Journal of Biosciences and Medicines, 2016, 4, 54-62
Published Online April 2016 in SciRes. http://www.scirp.org/journal/jbm
http://dx.doi.org/10.4236/jbm.2016.44008

Impact of Antibacterial Activity of Physical Storage Extracts on Pathogenic Bacteria

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Received 17 March 2016; accepted 19 April 2016; published 22 April 2016

Abstract

The overuse of antibiotics can lead to resistance among pathogenic bacteria. A new antibiotic that is effective against new and resistant bacterial strains is needed. Plants and marine organisms may offer such novel treatments. In this study, extracts of the seaweed *U. lactuca*, and the plant seeds *N. sativa* were tested against strains of Gram-positive cocci and Gram-negative bacilli *S. aureus*, and *P. aeruginosa*. The results of the bacterial inhibitor showed high activity in both extracts with inhibition of *S. aureus* growth up to 30 mm and 20 mm and *P. aeruginosa* growth inhibition was up to 12 mm and 15 mm, after the treated with 100 µl *U. lactuca* and *N. sativa* extracts, respectively. The MICs and MBCs were reflected with the growth inhibitor with values of 2 µl, 8 µl and 4 µl, 8 µl for *S. aureus* and *P. aeruginosa* after treated with *N. sativa* respectively. Kill-time increases as concentrations of *U. lactuca* and *N. sativa* extracts increase. Moreover, extracts stored in the transparent bottle decreased in effectiveness after one month of storage with percentage of 58.85%. After three months, heating the extracts of *U. lactuca* and *N. sativa* to 90˚C increased their antibacterial activity.

Keywords

Resistant Bacteria, *S. aureus*, *P. aeruginosa*, *U. lactuca*, *N. sativa*, Kill-Time

1. Introduction

Bacterial infections are usually treated with antibiotics. However, these drugs can be expensive. The continued use of antibiotics can also lead to bacterial resistance and thus decreased efficiency. Antibiotics can also cause

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How to cite this paper: Al-Zahrani, A., Omer, H. and Al-Judaibi, A. (2016) Impact of Antibacterial Activity of Physical Storage Extracts on Pathogenic Bacteria. *Journal of Biosciences and Medicines*, **4**, 54-62. http://dx.doi.org/10.4236/jbm.2016.44008

adverse effects, such as hypersensitivity and depletion of beneficial microbes in the gut [1]. For these reasons, the WHO has suggested the need for alternative treatments [2]. The increased demand for biodiversity in drug development has led to the identification of several compounds that can inhibit pathogenic bacterial growth and may provide new antibiotic medicines.

Marine organisms, especially seaweeds, have a broad range of antibacterial, antifungal, antitumor, and antioxidant behavior. The chemical structures of seaweed include sterols, isoprenoids, amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, fatty acids, and acrylic acid [3]-[5]. Seaweed-based products include alginate, carrageenan, and agar as phycocolloids. These products are abundantly available, renewable, and have been used for decades in medicine and pharmacy [6]. Seaweeds are also known to produce certain bioactive molecules [7]-[10], which interact with other organisms in the environment to inhibit bacterial or fungal growth [6] [11]. Seaweed's antimicrobial activity may also include the ability to synthesize bioactive secondary metabolites [12]-[14]. The extracts and active constituents of various marine seaweeds have been shown to have antibacterial activity against Gram-positive and Gram-negative bacteria [15]-[17].

The use of plant extracts in the treatment of diseases dates back to ancient times, and medicinal plants are widely used in contemporary medicine. Medicinal plants contain active compounds and essential oils. Si *et al.* [18] studied 66 essential oils and found that nine demonstrated particularly high efficacy against *S. typhimurium* DT104, *E. coli* O157: H7, and *E. coli* with K88 pili and significantly inhibited *E. coli* and coliform bacteria in the digestive system, with little effect on beneficial lactobacilli and anaerobic bacteria.

The coastlines of Jeddah in Saudi Arabia are abundant resources of many varieties of seaweeds and plant materials used in traditional medicine. The aim of the present study was to screen the effect of heat and storage on the antibacterial activity of *Ulva lactuca* and *Phoenix dactylifera* against *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

2. Materials and Methods

2.1. Study Bacteria

Bacteria were isolated and identified at King Abdulaziz University Hospital in Jeddah, Saudi Arabia as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, plates were prepared by inoculating 100 μl^{-1} of each sample (1×10^5 colony-forming units) onto Mueller-Hinton agar (OXOID CM 337) was as follows (g/L): beef, 300.00; casein acid hydrolysate 17.50; starch 1.50; agar 17.00. The final pH was 7.3 ± 0.1 at 25˚C.

