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## Antimicrobial properties of marine seaweed, *Sargassum muticum* against human pathogens

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### PEER REVIEW

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#### Comments

This is an important research work in which authors have demonstrated the antimicrobial activity of several extracts obtained from the seaweed *S. muticum* on human pathogens. *S. muticum* was found to be a promising natural source for developing antimicrobial drugs with diverse chemical structures and novel mechanism of action for new and reemerging human infectious diseases.

Details on Page 124

### ABSTRACT

**Objective:** To evaluate the antibacterial efficiency of the seaweed, *Sargassum muticum* (*S. muticum*) collected from Pudumadam, Ramanathapuram, Tamil Nadu, India.

**Methods:** Crude solvent extracts of *S. muticum* were obtained by using Soxhlet extraction and the solvents like acetone, methanol and chloroform. These different extracts were tested against different human bacterial pathogens such as *Micrococcus* sp., *Staphylococcus aureus* (methicillin resistance), *Salmonella paratyphi* B, *Staphylococcus epidermis* (3615), *Enterobacter aerogenus* (111), *Klebsiella pneumonia* (109), *Shigella fleschneri* (1457) (*S. fleschneri*), *Proteus vulgaris* (1771), *Staphylococcus aureus* (96) and *Salmonella typhimurium* (SP7) which were obtained from Microbial Type Culture Collection, Indian Institute of Microbial Technology, Chandigarh, India.

**Results:** The results revealed that acetone extract had unveiled the maximum of 11 mm zone of inhibition at 40 µL against *S. fleschneri*. Similar zone of inhibition (11 mm) was also observed at 50 µL against *Micrococcus* sp. and *S. fleschneri*. Followed by acetone extract, chloroform extract also contributed 11 mm zone of inhibition against *S. fleschneri* and *Salmonella paratyphi* B at 40 and 50 µL respectively. Besides, methanol extracts revealed meager antibacterial activity (9 mm).

**Conclusions:** The present investigation suggests that the phytochemical constituent of the *S. muticum* might be suitable agents for the control of human deadly diseases.

#### KEYWORDS

*Sargassum muticum*, Seaweed, Acetone extract, Chloroform extract, Methanol extract, Bacterial pathogens

## 1. Introduction

Seaweeds are macroscopic algae which attached to the bottom in relatively shallow coastal waters. They form one of the important living resources grouped under three divisions viz., Chlorophyceae, Phaeophyceae and Rhodophyceae. Seaweeds are considered as a potential source of bioactive compounds as they are able to produce a great variety of secondary metabolites characterized by a broad spectrum of biological activities including antibacterial activity[1].

They are excellent source of vitamins such as A, B<sub>1</sub>, B<sub>12</sub>, C, D and E, riboflavin, niacin, panthothanic acid, folic acid as well as minerals such as Ca, P, Na, K[2]. Among marine algae, brown algae have been reported to contain higher phlorotannin contents as marine phenolic compounds[3]. *Sargassum* species have been identified for such a purpose and are both very effective and easily available. Besides, the growing need for the development or discovery of highly potential bioactive compounds from natural sources is due to the resistance to chemical drugs which often leads to the reemergence of agents of air

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as well as water borne disease in a new phenotypic as well genotypic forms.

Recently, infections have become the leading cause of death worldwide one next to cancer which has led to an increase in antibacterial resistance, making it a global growing problem. Thus, there is an urgent need to discover new antimicrobial compounds from plants with diverse chemical structures and novel mechanism of action for new and reemerging infectious disease. The new therapeutic agents should be effective and have a novel mode of action that renders them impervious to existing resistance mechanism. Hence, curbing the genetically diverse pathogenic infectious diseases and searching the novel biologically active sources of seaweeds are considered now as a potential antimicrobial agent. Therefore, the present investigation aims to detect the bactericidal efficiency of the brown algae, *Sargassum muticum* (*S. muticum*).

