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Antibacterial activity of selected marine macro algae against vancomycin resistant *Enterococcus faecalis*

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PEER REVIEW

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Comments

The present investigation paves a way for the advanced research regarding the antimicrobial activity of seaweeds, stimulating the young researcher and academician in phytochemical screening and bio-effficacy studies.
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ABSTRACT

Objective: To evaluate the antibacterial activity of different extracts of *Caulerpa chemnitzia* (Epser) J.V. Lamououx, *Caulerpa racemosa* (Frosk.) Weber-van-Bosse (*C. racemosa*), *Caulerpa scalpelliformis* (R.Br.) Weber-van-Bosse, *Ulva lactuca* Lin, *Ulva fasciata* Dellie, *Ulva reticulata* Forsk, *Stoechospermum marginatum* (Ag.) Kutz (*S. marginatum*), *Sargassum wightii* Grev, *Gracilaria verrucosa* (Huds.) Papenfuss and *Gracilaria edulis* (S.G. Gemelin) P.C. Silva against *Enterococcus faecalis* (MTCC 439) (*E. faecalis*) and one clinical isolate of vancomycin resistant *E. faecalis*.

Methods: The selected marine macro algae were extracted with different solvents *viz*., hexane, chloroform, ethyl acetate, acetone and methanol. Antibacterial assay was carried out by using disc diffusion method, determination of minimum inhibitory concentration and minimum bactericidal concentration.

Results: The maximum antibacterial activity was recorded in the ethyl acetate extracts of *S. marginatum* and *C. racemosa* than the other extracts. The mean zone of inhibition produced by the extracts in agar diffusion assays against the tested bacterial strains ranged from 7.1 to 14.5 mm. The minimum inhibitory concentration was between 250 and 500 μg/mL, while the minimum bactericidal concentration was from 500 to 1 000 μg/mL. The ethyl acetate extracts of the seaweeds showed the presence of strong terpenoids, tannins and phenolic compounds compared with the other solvent extracts.

Conclusions: These findings suggest that ethyl acetate extracts of *S. marginatum* and *C. racemosa* can be used as an antibacterial substance for the treatment of infection caused by *E. faecalis*.

KEYWORDS

Marine macro algae, Antibacterial activity, Vancomycin resistant *Enterococcus faecalis* strains

1. Introduction

Marine macro algae, the most accessible marine resources of coastal zone, occupy a potentially important place as a source of biomedical compounds. They possess pharmacologically active substances that reduce level of epiphytism and prevent microbial attack[1]. Seaweeds with the valuable medicinal potentials can be used as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially who live in the coastal areas. From the literature, it is observed that the edible seaweeds contain a significant amount of the protein, vitamins and minerals essential for the human nutrition[2]. As a consequence of an increasing demand in screening for new therapeutic drugs from natural products, there is a greater interest towards marine organisms. Several marine organisms produce bioactive

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metabolites in response to ecological pressures such as competition for space, maintenance of unfouled surfaces, deterrence of predation and the ability to successfully reproduce[3]. Many substances obtained from marine algae such as alginate, carrageenan and agar as phycocolloids have been used for decades in medicine and pharmacy[4].

Enterococci are commensal bacteria of the animal and human gut but can also serve as dreaded nosocomial pathogens of life-threatening infections especially among the elderly, immuno-compromised or seriously ill patients. *Enterococcus faecalis* (*E. faecalis*) still earmarks the majority of enterococcal infections, mostly urinary tract infections, endocarditis and bacteraemia[5]. Some infections caused by vancomycin resistant enterococci are not effectively treated by any currently licensed and available antibiotic or antibiotic combination. The increase in vancomycin-resistant enterococcal infections seen over the past decade poses problems beyond the lack of available antimicrobial therapy for these organisms[6].

Hence, the present work was made to evaluvate the antibacterial activity of different extracts of *Caulerpa chemnitzia* (Epser) J.V. Lamououx (*C. chemnitzia*), *Caulerpa racemosa* (Frosk.) Weber-van-Bosse (*C. racemosa*), *Caulerpa scalpelliformis* (R.Br.) Weber-van-Bosse (*C. scalpelliformis*), *Ulva lactuca* Lin (*U. lactuca*), *Ulva fasciata* Dellie (*U. fasciata*), *Ulva reticulata* Forsk (*U. reticulata*), *Stoechospermum marginatum* (Ag.) Kutz (*S. marginatum*), *Sargassum wightii* Grev (*S. wightii*), *Gracilaria verrucosa* (Huds.) Papenfuss (*G. verrucosa*), *Gracilaria edulis* (S.G. Gemelin) P.C. Silva (*G. edulis*) against standard strain, *E. faecalis* (MTCC 439) and one clinical isolate of vancomycin resistant *E. faecalis* strain.

2. Materials and methods

2.1. Sample collection

C. chemnitzia, *C. racemosa*, *C. scalpelliformis*, *U. lactuca* Lin, *U. fasciata*, *U. reticulata*, *S. marginatum*, *S. wightii*, *G. verrucosa*, *G. edulis* were collected from various places of Mandapam (9°17.417′ N; 79°08.558′ E) of Rameshwaram District, Manappad (9°17.417′ N; 79°08.558′ E), Tuticorin District (8°45′ N; 78°10′ E) and Kanniyakumari District (9°11′ N; 79°24′ E), Gulf of Mannar Marine Biosphere Reserve, Tamil Nadu, India. The collections were made during the months of November and December 2011. The algae were identified by Dr. R. Selvaraj, Former Professor, Department of Botany, Annamalai University and the museum specimens were deposited in the Department of Botany, Annamalai University, Annamalainagar.

