

## Screening of the Antibacterial Activity of Some Marine Algae Against Fish and Human Pathogens

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**Abstract:** This study aims to investigate the effect of some algal extracts as antibacterial agents against some pathogenic bacteria. Thus the ethyl acetate and methanolic extracts of the marine algae *Ulva lactuca*, *Enteromorpha compressa*, *Ulva fasciata*, *Pterocladia capillacea*, *Corallina mediterranea*, *Hypnea musciformis* and *Padina pavonia*, collected from the coast of Alexandria (Egypt), were tested as antibacterial agents against fish and human pathogenic bacteria; *Aeromonas hydrophila*, *Vibrio anguillarum*, *Pseudomonas fluorescens*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*. The best activities were shown by the methanolic-L extract of *P. capillacea* against *P. fluorescens*, *V. anguillarum* and *P. aeruginosa*. The methanolic-L extract of *U. lactuca* showed high activity towards *A. hydrophila*, *V. anguillarum*, *P. fluorescens* and *S. aureus*. Cluster analysis was used to study the action of the crude algal extracts.

**Keywords:** Antibacterial Activity; Fish Pathogens; Marine Algae; *Pterocladia Capillacea*; *Ulva Lactuca*; Cluster Analysis

### INTRODUCTION

Bacterial infection causes high rate of complications, while *Salmonella* sp. causes diarrhea and typhoid fever.<sup>1,2</sup> *Aeromonas hydrophila* causes infection and several diseases like hemorrhagic septicemia, fin and tail rot in fishes.<sup>3,4</sup> *V.*

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*anguillarum* causes a bacteraemia in salmonoid fish that leads to internal haemorrhage and often death. It is of commercial importance due to loss of yield and quality of fish after infection.<sup>5</sup>

Microbially-derived diseases cause economic losses in aquaculture on global scale. The antibiotic resistant bacteria associated with fish disease are a worldwide problem in aquaculture and have received a considerable attention in recent years.<sup>6</sup> The search for new effective antimicrobial agents is necessary due to the appearance of microbial resistance and occurrence of fatal opportunistic infections. The development of antimicrobial resistance in many pathogenic microbes possesses one of the most serious problems in the control of infectious diseases.<sup>7</sup>

Using synthetic drugs are not recommended nowadays because of some ill effects to the organisms and human consumption. Therefore, research is focused on herbal medicines as alternate antibiotics. Marine macroalgae are good sources for new antimicrobial agents and they are able to produce a variety of secondary metabolites characterized by a broad spectrum of biological activities against human pathogens, fungi and yeast. However, there are a few reports concerning their effects against fish pathogens.<sup>8-10</sup> Some substances obtained from marine algae such as amino acids, terpenoids, phlorotannins, steroids, phenolic compounds, halogenated ketones and alkanes, cyclic polysulphides, fatty acids and acrylic acid have antimicrobial activity.<sup>11</sup>

The main objective of this study is to investigate the action of some algal extracts as antibacterial agents against some pathogenic bacteria.

#### MATERIAL AND METHODS

Seven species of marine algae from the divisions; Chlorophyta (*U. lactuca*, *E. compressa*, *U. fasciata*), Rhodophyta (*P. capillacea*, *C. mediterranea*, *H. musciformis*) and Phaeophyta (*P. pavonia*) [table 1] were collected during May and July, 2008 from sublittoral rocks at Abu Qir Bay and the Eastern Harbor (El-Manshia and El-Anfoushy) in the coast of Alexandria Fig.[1]. Their extracts were tested as antibacterial agents against fish (*A. hydrophila*, *V. anguillarum*, *P. fluorescens*), human (*S. aureus*, *S. typhimurium*) and human-fish (*P. aeruginosa*) pathogenic bacteria. The freshly collected algae were thoroughly washed with fresh water to remove sand

and epiphytes and then dried under shade.

#### Preparation of algal extracts:

The extraction procedure was performed according to Choudhury et al.<sup>12</sup> after modifications. The dried algae were powdered in a grinder and then soaked for two weeks in ethyl acetate followed by methanol. After filtration, organic layers were evaporated under vacuum at 45 °C. The methanolic extracts were separated as green residues (methanolic-R) and pale yellow liquids (methanolic-L). The crude extracts were stored at -20 °C until use.

