Available Online at http://www.recentscientific.com

ISSN: 0976-3031

International Journal of Recent Scientific Research
Vol. 4, Issue, 4, pp.376 - 380, April, 2013

International Journal of Recent Scientific Research

RESEARCH ARTICLE

INVESTIGATION OF *IN VITRO* ANTIBACTERIAL EVALUATION OF SOME MARINE ALGAE COLLECTED FROM GULF OF MANNAR, TAMIL NADU

[1]Shibu, A and [2]Dhanam, S

[1]PG Department of Botany, Government Thirumagal Mills College, Gudiyattam–632602, Thiruvalluvar University, Tamil Nadu, India
[2]PG and Research Department of Botany, Arignar Anna Arts College, Villupuram

ARTICLE INFO

Article History:

Received 12th, February, 2013
Received in revised form 15th, March, 2013
Accepted 27th, March, 2013
Published online 30th April, 2013

Key words:

Marine algae, Extracts, *in vitro*, Well diffusion, Antibacterial, Inhibitory activity.

ABSTRACT

Objective: To inquire the antibacterial efficacy of various solvent extracts of Marine algae collected from Gulf of Mannar. **Methods:** Hexane, Chloroform and Ethanol extracts of five Marine algal species at different concentrations were tested in vitro for their antibacterial activities against the following bacteria *Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Escherichia coli, Aeromonas hytrophila* with the well diffusion method. A suitable positive control was also maintained. **Results:** Among the five marine algae screened, the sample *Caulerpa recemosa, Turbinaria conoides,* and *Halimeda micronesica* have shown the maximum inhibitory activity. In which, the samples *Caulerpa recemosa* and *Turbinaria conoides* were found to be more active against the bacteria *Micrococcus luteus.* Whereas *Halimeda micronesica* have shown the maximum inhibitory activity against *E-coli*. Of all the solvent extracts, Hexane extract of the five marine algae showed higher inhibitory activity against all the selected bacterial species. **Conclusion:** The hexane extract of *Caulerpa recemosa* can be used as potential antibacterial sources. Further the investigations are now in progress towards the biological studies.

INTRODUCTION

Presently seaweeds constitute commercially important marine renewable resources which are providing valuable ideas for the development of new drugs against cancer, microbial infections and inflammations (Elena *et al.,* 2001; prince, 1980). Bacterial infection causes high rate of mortality in human population and aquaculture organisms (Kandhasamy and Arunachalam, 2008). Accordingly pharmaceutical industries are giving importance to compounds derived from traditional sources like soil, plants and marine organisms (Solomon and santhi, 2008). As marine algae have many medicinal and industrial uses that point to the power of nature. Even the dental industry includes algae as a component in the substance used to make dental impressions.

The objective of the present study is to bring into limelight the potential activities of crude extracts of these algae and to exploit these untapped resources in various ways for the benefit of mankind. As an efficient strategy of investigation, organic solvents have been used to extract the possible lipid soluble active principles from marine algae (Marasneh *et al.,* 1995). Chemically the bioactive metabolites of marine flora include brominated phenols, oxygen heterocyclics, nitrogen heterocyclics, sulphur nitrogen heterocyclics, sterolos, terpenoids, polysaccharides, peptides and proteins (Bhakuni and Rawat, 2005). Several studies have been undertaken to reveal the medicinal value of marine algae in different parts of the world as antitumorals (Espeche *et al.,* 1984; Maruyama and Yamamoto, 1984), anticoagulant (Athukorala *et al.,* 2006; Farias *et al.,* 2000), antifouling (Hellio *et al.,* 2004), antioxidant (Hong-Yu Luo *et al.,* 2010) and antimicrobial activities (Kolanjinathan and Stella, 2009), HIV antiviral agents (Schaeffer and Krylov, 2000; Luescher-Mattli, 2003; Wang *et al.,* 2007) and antibacterial activities (Valdebenito *et al.,* 1982; Xu *et al.,* 2003; Freile-Pelegrm and Morales, 2004).

