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ORIGINAL ARTICLE

ANTIMICROBIAL ACTIVITY OF SOME SEAWEED EXTRACTS AGAINST HUMAN PATHOGENS

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ABSTRACT

The current investigation was carried out to evaluate the antimicrobial potential of some seaweed extract commonly found in the coastal area of Karachi, Pakistan. Eight different species of seaweeds were used in the present study including two members of chlorophycota (*Chaetomorpha antennina* and *Codium iyengarii*), four members of phaeophycota (*Colpomenia sinousa*, *Iyengaria stellata*, *Padina tetrastromatica* and *Stoechospermum marginatum*) and two members of rhodophycota (*Gracilaria corticata* and *Sloeiria robusta*). Antimicrobial assessment of these extracts was performed by disc diffusion method against common human pathogens including gram negative bacterium (*Escherichia coli*), gram positive bacterium (*Staphylococcus aureus*) and fungal strain (*Candida albicans*). The experimental results reveal that the extract and colorant yield percentage were maximum in red seaweed, mediocre with brown seaweed and least in green seaweeds samples. The extract form *Iyengaria stellata* was highly effective against *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus*. The present finding reveals that the tested seaweed extracts were highly active against fungal strain than bacteria. The utilization of these seaweed extract could be used a promising source of pharmaceutical agent.

Keywords: Green seaweed, Brown seaweed, Red seaweed, Antimicrobial activity, Statistical analysis.

INTRODUCTION

Marine organisms are a rich source of active compounds, therefore, gaining interest in the development of new therapeutic drugs. Several antimicrobial compounds have been studied in marine organisms than those in the terrestrial one (Ireland *et al.,* 1988). Marine organisms are also a rich source of bioactive natural compounds with variety of pharmacological and biological activities (Schwartsmann *et al.,* 2001). Among the marine organisms, the marine algae or seaweed has been recognized as a source of bioactive compounds. Seaweeds also exhibits variety of pharmacological activities including antiviral, anti-oxidant, anticoagulant anti-inflammatory, antibacterial, antifungal and anticoagulant properties (Tuney *et al.,* 2006; Patra *et al.,* 2008). Moreover, these bioactive compounds also exhibit antimicrobial resistance against pathogenic bacteria (Kolanjinathan *et al.,* 2009). The marine seaweed or algae shows the antimicrobial activity due to their variety of bioactive compounds like terpenoids, aminoacids, acrylic acid, phenolic compounds, steroids, phlorotannins, halogenated ketones and alkanes fatty acids and cyclic polysulphides (Watson and Cruz-Rivera, 2003). Bioactive compounds in marine seaweeds also incorporated with medicines and pharmacotherapy while other compounds

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have bacteriostatic and bactericidal properties (Gorban *et al.,* 2003). Different diseases were treated with antibiotics, extracted from marine seaweed which were used as therapeutic agents along with commercial values (Smit, 2004).

Seaweed or algae are the abundantly available photosynthetic organisms distributed both in fresh and marine water environments. Seaweeds possess variety of photosynthetic pigments such as chlorophylls, carotenoids, phycobilins, phycocyanin and phycoerythrin and are classified them on the basis of these pigments into different groups, green seaweed has photosynthetic pigment chlorophyll b (El-Khatib *et al.,* 2016), fucoxanthin and carotenoids found in brown seaweed (Miyashita 2009; Peng *et al*. 2011) and red seaweeds have *β*-carotene and zeaxanthin as dominant photosynthetic pigment (Schubert *et al.,* 2006). Besides these pigments a rich source of structurally diverse secondary metabolites also found. These secondary metabolites develop a defence mechanism against herbivores, fouling organisms and pathogens, play and important role in protection from harmful UV radiation and also act as allelopathic agents (Hay, 1996; Watson and Cruz-Rivera, 2003).

The aim of current investigation is to evaluate the antimicrobial activity of some seaweeds extract against common human pathogens which were commonly found in hospitals and causes several diseases in human. Therefore, the utilization of seaweed extract could be used as effective alternative to new therapeutic drugs as well as promising source of pharmaceutical agent.