2.2. Preparation of seaweed extracts

The algal species were handpicked during low tide and manually cleaned to remove sand, epiphytes and animal waste. Then the samples were rinsed with sea water to remove associated debris, planktons and loosely attached microorganisms. Part of the macro algae was fixed in 4% formaldehyde for its taxonomic identification. Morphologically distinct thalluses of algae were placed separately in new polythene bags and kept in an ice box containing slush ice and transported to laboratory. Further, the material was washed thoroughly with tap water to remove the salt on the surface of the samples and the water was drained off from the alga and the samples were spread on the blotting paper to remove the excess water. The shade dried samples were again cleaned with sterile distilled water to remove the remaining salt on the surface of the samples to avoid pumping of the solvent during the extraction process. The algal samples were shade dried followed by oven drying at 50 °C for half an hour and milled in an electrical blender.

Five hundred grams of powdered samples were individually packed in Soxhlet apparatus and extracted with different solvents like hexane, chloroform, ethyl acetate, acetone and methanol for 72 h. The extracts were pooled and the solvents were evaporated under vacuum in rotary evaporator (Heidolph, Germany) at 40 °C and the dried extracts were stored at 4 °C for antibacterial assay.

2.3. Phytochemical screening

The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. chemnitzia*, *C. racemosa*, *C. scalpelliformis*, *U. lactuca*, *U. fasciata*, *U. reticulata*, *S. marginatum*, *S. wightii*, *G. verrucosa* and *G. edulis* were used for qualitative phytochemical studies. Screening of phytochemicals like terpenoids, tannins, cardic glycosides, steroids, alkaloids, and phenolic compounds were carried out according to the standard methods[7,8].

2.4. Bacterial strains used

The bacterial strain of *E. faecalis* (MTCC 439) was procured from Microbial Type Culture Collection, Chandigarh. One clinical isolate of vancomycin resistant *E. faecalis* strain was obtained from Department of Microbiology, Rajah Muthiah Medical College and Hospital, Annamalai University, Annamalainagar, Tamil Nadu, India. The stock cultures were maintained on nutrient agar medium at 4 °C.

In vitro antibacterial activity was determined by using Muller Hinton agar (MHA) and Muller Hinton broth (MHB) was obtained from Himedia, Mumbai.

2.5. Antibiotic susceptibility test

Antibiotic sensitivity of the bacterial strains was determined by standard Clinical and Laboratory Standards Institute disc diffusion method[9]. Antibacterial agents from different classes of antibiotics *viz*., methicillin (5 μg/disc),

oxacillin (1 μg/disc), linezolid (30 μg/disc), vancomycin (30 μg/disc), amikacin (30 μg/disc), ampicillin (10 μg/disc), cefixime (5 μg/disc), ceftazidime (30 μg/disc), ciprofloxacin (5 μg/disc), chloramphenicol (30 μg/disc), erythromycin (15 μg/disc), gentamycin (10 μg/disc), norfloxacin (10 μg/disc), nalidixic acid (30 μg/disc), ofloxacin (5 μg/disc), streptomycin (10 μg/disc) and tetracycline (30 μg/disc) were obtained from Himedia, Mumbai.

2.6. Antibacterial assays

2.6.1. Disc diffusion method

The antibacterial activity of crude algal extracts was determined by disc diffusion method according to Bauer *et al*. with modifications[10]. Petri plates were prepared by pouring 20 mL of MHA. Then the plates were allowed to solidify and used in susceptibility test. MHA plates were inoculated by streaking the swab over the entire agar surface using 100 μL of bacterial suspensions containing 10^8 CFU/mL and allowed to dry for 10 min. The crude extracts were dissolved in 10% dimethyl sulfoxide (DMSO) and under aseptic condition, sterile HiMedia paper discs (6 mm) were impregnated with 20 μL of different concentrations (500, 250 and 125 μg/disc) of crude extract. The discs with extract were placed on the surface of the medium with sterile forceps and gently pressed to ensure contact with inoculated agar surface. Vancomycin (30 μg/disc) for bacteria, were used as positive control and 10% DMSO was used as blind control in all the assays. Finally, the inoculated plates were incubated at 37 °C for 24–48 h. The zone of inhibition was observed and measured in millimeters. The assay in this experiment was repeated three times.

2.6.2. Antibiotic resistant profile

The antibiotic resistant profile of bacterial strains of both clinical and standard strains was confirmed by CLSI-M100-2012 method[9].

2.6.3. Determination of the minimum inhibitory concentration (MIC)

To determine the MIC of the crude algal extracts, a modified resazurin microtitre plate assay was carried out according to methods of Sarker *et al*[11]. Sterile 50 μL MHB of respective broth was transferred into each well of a sterile 96-well micro titer plate. The crude extracts were dissolved in 10% DMSO to obtain 2 000 μg/mL stock solution. A total of 50 μL of crude extract stock solution was added into the first well. After fine mixing of the crude extracts and broth, 50 μL of the solution was transferred to the second well and in this way, the serial dilution procedure was continued to a twofold dilution to obtain concentrations like 1 000 to 15.7 μg/mL of the extract in each well. To each well 10 μL of resazurin indicator solution was added. The resazurin solution was prepared by dissolving a 270 mg tablet in 40 mL of sterile distilled water. A vortex mixer was used to ensure that it was a well-dissolved and homogenous solution. Then 30 μL of MHB was added to each well. Finally, 10 μL of bacterial suspension was added to each well to achieve a concentration of approximately 5×10^5 CFU/mL. Each plate had a set of controls: a column with all solutions with the exception of the crude extracts; a column with all solutions with the exception of the bacterial solution adding 10 μL of MHB instead and a column with 10% DMSO solution as a negative control. The plates were incubated at 37 °C for 24–48 h. The color change was then assessed visually. The growth was indicated by color changes from blue (oxidized) to pink (reduced). The lowest concentration at which the color change occurred was taken as the MIC value.