Human pathogenic bacterial organisms such as *Bacillus cereus, E-coli, Staphylococcus aureus* are responsible for causing food borne diseases (Wijinands, 2008), mositis, abortion and upper respiratory complications (Jawetz *et al.,* 1995). These bacterial organisms were taken for the present study and Antibacterial inhibition towards these bacteria was explored using the selected seaweeds from Gulf of Mannar. Further work is in forward movement to analyse the biological activities that has been masked so far.

MATERIALS AND METHODS

Collection of Algal materials

Caulerpa recemosa, Turbinaria conoides, Halimeda micronesica, Padina gymnospora and *Sargassum polycystum* were collected in bulk quantity from coastal area of Gulf of Mannar, Tamil Nadu in India. Seaweed species exposed on sand and rocks were collected in sterile plastic bags under ice and brought to the laboratory. Each species was washed thoroughly with running water to remove epiphytes; animal castings, attached debris and sand particles and the final washings were done using fresh water and dried under shade.

* Corresponding author: Tel: +91–9944753138
E-mail address: shibugtmc9@gmail.com

The algal samples were identified in comparison with the herbarium collection under university of Madras.

Preparation of solvent extracts

The shade dried algal samples of 5g were placed in a soxhlet apparatus and was successively extracted using the following solvents Hexane, Chloroform and Ethanol. The crude extracts of whole part of algae at different concentrations were subjected to bioassay studies.

Antibacterial assay

The antibacterial activity is determined using the well diffusion method (Perez *et al.,* 1990). The different solvent extracts were dissolved in the DMSO to a final concentration of 100 mg/ml. Each bacterial strain was suspended in nutrient broth and incubated for 8 h at 37˚C. Nutrient Agar (NA) plates were seeded with 8 h broth culture of different bacteria. In each of these plates, wells were cut using sterile cork borer for 8 mm diameter. Using sterilized dropping pipettes, different concentrations (500, 1000, 1500 and 2000 µg/ml) of plant extract was carefully added into the wells and allowed to diffuse at room temperature for 2 h. The plates were then incubated at 37˚C for 18–24 h. The test was carried out by Triplicate method. Gentamicin (10µg/disc) was used as positive control and the solvent DMSO as negative control. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone.

Microorganism used

Antibacterial activity was tested against the standard culture of Gram positive and Gram negative bacterial strains. Gram positive strains such as methicillin resistant *Staphylococcus aureus* (MTCC 3381), *Bacillus cereus* (MTCC 430), *Micrococcus luteus* (MTCC 2470). Gram negative strains such as *Escherichia coli* (MTCC 739), *Aeromonas hydrophila* (MTCC 1739). These bacteria were obtained from Microbial Type Culture Collection, IMTECH, Chandigarh, India.

The present study revealed that, the overall antibacterial activity of *Caulerpa recemosa, Turbinaria conoides* and *Halimeda micronesica* being more active than *Padina gymnospora* and *Sargassum polycystum* against the human pathogenic bacteria in the control of their growth. Among the three solvents tested, Hexane and Chloroform extracts have exhibited the maximum inhibition against the growth of the bacterial species.

The observations have shown that the hexane extract of all the five marine seaweed have exhibited the highest inhibitory activity for the chosen bacterial strains. The maximum inhibitory activity recorded for the extracts Hexane and chloroform for the algae *Caulerpa recemosa* were (13.00±0.00 mm) and (12.00±0.00 mm) respectively. For all the test organisms, a lesser inhibitory effects were recorded in the algae *Padina gymnospora* and *Sargassum polycystum*. Also, Ethanol extract of *Sargassum polycystum* were not much effective against any of the tested pathogenic organisms. Of all the three solvent extracts of the marine algae *Caulerpa recemosa, Turbinaria conoides, Halimeda micronesica, Padina gymnospora* and *Sargassum polycystum* a minimum activity is shown against the Ethanolic extract of gram positive bacterial strain, the methicillin resistant *Staphylococcus aureus*.