2.2. Study Materials

The study materials were the green algae *Ulva lactuca* which was from Red Sea coastal areas around Jeddah, located between 21˚N and 39˚E, and the seeds of *Nigella sativa* and was collected from Jeddah markets. They were washed with distilled water several times, spread on plates and dried at 40˚C. After drying, they were ground and solubilized in methanol at the concentration of 100 g 100 ml^{-1}. The extracts were incubated on a 120 rpm shaker at 30˚C for 24 h and then filtered by using Whatman No. 1, after then it had dried under the reduced pressure at 40˚C, and the deposits were used as crude extracts [19].

2.3. Antibacterial Assays

Each crude extract was determined *in vitro* against the methicillin resistant *S. aureus* and *P. aeruginosa*. The activity of each extract was measured by the disc diffusion method following Clinical and Laboratory Standards Institute protocols [20]. Each extract was dissolved in dimethylsulfoxide (DMSO) with 3 $\mu g \cdot ml^{-1}$ and filtered by a 0.22 µm filter (Millipore, Billerica, MA). 100 µl of each solution were placed on 1 mm paper discs. Negative control was prepared with the solvent, and Augmentin XR (1 mg/ml) was used as the positive control. After inoculation, plates were incubated at 37˚C for 24 h and the inhibition zones were measured. All tests were performed in triplicate.

The extracts that inhibited the bacterial growth were tested to determine the minimum inhibitory concentrations (MICs) using serially diluted with Mueller-Hinton broth in a 96-well microplate [21]. Bacteria were cultured overnight on Mueller-Hinton agar and then suspended in 1 ml^{-1} of Mueller-Hinton broth (OXOID CM 405) to give a final concentration of 5×10^5 colony-forming units ml^{-1}. The microplate were inoculated with the bacteria and incubated at 37˚C for 16 - 20 h, and were evaluated for the visible presence or absence of bacterial growth. MICs were determined as the lowest concentration of an extract for which there was no visible growth

compared to the control [22].

The minimum bactericidal concentrations (MBCs) were determined by inoculating 0.1 ml of the negative growth wells in the MICs assays onto nutrient agar. Plates were incubated at 37˚C for 24 h. MBCs were considered to be the concentration that showed no growth of the tested bacteria, negative control was a plate that contained only medium [23]-[25].

2.4. Kill-Time Determination

This experiment was assayed to estimate the rate of killing bacteria by the crude methanol extract. Using the method of [26], the extract was incorporated into 10 ml Mueller Hinton broth in McCartney bottles at 1/2 × MIC, 1 × MIC, and 2 × MIC. Two controls, a Mueller-Hinton broth without extract inoculated with test organisms and a Mueller-Hinton broth incorporated with the extract at the test concentrations without the test organisms, were included. Inoculums density, approximately 10^5 cfu/ml further verified by total viable count, was used to inoculate 10 ml volumes of both in the McCartney bottles and control bottles. The bottles were incubated at 37˚C. A 100-µl aliquot was removed from the culture medium at 0, 2, 4, 8, 12, and 24 h to determine cfu/ml by using the Standard Plate Count technique [27]. After incubating at 37˚C for 24 h, the visible colonies were read by using an Interscience scan of 500 colony counters. Bacterial colonies were counted as cfu/ml and compared with the control count [28] [29]. Each treatment was performed in triplicate.

2.5. Effect of Storage on *U. lactuca* and *N. sativa* Methanol Extracts

To investigate the effect of storage time and the type of storage material on antibacterial activity of *U. lactuca* and *N. sativa*, extracts were divided into two major groups. In the first group, the extracts were stored in sterilized dark bottles. In the second group, the extracts were stored in sterilized transparent bottles. Each group was further subdivided into three portions that were stored for 1, 2, and 3 months before laboratory analyses. The positive control was crude of *U. lactuca* and *N. sativa* at 4˚C. Overnight nutrient broth cultures of *S. aureus* and *P. aeruginosa*. The cells were then suspended to the concentration of 5×10^5 colony-forming units ml^{-1}. By using holes in the agar, 100 µl of *S. aureus* or *P. aeruginosa* were inoculated in the plates, 100 µl of storage-treated extracts were added to the hole, and then the cultures were incubated at 30˚C for 24 h. Three replicates of each treatment were done. Results were recorded by measuring the diameter of the inhibition zone [30].