MATERIALS AND METHODS

Seaweed collection

Seaweed samples of green (*Chaetomorpha antennina* and *Codium iyengarii*), brown (*Colpomenia sinousa*, *Iyengaria stellata*, *Padina tetrastromatica* and *Stoechospermum marginatum*) and red (*Gracilaria corticata* and *Solieria robusta*) were collected from the Buleji costal area of Karachi, Pakistan in low tidal conditions during a period of October-March, 2015-16. Repeated attempts were performed to collect the desired samples in bulk amount. The collected samples were washed with tap water to remove unnecessary foreign particles and epiphytes attached on them than shade dried at room temperature. The dried specimen was ground into fine powder and utilize for extraction of natural colorant. The entire thalli of each specimen were mounted on herbarium sheets for identification purpose and their voucher specimens were deposited in Dr. Muhammad Afzal Hussain Qadri - Biological Research Centre (MAHQ-BRC) table I shows the seaweed name and their abbreviations. All the Samples were identified in Department of Botany, University of Karachi. Fig. 1 shows the different type of red, brown and green seaweeds.

Extraction of natural colorants form seaweed samples

Extraction of natural colorants were performed in Memmert 854 Schwabach water bath in basic medium. The extraction process was performed in Erlenmeyer flask using sodium hydroxide as solvent having material to liquor ratio of 20:1 at 80°C for 3 hrs. The mouth of flask was close with aluminum foil to prevent excess evaporation of extract. After completing the extraction process, flask were allowed to cool at room temperature and filtrate the extract by using a small piece of cotton. The filterate extract were kept on water bath at low temperature until a gummy paste obtained. The crude extract was used for antimicrobial assessment against desired test organisms. The extract and colorant yield of each extract were calculated by the following formula (Venkatasubramanian *et al.,* 2009),

$$Extract\ yield\ \% = \frac{Total\ extract\ obtained\ (G)}{Amount\ of\ seaweed + solvent\ used} \times 100$$

$$Colorant\ yield\ \% = \frac{Crude\ dye\ obtained\ (G)}{Amount\ of\ seaweed\ used} \times 100$$