The Hexane extract of the algae *Caulerpa recemosa* showed an excellent antibacterial activity specifically against the bacteria *Micrococcus luteus* and the methicillin resistant *Staphylococcus aureus*. A prominent inhibitory activity were shown by the hexane extract of the following algae *Caulerpa recemosa, Turbinaria conoides, Halimeda micronesica* and *Padina gymnospora* against the bacteria *Staphylococcus aureus, Bacillus cereus, Micrococcus luteus, Escherichia coli* and *Aeromonas hydrophila*. Also it was observed that the hexane extract of *Caulerpa recemosa* and *Turbinaria conoides*

Table 1 Antibacterial activity of *Caulerpa recemosa* crude extract against the tested pathogens

Test samples	Con (µg)	Zone of inhibition diameter(mm)				
		S. aureus	*B. cereus*	*M. luteus*	*E. coli*	*A. hydrophila*
Hexane	500	–	–	12.00±0.00	–	–
	1000	10.00±0.00	–	12.00±0.00	–	–
	1500	10.50±0.71	10.00±0.00	13.00±0.00	10.50±0.71	10.00±0.00
	2000	11.00±0.00	10.50±0.71	13.00±0.00	12.00±1.41	10.50±0.71
Chloroform	500	–	10.00±0.00	11.00±0.00	–	–
	1000	–	10.00±0.00	11.50±0.71	10.50±0.71	10.00±0.00
	1500	–	10.00±0.00	12.00±0.00	10.50±0.71	10.00±0.00
	2000	–	10.00±0.00	12.00±0.00	10.50±0.71	11.00±0.00
Ethanol	500	–	10.00±0.00	10.50±0.71	–	–
	1000	–	10.00±0.00	11.50±0.71	–	–
	1500	–	10.00±0.00	12.00±0.00	10.00±0.00	10.00±0.00
	2000	–	10.00±0.00	12.50±0.71	11.00±0.00	10.00±0.00
Gentamicin (positive control)	10	21.00±0.95	20.07±0.64	22.77±0.73	21.87±1.48	21.37±1.83

RESULTS

The different solvent extracts of *Caulerpa recemosa, Turbinaria conoides, Halimeda micronesica, Padina gymnospora* and *Sargassum polycystum* were tested for the antibacterial activity against five strains of Gram positive and Gram negative human pathogenic bacteria using Agar well diffusion method. The results of antibacterial activity against the tested pathogenic bacteria were tabulated in the table 1–5.

showed a wider spectrum of antibacterial activity against the methicillin resistant *Staphylococcus aureus, Bacillus cereus* and *Escherichia coli*. With reference to the table (1–5), the ethanolic extract of all the five marine algae exhibited a moderate activity against the bacteria *Aeromonas hydrophila*

A well profound inhibitory activity is being shown by all the algal samples for all the three extracts against the bacteria *Micrococcus luteus* (table 1–5). Under ethanolic extract, except *Padina gymnospora* the entire algal sample did not

Table 2 Antibacterial activity of *Turbinaria conoides* crude extract against the tested pathogens

Test samples	Con (µg)	Zone of inhibition diameter(mm)				
		S. aureus	*B. cereus*	*M. luteus*	*E. coli*	*A. hydrophila*
Hexane	500	–	–	11.00±0.00	–	–
	1000	10.00±0.00	–	11.50±0.71	–	–
	1500	10.00±0.00	10.00±0.00	11.50±0.71	10.00±0.00	10.00±0.00
	2000	10.50±0.71	10.00±0.00	12.00±0.00	10.00±0.00	10.00±0.00
Chloroform	500	–	–	10.00±0.00	10.00±0.00	–
	1000	10.00±0.00	–	10.50±0.71	10.50±0.71	10.00±0.00
	1500	10.00±0.00	10.00±0.00	11.00±0.00	10.50±0.71	10.00±0.00
	2000	11.00±1.41	10.00±0.00	11.00±0.00	11.00±0.00	10.00±0.00
Ethanol	500	–	–	10.00±0.00	–	–
	1000	–	–	10.00±0.00	10.00±0.00	10.00±0.00
	1500	–	–	10.00±0.00	10.00±0.00	10.00±0.00
	2000	–	–	10.00±0.00	10.00±0.00	10.00±0.00
Gentamicin (positive control)	10	21.00±0.95	20.07±0.64	22.77±0.73	21.87±1.48	21.37±1.83