2.6. Effect of Temperatures on *U. lactuca* and *N. sativa* Methanol Extracts

This study evaluates the effect of several temperature treatments on the antibacterial activity of *the U. lactuca* and *N. sativa* methanol extracts, Both *U. lactuca* and *N. sativa* were tested for their antibacterial activity after treated with −80˚C, 5˚C, 22˚C, 50˚C, and 90˚C. Overnight nutrient broth cultures of *S. aureus* and *P. aeruginosa* were centrifuged, and the cells were washed three times with sterilized deionized water. The cells were then suspended in a concentration of 5×10^5 colony-forming units ml^{-1}. Using holes in the agar, 100 µl of *S. aureus* or *P. aeruginosa* were inoculated in the plates, 100 µl of temperature-treated extracts were added to the hole, and then were incubated at 30˚C for 24 h. Three replicates of each treatment were done. Results were recorded by measuring the diameter of the inhibition zone [30].

2.7. Statistical Analysis

Results were analyzed by paired-samples *t*-test using the IBM SPSS 20 statistical software to compare the mean values of each treatment, and they are expressed as means ± SE. Probability levels of less than 0.01 were considered highly significant.

3. Results

In this study, the effect of the ethanol extracts of *U. lactuca* and *N. sativa* was estimated by the inhibition of the bacterial growth and the determination of MICs and MBCs. The MICs concentrations of the algae and plant extracts were then assayed to determine the ability of the extracts activity after storage and temperatures treatments. The results in **Table 1** showed the effect of *U. lactuca* and *N. sativa* ethanol extracts on the growth inhibition with the concentrations of 30, 50, 100, 150 and 200 mg/ml, *S. aureus* was sensitive to all the concentrations and

Table 1. Inhibition of bacterial growth (mm) after 24 hours of treated with 100 µl *U. lactuca* and *N. sativa* methanol extracts (Mean ± SD).

	U. lactuca					*N. sativa*					Augmentin XR
Bacteria	30 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	30 mg/ml	50 mg/ml	100 mg/ml	150 mg/ml	200 mg/ml	200 mg/ml
S. aureus	25 ± 1.527**	28 ± 1.000**	30 ± 1.155**	30 ± 1.000**	32 ± 4.000**	14 ± 2.517*	15 ± 1.000**	20 ± 2.000**	14 ± 1.732**	16 ± 3000*	12 ± 0.135**
P. aeruginosa	0	9 ± 1.527*	12 ± 1.000**	12 ± 1.000**	13 ± 4.000	0	14 ± 1.000**	15 ± 0.577**	14 ± 2.000**	15 ± 1.528**	17 ± 0.017**

**$P \leq 0.01$.

Table 2. MIC and MBC (µl/m) of bacterial growth after incubation with *U. lactuca* and *N. sativa* methanol extracts.

	MIC		MBC	
	U. lactuca	*N. sativa*	*U. lactuca*	*N. sativa*
S. aureus	4	2	>32	8
P. aeruginosa	2	4	32	8

the 100 µl were the best concentration with inhibition zone of 30 mm and 20 mm after the treated with the extract of *U. lactuca* and *N. sativa* respectively. Increasing the concentrations of the extracts to 150 mg/ml and 200 mg/ml resulted approximate effect with the concentration 100 mg/ml with inhibitions of 30 mm, 32 mm by the treated with *U. lactuca*, while the extract of *N. sativa* showed a different result in decreased of inhibition effect to 14 mm and 16 mm after treated with 150 mg/ml and 200 mg/ml respectively.

On other hand, *P. aeruginosa* showed more resistance than *S. aureus*, the concentration 30 mg/ml has no inhibitory effect. While the inhibition at the concentrations of 50, 100, 150 and 200 mg/ml were approximate with the average of 12 mm and 15 mm after the treated with *U. lactuca* and *N. sativa* respectively.

The growth inhibition reflected on the MICs and MBCs and the results shows in **Table 2**. The extract of *N. sativa* was more effective on the tested bacteria with MIC and MBC of 2 µl, 8 µl and 4 µl, 8 µl for *S. aureus* and *P. aeruginosa* respectively.

Table 3 shows the antibacterial activity of 1/2, 1, and 2 MIC concentrations of *U. lactuca* and *N. sativa* extracts on bacterial vitality during incubation. The cells were counted after 0, 2, 4, 8, 12, and 24 h. The results of *P. aeruginosa* showed different cell decreases after incubation with *U. lactuca* and *N. sativa*, with percentage of cells vitalities up to 48.04%, 74.02%, 9.22%, and 66.75% after incubation with 1×MIC of *U. lactuca* and *N. sativa* extracts for 2 and 4 h, respectively. The 2 × MIC concentration decreased cell vitality to 44.32% and 72.78% after incubation with *U. lactuca* extract for 2 and 4 h. Incubation with *N. sativa* extract decreased cell vitality to 31.07% after 2 h. The number of vital cells of *S. aureus* decreased at the concentration of 1/2 × MIC to 2.10%, 31.36%, and 65.30%, respectively, after 2, 4, and 8 h of incubation with *U. lactuca* extract. The decreasing percentages of cells incubated with *N. sativa* were 1.89%, 21.79%, and 76.33%, respectively.

