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Research Article

Antibacterial Activities of Selected Green Seaweeds from West African Coast

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Abstract

The continually increasing antibiotic resistance amongst microorganisms had steered up an increased intensity in the search for new drugs. The marine environment had been reported to be a great source of novel compounds with diverse biological activities and this had engendered the attention of researcher globally, but the West African Coast despite being blessed with a variety of Macro-algae remain untapped. This study was, therefore, embarked upon to investigate the antibacterial activities of selected green algal species from the West African coast. Crude extracts of *Ulva fasciata, Ulva lactuca, Chladophora vagabunda, Caulepa taxifolia, Chaetomorpha antennina* and *Chaetomorpha linum* were obtained by maceration using Dichloromethane/methanol (DCM/MeOH), chloroform/methanol (CHL/MeOH) 90% (v/v), Ethanol and Diethyl ether as solvents. Extracts were screened against some bacterial pathogens including Gram-positive bacteria- clinical strain (Sa I), *S. aureus* laboratory strain (Sa II), *S. aureus* ATCC 25922 (Sa III), *Bacillus subtilis* (Bs), *Streptococcus pneumonia* (Sp), *Streptococcus faecalis* (Sf) and *Mycobacterium aurum* (Ma). Gram-negative test bacteria were *Escherichia coli* clinical (Ec I) and *Escherichia coli* laboratory strain (Ec II), *Escherichia coli* NCTC 10418 (Ec III), *E. coli* ATCC 25923 (Ec IV), *Proteus vulgaris* (Pv), *Proteus mirabilis* (Pm), *Pseudomonas aeruginosa* (Pa) *Pseudomonas putida* (Pp), *Salmonella typhi* clinical strain (St I), Salmonella typhi NCTC 8385 (St II), *Serratia macerans* (Sm) and *Klebsiella pneumonia* (Kp). Crude extracts were obtained using Antibacterial screening was carried out by disc diffusion method. The result analysis was done by mean ± SD. The result showed that all the screened algae had antibacterial activity against at least one of the test organisms. Four (57%) of the seven algal species tested demonstrated inhibitory activities against the Gram-positive test bacteria while all the seven species tested showed inhibitory activities against the Gram-negative test bacteria. Highest inhibitory zone against Gram-negative bacterial species was observed in dichloromethane/methanol extract of *Caulepa taxifolia* (DCCT) against *E. coli* measuring 14.67 mm while the highest activity against Gram-positive bacterial strain was observed in diethyl acetate extract of *Chaetomorpha antennina* (DECA) against *S. aureus* (SaII) with an inhibitory zone of 17.67 mm. Extracts of *Chaetomorpha antennina* showed the highest inhibitory activities in this study. The result of this study showed that extracts from species of green macro-algae from the West African Coast possess antibacterial compounds that can serve as lead drug candidates in the quest for new antibacterial therapy if well explored.

Keywords: *Green Macro-Algae; Crude Extracts; Antibacterial Activity; West African Coast; Bacterial Pathogens*

Introduction

Seaweeds or marine algae are macroscopic, attached or freely floating plants. They form one of the essential marine living, renewable resources. They are primitive plants without any real root, stem and leaves. They belong to the division of Thallophyta in the plant kingdom. Marine algae are classified into four groups namely Chlorophyceae (green algae), Phaeophyceae (brown algae), Rhodophyceae

(red algae) and Cyanophyceae (blue-green algae) based on the type of pigments, morphological, anatomical and reproductive structures [1]. Green algae occur in a wide variety of shapes and sizes. Sea lettuce (Ulva sp.), sea grapes (Caulerpa) and turtle grass (Chlorodesmis) are some of the most abundant types of green algae on coral reefs where they play an essential role.