## 2. Materials and methods

### 2.1. Preparation of aqueous seaweed extract

*S. muticum* was collected by hand picking method at a depth of 1–2 m in Gulf of Mannar, Pudumadam in Rameshwaram, Tamil Nadu, India. The surface of seaweed was washed with tap water and subsequently roughly with sterile distilled water and then they were identified, shade dried for 15 d and powdered using mixer grinder. Later, aqueous extract was prepared by dissolving 200 mg of powdered seaweed in 100 mL of sterile distilled water. The extract was subject to heat at 60 °C for 20 min and centrifuged at 8000 r/min. The decanted supernatant was used throughout the study.

### 2.2. Solvent extraction and antibacterial activity

About 10 g of powdered seaweed was soaked in 100 mL of solvents such as chloroform, acetone and methanol and Soxhlet extraction was performed to obtain the crude extract. Then they were centrifuged at 5000 r/min for 20 min at 4 °C and filter sterilized using 0.2 µm membrane syringe. The seaweed extract was subjected to phytochemical screening as described by Harborne[4]. Bacterial cultures such as *Micrococcus* sp., *Staphylococcus aureus* (*S. aureus*) (methicillin resistance), *Salmonella paratyphi* B (*S. paratyphi*), *Staphylococcus epidermis* (3615) (*S. epidermis*), *Enterobacter aerogenus* (111) (*E. aerogenus*), *Klebsiella pneumonia* (109) (*K. pneumonia*), *Shigella fleschneri* (1457) (*S. fleschneri*), *Proteus vulgaris* (*P. vulgaris*) (1771), *S. aureus* (96) and *Salmonella typhimurium* (SP7) (*S. typhimurium*) were obtained from Microbial Type Culture Collection, Indian Institute of Microbial Technology, Chandigarh, India. The bioassay was carried out using the agar disc diffusion method and for each bacterial species, three replications were maintained[4].

## 3. Results

In the present investigation, the marine brown algae, *S. muticum*, was collected from Pudumadam (9°30' N and 78°97' E) by hand picking and was identified at Madurai Kamaraj University's Marine Ecotoxicology Field Research Facility, Pudumadam, Ramanathapuram

District, Tamil Nadu, India. As for the solvent extracts, acetone and chloroform exhibited higher antibacterial activity against tested human pathogenic bacteria compared to methanol extract. Solvent extracts such as acetone and chloroform had inhibited the bacterial pathogens such as *Micrococcus* sp., *S. paratyphi* B, *Salmonella typhi*, *S. aureus* and *S. fleschneri* accordingly by 11 mm. The concentration exhibited higher zone of inhibition in acetone and chloroform extract was 30 and 50 µL/mL respectively. It has been observed that the tested solvent extracts were highly efficient against Gram-negative bacteria compared to Gram-positive bacteria (Tables 1, 2 and 3). The phytochemical analysis also revealed that the active acetone extracts were endowed with flavonoids, alkaloids, sterols, phytosterols, tannins and coumarins (Table 4).

**Table 1**

Zone of inhibition of acetone extract of *S. muticum* against human pathogenic bacteria.

Organism	Zone of inhibition (mm)					
	Control	10 µL	20 µL	30 µL	40 µL	50 µL
<i>Micrococcus</i> sp.	-	9	9	11	11	11
<i>S. aureus</i> **	-	7	8	8	9	10
<i>S. paratyphi</i> B	-	10	10	11	11	11
<i>S. epidermis</i>	-	6	6	6	6	8
<i>E. aerogenus</i>	-	8	8	8	9	10
<i>K. pneumonia</i>	-	-	-	-	-	-
<i>S. fleschneri</i>	-	7	9	11	11	11
<i>P. vulgaris</i>	-	10	10	10	-	-
<i>S. aureus</i>	-	9	10	10	10	11
<i>S. typhimurium</i>	-	8	8	10	10	11

\*\* : Methicillin resistance.

**Table 2**

Zone of inhibition of chloroform extract of *S. muticum* against human pathogenic bacteria.