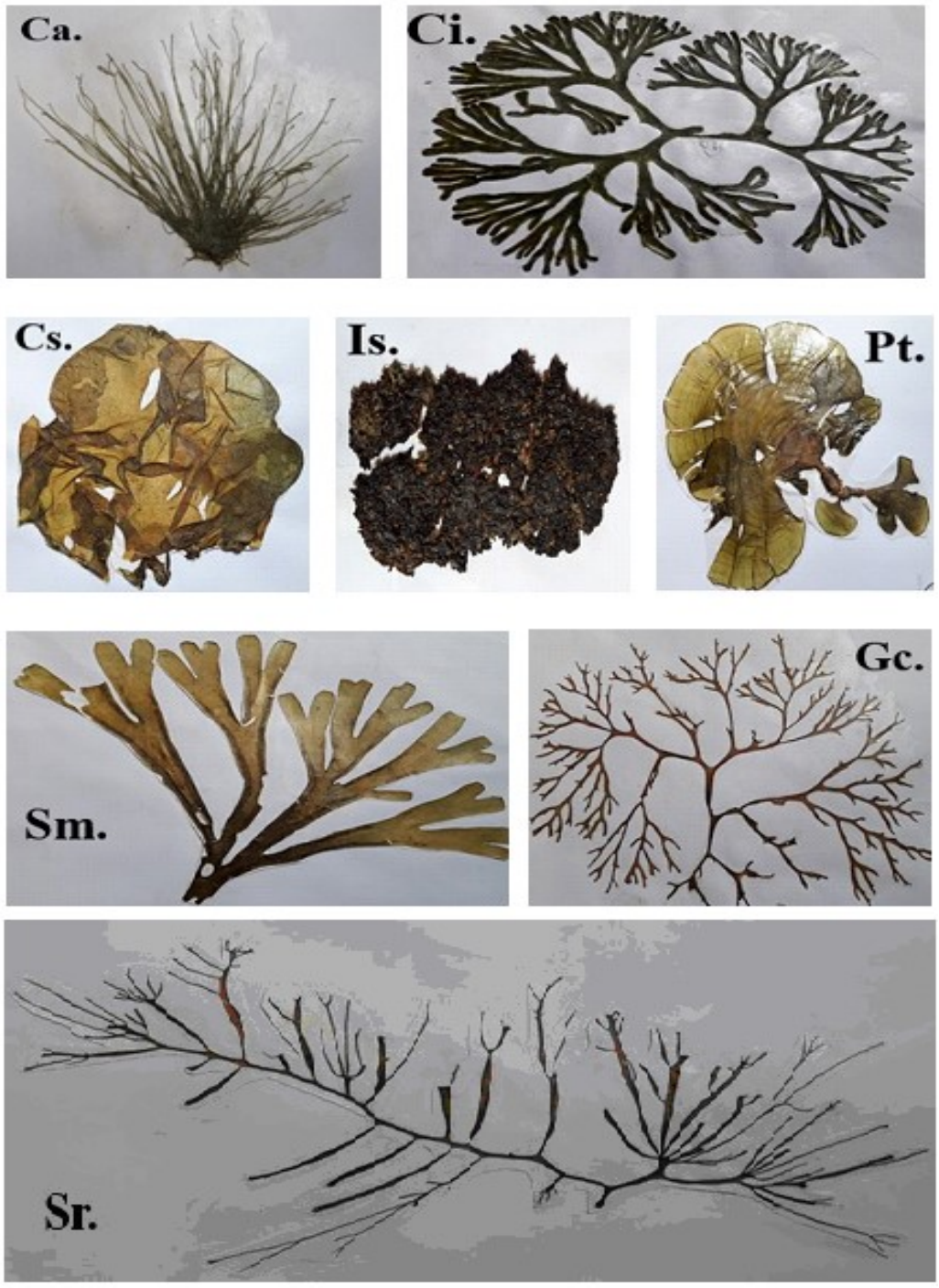

Fig. 1: Photograph of herbarium of *C. antennina* (Ca.), *C. iyengarii* (Ci.), *C. sinousa* (Cs.), *I. stellata* (Is.), *P. tetrastromatica* (Pt.), *S. marginatum* (Sm.), *G. coticata* (Gc.) and *S. robusta* (Sr.).

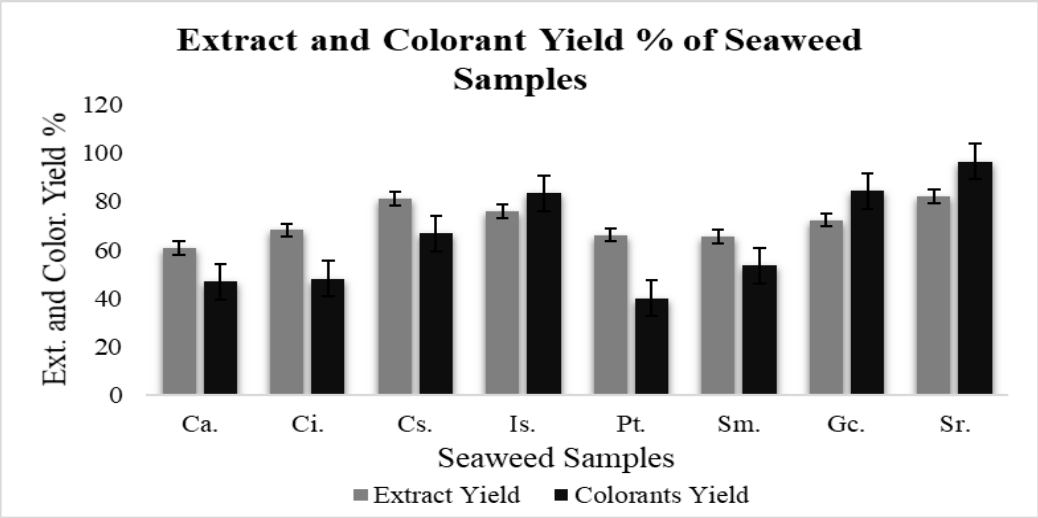

Fig. 2: Extract and colorant yield percentage of different seaweeds samples.