The use of seaweeds in medicine dates back to 300 BC with the Japanese and Chinese cultures using seaweed to treat goiter and other glandular problems. The Romans used seaweeds in the treatment of wounds, burns and rashes. The use of dried seaweeds in Scotland dates back to the 18th century when physicians used dried seaweed stem to drain abdominal wall abscesses successfully. Seaweeds were also reportedly used in the treatment of dysmenorrhea [2]. Seaweeds are one of the critical marine living resources in the world. These macroalgae have been a source of food, feed and medicine in the east as well as in the west, since ancient times [3,4]. Orient countries such as Japan, China and Korea nutritionally consume about (5%) of green algae, (66.5%) of brown algae and (33%) of red algae in daily diets [5-7]. Dietary seaweeds provide all essential minerals. Seaweeds can provide minerals often absent from freshwater and food crops grown on mineral-depleted soils and therefore serve as nutraceuticals [8]. Seaweeds are consumed as food, animal fodder, fertilizer, industrial material such as agar and minor medicines.

Marine algae produce a cocktail of metabolites with exciting biological activities (anti-infective, anti-inflammatory and anti-proliferative) and with potential commercial value [9-12]. The marine environment has reportedly been described as an exceptional reservoir of bioactive natural products, many of which exhibit structural/chemical features not found in terrestrial natural products [13]. Natural products remain the most prolific source of new antimicrobials and the chemical diversity of natural compounds is still unmatched by combinatorial chemistry approaches [14-15]. Seaweeds or marine algae have been reported to contain many substances such as alginate, carrragenean and agar as phycocolliods that have been used for decades in medicine and pharmacy. More chemists and biologists pay attention to the constituents of the algae; if their natural products are explored, they may give a qualified lead to the discovery of new drug molecules against several pathogens infectious diseases [16]. Kotimchencko and co-workers [17] reported that sulphated polysaccharides from marine algae inhibited tumor growth and the metastatic process by direct action on tumor cells and by enhancement of immune response. Oral administration of several seaweeds can cause a significant decrease in the incidence of carcinogenesis *in vivo*.

Reports have shown that a large number of algal extract products have antimicrobial activities [18]. Many of the structures were identified as fatty acids and hydroxyl unsaturated fatty acids, glycolipids, steroids, phenolics and terpenoids. Lauric acid, palmitic acid, linolenic acid, oleic acid, stearic acids are known to be potential antibiotic or antifungal agents. Most bioactive compounds extracted from seaweeds have been applied for their biocidal (anti-fungi, antibacterial) and pharmaceutical activities [16]. Reports have shown the antibacterial activities of some algal extracts against multidrug-resistant bacteria [19,20]. Many other reports of antioxidant activities of macroalgae have also been reported [19,21-23].

The use of marine natural products capable of inhibiting bacteria offers productive pharmacological potential and crude extracts obtained from various seaweeds using different solvents have been reported to exhibit inhibitory activities. This study was, therefore, aimed at screening selected species of green marine macro-algae for antibacterial activities against human pathogens.

Materials and Method

Sample Collection: Algal samples were obtained from the Ghana coasts between May 2013 and August 2014. Species of fresh algae were collected by detaching them manually from rock surfaces at low tides. Some other samples were collected using a knife at low tides to avoid intense wave action. These samples were immediately kept under the ice in an ice-box and transported to the laboratory where they were sorted out and then thoroughly cleaned with fresh water. Samples obtained were adequately identified as *Ulva fasciata, Ulva lactuca, Chladophora vagabunda, Caulepa taxifolia, Chaetomorpha antennina* and *Chaetomorpha linum*. Samples were properly labeled with voucher numbers- *Ulva fasciata* -PB0513UF, *Ulva lactuca* -SA0513UL, *Caulepa taxifolia* –PB0513CT, *Chaetomorpha antennina*-SA0513CA and *Chaetomorpha linum*- PB0513CL. They were kept frozen until the extraction period. Sample specimens were dropped in the Crawford University herbarium with their voucher numbers. The sampling locations were shown in the figure below (Figure 1).

