Research Article

Extraction of Marine Polyketides from Different Seaweeds and Analyzing its Antibacterial and Anti-Cancer Activity

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ABSTRACT

Aim: Three different seaweeds were collected in the present study and aimed to investigate its antibacterial activity against human bacterial pathogens and anticancer activity against human breast cancer cell lines (MCF-7) respectively.

Methods: Three seaweeds were collected and its bioactive compounds were extracted using soxhlet method to determine the minimal inhibitory concentration (MIC) and antibacterial activity against different pathogenic bacteria. Anti-cancer activity of the extracts in breast cancer cell lines (MCF-7) cells using MTT assay method was investigated.

Findings: MIC of all three seaweed extracts was found to be 300µg/ml with the inhibitory zones ranging from 8.6 ± 0.75mm to 16.3 ± 1.05mm against the respective bacteria *Escherichia coli* and *Staphylococcus aureus.* Antibacterial activity of three different seaweed extracts showed significant inhibitory zones ranging from 13.3 ± 1.05mm to 16.9 ± 0.57mm against the respective bacteria B1 - *Escherichia coli,* B2 - *Staphylococcus aureus,* B3 - *Staphylococcus epidermidis,* B4 - *Klebsiella pneumoniae,* B5 - *Enterobacter* sp. Anticancer activity test results revealed IC_{50} value of about 43.5µg/ml for the brown seaweed extracts after testing in MCF-7 cell lines. Increase in concentration of samples increased the cell cytotoxicity and proliferation of cancer cells were inhibited in a dose dependent manner.

Conclusion: Thus, the obtained results would contribute more and induce interest for the development of different medically significant seaweed farming and biotechnological use with growing interest.

Keywords: Marine Polyketides, seaweeds, antibacterial activity, breast cancer.

INTRODUCTION

Marine algae such as seaweeds harbor endophytes, like their terrestrial counterparts, which are a potential source of new secondary metabolites[1]. The natural products, especially in the field of anticancer drug development, has given importance to marine sources, particularly seaweeds. Anticancer compounds from seaweeds can induce cancer cell death via various signaling pathways and mechanisms[2]. Brown seaweeds are more highly enriched with polysaccharides and attracted great attention owing to their therapeutic effects on cancers.

Several studies have discussed the natural products from seaweeds and their anticancer potentials, as well as the signaling pathways involved in anticancer activity. *Fucus vesiculosus* contain a polysaccharide fucoidan, which showed anticancer activity by inducing apoptosis of colorectal cancer HCT-116 cells[3]. Fucoidan are the bioactive compounds of brown seaweeds act as an anticancer agent through various signaling pathways, including cell cycle arrest, apoptosis[4]; and anti-angiogenesis by inhibiting vascular endothelial growth factor formation, and natural killer cell activation[5]. Polar extract of *Sargassum oligocystum* inhibited the proliferation of leukemia cancer cells; and heterofucan of *Sargassum filipendula* showed the effect of anti-proliferation against cervical and prostate cancer cells[6].

Seaweeds are responsible for producing large number of secondary metabolites[7]. Many research articles cited those extracts from seaweeds contains divergent activities such as, anti-cancer or anti-tumor, antibacterial[8], antifungal, antiprotozoal[9], antiviral, antioxidant[10] and cytotoxic activity [11]. Significantly, antibacterial studies were reported by several researchers[12, 13, 14]. Bio-active compounds of seaweed extracts reported to be attributed for the presence of Lyengaroside, diterpene-benzoate, diterpenebenzoic acids, callophycols and eicosanoids[15].

Based on these citations, three different seaweeds were collected in the present study and aimed to investigate its antibacterial and anticancer activity. Following objectives were framed to fulfil the aim of the present research. To collect seaweed and extract the bioactive compounds from different types of seaweeds (Green, Red and brown seaweeds). To determine the minimal inhibitory concentration and antibacterial activity of extracts against different human pathogenic bacteria. To investigate the anti-cancer activity of seaweed extract in breast cancer cell lines (MCF-7) cells using MTT assay methods.

MATERIALS AND METHODS

Collection of seaweeds

The seaweeds (Green seaweed (*Ulva reticulata*), Brown seaweed (*Padina tetrastromatica*) and Red seaweed (*Kappaphycus terminara*)) were collected fresh from Tuticorin (Lat 8 45' N; Long 78 10'E), South east coast of

India. The seaweed samples were transported to the laboratory in sterile polythene bags immediately after collection for further analysis.