The results in **Table 4** and **Figure 1** show the effect of storage conditions; a transparent and dark storage bottle, on the activity of *U. lactuca* and *N. sativa* extracts against *S. aureus* and *P. aeruginosa*. Extracts stored in the transparent bottle decreased in effectiveness after one month of storage with percentage of 58.85%. After three months, the inhibition of the bacterial growth decreased to 38.90%. Further, after one month of storage, growth inhibition decreased after treated with *N. sativa* to 33.33%.

Extracts stored in the dark bottles performed better. *S. aureus* growth was inhibited to 53.70%, after stored for one month. While after three months, the growth inhibitor decreased to the percentage 46.30% by *U. lactuca* treatment.

As shown in **Table 5**, heating the extracts of *U. lactuca* and *N. sativa* to 90˚C increased their antibacterial activity. *U. lactuca* and *N. sativa* extracts stored at −08˚C decreased the growth of *S. aureus* and *P. aeruginosa*. *U. lactuca* and *N. sativa* extracts stored in transparent bottles for three months decreased the growth of *S. aureus*. Dark bottles reduced the percentage inhibitory of stored extracts for less than 10% in the *U. lactuca* extract and 20% in the *N. sativa* extract.

4. Discussion

The overuse of prescribed antibiotics can lead to resistance among these pathogenic bacteria. Thus, new antibiotics that are effective against new and resistant bacterial strains are needed. Plants and marine organisms may

Table 3. Kill-time determination of *S. aureus* and *P. aeruginosa* by cell vitality (cfu) after treatment with *U. lactuca* or *N. sativa* extracts for serial times and incubated for 24 h Mean ± SD.

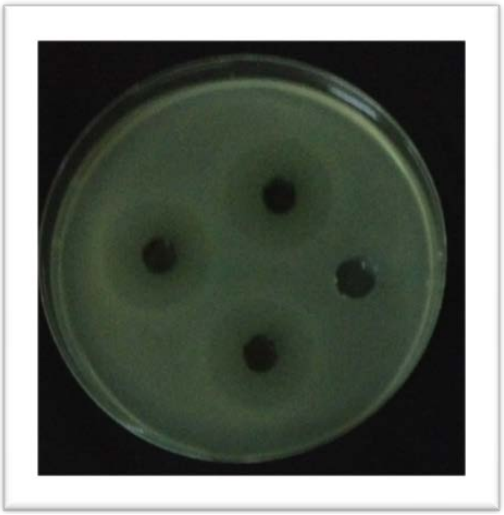
Tested bacteria		Control	1/2 × MIC					1 × MIC					2 × MIC				
			2 h	4 h	8 h	12 h	24 h	2 h	4 h	8 h	12 h	24 h	2 h	4 h	8 h	12 h	24 h
U. lactuca	*S. aureus*	3.89	3.81 ± 0.020**	2.67 ± 0.020**	1.35 ± 0.105**	0.0	0.0	2.84 ± 0.020**	2.52 ± 0.020**	1.28 ± 0.020**	0.0	0.0	2.77 ± 0.017**	1.35 ± 0.020**	0.0	0.0	0.0
	P. aeruginosa	4.85	2.54 ± 0.020**	2.44 ± 0.020**	1.30 ± 0.110**	1.26 ± 0.105**	0.0	2.52 ± 0.010**	1.26 ± 0.020**	0.00	0.0	0.0	2.70 ± 0.105**	1.32 ± 0.020**	0.0	0.0	0.0
N. sativa	*S. aureus*	5.28	5.18 ± 0.020**	4.12	1.25	0.00	0.00	3.77	2.49 ± 0.020**	0.00	0.00	0.00	2.77	1.33	0.00	0.00	0.00
	P. aeruginosa	4.12	3.89 ± 0.020**	2.72 ± 0.020**	1.37 ± 0.020**	0.00	0.00	3.74	1.37	0.00	0.00	0.00	2.84	0.00	0.00	0.00	0.00

**$P \leq 0.01$.

Table 4. Inhibition of bacterial growth (mm) after 24 h of incubation with 100 µl of *U. lactuca* or *N. sativa* extract stored for one, two and three months in two types of storage bottles Mean ± SD.

