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Antimicrobial activity of two marine algae *Ulva rigida* and *Ulva intestinalis* collected from Arzew gulf (Western Algeria)

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ABSTRACT

In this study, extracts of two marine cholophycean algae (*Ulva rigida* and *Ulva intestinalis*), harvested from Arzew gulf (Western Algeria) were investigated for their antimicrobial activity against Human pathogenic bacteria including antibiotic-resistant organisms and yeast were tested by using the paper disc agar diffusion method. Methanolic extract of *Ulva rigida* showed stronger anti-microbial activity compared to that of *Ulva intestinalis*. Both extracts inhibited but at different concentrations *Esherichia coli, Streptococcus pyogenes, methicillin-resistant Staphylococcus epidermidis (*MRSE), *Candida albicans* and *Aspergillus niger.* Methanolic extract of *Ulva rigida* elicited remarkable antibacterial activity against all Human pathogenic bacteria screened in this study. However no activity was detected by methanolic extract of *Ulva intestinalis* on multi-drug resistant(MDR) *Proteus mirabilis* and . Results of present study confirmed the potential usefulness of marine algae in the pharmaceutical and biotechnological industries.
KEY WORDS: marine algae, *Ulvarigida*, *Ulva intestinalis*, antimicrobial activity, Algeria

INTRODUCTION

Faced with escalating multidrug resistance in bacteria and the emergence of new infectious diseases, many researchers have focused on investigating natural and safe antimicrobial agents for both the food and medical industries [1]. Seaweeds or marine macroalgae are one of the abundant natural resources in marine ecosystems. They have recently received significant attention for their potential to supply new bioactive substances [2, 3, 4]. Their capacity to produce metabolites that exhibit various biological activities such as antibacterial, anti-inflammatory, antiviral [5, 6]; antifungal [7], anticancer, antidiabetic, antihypertensive, antihyperlipidemic, and antioxidant activities [8, 9, 10, 11, 12, 13] is increasingly recognized.

Chlorophyceaen seaweeds, popularly known as green algae, are widely distributed in both inter-tidal and deeper-water regions of the seas. The genus *Ulva (U.)* was first identified by Linnaeus in 1753 [14]. These algae have a potential for rapid and proliferous growth with a ubiquitous distribution with species living in a wide range of habitats and environments [15, 16]. They can proliferate in saline and brackish, freshwater habitats [17]. The chemical composition of these macroalgae was found to vary depending on geographical distribution and seasons and the principal environmental factors affecting the composition are water temperature, salinity, light, nutrients and minerals availability [15].

Our knowledge (obtained from literature) indicated that data related to antimicrobial activity of seaweeds throughout the west coast of Algeria are very limited. Hence, the aim of this study was to investigate the antibacterial and antifungal activity of two marine algae belonging to Chlorophyta (*Ulva rigida* and *U. intestinalis*) against Human pathogenic bacteria including antibiotic-resistant forms and three fungi (*Candida albicans*, *Aspergillus niger*, *Cryptococcus neoformans).*

MATERIALS AND METHODS

Sample Collection

Fresh samples of two species of marine algae. namely *U. rigida* and *U. intestinalis* were collected from Arzew gulf (Western Algeria) during March 2015. The identification of the investigated marine algae was kindly verified by Prof. ELKHIATI Laboratory of Biology and Plant Physiology Casablanca-Morocco. Once harvested, marine algae were stored in plastic bags and placed on ice for transport to the laboratory. They were washed several times with seawater to remove sand, mud and attached fauna. Then they were cleaned of epiphytes and necrotic parts were removed. The samples were dried in room temperature (28oC -30oC, low humidity) for three weeks. The dried algae materials were homogenized to fine powder before extraction.

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Preparation of algal extracts

Ten grams of dried material were added to 150 ml of methanol and left for 8 h at room temperature with stirring at 200 rpm. The solvent extracts were then filtered and the filtrate was concentrated by rotary evaporation at 45 - 50°C. The resulting extracts were then dissolved in dimethlysulfoxide (DMSO) and kept at +4°C until further use.