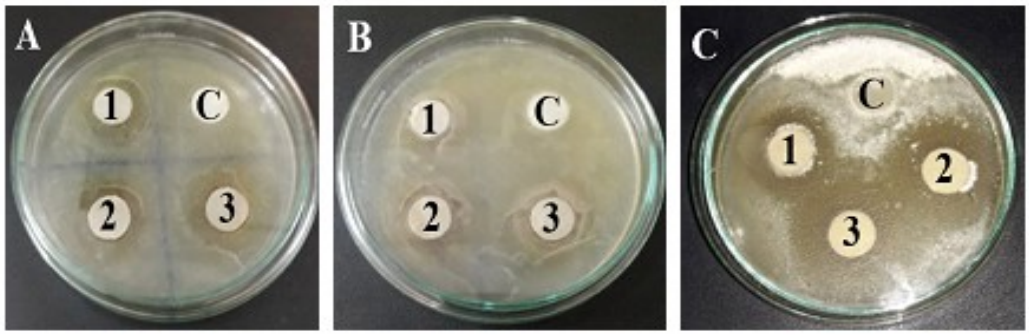

Fig. 3: Antimicrobial activity of brown seaweed *Iyengaria stellata* extract against *E. coli* (Panel-A), *S. aureus* (Panel-B) and *C. albicans* (Panel-C).

Table 1: Seaweed samples and their abbreviations used in this experiment

S. No.	Seaweed Name	Abbreviation
	Green seaweed	
1	*Chaetomorpha antennina*	Ca.
2	*Codium iyengarii*	Ci.
	Brown seaweed	
3	*Colpomenia sinousa*	Cs.
4	*Iyengaria stellata*	Is.
5	*Padina tetrastromatica*	Pt.
6	*Stoechospermum marginatum*	Sm.
	Red seaweed	
7	*Gracilaria coticata*	Gc.
8	*Soleria robusta*	Sr.

Table 2a: Antimicrobial assessment of seaweed extract against *E. coli*

Seaweed Name	Zone of Inhibition (Mean ± SEM)		
	1 g	2 g	3 g
Chaetomorpha antennina	14.86 ± 0.352^{b}	15.96 ± 0.317^{cd}	16.03 ± 0.145^{cd}
Codium iyengarii	16.06 ± 0.176^{cd}	16.96 ± 0.260^{de}	17.96 ± 0.202^{ef}
Padina tetrastromatica	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
Iyengaria stellata	21.36 ± 1.172^{h}	24.10 ± 0.264^{i}	27.03 ± 0.606^{j}
Colpomenia sinousa	18.50 ± 0.360^{f}	20.00 ± 0.288^{g}	23.93 ± 0.233^{i}
Stoechospermum marginatum	15.00 ± 0.230^{bc}	16.00 ± 404^{cd}	17.86 ± 0.185^{ef}
Solieria robusta	15.03 ± 0.202^{bc}	17.03 ± 0.145^{de}	17.00 ± 0.173^{de}
Gracilaria corticata	13.96 ± 202^{b}	16.03 ± 0.145^{cd}	17.90 ± 0.264^{ef}

Table 2b: Antimicrobial assessment of seaweed extract against *S. aureus*

Seaweed Name	Zone of Inhibition (Mean ± SEM)		
	1 g	2 g	3 g
Chaetomorpha antennina	14.83 ± 0.328^{c}	16.96 ± 0.317^{e}	21.03 ± 0.317^{i}
Codium iyengarii	0 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
Padina tetrastromatica	0 ± 0^{a}	14.00 ± 0.346^{b}	15.90 ± 0.378^{d}
Iyengaria stellata	19.06 ± 0.233^{g}	23.00 ± 0.288^{i}	25.96 ± 0.317^{j}
Colpomenia sinousa	15.03 ± 202^{c}	17.96 ± 0.260^{f}	22.00 ± 0.230^{h}
Stoechospermum marginatum	15.00 ± 0.288^{c}	15.83 ± 0.328^{d}	16.06 ± 0.176^{d}
Solieria robusta	16.10 ± 208^{d}	18.00 ± 0.230^{f}	20.00 ± 0.288^{h}
Gracilaria corticata	15.60 ± 0.378^{cd}	16.06 ± 0.176^{d}	17.96 ± 0.317^{f}