Storage periods		One month		Two months		Three months	
Type of bottles		Transparent bottles	Dark bottles	Transparent bottles	Dark bottles	Transparent bottles	Dark bottles
U. lactuca	*S. aureus*	28 ± 0.096**	29 ± 0.096**	27 ± 0.096**	28 ± 0.096**	21 ± 0.096**	25 ± 0.096**
	P. aeruginosa	28 ± 0.096**	30 ± 0.096**	32 ± 0.096**	35 ± 0.096**	36 ± 0.096**	37 ± 0.096**
N. sativa	*S. aureus*	18 ± 0.115**	19 ± 0.096**	13 ± 0.096**	15 ± 0.115**	12 ± 0.115**	13 ± 0.096**
	P. aeruginosa	23 ± 0.096**	25 ± 0.096**	27 ± 0.096**	30 ± 0.096**	32 ± 0.096**	32 ± 0.096**

**$P \leq 0.01$.



S. aureus treated with *U. lactuca* extract and stored for three month in transparent bottles

P. aeruginosa treated with *N. sativa* and stored for three months in transparent bottles

Figure 1. Bacterial inhibition growth after 24 h of incubation with *U. lactuca* or *N. sativa* extracts and stored for three months in transparent storage bottles.

offer such novel treatments. To that end, extracts of the seaweed *U. lactuca*, and the plant *P. dectylefera* were tested against strains of Gram-positive cocci and Gram-negative bacilli *S. aureus*, and *P. aeruginosa*. Seaweed metabolism is influenced by extreme marine environmental conditions, including high pressure, temperature, and osmolarity, as found in our study sites, Red Sea coastal areas in Jeddah. It is also affected by the presence of

Table 5. Inhibition of bacterial growth (mm) after 24 h of incubation with 100 µl of *U. lactuca* or *N. sativa* extract treated with serial temperatures Mean ± SD.

Temperatures		90˚C	50˚C	22˚C	5˚C	−80˚C
U. lactuca	*S. aureus*	30 ± 0.096**	28 ± 0.096**	27 ± 0.096**	23 ± 0.096**	20 ± 0.135**
	P. aeruginosa	13 ± 0.096**	13 ± 0.096**	12 ± 0.115**	12 ± 0.115**	11 ± 0.096**
N. sativa	*S. aureus*	22 ± 0.135**	20 ± 0.135**	20 ± 0.135**	19 ± 0.096**	17 ± 0.096**
	P. aeruginosa	17 ± 0.096**	16 ± 0.135**	16 ± 0.135**	16 ± 0.135**	15 ± 0.115**

**$P \leq 0.01$.

organic compounds, such as isonitrile, dichloramine, isocyanate, and halogenated compounds [31]. Bioactive compounds in antibacterial agents inhibit bacterial growth. These compounds include secondary metabolism components, such as alkaloids, peptides, terpenes, pigments, and sterols [32].

A study of antimicrobial activity of seaweeds *U. reticulata* and *U. lactuca* extracts showed promising antimicrobial activity against bacterial and fungal human pathogens [33]. In addition, *P. mirabilis* and *E. coli* were sensitive to the methanolic extract of *U. lactuca*, and *S. aureus* and *P. aeruginosa* were resistant to *U. lactuca* [34]. Selvin and Lipton [35] reported that the green alga *U. fasciata* exhibited broad spectrum antibacterial activity, these results agree with our results on *U. lactuca*. *U. fasciata* showed a broad spectrum of antibacterial activity, inhibiting the growth of both Gram-positive and Gram-negative tested organisms.

The effect of the bacterial inhibition growth reflected on the concentrations of both extracts's MICs and MBCs. MICs and MBCs were detected from methanol extract of *Stachytarpheta indica*, *Ligaria cuneifolia* and *Jodina rhombifolia*, and the MICs and MBCs were comparable to the results in this study [36] [37].

Kill-time is the time it takes for cells to recover after incubation with an antimicrobial agent. Kill-time increases as concentrations of *U. lactuca* and *N. sativa* extracts increase. This is likely due to the increase in bioactive compounds that exist in the extracts.

The ideal antibiotic should remain stable during extended storage periods and temperature fluctuations. *U. lactuca* and *N. sativa* extracts maintained high antibacterial activity against *S. aureus* and *P. aeruginosa* after three months of storage at 90˚C. This robustness may due to the concentrated and stability of its bioactive compounds. Other study results are consistent with ours [38]-[41]. Moreover, using dark bottles to store the extracts resulted in higher antibacterial activity, compared with the transparent bottles, this may due to biochemical light oxidation in the transparent bottles [42] [43].

5. Conclusion

The overuse of antibiotics can lead to resistance of pathogenic bacteria. A new antibiotic that is effective against new and resistant bacterial strains is needed. Medicinal plants and marine algae may offer such novel treatments. In this study, extracts of *U. lactuca*, and *N. sativa* were tested against *S. aureus*, and *P. aeruginosa*. The results of the bacterial inhibitor showed high activity in both extracts against *S. aureus* and *P. aeruginosa*. The MICs and MBCs were reflected with the growth inhibitor. Kill-time increases as concentrations of *U. lactuca* and *N. sativa* extracts increase. Moreover, extracts stored in the transparent bottle decreased in effectiveness after three months, and heating the extracts of *U. lactuca* and *N. sativa* to 90˚C increased their antibacterial activity.