Culture and Maintenance of microorganisms

The seaweed extracts were tested against a panel of clinical isolates viz: *Escherichia (E.) coli, Salmonella sp., Shigella (S.) dysentriae, Pseudomonas* (P.), *aeruginosa,* multi-drug resistant *(MDR) Proteus (P.) mirabilis* (*sensitive Streptococcus pyogenes, Staphylococcus aureus methicillin-resistant (MRSA), Staphylococcus epidermidis* (MRSE), *Klebsiella* (K.) *pneumoniae, vancomycin*resistant (VRE) *Enterococcus* (E.) *faecalis, Candida* (C.) *albicans, Aspergillus (A.) niger* and *Cryptococcus neoformans*.

Pure cultures of all experimental bacteria and fungi were obtained from Mascara Hospital-Algeria. The resistance patterns of these isolates were investigated according to criteria of National Committee for Clinical Laboratory Standards (NCCLS, 2002) and Manual of Antimicrobial Susceptibility Testing guidelines [18]. The pure bacterial cultures were maintained on nutrient agar medium and fungal culture on potato dextrose agar (PDA) medium. Each bacterial and fungal culture was further maintained by subculturing regularly on the same medium and stored at 4^{o} C.

Antibacterial assay

Antimicrobial activity was evaluated using the agar diffusion technique in Petri dishes [19]. Briefly, all bacterial isolates were suspended in saline to a turbidity equivalent to 0.5 McFarland (1.5 x 108 CFU/ml) and 0.1ml standardized inoculum suspension was swabbed uniformly on MHA plates. Sterile filter paper discs, 6 mm in diameters (Whatman No. 1), were loaded with 20 µl of the different extracts and air dried (the crude extracts were dissolved in 10% DMSO). Discs impregnated with methanol and with DMSO were used as negative controls. The discs were placed on Muller Hinton agar plates (Merck, Darmstadt, Germany) inoculated with each of the previously mentioned microorganisms (approximately 107-108 bacteria and fungus/ml). Plates were incubated for 24 h at 35°C. After incubation the clearance zones around the discs were measured and expressed in millimeter. The extracts were tested at different concentrations (200mg/ml, 100mg/ml, 50mg/ml, 25mg/ml and 12,5mg/ml).

Statistical analysis

All the experiments were carried out in triplicates and values were expressed as mean ± SD. Graphics were made using Microsoft Office Excel 2007.

RESULT AND DISCUSSION

In the present study, the methanol was chosen for extraction method because it was reported by many researchers that seaweeds extracts obtained with methanol have higher antimicrobial activity than that of extracts obtained with other organic solvents viz: n-hexane, acetone and ethyl acetate [20, 21].

As shown in Figure 1, results showed that methanolic extract of *U. rigida* elicited remarkable antibacterial activity against all Human pathogenic bacteria screened in this study.

Erturk and Taş [22], reported that ethanol extract of *U. rigida* collected from the coast of Vona Bay Perşembe, Ordu (TURKEY), exhibited inhibition activity against *Staphylococcus aureus*, *Bacillus cereus*, *Salmonella typhimurium*, *Listeria monocytogenes*, *Escherichia coli* and *Pseudomonas aeruginosa*. Also, Tuney *et al*. [23] indicated that diethyl ether extracts of *U. rigida* showed bactericidal activity against *some* pathogenic bacteria such as: *Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa.* In contrast, Taskin *et al*., [24]; Salvador *et al*. [25] and Cherif *et al*., [26] have reported very Low or no inhibitory activity by *U. rigida*. This variation between results may be explained by to several factors viz: extraction method, geographical sampling zone, algae species, collected season of the algae, algal growth phases and intraspecific variability in the production of secondary metabolit.e s. [27, 28, 29].