Table 2c: Antimicrobial assessment of seaweed extract against *C. albicans*

Seaweed Name	Zone of Inhibition (Mean ± SEM)		
	1g	2g	3g
Chaetomorpha antennina	17.93 ± 0.176^{e}	21.03 ± 0.260^{i}	24.96 ± 0.375^{l}
Codium iyengarii	14.93 ± 00.176^{b}	14.93 ± 0.405^{b}	$16.93 \pm 0.0.233^{d}$
Padina tetrastromatica	0 ± 0^{a}	17.86 ± 0.296^{e}	19.06 ± 0.233^{f}
Iyengaria stellata	22.03 ± 0.260^{j}	19.97 ± 0.260^{h}	28.10 ± 0.152^{m}
Colpomenia sinousa	15.06 ± 0.176^{b}	17.93 ± 0.233^{e}	20.70 ± 0.360^{hi}
Stoechospermum marginatum	15.10 ± 208^{bc}	17.96 ± 0.202^{e}	19.96 ± 0.317^{h}
Solieria robusta	18.00 ± 0.230^{e}	20.06 ± 0.176^{h}	24.13 ± 0.352^{k}
Gracilaria corticata	15.13 ± 0.352^{bc}	15.90 ± 0.321^{c}	17.96 ± 0.317^{e}

Test organisms

The antimicrobial potency of each seaweed extract was evaluated using bacterial strains of *Staphylococcus aureus* (gram positive), *Escherichia coli* (gram negative) and fungal strain of *Candida albicans* causing diseases in human. The bacterial and fungal strains were provided from the culture collection of Botany and Agriculture Departments, University of Karachi.

Inoculum and media preparation

The gram positive and gram negative bacterial strains were sub-culture in Nutrient agar medium overnight at 37°C in Laminar flow hood chamber under sterilized condition. Nutrient agar medium were purchase from Merck KGaA and prepared in 1 L Erlenmeyer flask containing 20 g medium powder and dissolved in 100 mL sterilized distilled water, allow to heat the medium flask on low heat at water bath until a clear solution obtained.

Maintained the desired volume of flask to 1 L by adding sterilized water. The mouth of flask was close with cotton plug and aluminum foil. Autoclave the prepared medium at 15 lbs. pressure at 121°C for 15 minutes.

Similarly, the fungal strain was prepared form the 72 hrs culture of the fungal isolate in Potato dextrose medium at 37°C in Laminar flow hood chamber under sterilized condition. Potato dextrose medium was purchased from Merck KGaA and prepared by following the same procedure as mention above. 39 g powder medium were prepared in 1 L sterilized distilled water and allowed to heat until a clear solution obtained. The autoclave medium was mixed well and pour onto sterilized petri plates (15 mL/plate) in laminar flow hood chamber and allowed to solidify it. The solidified media plates were used for antimicrobial screening of natural extract.

Antimicrobial screening of seaweed extract

Antimicrobial screening of extracts of different seaweeds was performed by disc diffusion method in solidified media plates. The seaweed extract residues of 1, 2 and 3g were re-dissolved in 65 mL sterilized distilled water. One loop full of test organisms were used with sterilized distilled water and inoculated on the solidified media plates. Sterilized Whatman-3 filter paper disc of 12 mm diameter was prepared by treating with 1, 2 and 3 g natural extract for 30 min and air dried in laminar flow hood chamber. Treated disc with natural extracts of seaweeds were placed in intimate contact with solidified medium which has been previously inoculated with test organisms. The plates thus prepared were left for 15 minutes allowing the diffusion of the extract into the medium. Then the plates were incubated 18-24 hrs. for bacterial and 72 hrs. for fungal assessment at 37°C. Triplicate set of each experiment were performed a control set was run along with. After incubation, antimicrobial potency of natural extracts were detected by measuring the zone of uninterrupted growth along the edge of disc. Distilled water were uses as solvent in our study and was employed as control.