Organism	Zone of inhibition (mm)					
	Control	10 µL	20 µL	30 µL	40 µL	50 µL
<i>Micrococcus</i> sp.	-	7	7	7	8	9
<i>S. aureus</i> **	-	7	8	9	9	9
<i>S. paratyphi</i> B	-	7	8	9	10	11
<i>S. epidermis</i>	-	6	7	8	9	10
<i>E. aerogenus</i>	-	8	8	8	9	10
<i>K. pneumonia</i>	-	8	8	8	9	10
<i>S. fleschneri</i>	-	7	8	9	10	11
<i>P. vulgaris</i>	-	-	-	8	9	10
<i>S. aureus</i>	-	-	-	-	-	8
<i>S. typhimurium</i>	-	8	8	9	9	9

\*\* : Methicillin resistance.

**Table 3**

Zone of inhibition of methanol extract of *S. muticum* against human pathogenic bacteria.

Organism	Zone of inhibition (mm)					
	Control	10 µL	20 µL	30 µL	40 µL	50 µL
<i>Micrococcus</i> sp.	-	6	6	7	7	9
<i>S. aureus</i> **	-	7	9	9	9	9
<i>S. paratyphi</i> B	-	-	-	-	-	-
<i>S. epidermis</i>	-	6	6	7	7	7
<i>E. aerogenus</i>	-	0	0	0	0	7
<i>K. pneumonia</i>	-	6	6	6	6	7
<i>S. fleschneri</i>	-	-	-	-	7	-
<i>P. vulgaris</i>	-	-	-	-	-	-
<i>S. aureus</i>	-	-	-	-	-	-
<i>S. typhimurium</i>	-	-	-	-	6	7

\*\* : Methicillin resistance.

**Table 4**

Phytochemical constituents of the methanol extract of the selected brown seaweed, *S. muticum*.

Phytochemical tests	<i>S. muticum</i>
Carbohydrate	+
Sterols, phytosterols, triterpenoid saponinins	+
Coumarins	+
Xanthoprotein	-
Aromatic acid	+
Essential oil	-
Tannins	+
Flavonoids	+
Alkaloids	+
Saponins	-
Reducing sugar	+

+: Present; -: Absent.

#### 4. Discussion

In the early, there are a number of reports evaluated the antibacterial activity of marine plants, marine algae or seaweeds[5-7]. In India, meager information was available on the antibacterial activity of marine algae. In the present investigation, acetone and chloroform extracts (11 mm zone of inhibition) of the *S. muticum* exhibited higher antibacterial activity compared to the other solvent extracts. Similar to the present investigation, Rosaline *et al.* reported that the acetone extract of *Sargassum wightii* showed antibacterial activity against several Gram-positive and Gram-negative bacteria[8]. Bioactive compounds of seaweeds have shown antimicrobial action against a number of Gram-positive and negative bacteria[9]. Vijayabaskar and Shiyamala reported the antimicrobial capacity of *Sargassum wightii* and *Turbinaria ornata* against *S. aureus*, *Aeromonas hydrophila*, *Escherichia coli* (*E. coli*), *Enterococcus faecalis*, *K. pneumonia*, *P. vulgaris*, *S. flexneri* and *Pseudomonas aeruginosa*[10]. Phlorotannins extracted from *Eisenia bicyclis* have been reported to have antibacterial activities against Gram-positive and negative bacteria, suggesting a potential use as natural preservatives in the food industry or as antibacterial drugs[11]. The extract of the brown alga *Taonia atomaria* has exhibited antifungal activity, due to the compound taondiol[12]. Rhimou *et al.* studied the antibacterial activity of extracts from 26 species of red algae against three Gram-positive and two Gram-negative bacteria[13]. Amongst all the seaweeds analyzed, 96% of the extracts were active against at least one of the five tested microorganisms with *S. aureus* being the most susceptible microorganism. An extract of *Hypnea musciformis* exhibited the highest antibacterial activity against all the bacteria tested[13]. Besides, they also found maximum activity against methicillin-resistance *S. aureus*. In addition, the acetone extracts and chloroform extracts also unveiled good antibacterial activity against tested bacterial human pathogens. It was well supported by Jebasingh *et al.* who reported potent bactericidal activity of chloroform extract of the seaweeds[14]. In the current study, only methanol extract of *S. muticum* showed little or no activity against tested human bacterial pathogens. It was in accordance with the report by Rosaline *et al.*[8]. In contrast to the present study, Alghazeer *et al.* have found broad array of antibacterial activity exhibited by green, red and brown seaweeds[15]. Lee *et al.* isolated an antibacterial compound, polyhydroxylated fucophlorethol[11]. Gupta *et al.* also reported 100% inhibition of common food spoilage and food pathogenic bacteria