Soxhlet extraction of Seaweeds[16, 17]

To extract the content from the seaweed, the Soxhlet method, which adheres to the infusion method's principles, was selected. The *seaweed* sample, which was finely pulverized, was put in a porous bag or "thimble" made of sturdy filter paper or cellulose, and then placed in the thimble chamber. The heat from the bottom flask causes the extraction solvent to evaporate into the sample thimble, condense in the condenser, and drip back. The process continues once the liquid is drained into the bottom flask when it reaches the siphon arm. The thimble was filled with powdered herbs, and it was then placed in the soxhlet extractor. An ethanol solvent solution was added to the extractor, which was heated to 60°C and left for six hours. Up to 100°C, the temperature was raised gradually and steadily. The extract from the thimble was collected by going via a side arm tube and into the round bottom flask housed in the heating mantle below.

Minimal Inhibitory Concentration seaweed extracts[18]

MIC of seaweed extracts was determined against the test organisms (*Escherichia coli* and *Staphylococcus aureus*) using well diffusion method. A sterile Nutrient broth (g/L) Peptone: 5g, Yeast extract: 5g, Beef extract: 3g, Sodium chloride: 5g, Final pH - 7.0 ± 0.2) was used to inoculate all of the test cultures, and they were then given 24 to 48 hours to proliferate. Plates of sterile Mueller-Hinton Agar (MHA) were made and allowed to set up. Swabs were used to evenly spread 0.1% inoculum suspensions of the test organism over the agar surface in each case. On the agar surface of each plate, 6mm wells were cut while maintaining sterility. Developed WBC fractions containing 100µg/ml, 200 µg/ml and 300 µg/ml was added to each well. Whereas 4µg/ml of gentamycin was added as standard. DMSO was used as negative control. The plates were then incubated at 37°C for 24 hours. The minimum inhibitory concentration (MIC) was determined to be the lowest dilution that expressed significant inhibitory zones against the test bacteria.

Antibacterial activity of seaweed extracts[19]

To determine the antibacterial activity of seaweed extracts against the pyogenic organisms, by the modified Kirby-Bauer method was studied. The antibacterial activity was evaluated against the five test organisms (B1 - *Escherichia coli,* B2 - *Staphylococcus aureus,* B3 - *Staphylococcus epidermidis,* B4 - *Klebsiella pneumoniae,* B5 - *Enterobacter* sp) by disc diffusion method. Sterile Nutrient Agar plates were prepared and allowed to solidify. About 0.1% inoculum suspensions of each of the bacterial cultures were swabbed with the sterile cotton swab three times by turning the plate at 60^{o} angle between each streaking. Under sterile conditions, film size of 20mm in diameter was cut and placed on the agar surface of each Nutrient Agar (NA) plates. Filter paper disc impregnated with standard antibiotic solution (Gentamicin – 4µg/ml) was also placed on the same plate to compare the efficacy of seaweed extracts. All the plates were incubated at 37ºC for 24 - 48h. The antibacterial activity was evaluated in terms of zone of inhibition around the wells of each extract in all the inoculated NA plates. The inhibition clear zones were measured and recorded in millimetre.

Anti-cancer activity of seaweed extracts in MCF-7 breast cancer cell lines[20]

Human breast cancer cell line (MCF-7) was used in the present study to determine the anticancer activity of the brown seaweed extract sample. Cytotoxicity activity of the sample on different concentrations (10, 20, 30, 40 and 50µg/mL) on the cancer cell line was evaluated *in-vitro.* It is based on the use of tetrazolium salt 3-[4,5-dimethylthiazolyl-2]-2,5-diphenyl tetrazolium bromide (MTT), which can be converted to an insoluble blue formazan product by mitochondrial enzymes in viable cells. The cell lines were cultivated in 12-well-microtitre plates to reach confluence growth. The provided samples were applied directly to the developed cell line monolayer. Before cell seeding, the specimens were pre-wetted in 70 % aqueous ethanol solution for 48 h, rinsed twice with ultrapure water and immersed in 1ml DMEM medium in 24-well plates for 2h in an incubator at 37°C. The specimens were then seeded with cell line at 10,000cells per well according to routine cell-culture methods. The plates were incubated at 37°C and 5% CO_2 for fifteen days.

The samples were taken from the 24-well plates and transferred into new plates for the MTT study. The MTT solution was prepared by dissolving the powder in phosphate buffered saline at a concentration 1mg/ml. After 1hr of incubation, the purple crystals were dissolved by adding sodium dodecylsulphate (SDS) in a 1:1 mixture of water and dimethyl formamide (DMF) at a concentration of 20% w/v. After adding 1ml of MTT medium (0.0005mg/ml) to each well, the plates were incubated for 3h, rinsed and desorbed in 100ul of 70% isopropanol. After being agitated rapidly at 400rpm/min for 40min, the dyed medium was transferred to 96-well plate, and read at 550nm. Cell proliferation and cell viability was calculated and observed under inverted microscopy. The difference between the control and sample (treated) cell line images were presented separately.

