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Determination of Minimum Inhibitory Concentrations (MICs) and Minimum Bactericidal Concentrations (MBCs) in Green Seaweeds against Diarrhea Causing Bacteria *E. Coli and S. Typhi*

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Abstract

Diarrhea is one of the most common complaints faced by internists and primary care physicians and accounts for many referrals to gastroenterologists. Acute infective diarrhea contributes to the high rates of morbidity and mortality worldwide. Diarrhea constitutes 70% of food borne disease. Seaweeds have been frequently used medicinally in past and present. These plants have antibacterial activity against diarrhea causing organisms Escherichia coli and Salmonella typhi. The aim of present work was to evaluated and compared the antimicrobial activity of ethanol and acetone extracts of eight green seaweeds species Caulerpa scalpelliformis, Codium iyengarii, C. shameelii, Enteromorpha compressa, Udotea indica, Ulva fasciata, U. lactuca and Valoniopsis pachynema against diarrhea and multidrug-resistance E. coli and S. typhi.

The average MICs values of tested green seaweeds both ethanol and acetone extract for tested bacteria E. coli and S. typhi ranged from 5mg/ml to 30mg/ml. While the average MBCs values of tested green seaweeds both ethanol and acetone extract for both tested bacteria E. coli and S. typhi ranged from 10mg/ml to 40mg/ml. To the best of my knowledge, this is the first report from Pakistan that studied seaweed containing inhibitory compounds. Data from this study show that green seaweeds are the potential antimicrobial agents against diarrhea causing organisms E. coli and S. typhi.

Keywords: *Antimicrobial activity, seaweeds, diarrhea, E. coli and S. typhi, Minimum inhibitory concentration (MIC) and Minimum bactericidal concentration (MBC)*

Introduction

Wendell Stanley was the first scientist who was inspired by virus when he worked on tobacco plant. A virus is a submicroscopic parasite that reproduce not by itself it replicates inside the living cell of any plants and animals even bacteria. They are microscopic, single-celled organisms that grow in different environments. They also live in human body.

Bacterial infection in plants, animals and humans are increasing day by day due to resistance developed by the bacteria [1]. Therefore alternative prevention and treatment are implied and new antimicrobial agents are being searched for the controlling of these infectious diseases from which mortality rates are increasing [2]. Diarrhea is also one of the major diseases linked with climate change [3]. Global climate change increase the global temperature, modified the quantity and timing of precipitation, rainfall patterns and raises sea level. These changes lead to flood especially in coastal areas of developing countries. These changes have great effect on health of million peoples.

MIC is defined as the lowest concentration of an antimicrobial that prevents the growth of a

microorganism following a specific incubation period. MIC is mostly used as a research tool to determine in-vitro activity of new antimicrobials whereas MBC is define as the lowest concentration of antibacterial agent needed to kill 99.9% of the initial inoculums. MBC determination is mostly reserved for accuracy of dosage of drug which will contribute to the treatment of infection [4]. Seaweeds are those marine plants that are used today as a one new and promising source of bioactive compounds in the field of medical research. *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* causing the infectious diseases like diarrhea and typhoid fever, mastitis, abortion and upper respiratiory tract complications [5]. Most of the seaweeds have been studied several times and Continuous research work is being carried out to determine many substances such as antibacterial, antiviral, antitumor, antifungal, antiprotozoa, mosquito and larva control and their broad spectrum antimicrobial effectiveness [6,7].

Indira et al. [8] confirmed the potential use of green seaweed *Halimeda tuna* extracts as a source of antimicrobial compound. Choi et al. [9] determined the level of MIC and MBC in fifty-seven species of common seaweed from the Coast of Korea.

The green seaweeds that are rich and have large diversity in coastal areas of Pakistan, approximately 200 genera and 500 species of marine plants reported by different authors Shameel and Tanaka [10] Qari and Khan [11], Qari, [12], Qari et al. [13], Shameel, Khan & Afaq-Hussain [14] Qari and Qasim [15], Qari and Qasim [16] The abundance of these species in Pakistan coast offers a great opportunity for researchers to look into their potential bioactive compounds. Thus, antibacterial activity of eight species of Chlorophyceae against two pathogenic bacteria (*E. coli and S. typhi*) were studied for the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) in present investigation. This study is initiated to assess the seaweed potential as a source of therapeutic agent that could be used clinically and industrially for future exploitation.