2.6.4. Determination of the minimum bactericidal concentration (MBC)

MBC was determined by plating a loop full of samples from each MIC assay well with growth inhibition into freshly prepared MHA. The plates were incubated at 37 °C for 24–48 h. The MBC was recorded as the lowest concentration of the extract that did not permit any visible bacterial growth after the period of incubation.

2.7. Statistical analysis

The results are expressed as mean±SD. All statistical analyses were performed using SPSS version 16.0 statistical software (SPSS Inc., Chicago, IL, USA). Student's *t*-test was performed to determine any significant difference between different extracts for *in vitro* antibacterial assays. Comparison of means for *in vitro* antibacterial assessment was carried out using One-way analysis of variance (ANOVA) and Duncan test. P value<0.05 was considered statistically significant.

3. Results

The hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. chemnitzia*, *C. racemosa*, *C. scalpelliformis*, *U. lactuca*, *U. fasciata*, *U. reticulata*, *S. marginatum*, *S. wightii*, *G. verrucosa* and *G. edulis* were analysed for the presence of phytochemicals, terpenoids, tannins, cardiac glycosides, steroids, alkaloids, phenolic compounds and coumarins. The ethyl acetate extracts of all the marine macro alga tested strongly showed the presence of phytochemicals, terpenoids, tannins and phenolic compounds than the other solvent extracts. Among the phytochemicals, cardiac glycosides were present in all the extracts of *C. chemnitzia*, *C. racemosa*, *C. scalpelliformis*, *U. lactuca*, *U. fasciata* and *U. reticulata* and absent in all the extracts of *G. edulis* and *G. verrucosa* and acetone, methanol and chloroform extracts of *S. marginatum* and *S. wightii*.

Steroids were present in all the extracts except extracts of *U. lactuca*, *U. fasciata* and *U. reticulata*. Alkaloids and coumarins were absent in all the extracts of *C. chemnitzia*, *C. racemosa*, *C. scalpelliformis*, *U. lactuca*, *U. fasciata*, *U. reticulata*, *S. marginatum* and *S. wightii* and alkaloids were present only in the chloroform and ethyl acetate extracts of *G. edulis* and *G. verrucosa*.

The standard strain, *E. faecalis* (MTCC 439) was found to be highly resistant to all the antibiotics tested except gentamycin, streptomycin, tetracycline, amikacin, erythromycin and chloramphenicol. One clinical isolate of *E. faecalis* was highly resistant to all the antibiotics tested except norfloxacin, cefixime, ceftazidime, nalidixic acid and ofloxacin.

In the present study, different solvents with increasing polarity *viz*., hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. chemnitzia*, *C. racemosa*, *C. scalpelliformis*, *U. lactuca*, *U. fasciata*, *U. reticulata*, *S. marginatum S. wightii*, *G. edulis* and *G. verrucosa* were tested against one clinical and standard strain of *E. faecalis* and all the extracts of seaweeds possessed antibacterial activity against all the bacterial strains tested when compared to the available antibiotics tested. The mean zones of inhibition and MIC and MBC values are presented in Tables 1 and 2. The mean zones of inhibition ranged between 7.1 and 14.5 mm. Vancomycin (30 μg/disc) was used as positive control and it produced mean zone of inhibition ranged from 7.1 to 12.8 mm. The blind control (10% DMSO) did not produce any zone of inhibition for all the bacterial strains tested. The results of MIC values of the different extracts of selected marine macro algae ranged from 250 to 500 μg/mL, while the MBC values were between 500 and 1 000 μg/mL.

Table 1

Antibacterial activity of selected marine macro algae against *E. faecalis* (MTCC 439).

Seaweed extracts prepared with different solvents		Mean zone of inhibition[a] (mm)[b]					
		500 (μg/disc)	250 (μg/disc)	125 (μg/disc)	Vancomycin (30 μg/disc)	MIC (μg/mL)	MBC (μg/mL)
C. chemnitzia	Hexane	11.00±0.28	9.60±0.76	7.50±0.50	9.30±0.57	500	1 000
	Chloroform	12.10±0.28	9.80±0.28	7.80±0.76	9.60±0.76	500	1 000
	Ethyl acetate	12.80±0.76	10.10±0.28	8.00±0.50	9.30±0.57	250	500
	Acetone	11.10±0.28	9.50±0.50	7.30±0.57	11.80±0.28	500	1 000
	Methanol	11.50±0.50	9.60±0.28	7.50±0.50	9.80±0.76	500	1 000
C. racemosa	Hexane	12.30±0.76	9.50±0.50	7.30±0.57	11.60±0.76	500	1 000
	Chloroform	13.00±0.50	10.10±0.28	7.80±0.76	10.80±0.76	500	1 000
	Ethyl acetate	13.60±0.76**	11.00±0.50	8.00±0.50	9.30±0.57	250	500
	Acetone	11.60±0.28	9.50±0.50	7.30±0.57	9.50±0.50	500	1 000
	Methanol	10.50±0.50	9.30±0.28	7.10±0.28	9.80±0.76	500	1 000
C. scapelliformis	Hexane	11.10±0.28	9.30±0.57	7.50±0.50	8.30±0.57	500	1 000
	Chloroform	12.10±0.28	9.60±0.28	7.80±0.76	8.00±0.50	500	1 000
	Ethyl acetate	12.50±0.50	10.10±0.28	8.00±0.50	9.30±0.57	250	500
	Acetone	10.60±0.76	9.50±0.50	7.10±0.28	11.60±0.76	500	1 000
	Methanol	10.10±0.28	9.00±0.50	7.10±0.28	8.80±0.76	500	1 000
U. lactuca	Hexane	11.50±0.50	9.50±0.50	7.10±0.28	9.30±0.57	500	1 000
	Chloroform	13.00±0.76	10.10±0.28	7.50±0.50	8.80±0.76	500	1 000
	Ethyl acetate	13.60±0.76**	10.50±0.50	8.50±0.50	9.30±0.57	250	500
	Acetone	12.50±0.50	9.80±0.76	7.80±0.76	10.60±0.76	500	1 000
	Methanol	11.00±0.50	9.30±0.76	7.10±0.28	9.80±0.76	500	1 000