Table 3 Antibacterial activity of *Halimeda micronesica* crude extract against the tested pathogens

Test samples	Con (µg)	Zone of inhibition diameter(mm)				
		S. aureus	*B. cereus*	*M. luteus*	*E. coli*	*A. hydrophila*
Hexane	500	–	10.00±0.00	10.50±0.71	–	10.00±0.00
	1000	–	10.00±0.00	11.00±0.00	10.00±0.00	10.00±0.00
	1500	10.00±0.00	10.00±0.00	11.50±0.71	10.50±0.71	10.00±0.00
	2000	11.00±0.00	10.50±0.71	11.50±0.71	12.00±0.00	10.50±0.71
Chloroform	500	–	–	10.00±0.00	–	–
	1000	10.00±0.00	–	10.00±0.00	10.00±0.00	–
	1500	10.00±0.00	–	10.50±0.71	10.00±0.00	10.00±0.00
	2000	10.00±0.00	–	11.00±1.41	10.50±0.71	10.00±0.00
Ethanol	500	–	–	10.50±0.71	10.00±0.00	10.00±0.00
	1000	–	10.00±0.00	10.50±0.71	10.00±0.00	10.00±0.00
	1500	–	10.00±0.00	11.00±1.41	10.00±0.00	10.00±0.00
	2000	–	10.00±0.00	11.00±1.41	10.00±0.00	10.00±0.00
Gentamicin (positive control)	10	21.00±0.95	20.07±0.64	22.77±0.73	21.87±1.48	21.37±1.83

Table 4 Antibacterial activity of *Padina gymnospora* crude extracs against the tested pathogens

Test samples	Con (µg)	Zone of inhibition diameter(mm)				
		S. aureus	*B. cereus*	*M. luteus*	*E. coli*	*A. hydrophila*
Hexane	500	10.00±0.00	–	10.50±0.71	10.00±0.00	10.00±0.00
	1000	10.00±0.00	–	11.00±0.00	10.00±0.00	10.00±0.00
	1500	10.00±0.00	10.00±0.00	11.00±0.00	10.00±0.00	10.00±0.00
	2000	10.00±0.00	10.00±0.00	11.50±0.71	10.00±0.00	10.00±0.00
Chloroform	500	10.00±0.00	–	10.00±0.00	–	–
	1000	10.00±0.00	–	10.00±0.00	–	–
	1500	10.00±0.00	10.00±0.00	10.00±0.00	–	–
	2000	10.00±0.00	10.00±0.00	11.00±0.00	–	–
Ethanol	500	–	–	10.00±0.00	10.00±0.00	–
	1000	–	10.00±0.00	10.00±0.00	10.00±0.00	–
	1500	10.50±0.71	10.00±0.00	10.00±1.00	10.00±0.00	–
	2000	10.50±0.71	11.00±0.00	10.50±0.71	10.50±0.71	10.50±0.71
Gentamicin (positive control)	10	21.00±0.95	20.07±0.64	22.77±0.73	21.87±1.48	21.37±1.83

Table 5 Antibacterial activity of *Sargassum polycystum* crude extracs against the tested pathogens

Test samples	Con (µg)	Zone of inhibition diameter(mm)				
		S. aureus	*B. cereus*	*M. luteus*	*E. coli*	*A. hydrophila*
Hexane	500	–	–	10.00±0071	–	–
	1000	10.00±0.00	–	10.00±0.00	–	–
	1500	10.00±0.00	–	10.00±0.00	–	–
	2000	10.00±0.00	–	10.00±0.00	–	–
Chloroform	500	11.00±0.00	10.00±0.00	10.00±0.00	–	10.00±0.00
	1000	11.50±0.71	10.00±0.00	10.00±0.00	10.00±0.00	10.00±0.00
	1500	11.50±0.71	10.00±0.00	10.00±0.00	11.00±1.41	10.50±0.71
	2000	12.00±0.00	10.00±0.00	10.00±0.00	12.00±1.41	11.00±0.00
Ethanol	500	–	–	10.00±0.00	–	10.00±0.00
	1000	–	–	10.00±0.00	–	10.00±0.00
	1500	–	–	10.00±0.00	–	10.00±0.00
	2000	–	–	10.00±0.00	–	10.00±0.00
Gentamicin (positive control)	10	21.00±0.95	20.07±0.64	22.77±0.73	21.87±1.48	21.37±1.83