STATISTICAL ANALYSIS

The zone of inhibition of each extract were examined statistically by using SPSS (Statistical package for social sciences) version 20. Each experiment was carried out in triplicate manner and the in triplicate manner and the zone of inhibition of treated and control were recorded in mean ± Standard error of means (SEM). One-way analysis of variance studies (ANOVA) were carried out to find out the difference ($P < 0.05$) among the antimicrobial activity of different seaweed extracts. Data has been examined with one way of ANOVA - Duncan's multiple range test, which shows that the mean difference is highly significant.

RESULTS AND DISCUSSION

Seaweed extract and colorant yield ercentage

The extract and colorant yield percentage of the employed seaweed are illustrated in fig. 2. The extract and colorant yield percentage were highest in red seaweed samples while it is mediocre in brown seaweed and least in green seaweed. The maximum colorant yield percentage of red seaweed *Gracilaria corticata* and *Solieria robusta*) due to presence of agar and carrageenan as cell water material in red seaweed and alginate in brown seaweed (*Colpomenia sinousa, Iyengaria stellata* and *Stoechospermum marginatum*) except Padina tetrastromatica because of less percentage of alginate in their cell wall composition while the green seaweed has no hydrocolloidal cell wall material and only comprises of cellulose. The effectiveness of extract and colorant yield can be expressed as *Solieria robusta* > *Gracilaria corticata* > *Iyengaria stellata* > *Colpomenia sinousa* > *Stoechospermum marginatum* > *Codium iyengarii* > *Chaetomorpha antennina* > *Padina tetrastromatica.*

Antimicrobial assay of seaweed extract

Evaluation of antimicrobial activity of eight seaweed extract was determined initially by disc diffusion method against human pathogens and the results are represented in

Table IIa, IIb and IIc. Zone of inhibition were increases as the amount of dye increases from 1, 2 and 3 g. The current study reveals that all seaweed extracts used in this investigation exhibited a varying degree of antimicrobial activity against all tested organisms. Experimental results showed that *Iyengaria stellata* was most effective among the eight seaweeds extract tested. Moreover, it possesses the highest antifungal activity against *C. albicans* and bacterial activity towards *S. aureus* (fig. 3).

Besides, *Codium iyengarii*, *Colpomenia sinousa* and *Stoechospermum* marginatum was found to be more effective against gram negative bacterium than gram positive. *Padina tetrastromatica* shows no activity against *E. coli* and *Codium iyengarii* towards gram positive bacterium S aureus.

The extract of *Solieria robusta*, *Gracilaria corticata* and *Chaetomorpha antennina* was found to be effective against gram positive bacterium than gram negative. The cell wall of gram negative bacterium composed of LPS that evades the saturation of antimicrobial agents on the cell membrane (Rabe and Staden, 1997; Ali-Shtayeh *et al*., 1998; Bezi, *et al*., 2003).

All the seaweed extract shows antifungal activity against *C. albicans*. Natural extract of seaweed possesses an effective antifungal activity than antibacterial.

The effectiveness of antifungal activity of eight seaweed samples can be express as *Iyengaria stellata* > *Colpomenia sinousa* > *Chaetomorpha antennina* > *Solieria robusta* > *Stoechospermum marginatum* > *Padina tetrastromatica* > *Gracilaria corticata* > *Codium iyengarii*. Distilled water were used as a solvent during the course of study and it shows no zone of inhibition against test organisms, therefore it is effectively concluded that the whole activity due to the dye molecules.

CONCLUSION

Our current investigation expresses that seaweed have a great potential as antimicrobial compound against human pathogens. Thus, they can be used in formation of antibiotic for treatment of infectious disease caused by pathogenic micro-organisms. The extract and colorant yield percentage of red seaweed higher than brown and green seaweed. Moreover, the brown seaweed possesses the higher antimicrobial potential than green and red seaweed. The natural extract of *Iyengaria stellata* exhibits the more effective antibacterial and antifungal activity than other extract. These findings indicate that the seaweed extract could be used for treatment of infectious disease caused by pathogenic microorganisms.

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