RESULTS AND DISCUSSION

MIC and MBC of seaweed extracts

Green seaweed

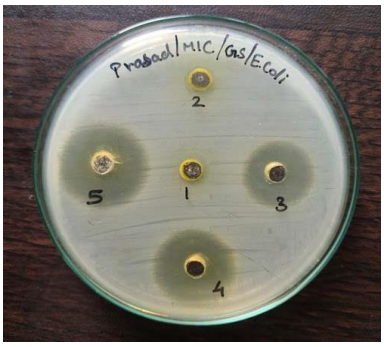
Minimal inhibitory concentration of green seaweed extracts was determined using standard agar well diffusion method. During the analysis, no inhibitory zones were found evident for 100µg/ml and 200µg/ml concentrations against any test bacteria (*Escherichia coli* and *Staphylococcus aureus*). For the concentration 300µg/ml, significant inhibitory zones of about 15.6 ± 0.75mm and

16.3 ± 1.05mm were found evident against both test bacteria and hence this concentration was selected as MIC of green seaweed extracts. The next concentration (400µg/ml) exhibited inhibitory zones of about 17.3 ± 1.05mm and 16.9 ± 0.57mm against the respective test bacteria. Higher concentration (500µg/ml) exhibited inhibitory zones of about 19.6 ± 0.75mm and 19.9 ± 0.57mm against *Escherichia coli* and *Staphylococcus aureus* respectively. In Table-1 and Fig. 1, the inhibitory zones of all seaweed extracts against test bacteria were presented separately.

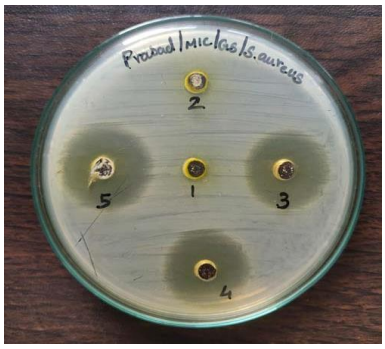
Table 1: MIC of different seaweed extracts against test organisms

Seaweed types	Test Organism	Zone of inhibition (millimetre)				
		1	2	3	4	5
Green	*Escherichia coli*	0 ± 0.0	0 ± 0.0	15.6 ± 0.75	17.3 ± 1.05	19.6 ± 0.75
	Staphylococcus aureus	0 ± 0.0	0 ± 0.0	16.3 ± 1.05	16.9 ± 0.57	19.9 ± 0.57
Brown	*Escherichia coli*	0 ± 0.0	0 ± 0.0	14.6 ± 0.75	16.6 ± 0.75	18.6 ± 0.75
	Staphylococcus aureus	0 ± 0.0	0 ± 0.0	15.9 ± 0.57	16.3 ± 1.05	18.9 ± 0.57
Red	*Escherichia coli*	0 ± 0.0	0 ± 0.0	8.6 ± 0.75	10.3 ± 1.05	13.6 ± 0.75
	Staphylococcus aureus	0 ± 0.0	0 ± 0.0	8.3 ± 1.05	9.9 ± 0.57	12.9 ± 0.57