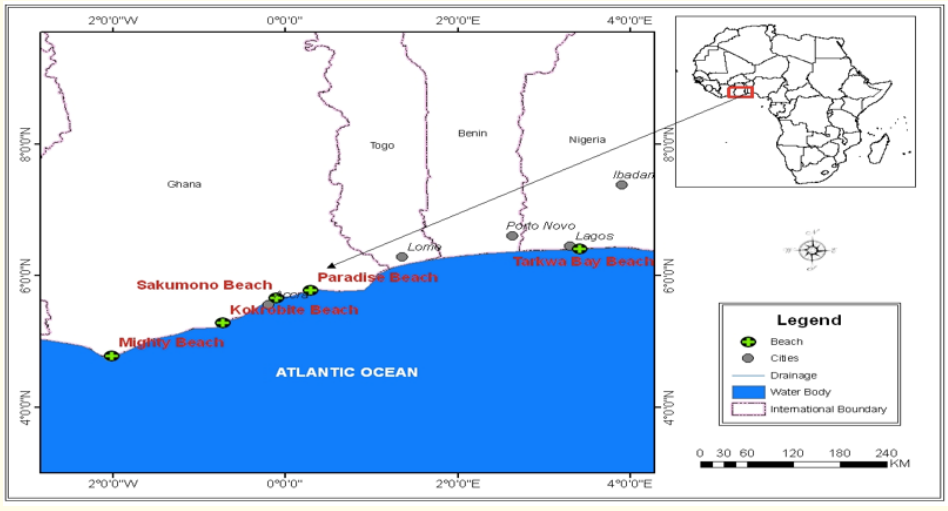

Figure 1: *Location map of sampling sites.*

Sampling sites

The different sampling sites were explored. Details of these sites are seen on the map. The green algae in this study were obtained from Mighty and Paradise Mighty Beaches at the shores of Ghanaian Coast.

Sample preparation and crude extraction

The algae were cleaned to remove epiphytes and debris and thereafter washed thoroughly in tap water and later with distilled water. Samples were then shade dried for seven days. Dried sample was pulverized using milling machine. Powdered sample was extracted with Diethyl ether, Dichloromethane/Methanol (ratio 2:1), 90% Ethanol, Chloroform/ Methanol [24]. Fifty grams of sample was extracted in 300ml of solvent. This was heated to 37°C and held for 30 minutes and the supernatant was filtered. The process was repeated twice. The extract was later concentrated using the Rotary evaporator (Buchi 200, Germany) at 40°C. Extract concentrate was then stored in the freezer till needed.

Antibacterial assay

Collection and preparation of test organisms

Bacterial strains

Bacterial strains were obtained from the Lagos University Teaching Hospital, (LUTH) (*Staphylococcus aureus* clinical and laboratory strains, *Bacillus subtilis, Streptococcus pneumoniae*, *Streptococcus faecalis,* Gram negative species *Escherichia coli* (clinical and laboratory strain), *E. coli* NCTC 10418*, Pseudomonas aeruginosa, Salmonella typhi* (clinical strain), *Salmonella typhi* NCTC 8385 *and Klebsiella pneumoniae*) and some others were obtained from the department of Life Sciences, Rhodes University, Grahamstown, South Africa comprising of both Gram-positive (*S. aureus* ATCC 25922, *Mycobacterium aurum* and Gram-negative strains including *E. coli* ATCC 25923, *Proteus vulgaris, Proteus mirabilis, Pseudomonas putida*). These bacterial samples were used as the test organisms and their identity was confirmed using appropriate biochemical tests according to standard procedures [25].

Disc diffusion method

The disc diffusion method was carried out using the method of Bauer and co workers [26] as adopted by other researchers [27,28]. The bacterial suspensions were standardized by MacFarland standard 0.5, which is equivalent to 10^8 CFU/ml. Plain discs of 6 mm were produced from Whatman's filter paper no. 1 and sterilized in the oven at 60°C overnight. These discs were soaked with known quantity

of extracts and allowed to dry completely. The dry discs containing the extracts were then placed on Petri plates containing solidified Mueller Hinton Agar seeded with test bacteria and incubated at 37°C for 48 hours. All tests were done in triplicates. Gentamycin (10 µg) and Ciprofloxacin (10 µg) were used as standard antibiotics.

Minimum inhibitory concentration (MIC)

The Minimum Inhibitory Concentration was carried out against the strains of *S. aureus* and *E. coli* tested [30]. Potency test was carried out using the disk diffusion method while MIC values of the extracts were obtained using the broth micro-dilution method. Sterile 96-well micro-plates were used for the assay (0.5 ml volume, Fisher Scientific).

Statistical analysis was done by Mean ± SD.