[a]: Diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]: Mean of three assays; **: Significant at $P<0.05$.

Table 1 continued

Antibacterial activity of selected marine macro algae against *E. faecalis* (MTCC 439).

Seaweed extracts prepared with different solvents		Mean zone of inhibition[a] (mm)[b]					
		500 (μg/disc)	250 (μg/disc)	125 (μg/disc)	Vancomycin (30 μg/disc)	MIC (μg/mL)	MBC (μg/mL)
U. fasciata	Hexane	10.50±0.50	9.30±0.57	7.10±0.28	8.80±0.76	500	1 000
	Chloroform	12.50±0.50	9.80±0.28	7.50±0.50	9.30±0.57	500	1 000
	Ethyl acetate	13.10±0.28	10.10±0.50	8.00±0.50	10.30±0.57	250	500
	Acetone	11.60±0.57	9.80±0.76	7.30±0.57	8.50±0.50	500	1 000
	Methanol	11.00±0.50	9.60±0.28	7.10±0.28	9.30±0.57	500	1 000
U. reticulata	Hexane	11.50±0.50	9.50±0.50	7.10±0.28	8.60±0.76	500	1 000
	Chloroform	12.10±0.28	9.80±0.28	8.00±0.50	9.30±0.57	500	500
	Ethyl acetate	13.00±0.50	11.10±0.28	8.50±0.50	12.80±0.28	250	500
	Acetone	11.30±0.57	9.60±0.50	7.30±0.57	9.00±0.50	500	1 000
	Methanol	11.00±0.50	9.30±0.57	7.10±0.28	9.30±0.57	500	1 000
S. wightii	Hexane	10.60±0.28	9.50±0.58	7.50±0.50	9.30±0.57	500	1 000
	Chloroform	11.50±0.50	9.50±0.50	7.50±0.50	11.60±0.76	500	1 000
	Ethyl acetate	13.00±0.50**	10.80±0.50	8.00±0.76	10.30±0.57	250	500
	Acetone	10.60±0.76	9.50±0.50	7.50±0.50	8.60±0.76	500	1 000
	Methanol	10.50±0.50	9.30±0.57	7.10±0.28	11.80±0.28	500	1 000
S. marginatum	Hexane	12.00±0.50	9.10±0.28	7.50±0.50	8.30±0.57	500	1 000
	Chloroform	13.10±0.76	10.50±0.50	7.80±0.76	8.60±0.76	250	500
	Ethyl acetate	13.80±0.28**	11.60±0.57	8.10±0.28	9.30±0.57	250	500
	Acetone	12.00±0.50	9.10±0.28	7.30±0.57	7.30±0.57	500	1 000
	Methanol	10.80±0.76	9.00±0.50	7.10±0.28	8.80±0.76	500	1 000
G. edulis	Hexane	11.50±0.50	9.10±0.28	7.30±0.57	9.30±0.57	500	1 000
	Chloroform	11.80±0.76	9.50±0.50	7.30±0.57	7.30±0.57	500	1 000
	Ethyl acetate	12.50±0.50	10.50±0.50	7.50±0.50	8.80±0.76	250	500
	Acetone	10.60±0.76	9.30±0.57	7.10±0.28	11.60±0.76	500	1 000
	Methanol	10.50±0.50	9.30±0.28	7.10±0.28	8.80±0.76	500	1 000
G. verrucosa	Hexane	10.60±0.28	9.10±0.28	7.10±0.28	9.30±0.57	500	1 000
	Chloroform	11.30±0.28	9.50±0.50	7.80±0.76	8.60±0.76	500	1 000
	Ethyl acetate	12.80±0.57	10.60±0.76	8.10±0.28	9.30±0.57	250	500
	Acetone	11.10±0.28	9.50±0.50	7.50±0.50	11.60±0.76	500	1 000
	Methanol	10.50±0.50	8.60±0.57	7.10±0.28	10.30±0.57	500	1 000

[a]: Diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]: mean of three assays; **: Significant at $P<0.05$.

Table 2

Antibacterial activities of selected marine macro algae against *E. faecalis* (clinical strain).

