenoids	-	-	-	-	-	-
Cardiac glycosides	-	-	+	+	+	+
Phlobatannins	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-

(+) – Presence, (-) – Absence

Table 2: Phytochemicals present in the sequentially extracted ethanol extracts

Pyhtochemicals	*C. fragile*	*S. polycystum* (Casuarina)	*S. tenerrimum*	*S. polycystum* (Point Pedro)	*G. crassa*	*T. ornata*
Glycosides	-	-	-	-	-	-
Alkaloids	-	+	-	-	-	-
Saponins	+	-	+	+	+	+
Flavonoids	+	-	+	+	+	+
Tannins	+	+	+	+	+	+
Terpenoids	-	-	-	-	-	-
Cardiac glycosides	-	-	+	+	+	+
Phlobatannins	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-

(+) – Presence, (-) – Absence

Table 3: Phytochemicals present in the direct ethanol extracts

Pyhtochemicals	*C.fragile*	*S. polycytum* (Casuarina)	*S. tenerrimum*	*S. polycystum* (Point Pedro)	*G. crassa*	*T. ornata*
Glycosides	-	-	-	-	-	-
Alkaloids	-	-	-	-	-	-
Saponins	+	+	-	+	+	+
Flavonoids	+	+	+	+	+	-
Tannins	-	+	-	+	+	+
Terpenoids	-	-	-	-	-	+
Cardiac glycosides	-	+	+	+	+	+
Phlobatannins	-	-	-	-	-	-
Anthraquinones	-	-	-	-	-	-

(+) – Presence, (-) – Absence

Table 4: Antibacterial activity of sequentially extracted algae

Solvent	Extracts	Diameter of inhibition zone (mm)* *E. coli*	*S. aureus*
Acetone	*C. fragile*	10.3^{de}	10.2^{d}
	S. polycystum (Casuarina)	11.2^{bc}	10.0^{d}
	S. tenerrimum	11.3^{bc}	--
	S. polycystum (Point Pedro)	11.2^{bc}	10.0^{d}
	G. crassa	11.8^{ab}	10.0^{d}
	T. ornata	11.2^{bc}	10.0^{d}
Ethanol	*C. fragile*	12.2^{a}	11.7^{b}
	S. polycystum (Casuarina)	9.8^{e}	11.5^{b}
	S. tenerrimum	9^{f}	12.2^{b}
	S. polycystum (Point Pedro)	9^{f}	10.8^{c}
	G. crassa	10.7^{cd}	11.8^{b}
	T. ornata	11.2^{bc}	13.3^{a}
Control		-	-
Streptomycin		28.5	20.3

*(-) - No activity; * Zone of inhibition includes the diameter of well (8mm), Values with different superscript on the same column are significantly (P < 0.05) different.*

Table 5: Antibacterial activity of direct ethanol extracts of algae

Extracts	Diameter of inhibition zone (mm)* *E. coli*	*S. aureus*
C. fragile	10^{b}	9.2^{e}
S. polycystum (Casuarina)	10^{b}	10.8^{d}
S. tenerrimum	10.2^{ab}	11.7^{bc}
S. polycystum (Point Pedro)	9.8^{b}	11^{cd}
G. crassa	11.5^{a}	12.3^{ab}
T. ornata	10.8^{ab}	12.8^{a}

*(-) - No activity; * Zone of inhibition includes the diameter of well (8mm), Values with different superscript on the same column are significantly (P < 0.05) different.*

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