In the current work, *E. coli, P. aeruginosa, S.dysentriae, Streptococcus pyogenes and Staphylococcus epidermidis (MRSE)* were inhibited by methanolic extract of *U. rigida* at different extract concentrations (Fig.1). *K.pneumoniae,* MDR *P.mirabilis, Salmonella* sp and *Staphylococcus aureus (*MRSA) were inhibited until 25mg/ml. However *E.faecalis* (VRE) was inhibited only at 200 and 100mg/ml (Fig. 1). *K.pneumonia and MDR P. mirabilis* exhibited higher sensitivity for *U. rigida* extract with an inhibition zone respectively of 25.66 ± 0. 23 mm and 24.16 ± 0.63 mm. The larger diameter of zone inhibition represents high sensitivity of the microorganisms to the seaweed extracts and the higher concentrations of extract.

Lim *et al*. [30] and Darah *et al*. [31], reported that the higher concentration of the extract is necessary to kill the microorganisms cells. According to Imbs *et al*. [32], antimicrobial activities of extracts varied considerably

between assay microorganisms and by variety of antimicrobial compounds. An increasing number of studies have recorded the mechanism of actions involved in bacterial killing process. Among them are the interactions of the antimicrobial compound with the cell membrane, interference with the production of essential proteins or with the transformation (metabolism) of nucleic acid [33, 22].

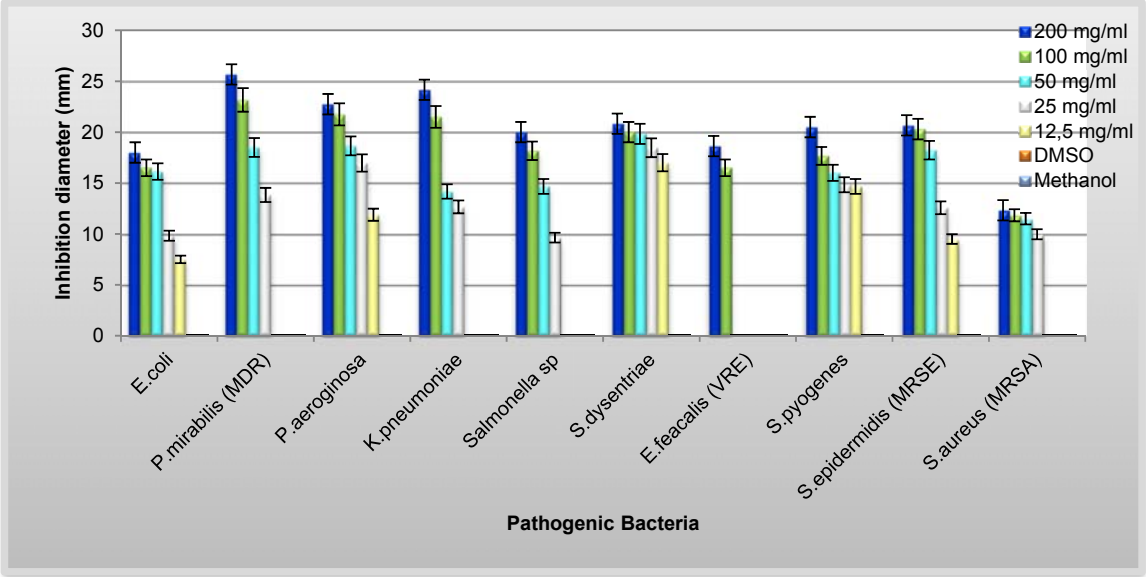

Figure 1. Antibacterial activity of extracts of *U. rigida* collected from Arzew gulf (Western Algeria) during March 2015.