Seaweed extracts prepared with different solvents		Mean zone of inhibition[a] (mm)[b]					
		500 (μg/disc)	250 (μg/disc)	125 (μg/disc)	Vancomycin (30 μg/disc)	MIC (μg/mL)	MBC (μg/mL)
C. chemnitzia	Hexane	11.60±0.28	10.10±0.28	7.50±0.50	9.30±0.57	500	1 000
	Chloroform	11.80±0.76	9.80±0.76	7.80±0.76	8.60±0.76	500	1 000
	Ethyl acetate	12.60±0.28	10.10±0.28	8.30±0.57	9.30±0.57	250	500
	Acetone	11.50±0.50	9.50±0.50	7.50±0.50	9.60±0.50	500	1 000
	Methanol	11.10±0.28	9.30±0.76	7.10±0.28	8.80±0.76	500	1 000
C. racemosa	Hexane	12.30±0.57	9.30±0.57	7.50±0.50	10.30±0.57	250	500
	Chloroform	13.10±0.28	10.00±0.50	7.80±0.76	10.80±0.76	250	500
	Ethyl acetate	14.00±0.76**	10.60±0.28	8.80±0.76	9.30±0.57	250	500
	Acetone	11.80±0.28	9.80±0.76	7.10±0.28	10.00±0.50	500	1 000
	Methanol	11.50±0.50	9.50±0.50	7.10±0.28	8.80±0.76	500	1 000
C. scapelliformis	Hexane	11.00±0.50	9.80±0.76	7.10±0.28	8.30±0.57	500	1 000
	Chloroform	12.10±0.28	10.10±0.28	7.50±0.50	8.00±0.50	500	1 000
	Ethyl acetate	13.50±0.50	10.80±0.28	8.10±0.28	9.30±0.57	250	500
	Acetone	11.00±0.50	9.50±0.50	7.10±0.28	9.10±0.28	500	1 000
	Methanol	11.10±0.28	9.60±0.28	7.30±0.57	8.80±0.76	500	1 000
U. lactuca	Hexane	11.80±0.76	9.60±0.50	7.30±0.57	7.30±0.57	500	1 000
	Chloroform	12.00±0.50	10.00±0.28	7.50±0.50	8.80±0.76	500	1 000
	Ethyl acetate	13.80±0.28**	10.10±0.28	7.80±0.76	9.30±0.57	250	500
	Acetone	12.30±0.57	9.60±0.57	7.30±0.57	8.60±0.76	500	1 000
	Methanol	10.00±0.50	9.30±0.57	7.10±0.28	8.80±0.76	500	1 000
U. fasciata	Hexane	11.80±0.76	9.50±0.50	7.30±0.57	7.80±0.76	500	1 000
	Chloroform	12.60±0.57	10.00±0.50	8.00±0.50	8.80±0.57	250	500
	Ethyl acetate	13.50±0.50	10.80±0.76	8.50±0.50	8.30±0.28	250	500
	Acetone	11.50±0.50	9.60±0.57	7.30±0.57	8.10±0.28	500	1 000
	Methanol	11.10±0.28	9.50±0.50	7.10±0.28	9.30±0.57	500	1 000

[a]: Diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]: Mean of three assays; **: Significant at $P<0.05$.

Table 2 continued

Antibacterial activities of selected marine macro algae against *E. faecalis* (clinical strain).

Seaweed extracts prepared with different solvents		Mean zone of inhibition[a] (mm)[b]					
		500 (μg/disc)	250 (μg/disc)	125 (μg/disc)	Vancomycin (30 μg/disc)	MIC (μg/mL)	MBC (μg/mL)
U. reticulata	Hexane	12.10±0.28	9.60±0.58	7.30±0.57	11.60±0.76	500	1 000
	Chloroform	12.30±0.57	10.00±0.56	8.00±0.50	10.80±0.76	500	1 000
	Ethyl acetate	13.00±0.50	11.00±0.50	8.50±0.50	10.30±0.57	250	500
	Acetone	11.00±0.50	9.50±0.50	7.10±0.28	12.10±0.28	500	1 000
	Methanol	10.30±0.57	9.50±0.57	7.00±0.50	11.60±0.76	500	1 000
S. wightii	Hexane	10.80±0.28	9.50±0.50	7.50±0.50	10.30±0.57	500	1 000
	Chloroform	11.00±0.50	10.00±0.76	7.80±0.76	10.80±0.76	500	1 000
	Ethyl acetate	14.00±0.50**	10.50±0.50	8.00±0.50	11.50±0.57	250	500
	Acetone	10.60±0.76	9.50±0.50	7.10±0.28	11.60±0.50	500	1 000
	Methanol	11.00±0.50	9.50±0.50	7.30±0.57	12.80±0.28	500	1 000
S. marginatum	Hexane	12.50±0.50	9.50±0.50	7.50±0.50	9.30±0.57	250	500
	Chloroform	13.50±0.50	10.00±0.50	8.10±0.28	8.60±0.76	250	500
	Ethyl acetate	14.50±0.50**	11.30±0.57	8.60±0.57	9.10±0.28	250	500
	Acetone	12.30±0.28	9.80±0.76	7.50±0.50	8.80±0.76	500	1 000
	Methanol	11.00±0.50	9.50±0.50	7.10±0.28	8.60±0.76	500	1 000
G. edulis	Hexane	10.50±0.50	9.60±0.76	7.10±0.28	10.30±0.58	500	1 000
	Chloroform	12.10±0.28	10.50±0.86	7.80±0.76	11.60±0.76	500	1 000
	Ethyl acetate	13.10±0.28	11.00±0.50	8.00±0.50	12.10±0.50	250	500
	Acetone	11.00±0.50	9.80±0.28	7.10±0.28	11.50±0.50	500	1 000
	Methanol	10.30±0.28	8.80±0.76	7.00±0.50	9.00±0.50	500	1 000
G. verrucosa	Hexane	10.60±0.28	9.10±0.28	7.50±0.50	8.80±0.76	500	1 000
	Chloroform	11.30±0.57	9.80±0.76	7.80±0.76	8.60±0.76	500	1 000
	Ethyl acetate	13.00±0.57	10.60±0.76	8.10±0.28	9.30±0.57	250	500
	Acetone	11.10±0.28	9.10±0.28	7.50±0.50	11.60±0.76	500	1 000
	Methanol	10.50±0.50	8.60±0.57	7.10±0.28	11.00±0.50	500	1 000

[a]: Diameter of zone of inhibition (mm) including the disc diameter of 6 mm; [b]: Mean of three assays; **: Significant at $P<0.05$.