Figure 1: MIC of green seaweed extracts against test organisms

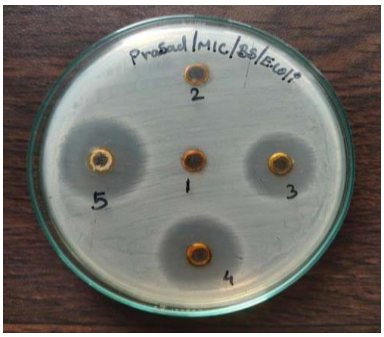


Escherichia coli

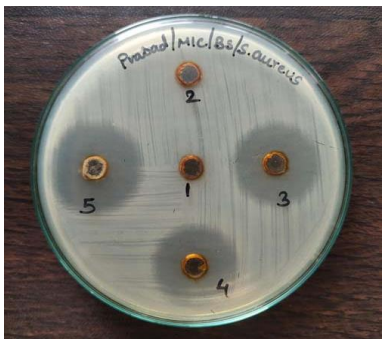


Staphylococcus aureus

Figure 2: MIC of brown seaweed extracts against test organisms

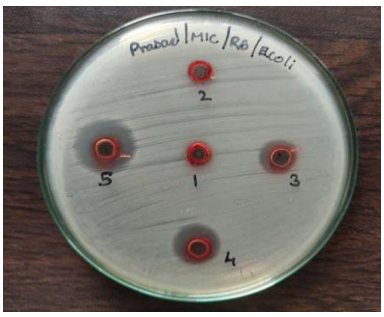


Escherichia coli

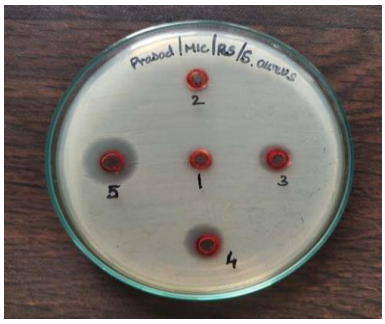


Staphylococcus aureus

Figure 3: MIC of red seaweed extracts against test organisms



Escherichia coli



Staphylococcus aureus

Brown seaweed

Minimal inhibitory concentration of brown seaweed extracts was determined using standard agar well diffusion method. During the analysis, no inhibitory zones were found evident for 100µg/ml and 200µg/ml concentrations against any test bacteria (*Escherichia coli* and *Staphylococcus aureus*). For the concentration 300µg/ml, significant inhibitory zones of about 14.6 ± 0.75mm and 16.6 ± 0.75mm were found evident against both test bacteria and hence this concentration was selected as MIC of brown seaweed extracts. The next concentration (400µg/ml) exhibited inhibitory zones of about 15.9 ± 0.57mm and 16.3 ± 1.05mm against the respective test bacteria. Higher concentration (500µg/ml) exhibited inhibitory zones of about 18.6 ± 0.75mm and 18.9 ± 0.57mm against *Escherichia coli* and *Staphylococcus aureus* respectively. In Table-1 and Fig. 2, the inhibitory zones of all seaweed extracts against test bacteria were presented separately.

Red seaweed

Minimal inhibitory concentration of red seaweed extracts was determined using standard agar well diffusion method. During the analysis, no inhibitory zones were found evident for 100µg/ml and 200µg/ml concentrations against any test bacteria (*Escherichia coli* and *Staphylococcus aureus*). For the concentration 300µg/ml, significant inhibitory zones of about 8.6 ± 0.75mm and 8.3 ± 1.05mm were found evident against both test bacteria and hence this concentration was selected as MIC of red seaweed extracts. The next concentration (400µg/ml) exhibited inhibitory zones of about 10.3 ± 1.05mm and 9.9 ± 0.57mm against the respective test bacteria. Higher concentration (500µg/ml) exhibited inhibitory zones of about 13.6 ± 0.75mm and 12.9 ± 0.57mm against *Escherichia coli* and *Staphylococcus aureus* respectively. In Table-1 and Fig. 3, the inhibitory zones of all seaweed extracts against test bacteria were presented separately.

Antibacterial activity of seaweed extracts

Antibacterial activity of three different seaweed extracts showed significant inhibitory zones against all test bacteria (B1 - *Escherichia coli,* B2 - *Staphylococcus aureus,* B3 - *Staphylococcus epidermidis,* B4 - *Klebsiella pneumoniae,* B5 - *Enterobacter* sp).

Green seaweed

Antibacterial activity of green seaweed extracts showed maximum inhibitory zone of about 14.9 ± 0.57mm against B1-*Escherichia coli,* 13.9 ± 0.57mm against B2 - *Staphylococcus aureus,* 14.3 ± 1.05mm against B3 - *Staphylococcus epidermidis,* 14.9 ± 0.57mm against B4 - *Klebsiella pneumoniae* and 15.3 ± 1.05mm B5 - *Enterobacter* sp. No inhibitory zones were found for negative control (DMSO) for any test bacteria. The obtained values were found significant to retard or prevent the growth of test bacteria using the green seaweed extracts (Table-2; Fig. 4). This was confirmed when compared with inhibitory zones obtained for standard antibiotic tested in parallel against all test bacteria; the standard antibiotic showed slightly better activity ranging from 14.9 ± 0.57mm to 17.9 ± 0.57mm than all the other seaweed extracts.