using extracts from brown seaweed, *Heliophora elongata*, *Laminaria saccharina* and *Laminaria digitata*[9]. Tetracyclic brominated diterpenes isolated from the organic extract of *Sphaerococcus coronopifolius* and collected from the rocky coasts of Corfu Island showed minimum inhibitory concentrations between 16 and 128 µg/mL against a range of bacteria including multidrug-resistant and methicillin-resistant *S. aureus*[16]. Five bromophenols were isolated from the methanolic extract of the marine alga, *Rhodomela confervoides*. The compound bis (2,3-dibromo-4,5-dihydroxybenzyl) ether was the most active against several strains of *Staphylococcus coli*, *Pseudomonas coli* and *E. coli* with minimal inhibitory concentration being less than 70 µg/mL[17]. Two bromoditerpenes, 12 S-hydroxybromospha-erodiol and bromosphaerone, isolated from red seaweed and *Sphaerococcus coronopifolius* showed an antibacterial activity against *S. aureus* with a minimal inhibitory concentration of 0.104 and 0.146 µmol/L, respectively[18]. Wang *et al.* reported that phlorotannin from *Ascophyllum nodosum* was bacteriostatic at concentrations of 25 µg/mL or higher and was bactericidal above 50 µg/mL against *E. coli*[19]. Thus, the present investigation unveiled the highly potent antimicrobial substance containing brown seaweed, *S. muticum*.

The antimicrobial substance is considered as the inhabitants of seaweeds. The *S. muticum* used in the present investigation derailed the tested human pathogenic bacteria including methicillin-resistance *S. aureus*. Brown seaweeds including *S. muticum* are source of phenolic compounds that have exhibited its potent antibacterial activity. It could be further evaluated for its medicinal applications for the welfare of human beings.

#### Conflict of interest statement

We declare that we have no conflict of interest.

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#### Comments

##### Background

Infections have become one of the leading causes of death worldwide and antibacterial resistance has emerged as a global growing problem. Therefore, there is an urgent need to develop new antimicrobial compounds. Seaweeds are considered as potential sources of novel biologically active antimicrobial agents with promising mechanisms of action.

##### Research frontiers

The present research depicts the antibacterial properties of crude extracts of *S. muticum* prepared using different solvents. The extracts were tested *in vitro* against distinct human bacterial pathogens. The

phytochemical constituents of *S. muticum* were also determined.

### Related reports

Earlier reports documented the antibacterial activity of marine plants, marine algae or seaweeds. Specially, seaweeds are considered as a potential source of bioactive compounds, including a great variety of secondary metabolites with a broad spectrum of biological activities such as antibacterial activity.

### Innovations and breakthroughs

The authors open new insights into the knowledge of metabolites contained on the antibacterial activity of the brown seaweed *S. muticum*, including the effects on antibiotic resistant strains.

### Applications

This scientific study suggests the use of *S. muticum* extracts as antimicrobial agents. Further evaluations will support its medicinal applications for the welfare of human beings.

### Peer review

This is an important research work in which authors have demonstrated the antimicrobial activity of several extracts obtained from the seaweed *S. muticum* on human pathogens. *S. muticum* was found to be a promising natural source for developing antimicrobial drugs with diverse chemical structures and novel mechanism of action for new and reemerging human infectious diseases.

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