#### Antibacterial activity of algal extracts:

Antibacterial activity was evaluated using well-cut diffusion technique.<sup>13</sup> Wells were punched out using a sterile 0.7 cm cork borer in nutrient agar plates inoculated with the test microorganisms (*A. hydrophila*, *V. anguillarum*, *P.*

*fluorescens*, *S. aureus*, *P. aeruginosa* and *S. typhimurium*) and each of the well bottoms was sealed with two drops of sterile water agar. Then 100 µl of algal extract were transferred into each well. Wells loaded with dimethyl formamide (DMF) were used as controls. All plates were incubated at 30 °C for 24 h. The inhibition zone was determined for each well and expressed in millimeter.

#### **Statistical analysis:**

Statistical analyses were performed by Minitab statistical software Version 14, using cluster analysis for data upon complete linkage level.

## **RESULTS**

#### **Antibacterial activity:**

The results of the antibacterial activities of the selected algal species extracts [table 2] generally indicated that the methanolic extracts showed more growth inhibitory effects

against most of the tested bacteria, except *S. typhimurium*, than ethyl acetate extracts. The strongest antibacterial activities were achieved by the methanolic-L extract of *P. capillacea* against *P. fluorescens*, *V. anguillarum* and *P. aeruginosa* with inhibition zones (IZ) of 29 mm, 28 mm and 27 mm, respectively. The methanolic-L extract of *U. lactuca* strongly inhibited the growth of *A. hydrophila* (27 mm), *V. anguillarum* (24 mm), *S. aureus* (20 mm) and *P. fluorescens* (20 mm) but no activity was exerted against *P. aeruginosa* or *S. typhimurium*. The methanolic extract of *E. compressa* showed good inhibitory activity against *A. hydrophila*, and *P. fluorescens* with IZ = 19 mm and 17 mm, respectively, and a moderate inhibitory activity against *V. anguillarum* (IZ = 13 mm). Furthermore, the methanolic-R of *P. pavonia* inhibited the growth of *A.*

*hydropila*, and *P. fluorescens* with equal inhibition zone (17 mm), but the methanolic-L extract of this alga inhibited only the growth of *P. fluorescens* (IZ = 18 mm). On the other hand, the species *U. fasciata* showed no antibacterial activity against tested pathogenic of bacteria. fish (*A. hydropila*, *V. anguillaum*, *P. fluorescens*), human (*S. aureus*, *S. typhimurium*) or human-fish (*P. aeruginosa*)

The ethyl acetate extracts of *U. lactuca*, *P. capillacea* and *C. mediterranea* showed antibacterial activities against *A. hydropila*, *V. anguillaum*, *P. fluorescens* and *P. aeruginosa*. The tested pathogens were varied in their response to the antibacterial action of the different extracts. The most susceptible organisms were *A. hydropila* and *P. fluorescens*. The growth of *A. hydropila*

was strongly inhibited by the methanolic-L extracts of *U. lactuca* (IZ = 27 mm) and *H. musciformis* (IZ = 21 mm) while *P. fluorescens* was strongly inhibited by the methanolic-L extracts of *P. capillacea* (IZ = 29) and *U. lactuca* (IZ = 20 mm).

#### **Cluster analysis:**

The action of the different algal extracts on the tested pathogens was studied using the cluster analysis as shown in Fig. [2]. They were clustered into 5 groups (A, B, C, D and E) in addition to two single clusters representing the ethyl acetate extract of *U. lactuca* and methanolic-L extract of *P. pavonia* at similarity level 64%. Group A represented (methanolic-R extract of *U. lactuca* and ethyl acetate extract of *C. mediterranea*) with similarity (100%), group B represented (ethyl acetate extract of *P. capillacea* and methanolic-R extract of *P. pavonia* with

similarity (94%), group C included (methanolic-L extract of *H. musciformis* and methanolic extract of *E. compressa*) with similarity (95%) and group D represented the major group which comprised (methanolic-R extract of *P. capillacea*, ethyl acetate extract of *H. musciformis*, methanolic-R extract of *H. musciformis* and ethyl acetate extracts of *U. fasciata* (A) and *U. fasciata* (B) with similarity 100%. Group D represented the most closely related extracts with respect to their action on the tested pathogens as group A. Furthermore, group E (methanolic-L extracts of *U. lactuca* and *P. capillacea*) was separated at 41% similarity level with (67%) similarity.

## DISCUSSION

The occurrence of antibiotic resistant bacteria associated with fish diseases is A worldwide problem in aquaculture.