Results

The algal yield ranged from 2.22% in *Caulepa taxifolia* to 8.88% in *Chaetomorpha anteninna*. Antibacterial activities of the crude extracts are shown in figure 2-5. Inhibitory activity was observed in all the extract against at least two pathogens except for ethanol extract, which showed inhibitory activity in *C. taxifolia* (ETCT) against Gram-negative bacteria. Diethyl extracts of *U. fasciata* (DEUF) and *C. anteninna* (DECA) showed inhibitory activities against the strains of *S. aureus* tested with zones of inhibition ranging from 9.33 mm in *U. fasciata* and 17.67 mm in *C. anteninna*. The diethyl ether extract of the alga *Ulva fasciata* (DEUF) showed a zone of inhibition of (13.67 mm) against *Proteus mirabilis*, while 9.33 mm, 11.17 mm and 12 mm zones of inhibition were observed against Sa I, Sa II and Sa III, the different strains of *S. aureus* respectively. The inhibitory activities of extracts of this solvent against Gram-negative bacteria ranged between 8.00 mm in *C. vagabunda* (DECV) and 15.33 mm in *C. anteninna* (DECA). The Diethyl ether of *C. vagabunda* only showed inhibitory activity against Gram-negative bacterial strains. For the chloroform/methanol extracts, the algae *C. vagabunda* (CHCV) and *C. taxifolia* (CHCT) showed no inhibitory activities against the tested Gram-positive pathogens but *C. taxifolia* (CHCT) showed a zone of inhibition ranging from 11.17 mm to 14.67 mm against the *E. coli* strains. The chloroform/methanol extract of *C. anteninna* showed broad-spectrum activities inhibiting both Gram-positive and Gram-negative pathogens with zones of inhibition ranging from 10.00 mm to 11.17 mm against *S. aureus* strains and 7.33 mm to 10.67 mm against *E. coli* strains. The minimum inhibitory concentration (MIC) of the extracts was obtained against strains of *E. coli* and *S. aureus* (Table 2) and the lowest MIC value obtained is 640 µg as observed in *U. fasciata*, *C. vagabunda* and *C. taxifolia* against *E. coli* as well as in *U. lactuca* and *C. anteninna* against *S. aureus*.