Brown seaweed

Antibacterial activity of brown seaweed extracts showed maximum inhibitory zone of about 16.9 ± 0.57mm against B1-*Escherichia coli,* 15.9 ± 0.57mm against B2 - *Staphylococcus aureus,* 14.3 ± 1.05mm against B3 - *Staphylococcus epidermidis,* 14.9 ± 0.57mm against B4 - *Klebsiella pneumoniae* and 15.3 ± 1.05mm B5 - *Enterobacter* sp. No inhibitory zones were found for negative control (DMSO) for any test bacteria. The obtained values were found significant to retard or prevent the growth of test bacteria using the brown seaweed extracts (Table-2; Fig. 4). This was confirmed when compared with inhibitory zones obtained for standard antibiotic tested in parallel against all test bacteria; the standard antibiotic showed slightly better activity ranging from 14.9 ± 0.57mm to 17.9 ± 0.57mm than all the other seaweed extracts.

Red seaweed

Antibacterial activity of red seaweed extracts showed maximum inhibitory zone of about 15.9 ± 0.57mm against B1-*Escherichia coli,* 14.9 ± 0.57mm against B2 - *Staphylococcus aureus,* 14.3 ± 1.05mm against B3 - *Staphylococcus epidermidis,* 13.9 ± 0.57mm against B4 - *Klebsiella pneumoniae* and 13.3 ± 1.05mm B5 - *Enterobacter* sp. No inhibitory zones were found for negative control (DMSO) for any test bacteria.

Table 2: Antibacterial activity of different seaweed extracts against test organisms

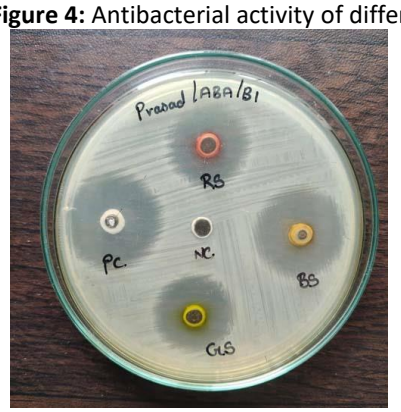
S. No	Test Bacteria	Zone of inhibition (mm)				
		Green seaweed extracts	Brown seaweed extracts	Red seaweed extracts	NC	PC
1	*Escherichia coli*	14.9 ± 0.57	16.9 ± 0.57	15.9 ± 0.57	0 ± 0.0	17.9 ± 0.57
2	*Staphylococcus aureus*	13.9 ± 0.57	15.9 ± 0.57	14.9 ± 0.57	0 ± 0.0	17.3 ± 1.05
3	*Staphylococcus epidermidis*	14.3 ± 1.05	14.3 ± 1.05	14.3 ± 1.05	0 ± 0.0	16.9 ± 0.57
4	*Klebsiella pneumoniae*	14.9 ± 0.57	14.9 ± 0.57	13.9 ± 0.57	0 ± 0.0	14.9 ± 0.57
5	*Enterobacter* sp	15.3 ± 1.05	15.3 ± 1.05	13.3 ± 1.05	0 ± 0.0	15.3 ± 1.05

NC: Negative control, PC: Positive control

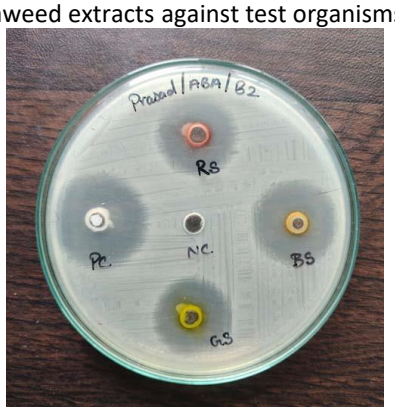
Table 3: MTT assay of Brown seaweed extracts in MCF-7 cell lines

S. No	Fibroblast cell lines – MTT Assay			IC_{50}
	Concentration of seaweed extracts (µg/ml)	Cytotoxicity (%)	Cell viability (%)	
1	0 (Control)	0	>99	No cytotoxicity
2	10	14.6 ± 0.57	85.6 ± 0.75	IC_{50} = 43.5µg/ml
3	20	27.3 ± 0.75	72.9 ± 0.57	
4	30	36.6 ± 0.75	63.6 ± 0.75	
5	40	46.3 ± 1.05	53.9 ± 0.57	
6	50	58.6 ± 0.75	49.6 ± 0.75	

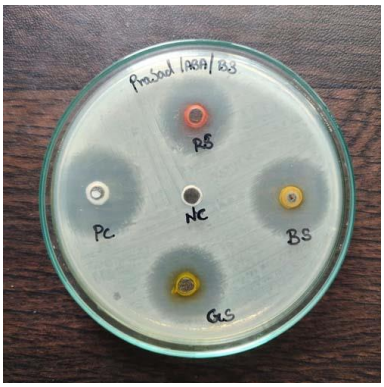
Figure 4: Antibacterial activity of different seaweed extracts against test organisms