4. Discussion

In the present study, different solvents with increasing polarity *viz*., hexane, chloroform, ethyl acetate, acetone and methanol extracts of *C. chemnitzia*, *C. racemosa*, *C. scalpelliformis*, *U. lactuca*, *U. fasciata*, *U. reticulata*, *S. marginatum*, *S. wightii*, *G. edulis* and *G. verrucosa* were tested against one clinical and standard strains of *E. faecalis*. The highest mean zone of inhibition (14.5 mm) was observed in ethyl acetate extracts of *S. marginatum* against clinical isolate of *E. faecalis*. The lowest MIC (250 μg/mL) and MBC (500 μg/mL) values were observed in ethyl acetate extracts of all the marine macro algae tested against both standard and clinical strains of *E. faecalis*. Likewise, lowest MIC (250 μg/mL) and MBC (500 μg/mL) values of chloroform extract of *S. marginatum* against standard strain, *E. faecalis* and hexane, chloroform extracts of *S. marginatum* and *C. racemosa* against clinical isolate of *E. faecalis* were observed.

A similar report was given by Salem *et al*., in which they stated that higher antibacterial activity was recorded in the ethyl acetate extracts of *C. racemosa*, *Sargassum dentifolium* (*S. dentifolium*) and *Padina gymnospora* and methanol extracts of *Sargassum hystrix*, *C. racemosa*, *Codium fragile*, *S. dentifolium* and *Cystoseria myrica*[12]. Kasinathan *et al*. reported that the ethyl acetate extract of *U. lactuca* and *G. verrucosa* showed the highest antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus* and *Bacillus subtilis* and also identified the presence of myristic and palmitic acid, linoleic acid, oleic acid, lauric, stearic and myristic acid, from ethyl acetate extracts[13]. Karthikaidevi *et al*. used seven different solvents including methanol and ethyl acetate for extraction of antibacterial substances from *Codium adherens*, *U. reticulata* and *Halimeda tuna*[14].

In present study, results of different extracts of *C. chemnitzia*, *C. racemosa*, *C. scalpelliformis*, *U. lactuca*, *U. fasciata*, *U. reticulata*, *S. marginatum S. wightii*, *G. edulis* and *G. verrucosa* against *E. faecalis* showed that brown algae were more active compared to other groups of algae tested. Similar results were also obtained by other researchers[14,15]. These strong activities related to brown algae may be due to the phenolic compounds such as phlorotannins, eckol and eckol- related compounds that have strong bactericidal activity[16]. Previous studies have demonstrated that brown algae produce a wide variety of isoprenoid metabolites as defences against herbivores as well as to prevent biofouling[17]. From the evolutionary perspective, it would be important to determine if the same molecules that daunt herbivores and/or prevent biofouling also function as antimicrobial chemical defences. Yamashita *et al*. attributed the antimicrobial activity to the high concentration of polysaccharides in these species which are known to have antimicrobial properties[18]. Berteau and Mulloy reported that the antimicrobial activity of the polysaccharides was related to their chemical structure and ester sulfate groups[19]. On the other hand, several species of brown seaweed that have a high sulfate content have been reported to show differences in antimicrobial activities[20]. The polysaccharides were extracted from *Sargassum stenophyllum*, which had fucose, galactose, mannose, xylose, glucose and uronic acid as the main components[21].

In this study, the antibacterial activity of ethyl acetate extracts of *C. chemnitzia*, *C. racemosa*, *C. scalpelliformis*, *U. lactuca*, *U. fasciata*, *U. reticulata*, *S. marginatum*, *S. wightii*, *G. edulis* and *G. verrucosa* may be due to the presence of phytochemicals, terpenoids, tannins and phenolic compounds. Phenolic compounds may affect growth and metabolism of bacteria. They could have an activating or inhibiting effect on microbial growth according to their constitution and concentration[22]. Zapata and McMillan reported that the phenolic compounds present in seagrasses could also enhance the antimicrobial activity[23]. Steroid glycosides are a class of widespread natural products having either terrestrial or marine origins. Several cardiac glycosides are used therapeutically in the treatment of cardiac failure and arrhythmias; and many glycoside compounds, belonging to other structural groups, have cytotoxic, antimicrobial, hypocholesterolemic and other biological activities[24]. Tannins were used therapeutically as antiviral, antibacterial, antiulcer and antioxidant agents. Many drugs containing tannin are used in the treatment of piles, inflammation, burns and as astringent[25].

In this prsent study, the ethyl acetate extracts of *S. marginatum* and *C. racemosa* showed the highest antibacterial activity than other extracts against clinical and standard bacterial strains tested.