Materials and Methods

Total eight species of green seaweed were collected from Buleji, seven from Paradise Point and six from Manora site of Karachi coast for the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against diarrhea causing bacteria (*E. coli* and *S. typhi*). Gentamicin, Erythrocin, Ceftrioxone and Cefaxime were used as a control. Gentamicin is active against a wide range of bacterial infections mostly gram negative bacteria including *Pseudomonas*, *Proteus, E. coli*, *Klebsiella pneumonia*, *Enterobacter aerogenes*, *Serratia* and gram positive *Staphylococcus* [17]. Erythrocin, Ceftrioxone and Cefixime were also killed bacteria (*E. coli*) with a higher potency. The two reagents ethanol and acetone were used for the extractions of seaweed species. MIC was determined by macrodilution agar method and MBC determined by broth methods [18, 19]. Both MIC and MBC values were recorded as mg/Land each treatment was performed in triplicate.

Results and Discussion

The efficacies of eight therapeutic agents for controlling bacterial diarrhea disease were evaluated.against *E. coli* and *S. typhi*. MIC of tested green seaweeds extracts ranging from 5mg/ml to 30mg/ml. Among the ethanol extractions of green seaweed the lowest MIC value of 10 mg/ml was recorded in *Caulerpa scalpelliformis* (Buleji and Manora), *Enteromorpha compressa* (Buleji), *Codium shameelii*, *Ulva lactuca* (Paradise Point) and *Ulva fasciata* (Manora) *against E. coli and* the lowest MIC value 5mg/ml against *S. typhi* recorded in *C. scalpelliformis* (Paradise Point) (Tables 1-3). In acetone extractions, the lowest MIC 5mg/ml was recorded in *C. iyengarii* (Buleji) against *E. coli* and in *U. fasciata* (Buleji) and *C. iyengarii* (Manora) against *S. typhi* (Tables1 and 3).

MBC of tested seaweeds extracts ranging from 10mg/ml to 40mg/ml. Highest MBC value (40mg/ml) was recorded in the ethanol extraction of *Udotea indica* and *U. lactuca* (Buleji), *Udotea indica* (Paradise Point), *C. shameelii* and *Valoniopsis pachynema* (Manora) against *E. coli* whereas against *S. typhi* the highest MBC value was recorded in *Codium iyengarii* (Buleji), *C. shameelii* (Buleji and Manora), *Udotea indica* and *Ulva fasciata* (Paradise Point) (Tables1 and 3).

In acetone extractions, the highest MBC (40mg/ml) was recorded in *Ulva fasciata* (Buleji), *C. shameelii* and *Enteromorpha compressa* (Paradise Point) and *Caulerpa scalpelliformis* (Manora) against *E. coli* whereas against *S. typhi* the highest MBC value

(40mg/ml) recorded in *Udotea indica* (Buleji), *Codium iyengarii* and *Enteromorpha compressa* (Paradise Point) and *C. shameelii* (Paradise Point and Manora) (Tables1 and 3).

Table1. *Minimum inhibitory concentration and minimum bactericidal concentration of green seaweed extracts collected from Buleji against E. coli and S. typhi.*

S. No.	Name of Extracts	MIC (mg/ml)		MBC (mg/ml)	
		E. coli	*S. typhi*	*E. coli*	*S. typhi*
1	Control	0	0	0	0
2	Gentamicin sulfate	5	5	40	40
3	Erythromycin	5	5	40	40
4	Ceftriaxone sodium	5	5	40	40
5	Cefotaxime sodium	5	5	40	40
Ethanolic Extracts					
1	*Caulerpa scalpelliformis*	10	15	20	20
2	*Codium iyengarii*	20	30	30	40
3	*C. shameelii*	20	30	30	40
4	*Enteromorpha compressa*	10	15	20	20
5	*Udotea indica*	15	10	40	30
6	*Ulva fasciata*	30	15	30	25
7	*U. lactuca*	25	10	40	30
8	*Valoniopsis pachynema*	20	10	30	15
Acetone Extract					
1	*Caulerpa scalpelliformis*	10	25	20	30
2	*Codium iyengarii*	5	10	10	20
3	*C. shameelii*	10	15	20	30
4	*Enteromorpha compressa*	20	10	30	20
5	*Udotea indica*	15	30	20	40
6	*Ulva fasciata*	20	5	40	15
7	*U. lactuca*	10	25	20	30
8	*Valoniopsis pachynema*	10	25	20	30

Table2. *Minimum inhibitory concentration and minimum bactericidal concentration of green seaweed extracts collected from Paradise Point against E. coli and S. typhi.*