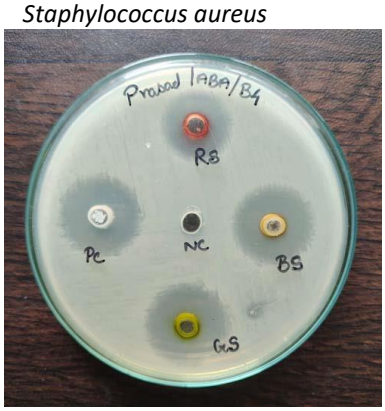
Escherichia coli



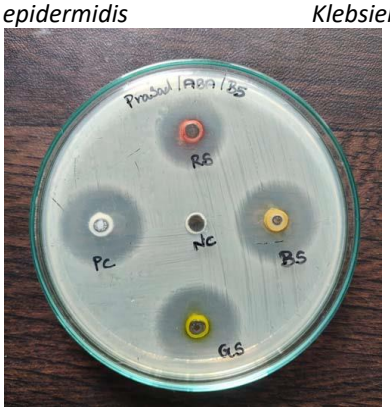
Staphylococcus aureus



Staphylococcus epidermidis



Klebsiella pneumoniae



Enterobacter sp

The obtained values were found significant to retard or prevent the growth of test bacteria using the red seaweed extracts (Table-2; Fig. 4). This was confirmed when compared with inhibitory zones obtained for standard antibiotic tested in parallel against all test bacteria; the standard antibiotic showed slightly better activity ranging from 14.9 ± 0.57mm to 17.9 ± 0.57mm than all the other seaweed extracts.

During the literature survey, it was found several antibacterial activity works were carried out against Gram positive and Gram negative organisms. All the works were

found supportive to the present findings in terms of antibacterial activity.

Antibacterial activity using the crude extracts of *Gracilaria edulis*, *Calorpha peltada* and *Hydroclothres* sp. against six bacterial pathogens *Escherichia coli*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus faecalis* and *Bacillus cereus* was analysed in research. The results showed that ethanol extract of *Gracilaria edulis* inhibited growth of all the test organisms except *Bacillus cereus* and *Enterobacter aerogenes*. Seaweed extract of *Calorpha peltada* was found effective against a number of Gram negative and Gram positive bacteria such as *Escherichia coli*, *Staphylococcus aureus* and *Streptococcus faecalis*. *Hydroclothres* sp. extract inhibited the growth of *Pseudomonas aeruginosa* only out of the six tested pathogens[18].

In another study, the crude extracts from *Ulva lactuca*, *Enteromorpha compressa* (Chlorophyta), *Padina pavonica* (Phaeophyta) and *Jania rubens* (Rhodophyta) which were collected from the coast of Gaza strip, Palestine was studied. Findings revealed that all crude extracts showed excellent antibacterial activity against all the test bacteria, the extracts were prepared using the solvent methanol and evaluated for antibacterial activity by well diffusion method against both Gram negative (*Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris* and *Klebsiella pneumoniae*) and Gram-positive bacteria (*Staphylococcus aureus and Bacillus subtilis*[19].

Antibacterial activity against Gram negative *Proteus mirabilis* causing urinary tract infections was analysed in another research. The activity was tested using the extracts of different seaweeds such as *Egregia menziesii, Codium fragile, Sargassum muticum, Endarachne binghamiae, Centroceras clavulatum* and *Laurencia pacifica* collected from Todos Santos Bay, Mexico. All seaweeds showed promising antibacterial activity against the selected *Proteus* species[21].

Anti-cancer activity of brown seaweed extracts in MCF-7 breast cancer cell lines

Cytotoxic activity of brown seaweed on the growth and multiplication of MCF-7

MCF-7 cells were exposed with different concentrations of the sample - brown seaweed (10, 20, 30, 40 and 50µg/mL). Increase in concentration of samples increased the cell cytotoxicity. This is evident from Table-3 values. IC_{50} value for the given sample (brown seaweed extract was found to be 43.5µg/ml (Fig. 5). The proliferation of cancer cells were inhibited in a dose dependent manner.

Cell viability/cytotoxicity of MCF-7 after treating with brown seaweed

Inverted microscopic images of treated samples revealed that, brown seaweed extract inhibited the cell multiplication of MCF-7 cells and hence significant difference was evident in cell viability between the Control (Fig. 6A) and brown seaweed treated cells (Fig. 6B). In the treated samples, the MCF-7 cells were found to be ovoid, tiny and round in shapes. Red arrow marks are indicated in the image (Fig. 6B) to differentiate the cells.