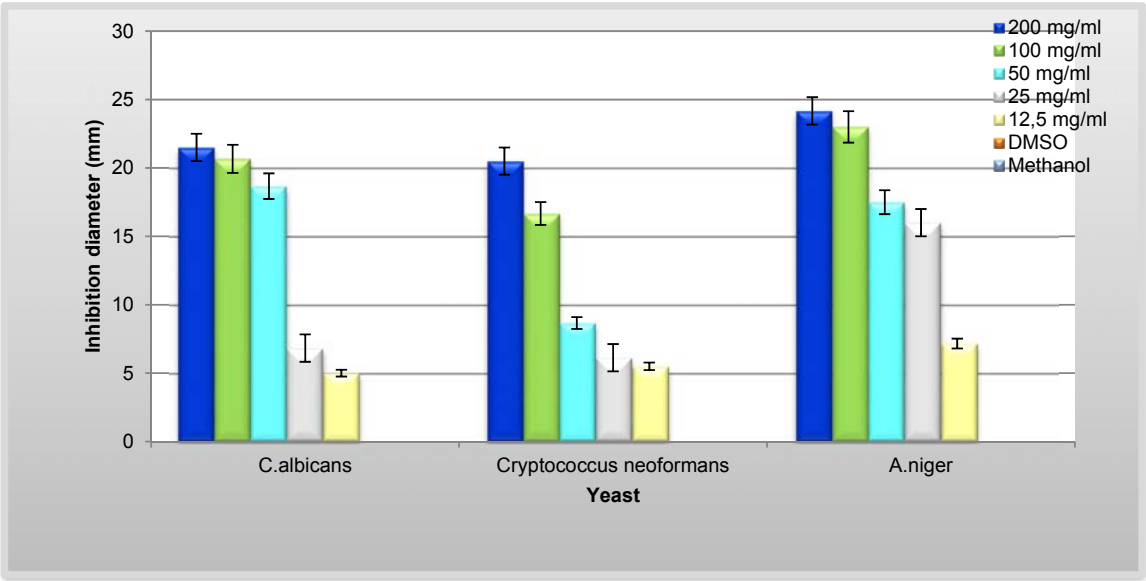

Figure 2. Antifungal activity of extracts of *U. rigida* collected from Arzew gulf (Western Algeria) during March 2015.

Another significant result of the present study was that the methanolic extract of *U. rigida* showed good antifungal activity against *A. niger* (*24,02 ± 0.84),C. albicans (21.5±1.08) and Cryptococcus neoformans* (20.5± 0.70) (Fig. 2). A similar observation was recorded by Madalena *et al*., [17] and Ertürk and Taş [22].

On the other hand, the results obtained show that methanolic extract of *U. intestinalis* has a lower antibacterial activity than that observed by methanolic extract of *U. rigida.* Indeed, only *E. coli, Streptococcus pyogenes* and *Staphylococcus epidermidis* (MRSE) were inhibited at different extract concentrations (Fig. 3). However, *P. aeruginosa* was inhibited only at 200 mg/ml. In this study it was found that, MDR *P. mirabilis* and *Staphylococcus aureus* (MRSA) were more resistant to methanolic of *U. intestinalis*. A possible explanation for these observations may be attributed to masking antibacterial activity by the presence of some inhibitory

compounds in the extract [31]. Referring to the literature, bacterial resistance, may be ascribed to 2 distinct pathways: passive, which involves alterations of outer membrane proteins, the porins, which decrease the rate of entry of antimicrobial compound into the bacteria by diminution of the pore size and active, which involves overexpression of an indigenous efflux pump that exports the antimicrobial compound outside the cell after a regulatory mutation [34].

In the current work, the maximum activity was observed in *K.pneumoniae* (22. 77 ± 0.34 mm). A similar observation was recorded by Maruthupandian *et al*., [35]. In contrast, our results are partially in agreement with those obtained by Berber *et al*., [36] who demonstrated that metabolic extract *U. intestinalis* collected from the coastal region of Sinop (Turkey) showed maximum activity against *Staphylococcus epidermidis* but no activity was observed towards *Escherichia coli.* Pandidurai and Perumal, [37] demonstrated that *U.intestinalis* showed maximum activity against *P. mirabilis.*

Furthermore, we note that diameters of the inhibition zones recorded in our research are more important than indicated in the three aforementioned studies. These differences can be explained by the seasonal variation, sampling location or the higher concentrations of extract as demonstrated by several researchers mentioned above.