References

[1] Badea, V., Balaban, D.P., Rapeanu, G., Amariei, C. and Badea, C.F. (2009) The Antibacterial Activity Evaluation of *Cystoseira barbata* Biomass and Some Alginates upon Bacteria from Oropharyngeal Cavity. *Romanian Biotechnological Letters*, **14**, 4851-4857.

[2] Abdul Hannan, S., Chaudhary, S., Barkaat, M. and Arshad, M. (2008) Anti Bacterial Activity of *Nigella sativa* against Clinical Isolates of Methicillin Resistant *Staphylococcus aureus*. *Journal of Ayub Medical College Abbottabad*, **20**, 72-74.

[3] Ahmad, V.U., Aliya, R., Perveen, S. and Shameel, M. (1993) Sterols from Marine Green Alga *Codium decorticatum*. *Phytochemistry*, **33**, 1189-1192. http://dx.doi.org/10.1016/0031-9422(93)85047-U

[4] Taskin, E., Ozturuk, M. and Kurt, O. (2001) Turkiye'nin yararlanilabilir denizel alg potansiyeli. *XI. Ulusal Su Urunleri Sempozyumu*, 225-232. http://dx.doi.org/10.3923/jas.2010.3007.3013

[5] Nor Afifah, S., Darah, I., Shaida Far, S., Jain Nordi, M.K. and Nurul Aili, Z. (2010) Antimicrobial Activity of Various Extracts of a Tropical *Chlorophyta macroalgae*, *Halimeda discoidea*. *Journal of Applied Sciences*, **10**, 3007-3013.

[6] Singh, A. and Chaudhary, B. (2010) Preliminary Phycochemical Analysis and *in Vitro* Antibacterial Screening of *Pithophora oedogonia* (Mont.) Wittrock: A Fresh Water Green Alga Forming Mats in the Water Bodies. *Journal of Algal Biomass Utilization*, **1**, 33-41.

[7] Yuan, Y.V. and Walsh, N.A. (2006) Antioxidant and Antiproliferative Activities of Extracts from a Variety of Edible Seaweeds. *Food and Chemical Toxicology*, **44**, 1144-1150. http://dx.doi.org/10.1016/j.fct.2006.02.002

[8] Thirumaran, G., Vijayabaskar, P. and Anantharaman, P. (2006) Antibacterial and Antifungal Activities of Brown Marine Macro Alaga *Dictyota dichotoma* from the Gulf of Mannar Biosphere Reserve. *Environ. Ecol*, **24**, 37-40.

[9] Vairappan, C.S., Ishii, T., Lee, T.K., Suzuki, M. and Zhaoqi, Z. (2010) Antibacterial Activities of a New Brominated Diterpene from Borneon *Laurencia* spp. *Marine Drugs*, **8**, 1743-1749. http://dx.doi.org/10.3390/md8061743

[10] Zbakh, H., Chiheb, H., Bouziane, H., Sanchez, V.M. and Riadi, H. (2012) Antibacterial Activity of Benthic Marine Algae Extracts from the Mediterranean Coast of Morocco. *Journal of Microbiology, Biotechnology and Food Sciences*, **2**, 219-228.

[11] Pushpamali, W.A., Nikapitiya, C., De Zoysa, M., Whang, I., Kim, S.J. and Lee, J. (2008) Isolation and Purification of an Anticoagulant from Fermented Red Seaweed *Lomentaria catenata*. *Carbohydrate Polymers*, **73**, 274-279. http://dx.doi.org/10.1016/j.carbpol.2007.11.029

[12] Siddhanta, A.K., Mody, K.H., Ramavat, B.K., Chauhan, V.D., Garg, H.S., Goel, A.K., Doss, M.J., Srivastava, M.N., Patnaik, G.K. and Kamboj, V.P. (1997) Bioactivity of Marine Organisms: Part VIII—Screening of Some Marine flora of Western Coast of India. *Indian Journal of Experimental Biology*, **35**, 638-643.

[13] Villarreal-Gómez, L.J., Soria-Mercado, I.E., Guerra-Rivas, G. and Ayala-Sánchez, N.E. (2010) Antibacterial and Anticancer Activity of Seaweeds and Bacteria Associated with Their Surface. *Revista de Biology Marinay Oceanografa*, **45**, 267-275. http://dx.doi.org/10.4067/s0718-19572010000200008

[14] Vijayabaskar, P. and Shiyamala, V. (2011) Antibacterial Activities of Brown Marine Algae (*Sargassum wightii* and *Turbinaria ornata*) from the Gulf of Mannar Biosphere Reserve. *Advances in Biological Research*, **5**, 99-102.