Figure 5: IC_{50} value of brown seaweed extracts on MCF-7 cells

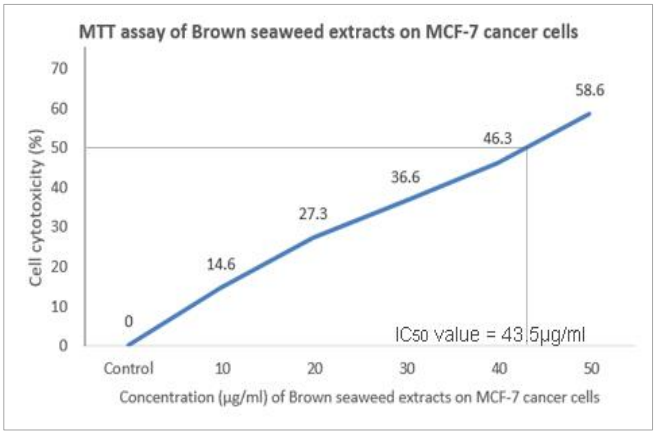
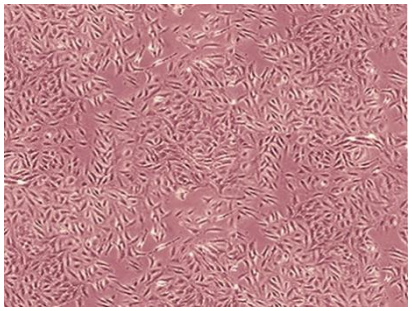
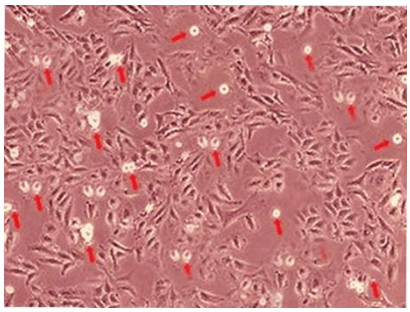


Figure 6: Cell viability/cytotoxicity of MCF-7 after treating with brown seaweed extracts



6A. Control



6B. Brown seaweed extract treated (50µg/mL)

Literature survey also revealed similar findings to Ade arsianti et al[20]. The researchers studied the phytochemical composition and anticancer activity of seaweeds *Ulva lactuca* and *Eucheuma cottonii* against breast MCF-7 and colon HCT-116 cells [21]. In another study, Luis et al[21] analysed anticancer activity in HCT-116 colon cancer cells against different seaweeds such as *Egregia menziesii, Codium fragile, Sargassum muticum, Endarachne binghamiae, Centroceras clavulatum* and *Laurencia pacifica* collected from Todos Santos Bay, Mexico. In both these studies the proliferation of cancer cells were inhibited in a dose dependent manner and hence our present findings were found supportive to these earlier works.

CONCLUSION

In the present study, three different seaweed extracts were investigated for its antibacterial activity and anticancer

activity. During the analysis, promising results were confirmed for green and brown seaweed extracts with more antibacterial activity and good anticancer activity. Antibacterial activity of three different seaweed extracts showed significant inhibitory zones ranging from 13.3 ± 1.05mm to 16.9 ± 0.57mm against the respective bacteria B1 - *Escherichia coli,* B2 - *Staphylococcus aureus,* B3 - *Staphylococcus epidermidis,* B4 - *Klebsiella pneumoniae,* B5 - *Enterobacter* sp. Considering these results, green and brown seaweed extracts seem to be very promising agents in the potentiation of antibacterial and anticancer drugs. Anticancer activity test results revealed IC_{50} value of about 43.5µg/ml for the brown seaweed extracts after testing in MCF-7 cell lines. Increase in concentration of samples increased the cell cytotoxicity and proliferation of cancer cells were inhibited in a dose dependent manner. However, the exact mechanisms involved need to be further explored with the addition of other assays such as assessment of DNA fragmentation analysis, cell cycle arrest, cytochrome c release, and mitochondrial membrane potential. Thus the obtained results would contribute more and induce interest for the development of different medically significant seaweed farming and biotechnological use with growing interest.

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REFERENCES

1. Strobel G, Daisy B, Castillo U and Harper J. Natural products from endophytic microorganisms. J. Nat. 2008; 67(2): 257-268.

2. Moussavou G, Kwak DH, Obiang-Obonou BW, Maranguy CA, Dinzouna-Boutamba SD, Lee DH, Pissibanganga OGM, Ko K, Seo J and Choo YK. Anticancer effects of different seaweeds on human colon and breast cancers. Mar. Drugs. 2014; 12: 4898–4911.