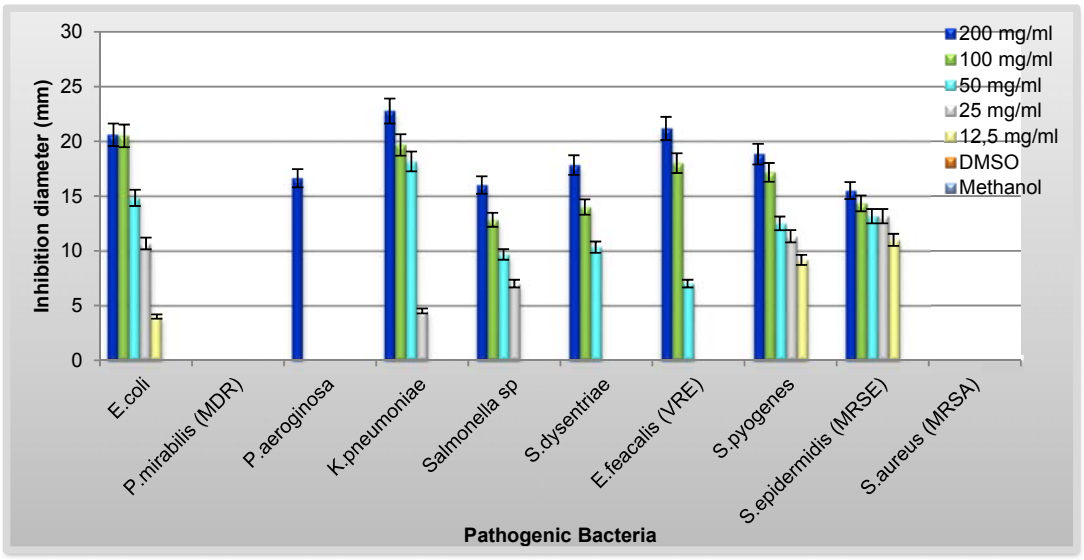

Figure 3. Antibacterial activity of extract of *U. intestinalis* collected from Arzew gulf (Western Algeria) during March 2015.

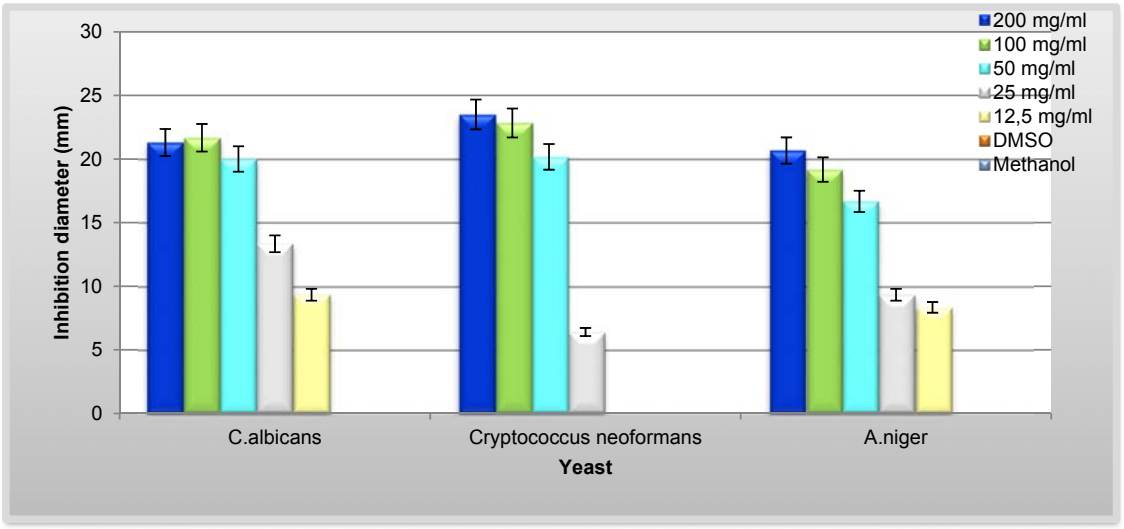

Figure 4. Antifungal activity of extracts of *U. intestinalis* collected from Arzew gulf (Western Algeria) during March 2015.