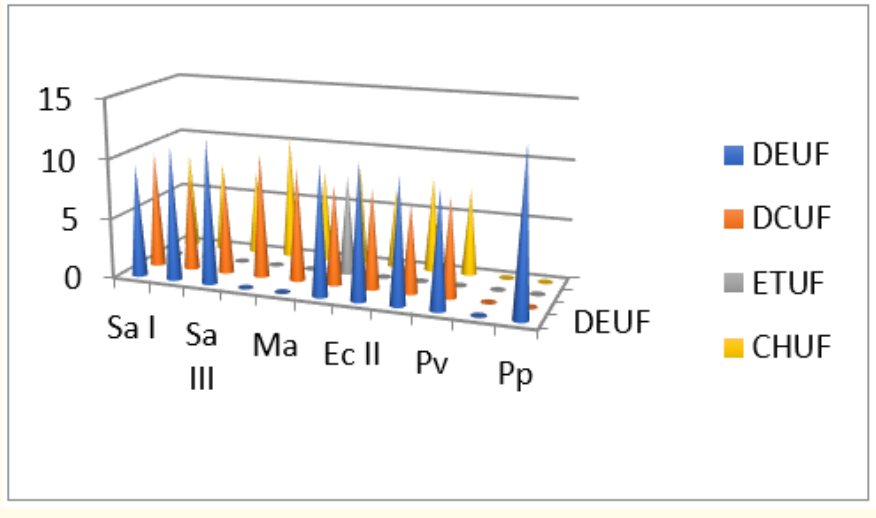

Figure 2: *Antibacterial activities of Ulva fasciata extracts*

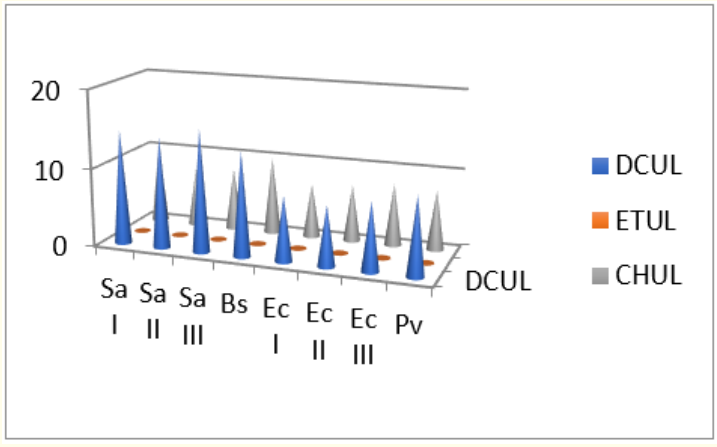

Figure 3: *Antibacterial Activities of Ulva lactuca extracts.*

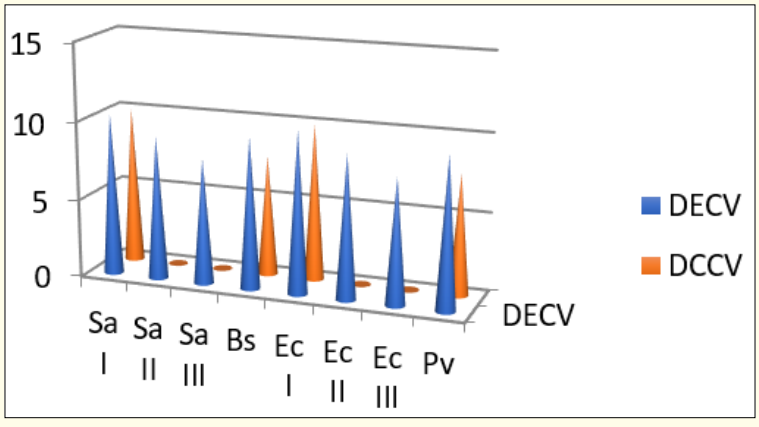

Figure 4: *Antibacterial activities of C. vagabunda extracts.*

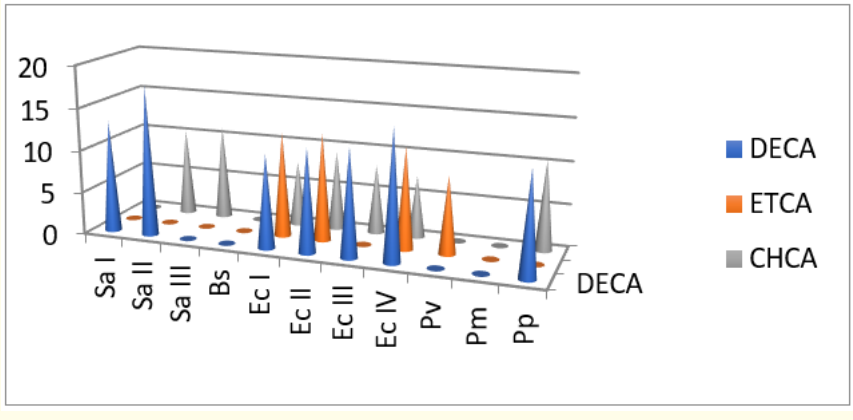

Figure 5: *Antibacterial activities of C. antennina extracts.*

Algal species	Location	Weight of Algae (g)	Weight of extract (g)	Extract yield %	Extract color
Ulva fasciata	Ghana	500	43.35	8.67	Greyish green
Ulva lactuca	Ghana	100	8.50	8.50	Light green
Chladophora vagabunda	Ghana	20	1.58	7.92	Deep green
Caulepa taxifolia	Ghana	100	2.22	2.22	Dirty green
Chaetomorpha antennina	Ghana	50	4.44	8.88	Light green
Chaetomorpha linum	Ghana	50	3.83	7.65	Light green

Table 1: *Extract yield of algal samples.*

Algal species and standard antibiotics	*E. coli* I (µg)	*S. aureus* I (µg)
Ulva fasciata	640	> 640
Ulva lactuca	> 640	640
Cladophora vagabunda	640	ND
Caulepa taxifolia	640	No activity
Chaetomorpha anteninna	640	640
Chaetomorpha linum	> 640	No activity
Ciprofloxacin	5	5
Gentamycin	5	5

Table 2: *Minimum Inhibitory Concentration of some of the dichloromethane/methanol extracts against E. coli and S. aureus strains.*