Antibiotics used in both human as well as veterinary medicines have been tried experimentally to treat bacterial infections of fish. Problems including solubility, toxicity, cost, delivery and governmental restrictions have limited the available antibiotics to a select few, especially in food fish culture. Decreased efficacy and resistance of pathogens to antibiotics have necessitated development of new alternatives.<sup>14</sup> The interest in marine organisms as potential and promising alternatives has been increased during the last years.<sup>15-17</sup>

Some species of marine algal crude extracts showed inhibitory activity against pathogenic bacteria.<sup>18</sup> However, variation in antibacterial activity may be due to the production of bioactive materials which is related to the method of extraction, organic solvents used for the extraction

of bioactive compounds, season at which samples were collected and differences in assay methods. Some studies revealed that methanol extraction produces higher antimicrobial activity than n-hexane and ethyl acetate.<sup>19-21</sup> The present data revealed that the significant inhibition zones were observed for methanolic extracts.

Tuney et al.<sup>22</sup> found that the acetone, methanolic and diethyl ether extracts of *P. pavonia* had no antibacterial activity; however, in this study the methanolic extract of *P. pavonia* inhibited the growth of *A. hydrophila* and *P. fluorescens*. Gonzalez del Val et al.<sup>8</sup> found that the methanolic extracts of *P. pavonia* show antibacterial activity only against *B. subtilis*. It was reported that no antibacterial activity of the dichloromethane extract of *E. compressa* was observed against fish pathogenic

bacteria *V. anguillarum* or *A. hydrophila*.<sup>6</sup> Choudhury et al.<sup>12</sup> showed that the methanolic extract of *E. compressa* and *U. fasciata* had no antibacterial activity against *P. aeruginosa*, *A. hydrophila* or *P. fluorescens* while weak activity was obtained by *U. fasciata* against *P. fluorescens*.

Tuney et al.<sup>22</sup> demonstrated that the diethyl ether extract of *C. mediterranea* significantly inhibited the growth of *S. aureus* and *P. aeruginosa*. This study showed that the ethyl acetate extract of *C. mediterranea* had high antibacterial activity against *P. aeruginosa* and *A. hydrophila* with inhibition zones of 22 mm and 19 mm, respectively but no activity was observed against *S. aureus*.

Moreover, the ethyl acetate extract of *U. fasciata* had no antibacterial activity while the methanolic extract of *E. compressa* showed good inhibitory activity against *A. hydrophila* and *P.*

*fluorescens* with IZ values of 19 mm and 17 mm, respectively and a moderate activity against *V. anguillarum* (IZ = 13 mm). Kim and Lee<sup>23</sup> found that the methanolic extract of *E. compressa* had no antibacterial activity against *S. aureus*, *P. aeruginosa* or *S. typhimurium* and these results agreed with our finding.

This work showed that *Ulva lactuca* possessed significant antibacterial activity against different pathogenic bacteria in contrast to the results of Perez et al.<sup>24</sup> which may be due to time and place of sample collection. Also, it was in contrast with Wefky and Ghobrial<sup>25</sup> who reported that the acetone and ethanolic extracts of *P. capillacea* had strong antimicrobial activity against fish pathogens while no activity was observed by the methanolic extract.

The present results are in consistence with the findings of previous studies for the crude extracts of some

green, red and brown algae that have great antibacterial activity against Gram positive and Gram negative bacteria.<sup>26,27</sup> The results also revealed that the algal extracts showed significant activity against some of the tested pathogenic bacteria, however, other strains did not respond to these extracts. This might be due to masking of the antibacterial activity by the presence of some inhibitory compounds in the extract<sup>21</sup> as well as the distribution of antimicrobial substances which varied from species to species.<sup>28</sup>

Cluster analysis showed that high linkage (100%) exists between the antibacterial actions of the methanolic-R extract of *U. lactuca* and ethyl acetate extract of *C. mediterranea*. This may be attributed to their collection from the same site (Abu Qir) and during the same season. High linkages also observed between the methanolic-R extracts of *P.*

*capillacea* and *H. musciformis* as well as the ethyl acetate extracts of *H. musciformis*, and both of *U. fasciata* (A) and (B) while lower linkage was found between the methanolic-L extracts of *U. lactuca* and *P. capillacea*. This could be explained by the qualitative and quantitative distribution of allelochemicals which varied and influenced by the growth conditions and physiological state of the algae.<sup>29</sup>

## CONCLUSIONS

Macroalgae from the Egyptian coast are potential sources for bioactive compounds that may be useful as therapeutic agents for fish populations with bacterial diseases, and should be

investigated for natural antibiotics. The results also showed that methanol is an efficient solvent for extracting the bioactive materials and the significant inhibition zones were observed for methanolic extracts.