Antifungal activity results of *U. intestinalis* were more significant than those observed by methanolic extract of *U. rigida* (Figure 4). The highest inhibition activity was observed in *Cryptococcus neoformans* (23. 5± 1.08 mm) and *C. albicans* 21. 2 ± 0,62 mm). These results are not in agreement with those obtained by Darah and Lim [38]. No activity was exhibited by methanolic extract of *E. intestinalis* on tested fungi and yeast according to these researchers.

Our results highlighted the strongly in vitro antifungal activity of the tested algal extracts. However, it is important to note that Saidani *et al*., [39] reported methanolic extracts of four species of marine algae *(Rhodomella confervoides*, *U. lactuca*, *Cystoseira tamaricifolia* and *Padina pavonica*) collected from Bejaia coast (Algeria) showed antifungal activity against three fungal species (*A. niger*, *C. albicans* and *Mucor ramaniannus*).

In all case, the antimicrobial effect of both methanol extracts were found to be dose-dependent as it was observed that the inhibition zone increased as the concentration of methanol extracts increased. Also, no inhibition of test microorganisms growth was observed in the presence of 10% DMSO in disc diffusion method.

Hence, results obtained from this study demonstrated that methanolic extracts of *U. rigida* and *U. intestinalis* showed clear and important antimicrobial activities against Human pathogenic microorganisms including antibiotic-resistant bacteria. The present results agreed with the findings of Kandhasamy and Arunachalam [40], who concluded that Chlorophyceaen members showed higher antimicrobial activity than other algal groups. In fact, a large number of *Ulva extractions* products have been found to have antimicrobial activity, many of these compounds have been identified as fatty acids, hydroxyl unsaturated fatty acids, ghycolipids, steroids, phenolics and terpenoids [41].

Conclusion

Our findings, using green macroalgae from the Algerian coast, show that they are potential sources of bioactive compounds. These algae may be a valuable and renewable source for many active substances with a wide range of applications in agriculture, medical or food industry. Therefore screening these natural products will be of great interest and further studies should be undertaken to characterize the active compounds residing in species of algae. Moreover, more toxicological studies need to be performed.

REFERENCES

1. Silver, LL., and KA. Bostian, 1993. Discovery and development of new antibiotics: the problem of antibiotic resistance. Antimicrob. Agents Chemother, 37: 377-383.

2. Gamal, AAE., 2010. Biological importance of marine algae. *Saudi Pharmaceut. J.,* 18: 1-25.

3. Mohamed, S., SN. Hashim and AH. Rahman, 2012. Seaweeds: A sustainable functional food for complementary and alternative therapy. *Trends in Food Sc.* and *Technol.,* 23: 83-96.

4. Roussis V., T. Rapti, C. Vagias, C. Harvala and H. Caberi, 2000. Volatile metabolites of the green alga *U. rigida* (CAg). J. of *Essential Oil Res.,* 12: 201-204.

5. Barbosa JP., RC. Pereira, JL. Abrantes, CC. Cirne dos Santos, MA. Rebello, IC. Frugulhetti and VL. Texeira, 2004. In vitro antiviral diterpenes from the Brazilian brown alga Dictyotapfaffii. Planta Medica, 70: 856–860.

6. Seenivasan R., H. Indu, G. Archana and S. Geetha, 2010. The antibacterial activity of some marine algae from south east coast of India. J.of Pharm. Res., 3: 1907-1912.

7. De Felicio, R., S. De Albuquerque, M.C.Young, NS. Yokoya and HM. Debonsi, 2010. Trypanocidal, leishmanicidal and antifungal potential from marine red alga Bostrychia tenella, J.Agardh (Rhodomelaceae, Ceramiales). J. of Pharmaceut. and Biomed. Analysis, 52: 763-769.

8. Devi, G., K. Manivannan, G. Thirumaran, F.A. Rajath, and P. Anantharaman , 2011. *In vitro* antioxidant activities of selected Seaweeds from southeast coast of India. Asian. Pac. J. Trop. Med., 4: 205-211.