[15] Lima-Filho, J.V.M., Carvalho, A.F.F.U., Freitas, S.M. and Melo, V.N.M.M. (2002) Antibacterial Activity of Extracts of Six Macroalgae from the Northeastern Brazilian Coast. *Brazilian Journal of Microbiology*, **33**, 311-314. http://dx.doi.org/10.1590/S1517-83822002000400006

[16] Paul, G., Yusuf, S. and Sharma, S. (2006) Unmasking of the Brugada Syndrome Phenotype during the Acute Phase of Amiodarone Infusion. *Circulation*, **114**, e489-e491. http://dx.doi.org/10.1161/CIRCULATIONAHA.106.620799

[17] Devi, G.K., Manivannan, K., Thirumaran, G., Rajathi, F.A.A. and Anantharaman, P. (2011) *In Vitro* Antioxidant Activities of Selected Seaweeds from Southeast Coast of India. *Asian Pacific Journal of Tropical Medicine*, **4**, 205-211. http://dx.doi.org/10.1016/S1995-7645(11)60070-9

[18] Si, W., Gong, J., Tsao, R., Zhou, T., Yu, H., Poppe, C., Johnson, R. and Du, Z. (2006) Antimicrobial Activity of Essential Oils and Structurally Related Synthetic Food Additives towards Selected Pathogenic and Beneficial Gut Bacteria. *Journal of Applied Microbiology*, **100**, 296-305. http://dx.doi.org/10.1111/j.1365-2672.2005.02789.x

[19] Vijayakumar, M., Priya, K., Nancy, F.T., Noorlidah, A. and Ahmed, A.B.A. (2013) Biosynthesis, Characterisation and Antibacterial Effect of Plantmediated Silver Nanoparticles Using *Artemisia nilagirica*. Indust. *Crops Products*, **41**, 235-240. http://dx.doi.org/10.1016/j.indcrop.2012.04.017

[20] Wikler, M.A. (2007) Performance Standards for Antimicrobial Susceptibility Testing: Seventeenth Informational Supplement. Clinical and Laboratory Standards Institute (CLSI), Wayne.

[21] CLSI (2012) Performance Standards for Antimicrobial Disk Susceptibility Tests; Approved Standard—Eleventh Edition. CLSI Document M02-A11. Vol. 32, Clinical and Laboratory Standards Institute, Wayne.

[22] CLSI (2007) Performance Standards for Antimicrobial Susceptibility Testing; Seventeenth Information Supplement. CLSI Document M100-S17 (M2-A7 and M7-A7). Vol. 27, Clinical and Laboratory Standards Institute, Wayne.

[23] Wiegand, I., Hilpert, K. and Hancock, R.E.W. (2008) Agar and Broth Dilution Methods to Determine the Minimal Inhibitory Concentration (MIC) of Antimicrobial Substances. *Nature Protocols*, **3**, 163-175. http://dx.doi.org/10.1038/nprot.2007.521

[24] Hernandes, C., Coppede, J.D.S., Bertoni, B.W., França, S.D.C. and Pereira, A.M.S. (2013) Flash Microbiocide: A Rapid and Economic Method for Determination of MBC and MFC. *American Journal of Plant Sciences*, **4**, 850-852. http://dx.doi.org/10.4236/ajps.2013.44104

[25] Joshua, M. and Takudzwa, M. (2013) Antibacterial Properties of Mangifera Indica on *Staphylococcus aureus*. *African Journal of Clinical and Experimental Microbiology*, **14**, 62-74.

[26] Madigan, M.T., Martinko, J.M. and Parker, J. (1997) Brock Biology of Microorganisms. 8th Edition, Prentice Hall In-

ternational, Inc., New York.

[27] Rocha-Martin, J., Harrington, C., Dobson, A.D.W. and Ogara, F. (2014) Emerging Strategies and Integrated Systems Microbiology Technologies for Biodiscovery of Marine Bioactive Compounds. *Marine Drugs*, **12**, 3516-3559. http://dx.doi.org/10.3390/md12063516

[28] Wu, D. and Sun, D.W. (2013) Potential of Time Series-Hyperspectral Imaging (TS-HSI) for Non-Invasive Determination of Microbial Spoilage of Salmon Flesh. *Talanta*, **111**, 39-46. http://dx.doi.org/10.1016/j.talanta.2013.03.041

[29] Barbosa, M., Valentao, P. and Andrade, P.B. (2014) Bioactive Compounds from Macroalgae in the New Millennium: Implications for Neurodegenerative Diseases. *Marine Drugs*, **12**, 4934-4972. http://dx.doi.org/10.3390/md12094934

[30] Al-Awadi, A. and Al-Judaibi, A. (2014) Effects of Heating and Storage on the Antifungal Activity of Camel Urine. *Clinical Microbiology*, **3**, 179.