S. No.	Name of Extracts	MIC (mg/ml)		MBC (mg/ml)	
		E. coli	*S. typhi*	*E. coli*	*S. typhi*
1	Control	0	0	0	0
2	Gentamicin sulfate	5	5	40	40
3	Erythromycin	5	5	40	40
4	Ceftriaxone sodium	5	5	40	40
5	Cefotaxime sodium	5	5	40	40
Ethanolic Extracts					
1	*Caulerpa scalpelliformis*	15	5	30	10
2	*Codium iyengarii*	30	10	20	20
3	*C. shameelii*	10	10	20	20
4	*Enteromorpha compressa*	15	10	30	30
5	*Udotea indica*	20	30	40	40
6	*Ulva fasciata*	20	30	30	40
7	*U. lactuca*	10	25	20	30
8	*Valoniopsis pachynema*	20	20	30	20

Acetone Extract					
1	*Caulerpa scalpelliformis*	15	15	20	30
2	*Codium iyengarii*	20	30	30	40
3	*C. shameelii*	30	25	40	40
4	*Enteromorpha compressa*	30	30	40	40
5	*Udotea indica*	10	15	20	30
6	*Ulva fasciata*	10	20	20	30
7	*U. lactuca*	10	10	15	25
8	*Valoniopsis pachynema*	20	15	20	30

Table3. *Minimum inhibitory concentration and minimum bactericidal concentration of green seaweed extracts collected from Manora against E. coli and S. typhi.*

S. No.	Name of Extracts	MIC (mg/ml)		MBC (mg/ml)	
		E. coli	*S. typhi*	*E. coli*	*S. typhi*
1	Control	0	0	0	0
2	Gentamicin sulfate	5	5	40	40
3	Erythromycin	5	5	40	40
4	Ceftriaxone sodium	5	5	40	40
5	Cefotaxime sodium	5	5	40	40
Ethanolic Extracts					
1	*Caulerpa scalpelliformis*	10	20	20	30
2	*Codium iyengarii*	15	10	30	25
3	*C. shameelii*	30	30	40	40
4	*Enteromorpha compressa*	20	10	25	20
5	*Udotea indica*	15	20	20	40
6	*Ulva fasciata*	10	15	20	20
7	*U. lactuca*	10	20	20	30
8	*Valoniopsis pachynema*	25	30	40	30
Acetone Extract					
1	*Caulerpa scalpelliformis*	30	10	40	20
2	*Codium iyengarii*	10	5	20	10
3	*C. shameelii*	20	30	25	40
4	*Enteromorpha compressa*	10	15	20	20
5	*Udotea indica*	15	25	30	40
6	*Ulva fasciata*	20	25	30	30
7	*U. lactuca*	10	20	20	20
8	*Valoniopsis pachynema*	15	10	20	20

By comparing the MIC and MBC values obtained from all studied species of seaweed collected from four different coasts, the species of seaweed collected and evaluated in this present study showed better bacteriostatic potential. A great variation was observed in the MIC and MBC value in seaweeds collected from four different coasts. Chiao-Wei et al. [20] reported in their experimental study that these variations may be due to the physical environmental parameters such as salinity, dissolve oxygen, temperature, light, nutrients, depth and pH. The effects of these abiotic characteristic on the metabolic activities of the seaweeds resulted to this better bacteriostatic potential found in most of the studied seaweed [21]. The result of MIC values obtained in this present study is not in agreement with the data recorded by Choi et al. [9] and Chiao-Wei et al. [20]. The green seaweeds evaluated in this present study are commonly used as human food, fodder and in pharmaceutics in developed and developing countries [22].

As reported by Andrew et al. [23] MIC assay is used to judge the performance of all other methods of susceptibility testing and are used in diagnostic laboratories to give a definitive answer when a borderline result is obtained by other methods

of testing or when disc diffusion methods are not appropriate. MIC and MBC assays were conducted to assess the bacteriostatic and bactericidal concentration of the seaweed extracts against the respective pathogenic bacterial strains. The lower the MIC and MBC value, the higher the antibacterial potential of the plant extracts.

Conclusion

It is concluded that antibacterial activity of the seaweeds varies according to their species and extraction solvents. The ethanol and acetone extract of green seaweed exhibited promising bacteriostatic activity against *E. coli* and *S. typhi*. This is due to the presence of higher potential of antimicrobial compounds in studied seaweeds. It is also noted that the sites of Karachi (Buleji, Paradise Point and Manora) have variety of marine plants of potential source that can be used as a medicine clinically and industrially for future exploitation.

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