9. Kim, S.K., NV. Thomas and X. Li, 2011. Anticancer compounds from marine macroalgae and their application as medicinal foods. Adv. Food and Nut. Res., 64: 213-224.

10. Ren, D., H. Noda, H. Amano, T. Nishino and K. Nisizawa, 1994. Study on antihypertensive and antihyperlipidemic effects of marine algae. Fish. *Sc.,* 60: 83-88.

11. Samarakoon, K. and YJ. Jeon, 2012. Bio-functionalities of proteins derived from marine algae- A review. *Food Res. Intern.*, *48*: 948-960.

12. Santoso, J., Y. Yoshie and T. Suzuki, 2004. Polyphenolic compounds from seaweeds: distribution and their antioxidative effect. Develop. in Food Sc., 42: 169-177.

13. Xu, Hl., C. Kitajima, H. Ito, T. Miyazaki, M. Baba, T. Okuyama and Y. Okada, 2012. Antidiabetic effect of polyphenols from brown alga *Ecklonia kurome* in genetically diabetic KK-Ay mice. *Pharmaceut. Biol.,* 50: 393–400.

14. Kong, F., Y.Mao, F. Cui, X. Z.Zhang and Z. Gao, 2011. Morphology and Molecular Identification of U. Forming Green Tides in Qingdao China. J. of *Ocean Univ. of China,* 10: 73-79.

15. Messyasz, B. and A. Rybak, 2010. Abiotic factors affecting the development of *U.* sp. (Ulvophyceae, Chlorophyta) in freshwater ecosystems. *Aquatic Ecol.*, 45: 75-87.

16. Wolf, MA., K. Sciuto, C. Andreoli and I. Moro , 2012. *Ulva* (Chlorophyta, Ulvales) Biodiversity in the North Adriatic Sea (Mediterranean, Italy): Cryptic Species and New Introductions. *J. of Phycol.*, 48: 1510-1521.

17. Madalena, S., M. Luís, M. Vieira, AP. Almeida, M. Artur, S. Silva, ML. Ana, M. Seca, M. Carmo Barreto, IA. Neto, P.Madalenao, E. Pinto and A. Kijjoa, 2013. Chemical Study and Biological Activity Evaluation of Two Azorean Macroalgae: *Ulva rigida* and *Gelidium microdon*. Oceanography, 1:1

18. Coyle, MB., 2005. Manual of Antimicrobial Susceptibility Testing, American Society for Microbiology Press, D.C. MB Washington, pp. 25-39.

19. Bauer, AW., WM. Kirby, JC. Scherris and M.Turck, 1966. Antibiotic susceptibility testing by a standardized single disk method. *Americ. J.* of *Clin. Pathol.,* 45: 493-496.

20. Sastry, VMVS. and GRK. Rao, 1994. Antibacterial substances from marine algae: successive extraction using benzene, chloroform and methanol. Botanica Marina, 37: 357-360.

21. Lavanya, R. and N.Veerappan, 2011. Antibacterial potential of six seaweeds collected from Gulf of Mannar of Southeast Coast of India. Adv. in Biolog. Res., 5(1): 38-44.

22. Ertürk, Ö. and B. Taş, 2011. Antibacterial and Antifungal Effects of Some Marine Algae. Kafkas Univ Vet Fak Derg. 17 (Suppl A): S121-S124.

23. Tüney, I., BH. Adirci, D. Nal and A. Sukatar,2006. Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey).Turk. J. Of Biol., 30: 171-175.

24. Taskin, E., M. Ozturk, E. Taskin and O. Kurt, 2007. Antibacterial activities of some marine algae from the Aegean Sea (Turkey). Afr. J. of Biotechnol., 6 (24): 2746-2751.

25. Salvador, N., A.Gomez-Garreta, L. Lavelli and MA. Ribera, 2007. Antimicrobial activity of Iberian acroalgae. Sci. Mar., 71:101-113.

26. Chérif, W., M. El Bour, O. Dali Yahia-Kefi and L. Ktari, 2011. Screening de l'activité anti-microfouling d'algues vertes récoltées sur la côte nord Tunisienne. Bull. Inst. Natn. Scien. Tech. Mer de Salammbô, 38: 131-138.