[31] Kolanjinathan, K. and Stella, D. (2011) Pharmacological Effect of *Gracilaria corticata* Solvent Extracts against Human Pathogenic Bacteria and Fungi. *International Journal of Pharmaceutical & Biological Archive*, **2**, 1722-1728.

[32] Pushparaj, A., Raubbin, R.S. and Balasankar, T. (2014) Antibacterial Activity of *Kappaphycus alvarezii* and *Ulva lactuca* Extracts against Human Pathogenic Bacteria. *International Journal of Current Microbiology and Applied Sciences*, **3**, 432-436.

[33] Christabell, J., Lipton, A.P., Aishwarya, M.S., Sarika, A.R. and Udayakumar, A. (2011) Antibacterial Activity of Aqueous Extract from Selected Macroalgae of Southwest Coast of India. *Seaweed Research Utilization*, **33**, 67-75.

[34] Chandrasekaran, M., Venkatesalu, V. and Raj, G.A. (2014) Antibacterial Activity of Selected Marine Macro Algae against Vancomycin Resistant *Enterococcus faecalis*. *Journal of Coastal Life Medicine*, **2**, 940-946.

[35] Selvin, J. and Lipton, A.P. (2004) Biopotentials of *Ulva fasciata* and *Hypnea musciformis* Collected from the Peninsular Coast of India. *Journal of Marine Science and Technology*, **12**, 1-6.

[36] Amenu, D. (2014) Antimicrobial Resistance for Enteric Pathogens Isolated from Acute Gastroenteritis Patients. *World Journal of Natural and Applied Sciences*, **1**, 1-14.

[37] Soberón, J.R., Sgariglia, M.A., Dip Maderuelo, M.R., Andina, M.L., Sampietro, D.A. and Vattuone, M.A. (2014) Antibacterial Activities of *Ligaria cuneifolia* and *Jodina rhombifolia* Leaf Extracts against Phytopathogenic and Clinical Bacteria. *Journal of Bioscience and Bioengineering*, **118**, 599-605. http://dx.doi.org/10.1016/j.jbiosc.2014.04.018

[38] Bukvicki, D., Gottardi, D., Tyagi, A.K., Veljic, M., Marin, P.D., Vujisic, L., Guerzoni, M.E. and Vannini, L. (2014) *Scapania nemorea* Liverwort Extracts: Investigation on Volatile Compounds, *in Vitro* Antimicrobial Activity and Control of *Saccharomyces cerevisiae* in Fruit Juice. *LWT-Food Science and Technology*, **55**, 452-458. http://dx.doi.org/10.1016/j.lwt.2013.09.029

[39] Ocheng, F., Bwanga, F., Joloba, M., Borg-Karlson, A.-K., Gustafsson, A. and Obua, C. (2014) Antibacterial Activities of Extracts from Ugandan Medicinal Plants Used for Oral Care. *Journal of Ethnopharmacology*, **155**, 852-855. http://dx.doi.org/10.1016/j.jep.2014.06.027

[40] Friedman, M., Henika, P.R. and Levin, C.E. (2015) Antimicrobial Activities of Red Wine-Based Formulations Containing Plant Extracts against *Escherichia coli* O157:H7 and *Salmonella enterica* Serovar Hadar. *Food Control*, **50**, 652-658. http://dx.doi.org/10.1016/j.foodcont.2014.10.005

[41] Adegbolagun, O.M. and Olukemi, O.O. (2010) Effect of Light Irradiation on the Antimicrobial Activity of *Zanthoxylum zanthoxyloides* (lam) Methanolic Extract. *African Journal of Pharmacy and Pharmacology*, **4**, 145-150.

[42] Akhtar, M.S., Bashir, S. and Sial, N.T. (2012) Antimicrobial Screening of *Heterophragma adenophyllum* Extracts and Effects of Light Irradiation. *Canadian Journal of Applied Sciences*, **3**, 304-313.

[43] Tanaka, K., Ohgo, Y., Katayanagi, Y., Yasui, K., Hiramoto, S., Ikemoto, H., Nakata, Y., Miyoshi, N., Isemura, M. and Ohashi, N. (2014) Anti-Inflammatory Effects of Green Soybean Extract Irradiated with Visible Light. *Scientific Reports*, **4**, Article No. 4732. http://dx.doi.org/10.1038/srep04732