3. Kim EJ, Park SY, Lee JY, Park JH. Fucoidan present in brown algae induces apoptosis of human colon cancer cells. BMC Gastroenterol. 2010; 10: 96-103.

4. Jin JO, Zhang W, Du JY, Wong KW, Oda T and Yu Q. Fucoidan can function as an adjuvant *in vivo* to enhance dendritic cell maturation and function and promote antigen-specific t cell immune responses. PLoS ONE. 2014; 9: e99396.

5. Ganesan AR, Tiwari U and Rajauria G. Seaweed nutraceuticals and their therapeutic role in disease prevention. Food Sci. Hum. Wellness. 2019; 8: 252–263.

6. Lee JC, Hou MF, Huang HW, Chang FR, Yeh CC, Tang JY. Marine algal natural products with anti-oxidative, anti-inflammatory, and anti-cancer properties. Cancer. Cell. Int. 2013; 13(1): 55-63.

7. Maximilien R, de Nys R, Holmström C, Gram L, Givskov M, Crass K, Kjelleberg S and Steinberg PD. Chemical mediation of bacterial surface colonisation by secondary metabolites from the red alga *Delisea pulchra*. Aquat. Microb. Ecol. 1998; 15: 233-246.

8. Seenivasan R, Indu H, Archana G and Geetha S. The Antibacterial Activity of Some Marine Algae from South East Coast of India. American- Eu. J. Agri. Environ. Sci. 2010; 9(5): 480-489.

9. Allmendinger A, Spavieri J, Kaiser M, Casey R, Hingley-Wilson S, Lalvani A, Guiry M, Blunden G and Tasdemir D. Antiprotozoal, antimycobacterial and cytotoxic potential of twenty-three British and Irish red algae. Phyto. Res. 2010; 24: 1099-1103.

10. Cox S, Abu-Ghannam N and Gupta S. An assessment of the antioxidant and antimicrobial activity of six species of edible Irish seaweeds. Int. Food Res. J. 2010; 17: 205-220.

11. Taskin E, Caki Z, Ozturk M and Taskin E. Assessment of in vitro anti-tumoral and antimicrobial activities of marine algae harvested from the eastern Mediterranean Sea. Afr. J. Biotechnol. 2010; 9(27): 4272-4277.

12. Kolanjinathan K and Stella D. Antibacterial activity of ethanol extracts of seaweeds against human bacterial pathogens. Recent Res. Sci. Tech. 2009; 1(1): 20-22.

13. Lavanya R and Veerappan N. Antibacterial Potential of six seaweeds collected from Gulf of Mannar of Southeast Coast of India. Adv. Biol. Res. 2011; 5(1): 38-44.

14. Osman MEH, Abushady AM and Elshobary ME. In vitro screening of antimicrobial activity of extracts of some macroalgae collected from Abu-Qir bay Alexandria, Egy. Afr. J. Biotechnol. 2010; 9(12): 7203-7208.

15. El Gamal AA. Biological importance of marine algae. Saudi Pharm. J. 2010; 18: 1-25.

16. Elmanamam AA, Alyazji AA and Abu Gheneima NA. Antibacterial, Antifungal and Synergistic Effect of *Lawsonia inermis, Punica granatum* and *Hibiscus sabdariffa*. Ann. Alquds Med. 2011; 7: 33-41.

17. Sreenivasa-Rao PP and Parekh KS. Antibacterial activity of Indian seaweeds. Phykos. 1981; 23: 216-221.

18. Kolanjinathan K, Ganesh P, Govindarajan M. Antibacterial activity of ethanol extracts of seaweeds against fish bacterial pathogens. European Rev. Med. Pharmacol. Sci. 2009; 13: 173-177.

19. Elnabris KJ, Elmanamam AA and Chihadeh WN. Antibacterial activity of four marine seaweeds collected from the coast of Gaza Strip, Palestine. Mesopot. J. Mar. Sci. 2013; 28(1): 81 – 92.

20. Ade arsianti, Fadilah, Fatmawaty. Phytochemical composition and anticancer activity of seaweeds *Ulva lactuca* and *Eucheuma cottonii* against breast MCF-7 and colon HCT-116 cells. Asian J Pharm Clin Res. 2016; 9(6):115-119.

21. Luis JV, Irma E, Graciela GR and Nahara EA. Antibacterial and anticancer activity of seaweeds and bacteria associated with their surface. Revista de Biología Marina y Oceanografía. 2010; 45(2): 267-275.