27. Moreau, J., D. Pesando and B. Caram, 1984. Antifungal and antibacterial screening of dictyotales from the French Mediterranean Coast. Hydrobiologia, 11: 521-5241.

28. Rajasulochana, P., R. Dhamotharan, P. Krishnamoorthy and, S. Murugesan, 2009. Antibacterial activity of the extracts of marine red and brown algae. J. of Amer. Sc., 5: 20-25.

29. Rebecca, LJ., V. Dhanalakshmi and S. Chandra, 2012. Antibacterial activity of *Sargassum ilicifolium* and *Kappaphycus alvarezii*. J. of *Chemic.* and *Pharmaceu.* Res., 4(1): 700-705.

30. Lim, SH, I. Darah, K. Jain and S. Suraya, 2011. Gallic acid An anticandidal compound in hydrolysable tannin extracted from the barks of *Rhizophora apiculata*. Blume. J. of *Appl.* Pharmaceut. *Sc.,* 1: 75-79.

31. Darah, I., WY. Tong, S. Nor-Afifah, Z.Nurul-Aili and SH. Lim, 2014. Antimicrobial effects of Caulerpa sertularioides extract on foodborne diarrhea-caused bacteria. Europ. Rev. for med. and pharmacol. Sc., 18: 171-178.

32. Imbs, TI, EL. Chaykina, LA. Dega, AP. Vashchenko and MM. Anisimon, 2011. Comparative study of the chemical composition of ethanol extract from brown algae and their effects on seedling growth and productivity of soya Clycine max (L.) MERR. *Russ.* J. of *Bioorganic Chemistry*, 37(7): 871-876.

33. Lorian, V. and CG. Gemmell, 1991. Effect of low antibiotic concentration on bacteria: effect on ultrastructure, virulence, and susceptibility to immune defence. *In*: Lorian V, editor. Antibiotic in laboratory medicine. United Kingdom: Williams and Wilkins, 493-547.

34. Courvalin, P., 2005. Antimicrobial Drug Resistance: "Prediction Is Very Difficult, Especially about the Future". Emerg Infect Dis. 11(10):1503-1506.

35. Maruthupandian, A., P. Munirasu and L. Archana, 2015. Anti-pathogenic activity of green seaweeds (Chlorophyceae) from the coast of Ramanathapuram,Tamilnadu. Internat. J. of Curr. Innov. Res., 1:2, 58-62.

36. Berber, I. C., Avşar and H. Koyuncu, 2015. Antimicrobial and antioxidant activities of *Cystoseira crinita* Duby and *Ulva intestinalis* Linnaeus from the coastal region of Sinop, Turkey. J. of Coast. Life Medic., 3(6): 441-445.

37. Pandidurai, NSR., K. Kalavathi and P. Perumal, 2014. Studies on antibacterial activity of seaweeds, *Enteromorpha intestinalis* (Linnaeus) and *Gelidiella acerosa* (forsskal) from Puducherry and rameswaram (southeast coast of India). *Internat. J.* of *Pharma and Bio Sc.,* 5(1):1139-1147.

38. Darah, I., SH. Lim, 2015.In vitro antimicrobial activities of methanolic extract from marine alga Enteromorpha intestinalis. Asian Pac J Trop Biomed 2015; 5(9): 785–788.

39. Saidani, K., F. Bedjou, F. Benabdesselam and N. Touati, 2012. Antifungal activity of methanolic extracts of four Algerian marine algae species. Afri. J. of Biotechnol., 11(39):9496-9500

40. Kandhasamy, M. and KD.Arunachalam, 2008. Evaluation of *in vitro* antibacterial property of seaweeds of southeast coast of India. Afri. J. of Biotechnol 7(12): 1958-1961.

41. Abd El Baky, HH., FK . El Baz and GS. El Baroty , 2008. Evaluation of marine alga *U. lactuca* as a source of natural preservative ingredient. American-Eurasian J. Agric. & Environ. Sci., 3 (3): 434-444.