Four diterpenes such as 19-acetoxy-5 (R), 15, 18 (R and S)-tetrahydroxyspata-13, 16 (E)-diene, 5 (R), 15, 18 (R and S), 19-tetrahydrowspata-13, 16(E)-diene[5], 5 (R), 18-dihydroxyspata-13, 16 (E)-dime and 5 (R), 16-dihydroxyspata-13, 17-diene were isolated from the chloroform and methanol (1:1) extracts of *S. marginatum*[6,7]. All the diterpenes showed strong antibacterial activity against two Gram positive and four Gram negative bacteria[26]. The genus *Caulerpa* has been widely studied, and the structures of many new compounds, such as di-, sesqui- and mono-terpenes with the terminal 1,4-diacetoxybutadiene moiety and the nitrogen-containing compounds bisindole alkaloids (exemplified by caulerpin) and caulerpicin have been reported[27]. Recently, the genus *Caulerpa* has attracted the attention of researchers due to its important secondary metabolite caulerpenyne that is reported to exhibit the antineoplastic, antibacterial and antiproliferative activities[28].

In this study, three control drugs, namely, methicillin, oxacillin and vancomycin were used. Vancomycin, a tricyclic glycopeptide antibiotic, is used to treat methicillin-resistant *Staphylococcus aureus* infections[29]. Vancomycin interferes with bacterial cell wall synthesis, as penicillin does, eventually leading to cell lysis[30]. Most Gram-negative bacteria are less sensitive to vancomycin than Gram-positives[29]. The search for new ways to treat vancomycin-resistant *E. faecalis* infections stimulates the investigation of natural compounds as an alternative treatment of these infections.

In this study, as the ethyl acetate extracts of *S. marginatum* and *C. racemosa* were found to be the most effective anti-vancomycin resistant *E. faecalis* agents, it can be recommended that these two species can be used as antibacterial substance for treating infection caused by *E. faecalis*.

Conflict of interest statement

We declare that we have no conflict of interest.

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Comments

Background

Seaweeds with the valuable medicinal potentials can be used as antibiotics, laxatives, anticoagulants, anti-ulcer products and suspending agents in radiological preparations. Fresh and dry seaweeds are extensively consumed by people especially who live in the coastal areas. As a consequence of an increasing demand in screening for new therapeutic drugs from natural products, there is a greater interest towards marine organisms.

Research frontiers

In the present study, different extracts (hexane, chloroform, ethyl acetate, acetone and methanolic extracts) of *C. chemnitzia*, *C. racemosa*, *C. scalpelliformis*, *U. lactuca*, *U. fasciata*, *U. reticulata*, *S. marginatum*, *S. wightii*, *G. edulis* and *G. verrucosa* showed varied degree of antibacterial activity against all the tested bacterial strains.

Related reports

Salem *et al*. reported higher antibacterial activity of the ethyl acetate extracts of *C. racemosa*, *S. dentifolium* and *Padina gymnospora* and methanol extracts of *Sargassum hystrix*, *C. racemosa*, *C. fragile*, *S. dentifolium* and *Cystoseria myrica*. Kasinathan *et al*. reported that the ethyl acetate extract of *U. lactuca* and *G. verrucosa* showed the highest antimicrobial activity against *Escherichia coli*, *Klebsiella pneumoniae*, methicillin-resistant *Staphylococcus aureus* and *Bacillus subtilis*.

Innovations and breakthroughs

The present study showed that, brown algae were more active compared to other groups of algae tested. The ethyl acetate extracts of *C. chemnitzia*, *C. racemosa*, *C. scalpelliformis*, *U. lactuca*, *U. fasciata*, *U. reticulata*, *S. marginatum*, *S. wightii*, *G. edulis* and *G. verrucosa* showed higher antibacterial activity may due to the presence of phytochemicals *viz*., terpenoids, tannins and phenolic compounds.

Applications

The ethyl acetate extracts of *S. marginatum* and *C. racemosa* were found to be the most effective anti-vancomycin resistant *E. faecalis* agents. It can be recommended that these two species can be used as antibacterial agents to treat infection caused by *E. faecalis*.

Peer review

The present investigation paves a way for the advanced research regarding the antimicrobial activity of seaweeds,

stimulating the young researcher and academician in phytochemical screening and bio-effficacy studies.

References

[1] Arun Kumar K, Rengasamy R. Antibacterial activity of seaweed extracts/fractions obtained through a TLC profile against the phytopathogenic bacterium *Xanthomonas oryzae*. *Bot Mar* 2000; **43**: 417-421.

[2] Fayaz M, Namitha KK, Murthy KN, Swamy MM, Sarada R, Khanam S, et al. Chemical composition, iron bioavailability and antioxidant activity of *Kappaphycus alvarezzi* (Doty). *J Agric Food Chem* 2005; **53**: 792-797.

[3] Konig GM, Wright AD, Stiche O, Angerhofer CK, Pezzuto JM. Biological activities of selected marine natural products. *Planta Med* 1994; **60**: 532-537.

[4] Kaliaperumal N, Kalimuthu S, Ramalingam JR. Present scenario of seaweed exploitation and industry in India. *Seaweed Res Utiln* 2004; **26**: 47-53.

[5] Top J, Willems RJ, Blok H, de Regt M, Jalink K, Troelstra A, et al. Ecological replacement of *Enterococcus faecalis* by multiresistant clonal complex 17 *Enterococcus faecium*. *Clin Microbiol Infect* 2007; **13**: 316-319.

[6] Clark NC, Cooksey RC, Hill BC, Swenson JM, Tenover FC. Characterization of glycopeptide-resistant enterococci from U.S. hospitals. *Antimicrob Agents Chemother* 1993; **37**: 2311-2317.