Discussion

The yield percentage of the algae ranged between 2.22% and 8.88%. Virtually all the algal species showed antibacterial activities against several of the test bacterial strains tested. All solvent extracts were positive for activity displaying either broad or narrow spectrum activities. The alga, *Ulva fasciata* exhibited broad-spectrum antibacterial activity. Activities were observed against five out of seven test Gram-positive bacteria strains while it showed inhibitory activity against five out of eleven Gram-negative bacterial strains. The inhibitory zones ranged from 6.67 to 13.67 mm. Priyadshini., *et al*. [29] reported inhibitory activities of this alga against *V. alginolyticus* and *Enterobacter* sp. while Paulert., *et al.* [30] reported that extract of *U. fasciata* was inhibitory against *P. aeruginosa, X. campestris* and *Ewinia carotovora*. In this study, it was observed that the different solvents extracts of *U. fasciata* (DEUF, DCUF and CHUF) all inhibited the three strains of *Staphylococcus aureus*, *Bacillus subtilis* and *Mycobacterium aurium* as well as the *E. coli* strains and *P. mirabilis* tested.

The activities of extracts of *U. fasciata* had been reported against multidrug-resistant organisms. Pramintha and Lipton [31] reported the antibacterial activities of *U. fasciata* against multidrug resistant *S. aureus, P. aeruginosa* and *V. alginoluticus* with inhibitory zones ranging between 15 mm and 18 mm. The report of Chandrasekaran., *et al*. [32] also revealed the inhibitory activities of *U. fasciata* against *B. substilis* with the mean zones of inhibition (15.0 mm), *S. pyogenes* (14.0 mm), *E. coli* (13.6 mm) and *P. mirabilis* (13.3 mm). This is comparable to the result of this study in which *B. substilis* has a mean zone of inhibition (13.33 mm), *S. aureus* (8.00-12.0 mm), *E. coli* (6.67 - 11.17 mm) and *P. mirabilis* (13.67 mm). *U. fasciata* from the West African coast could be a source of novel bioactive compounds that could of therapeutic intervention against pathogenic bacteria and even multidrug-resistant bacteria.

In this study, the macro-algae *Ulva lactuca* and *Chaetomorpha antennina* both displayed broad-spectrum antibacterial activities against bacterial test strains. *Ulva lactuca* extracts in this study positive bacterial strains tested with the lowest inhibitory zone of 7.17 mm observed in chloroform: methanol extract against Sa III and the highest zone of inhibition of 15.67 mm observed against Sa III while 6.67 mm and 12.67 mm were the lowest and highest inhibitory zones observed against Gram-negative strains. The family Ulvaceae had previously been reported for different antimicrobial activities against human pathogens [33] and such reports corroborate this result.

The activities of this *C. anteninna* against multidrug-resistant bacteria had also been reported [34]. Activity was observed against strains of *E. coli, S. aureus, P. vulgaris* and *P. putida* with inhibitory zones ranging from 7.33 mm in *E. coli* I to 17.67 mm in *S. aureus* (Sa II) at a concentration of 100 µg/ml. Sivarkumar., *et al*. [35] reported inhibitory activities of this algal extract against *S. aureus, E. coli* and by *P. aeruginosa* at the concentration of 50 µg/ml with zones of inhibition of 7.3 ± 0.8 mm against *S. aureus*, 8 ± 0.6 mm against *E. coli* and 7.3 ± 1 mm for *P. aeruginosa*. The observed activity in this study even revealed much higher inhibitory zones than those reported, *S. aureus* -Sa II strain tested showed a zone of 17.67 mm as against 7.3 mm reported by Sivarkumar and co-workers [37].

The extracts of *C. taxiofolia, C. vagabunda and C. linum* displayed a narrow-spectrum activity by inhibitory activity against Gram-negative. The report of Muha mmad and Abu-Dobara is at variance with this present study because no activity was observed from *C. taxifolia* against their test bacteria. The report of Chandrasekaran., *et al*. [32] revealed that productive inhibitory activity of a species of the genus *Caulepa* (*C. racemosa*) was active against vancomycin resistant *E. faecalis*. Also, Etcherla and Rao reported the inhibitory activities of *C. taxifolia* against *Bacillus subtillis* and *Micrococcus luteus* and the fungus *Candida albicans*. The selective activity of these three green algae against Gram-negative test bacteria may be a function of the mechanism of action which could be cell wall-related. The MIC results of the tested crude extracts revealed that most of the extracts had a MIC of 640 µg. For crude extract to show a MIC as low as 640 µg means the algae are promising and should, therefore, be further researched [36,37].