## RECOMMENDATION

Further investigations should be undertaken for isolation and identification of the bioactive compounds which are responsible for the antibacterial effect.

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**Table 1: Egyptian algae collected from Alexandria coast**

Division	Species	Collection Date	Location
Chlorophyta	<i>U. lactuca</i> (Linnaeus) <i>E. compressa</i> (Linnaeus) Nees <i>U. fasciata</i> (Delile)	May 24, 2008 July 10, 2008 July 30, 2008 July 30, 2008	Abu Qir Bay Abu Qir Bay El-Manshia (EH) El-Anfoushy (EH)
Rhodophyta	<i>P. capillacea</i> (Gmelin) <i>C. mediterranea</i> (Areschoug) <i>H. musciformis</i> (Wulfen)	May 24, 2008 May 24, 2008 July 10, 2008	Abu Qir Bay Abu Qir Bay Abu Qir Bay
Phaeophyta	<i>P. pavonia</i> (Linnaeus) Thivy	July 10, 2008	Abu Qir Bay

EH: Eastern Harbor

**Table 2: Antibacterial activities of crude algal extracts**

Species	Extract	Inhibition Zone (mm)					
		<i>A. hydrophila</i>	<i>V. anguillarum</i>	<i>P. fluorescens</i>	<i>P. aeruginosa</i>	<i>S. aureus</i>	<i>S. typhimurium</i>
<i>U. lactuca</i>	1 Ethyl acetate	18	17	0	15	0	0
	2 Methanolic-R	19	0	0	19	0	0
	3 Methanolic-L	27	24	20	0	20	0
<i>P. capillacea</i>	4 Ethyl acetate	19	0	15	18	0	0
	5Methanolic-R	0	0	0	17	0	0
	6Methanolic-L	15	28	29	27	15	0
<i>C. mediterranea</i>	7 Ethyl acetate	19	0	0	22	0	0
<i>H. musciformis</i>	8 Ethyl acetate	0	0	0	0	0	0
	9 Methanolic-R	0	0	0	0	0	0
	10 Methanolic-L	21	13	18	0	0	0
<i>P. pavonia</i>	11 Methanolic-R	17	0	17	0	0	0
	12 Methanolic-L	0	0	18	0	0	0
<i>E. compressa</i>	13 Methanolic	19	13	17	0	0	0
<i>U. fasciata</i> (A)	14 Ethyl acetate	0	0	0	0	0	0
<i>U. fasciata</i> (B)	15 Ethyl acetate	0	0	0	0	0	0

A: The alga collected from El-Manshia; B: The alga collected from El-Anfoushy.