[7] Harborne JB. *Phytochemical methods: a guide to modern techniques of plant analysis*. 2nd ed. New York: Chapman and Hall; 1973, p. 88-185.

[8] Trease GE, Evans WC. *Text book of pharmacognosy*. 12th ed. London: Balliese Tindall and Company Publisher; 1983, p. 343-383.

[9] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility tests: twenty-second informational supplement. Wayne: Clinical and Laboratory Standards Institute; 2012. [Online] Availabel from: http://antimicrobianos.com.ar/ATB/wp-content/uploads/2012/11/M100S22E.pdf [Accessed on 7th March, 2014]

[10] Bauer AW, Kirby WM, Scherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 1966; **45**: 493-496.

[11] Sarker SD, Nahar L, Kumarasamy Y. Microtitre plate-based antibacterial assay incorporating resazurin as an indicator of cell growth, and its application in the *in vitro* antibacterial screening of phytochemicals. *Methods* 2007; **42**: 321-324.

[12] Salem WM, Galal H, Nasr El-deen F. Screening for antibacterial activities in some marine algae from the red sea (Hurghada, Egypt). *Afr J Microbiol Res* 2011; **5**(15): 2160-2167.

[13] Kasinathan T, Veeramuthu D, Perumal P, Savarimthu I. Antimicrobial activity of *Trichodesmium erythraeum* (Ehr) (microalga) from south east coast of Tamil Nadu, India. *Int J Integr Biol* 2009; **5**: 167-170.

[14] Karthikaidevi G, Manivannan K, Thirumaran G, Anantharaman P, Balasubaramanian T. Antibacterial properties of selected green seaweeds from Vedalai coastal waters; Gulf of Mannar Marine Biosphere Reserve. *Global J Pharmacol* 2009; **3**(2): 107-112.

[15] Kandhasamy M, Arunachalam KD. Evaluation of *in vitro* antibacterial property of seaweeds of southeast coast of India. *Afr J Biotechnol* 2008; **7**(12): 1958-1961.

[16] Nagayama K, Iwamura Y, Shibata T, Hirayama I, Nakamura T. Bactericidal activity of phlorotannins from the brown alga *Ecklonia kurome*. *J Antimicrob Chemother* 2002; **50**: 889-893.

[17] Paul VJ, Puglisi MP. Chemical mediation of interactions among marine organisms. *Nat Prod Rep* 2004; **21**: 189-209.

[18] Yamashita S, Sugita-Konishi Y, Shimizu M. *In vitro* bacteriostatic effects on dietary polysaccharides. *Food Sci Technol Res* 2001; **7**(3): 262-264.

[19] Berteau O, Mulloy B. Sulfated fucans, fresh perspectives: structures, functions and biological properties of sulfated fucans and an overview of enzymes active toward this class of polysaccharide. *Glycobiology* 2003; **13**: 29R-40R.

[20] Asker MMS, Mohamed SF, Ali FM, El-Sayed OH. Chemical structure and antiviral activity of water-soluble sulfated polysaccharides from *Sargassum latifolium*. *J Appl Sci Res* 2007; **3**: 1178-1185.

[21] Dias PF, Siqueira JM Jr, Maraschin M, Ferreira AG, Gagliardi AR, Ribeiro-do-Valle RM. A polysaccharide isolated from the brown seaweed *Sargassum stenophyllum* exerts antivasculogenic effects evidenced by modified morphogenesis. *Microvasc Res* 2008; **75**(1): 34-44.

[22] Reguant C, Bordons A, Arola L, Roze N. Influence of phenolic compounds on the physiology of *Oenococcus oeni* from wine. *J Appl Microbiol* 2000; **88**: 1065-1071.

[23] Zapata O, McMillan C. Phenolic acid in sea grasses. *Aquat Bot* 1979; **7**: 307-317.

[24] Ivanchina NV, Kicha AA, Stonik VA. Steroid glycosides from marine organisms. *Steroids* 2011; **76**: 425-454.

[25] Kolodziej H, Kiderlen AF. Antileishmanial activity and immune modulatory effects of tannins and related compounds on *Leishmania* parasitized RAW 264.7 cells. *Phytochemistry* 2005; **66**(17): 2056-2071.

[26] Shiaikh W, Shameel M, Hayee-Memon A, Usmanghani K, Bano S, Ahmad VU. Isolation and characterization of chemical constituents of *Stoechospermum marginatum* (Dictyotales, Phaeophyta) and their antimicrobial activity. *Pak J Pharm Sci* 1990; **3**(2): 1-9.

[27] Mao SC, Guo YW, Shen X. Two novel aromatic valerenane-type sesquiterpenes from the Chinese green alga *Caulerpa taxifolia*. *Bioorg Med Chem Lett* 2006; **16**: 2947-2950.

[28] Cavas L, Baskin Y, Yurdakoc K, Olgun N. Antiproliferative and newly attributed apoptotic activities from a marina alga: *Caulerpa racemosa* var. *cylindracea*. *J Exp Mar Biol Ecol* 2006; **339**: 111-119.

[29] Lee DS, Kang MS, Hwang HJ, Eom SH, Yang JY, Lee MS, et al. Synergistic effect between dieckol from *Ecklonia stolonifera* and β-lactams against methicillin-resistant *Staphylococcus aureus*. *Biotechnol Bioprocess Eng* 2008; **13**: 758-764.

[30] Barna JC, Williams DH. The structure and mode of action of glycopeptide antibiotics of the vancomycin group. *Annu Rev Microbiol* 1984; **38**: 339-357.