Significant Statement

Various species are available at this coast with little attention regarding utilization and exploration. This study discovered that some green algal species from the West African Coast could be beneficial in the quest for new antimicrobials if well explored. This study will help project the possible health benefits that can be obtained from these species as regards medicinal effects. Hence, forming a basis for more exploration and critical studies of these species.

Conclusion

This study revealed that the crude extracts of the selected green algae all have potential inhibitory activities against selected bacterial strains tested. The West African Coast is having so much of these algal species and as such should be a center of attraction for future studies of these macroalgae with the inclusion of isolation of active principle, which may be readily utilized for the development of new drugs.

Bibliography

1. Kolanjinathan K., *et al*. "Pharmacological Importance of Seaweeds: A Review". *World Journal of Fish and Marine Sciences* 6.1 (2014): 1-15.
2. Hocman G. "Prevention of cancer: vegetables and plants". *Comparative Biochemistry and Physiology* 93.2 (1989): 201-212.
3. Chapman VJ and Chapman DJ. "Seaweeds and Their Uses". (3rd edition). Chapman and Hall, New York (1980): 334.
4. Mohammadi M., *et al*. "Nutritional composition of seaweeds from the Northern Persian Gulf Iranian". *Journal of Fisheries Sciences* 12.1 (2013): 232-240.
5. Gade R., *et al*. "Seaweeds: a novel biomaterial". *International Journal of Pharmacy and Pharmaceutical Sciences* 5 (2013): 40-44.
6. Valentina J., *et al*. "Estimation of protein, carbohydrate and mineral content in selected seaweeds". *International Journal of Current Research* 7.1 (2015): 11329-11333.
7. Ismail GA. "Biochemical composition of some Egyptian seaweeds with potent nutritive and antioxidant properties". *Food Science and Technology* 37.2 (2017): 294-302.

8. Mišurcová L., *et al*. "Seaweed minerals as nutraceuticals". *Advances in Food and Nutrition Research* 64 (2011): 371-390.

9. Cardozo KHM., *et al*. "Metabolites from algae with economical impact". *Comparative Biochemistry and Physiology - Part C* 146.1-2 (2007): 60-78.

10. Nair R., *et al*. "Marine algae: screening for a potent antibacterial agent". *Journal of Herbal Pharmacotherapy* 7.1 (2007): 73-86.

11. El-Gamal AA. "Biological importance of marine algae". *Saudi Pharmaceutical Journal* 18.1 (2010): 1-25.

12. Nunnery JK., *et al*. "Biologically active secondary metabolites from marine cyanobacteria". *Current Opinion in Biotechnology* 21.6 (2010):787-793.

13. Carté BK. "Biomedical Potential of Marine Natural Products". *Biosciences* (1996): 271-286.

14. Newman DJ and Cragg GM. "Natural products as sources of new drugs over the last 25 years". *Journal of Natural Products* 70.3 (2007): 461-477.

15. Craig GM., *et al*. "Impact of natural products on developing new anti-cancer agents". *Chemical Reviews* 109.7 (2009): 3012-3043.

16. Zerrifi SE., *et al*. "Review-Seaweed Bioactive Compounds against Pathogens and Microalgae: Potential Uses on Pharmacology and Harmful Algae Bloom Control". *Marine Drugs* 16.2 (2018): 55.

17. Kotimchencko YS., *et al*. "Carrageens as a new source of drugs with metal binding properties". *Marine Drugs* 8.4 (2010): 1106-1121.

18. Plaza M., *et al*. "Screening for bioactive compounds from algae". *Journal of Pharmaceutical and Biomedical Analysis* 51.2 (2010): 450-455.

19. Alghazeer R., *et al*. "In Vitro Antibacterial Activity of Flavonoid Extracts of Two Selected Libyan Algae against Multi-Drug Resistant Bacteria Isolated from Food Products". *Journal of Biosciences and Medicines* 5.1 (2017): 27-48.