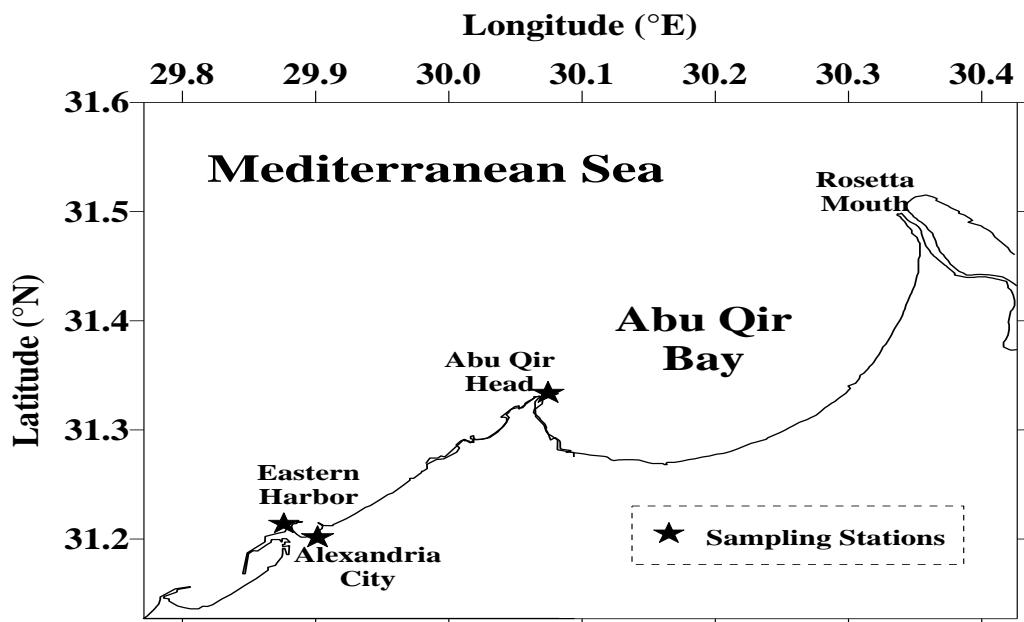


Figure (1): Location of Study Area

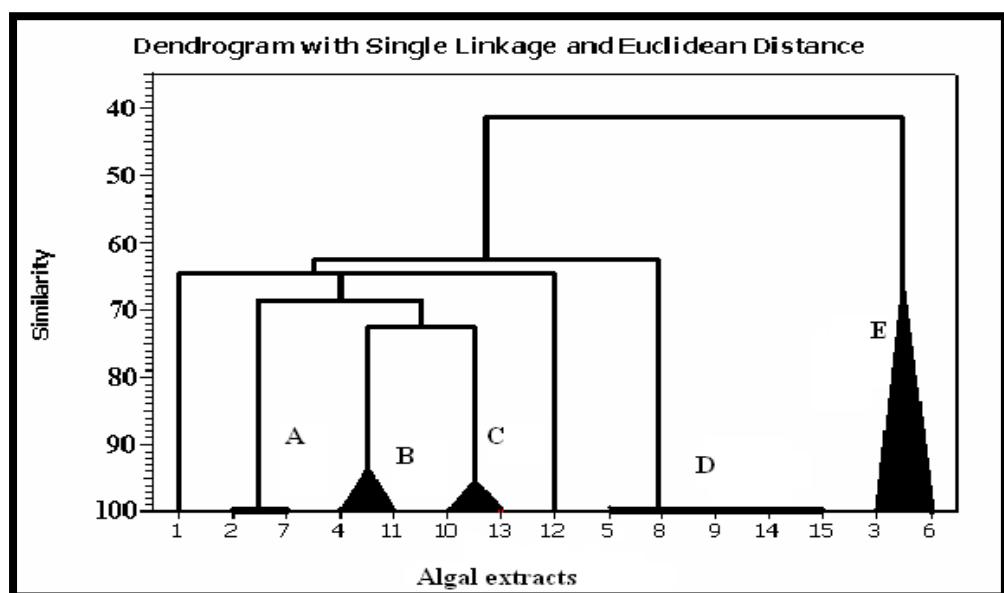


Figure (2): Dendrogram of the Action of Different Algal Extracts (1-15) Against Tested Pathogens