20. Agbaje-Daniels F., *et al*. "Evaluation of Antibacterial Activity of Bryopsis pennata and Chaetomorpha antennina against Multidrug Resistant Morganella morganii and Salmonella species Isolated from Healthy Individuals". *Journal of Pharmaceutical Research International* 18.4 (2017): 1-7.

21. Farasat M., *et al*. "Antioxidant Properties of two Edible Green Seaweeds from northern coasts of the Persian Gulf". *Jundishapur Journal of Natural Pharmaceutical Products* 8.1 (2013): 47-52.

22. Chai TT., *et al*. "Antioxidant Activities of Methanol Extract and Solvent Fractions of Marine Macroalga, Avrainvillea Erecta (Berkeley) a. Gepp and E.S. Gepp (Dichotomosiphonaceae)". *Tropical Journal of Pharmaceutical Research* 14.3 (2015): 503-509.

23. Pinteus S., *et al*. "Cytoprotective effect of seaweeds with high antioxidant activity from the Peniche coast (Portugal)". *Food Chemistry* 218 (2017): 591-599.

24. Afolayan AF., *et al*. "Fucoxanthin, tetraprenylated toluhydroquinone metabolites from Sargassum heterrophyllum inhibit the in vitro growth of malariaparasite, Plasmodium falciparum". *Zeitschrift fur Naturforschung* 63.11-12 (2008): 848-852.

25. Cheesbrough M. "District Laboratory Practice in Tropical Countries". Cambridge University Press (2006): 434.

26. Bauer AW., *et al*. "Antibiotic susceptibility testing by a standardized single disk method". *American Journal of Clinical Pathology* 45.4 (1966): 493-496.

27. Adeleye AI., *et al*. "Screening of crude extracts of twelve medicinal plants in wonder cure concoction used in Nigeria as unorthodox medicine for activity on Mycobacterium tuberculosis isolated from HIV patients' sputum". *African Journal of Bacteriology* 7.18 (2008).

28. NCCLS. "Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically". Approved standard, 5th edition. NCCLS document M7-A5. NCCLS, Wayne, Pa (2000).

29. Priyadharshini S., *et al*. "Antimicrobial and hemolytic activity of extracts of Ulva fasciata (Delile 1813) from Mandapam, Southeast coast of India". *Asian Pacific Journal of Tropical Biomedicine* 1.1 (2011): S38-S39.

30. Paulet R., *et al*. "Antimicrobial properties of extracts from green seaweed Ulve fasciata against pathogenic bacteria and fungi". *Algological Studies* 123 (2007): 123-130.

31. Praminantha VS and Lipton AP. "Antimicrobial effect of Ulva fasciata extract on multidrug resistant human and pathogens". *Indian journal of Geo-marine Science* 43.11 (2014): 1-10.

32. Chandrasekaran M., *et al*. "Antibacterial activity of selected marine macro algae against vancomycin resistant Enterococcus faecalis". *Journal of Coastal Life Medicine* 2.12 (2014): 940-946.

33. Al-Zahrani A., *et al*. "Impact of Extracts of Marine Macroalgae on Multidrug-Resistant Bacteria". *Journal of Microbiology Research* 4.6 (2014): 18-24.

34. Shanmughapriya S., *et al*. "Antimicrobial activity of seaweeds extracts against multiresistant pathogens". *Annals of Microbiology* 58.3 (2008): 535-541.

35. Sivakumar SM and Safhi MM. "Isolation and screening of bioactive principle from Chaetomorpha antennina against certain bacterial strains". *Saudi Pharmaceutical Journal* 21.1 (2013): 119-121.

36. Mohamed AD and Abou-Dobara MI. "Antibacterial Activity of Some Marine Algal Extracts Against Most Nosocomial Bacterial Infections". *The Egyptian Journal of Experimental Biology* 9.2 (2010): 281-286.

37. Etcherla M and Rao GM. "In Vitro Study of Antimicrobial Activity in Marine Algae Caulerpa Taxifolia and Caulerpa Racemosa (C. Agardh)". *International Journal of Applied Biology and Pharmaceutical Technology* 5.2 (2014): 57-62.

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