## REFERENCES

1. Leven MM. *Escherichia coli* that causes diarrhea: Enterotoxigenic, enteropathogenic, enteroinvasive, enterohemorrhagic and enteroadherent. *J Infect Dis* 1987; 155: 377-89.
2. Jawetz E, Mellnick JL, Adelberg EA. Review of medical microbiology, 20th edition. Applebaum Lange Norwalk, Connecticut; 1995. P. 139-218.
3. Pulliam JK, Dillehay DL, Webb S. High mortality in zebrafish (*Danio rerio*). *Contemp Top Lab Anim Sci* 1999; 38: 80-3.
4. Qian D, Chen Y, Shen J, Shen Z. Serogroups, virulence and hemolytic activity of *Aeromonas hydrophila* which caused fish bacterial septicaemia. *Wei Sheng Wu Xue Bao* 1995; 35: 460-4.
5. Stroehner UH, Jedani KE, Manning PA. Genetic organization of the regions associated with surface polysaccharide synthesis in *Vibrio cholerae* O1, O139 and *Vibrio anguillarum* O1 and O2: a review. *Gene* 1998; 223: 269-82.
6. Bansemir A, Blume M, Schröder S, Lindequist, U. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture* 2006; 252: 79-84.
7. Atta-ur-Rahman, Choudhary MI, Thomsen WJ. Bench-Top and Primary Bioassay Screening. In: *Bioassay Techniques for Drug Development*. Harwood Academic Publishers; 2001. P. 14-25.
8. Gonzalez del Val A, Platas G, Basilio A, Cabello A, Gorrochategui J, Suay I et al. Screening of antimicrobial activities in red, green and brown macroalgae from Gran Canaria (Canary Islands, Spain). *Int Microbiol* 2001; 4: 35-40.
9. Kubanek J, Jensen PR, Keifer PA, Sullards C, Collins DO, Fencial W. Seaweed resistance to microbial attack: A targeted chemical defense against marine fungi. *Proc Natl Acad Sci USA* 2003; 100: 6916-21.
10. Mahasneh I, Jamal M, Kashashneh M, Zibdeh M. Antibiotic activity of marine algae against multi-antibiotic resistant bacteria. *Microbios* 1995; 83: 23-6.
11. Mtolera MSP, Semesi AK. Antimicrobial activity of extracts from six green algae from Tanzania. In: Björk M, Semesi A, Pedersén M, Bergman B. eds. *Current trends in marine botanical research in the east African region*. Uppsala, Sweden: Gotab AB; 1996. P 211-7.
12. Choudhury S, Sree A, Mukherjee SC, Pattnaik P, Bapuji M. In vitro antibacterial activity of extracts of selected marine algae and mangroves against fish pathogens. *Asian Fish Sci* 2005; 18: 285-94.
13. El-Masry MH, Khalil AI, Hassouna MS, Ibrahim HAH. In situ and in vitro suppressive effect of agricultural composts and their water extracts on some phytopathogenic fungi. *World J Microbiol Biotechnol* 2002; 18: 551-8.
14. Smith P, Hiney MP, Samuels OB. Bacterial resistance to antimicrobial agents used in fish farming. *Annu Rev Fish Dis* 1994; 4: 273-313.
15. Lindequist U, Schweder T. Marine biotechnology. In: Rehm HJ, Reed G, eds. *Biotechnology 10*. Weinheim: Wiley-VCH; 2001. P. 441-84.
16. Mayer AMS, Hamann MT. Marine pharmacology in 1999: compounds with antibacterial, anticoagulant, antifungal, anthelmintic, anti-inflammatory, antiplatelet, antiprotozoal and antiviral activities affecting the cardiovascular, endocrine, immune and nervous systems, and other miscellaneous mechanism of

- action. *Comp Biochem Physiol Part C* 2002; 132: 315–39.
17. Newman DJ, Cragg GM, Snader KM. Natural products as source of new drugs over the period 1981–2002. *J Nat Prod* 2003; 66: 1022–37.
18. Hornsey IS, Hide D. The production of antimicrobial compounds by British marine algae. IV Variation of antimicrobial activity with algal generation. *Br Phycol J* 1985; 20: 21–5.
19. Moreau J, Pesando D, Bernad P, Caram B, Pionnat JC. Seasonal variations in the production of antifungal substances by some dictyotales (brown algae) from the French mediterranean coast. *Hydrobiologia* 1988; 162: 157–62.
20. Rosell KG, Srivastava LM. Fatty acids as antimicrobial substances in brown algae. *Hydrobiologia* 1987; 151/152: 471–5.
21. Sastry VMVS, Rao GRK. Antibacterial substances from marine algae: successive extraction using benzene, chloroform and methanol. *Bot Mar* 1994; 37: 357–60.
22. Tuney I, Cadirci BH, Unal D, Sukatar A. Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey). *Turk J Biol* 2006; 30: 171–5.
23. Kim IH, Lee JH. Antimicrobial activities against methicillin-resistant *Staphylococcus aureus* from macroalgae. *J Ind Eng Chem* 2008; 14: 568–72.
24. Perez RM, Avila JG, Perez G. Antimicrobial activity of some American algae. *J Ethnopharmacol* 1990; 29: 111–118.
25. Wefky S, Ghobrial M. Studies on the bioactivity of different solvents extracts of selected marine macro-algae against fish pathogens. *Res J Microbiol* 2008; 3: 673–82.
26. Rao PS, Parekh KS. Antibacterial activity of Indian seaweed extracts. *Bot Mar* 1981; 24: 577–82.
27. Rao PPS. Biological investigation of Indian marine algae, screening of some green, red and brown seaweeds for their antimicrobial activity. *Seaweed Res Util* 1991; 14: 37–43.
28. Lustigman B, Brown C. Antibiotic production by marine algae isolated from the New York/New Jersey coast. *Bull Environ Contam Toxicol* 1991; 46: 329–35.
29. Levin DA. Plants phenolics: An ecological perspective. *Am Nat* 1971; 105: 157–81.