show any inhibitory activity against the methicillin resistant *Staphylococcus aureus* (Table 4). In contrast, under chloroform extract, all the algal samples except *Caulerpa recemosa* were resulted with an excellent inhibitory activity against the methicillin resistant *Staphylococcus aureus* (MRSA) (Table 2–4).

DISCUSSIONS

The main objective of this work is to evaluate and compare the ability of different marine algae or seaweed species from Gulf of Mannar , Tamil Nadu to produce bioactive compounds of potential therapeutic interest. The marine algae have an effective antibacterial activity against most of the human pathogenic bacteria (Hornsey and Hide, 1985). Thus the therapeutic value of marine algae lies in the various chemical constituents present in it. There have been number of reports that demonstrating the antimicrobial activity of marine algae or seaweeds (Devi *et al.,* 2008; Haliki *et al.,* 2005; Tuney *et al.,* 2006; Tuney *et al.,* 2007; Ozdemir *et al.,* 2006; Karabay-Yavasoglu *et al.,* 2007; Nair *et al.,* 2007). Still in India only limited information is available on marine algae. Hence it was intended to evaluate and compare the ability of some abundantly available marine algae from the coastal regions of Tamil Nadu.

The present learn also made an effort, that the seaweed *Caulerpa recemosa* have composed the maximum inhibitory activity against *Micrococcus luteus* under Hexane extract (Plazaa *et al.,* 2010). Also it is proved through the present work that *Caulerpa recemosa, Halimeda micronesica* and *Sargassum polycystum* have initiated a greater inhibitory activity against the flesh eating bacteria *Aeromonas hydrophila* under chloroform and ethanolic extract (Rapini *et al.,* 2007). Again, the Hexane and chloroform extract of the algae *Turbinaria conoides* have developed the best inhibitory activity against all the pathogenic organisms tested. An excellent inhibitory activity was shown by *Halimeda micronesica* of Hexane extract against all the five pathogenic organisms.

With the present antibacterial inspection, the future work is in development towards the detection of biomedicine against these human pathogenic bacteria.

Acknowledgement

I am immensely thankful to Dr. S. Dhanam for her perfect guidance towards my work.

References

Elena, M., Francisco, Y., and Erickson, K.L. 2001. Mailiohydrin a cytotoxic chamigrene

Dibromohydrin from a phillippine. Laurencia species. J.Nat. Prod., 64(6): 790–791.

Prince, J.S., 1980. The ecology of *Sargassum pteropleuron* Grunow (Phaeophyceae, Fucales) in the waters off South Florida. II. Seasonal photosynthesis and respiration of *S.pteropleuron* and comparison of its phenology with that of *S. polyceratium* Montagne. Phycologia., 19: 190–193.

Kandhasamy, M. and Arunachalam, K.D. 2008. Evaluation of *in vitro* antibacterial property of seaweeds of southeast coast of India. African J. Biotech., 7(12): 1958–1961.

Solomon, RDJ. and Santhi, VS. 2008. Purification of bioactive natural product against human microbial pathogens from marine seaweed *Dictyota acutiloba.* J. Ag. World J. Microbiol. Biotechnol., 24: 1747–1752.

Marasneh, I., Jamal, M., Kashasneh, M. and Zibdeh, M.1995. Antibiotic activity of marine algae against multi-antibiotic resistant bacteria. Microb., 83: 23–26.

Bhakuni, DS and Rawat, DS. 2005. Bioactive Marine Natural Products., New Delhi: Anamaya Publishers.

Espeche, ME., Fraile, ER. and Mayer, AMS.1984. Screening of Argentine marine algae from antimicrobial activity. Hydrobiologia., 116/117: 525–582.

Maruyama, H. and Yamamoto, I.1984. An antitumor fucoidan fraction from edible brown seaweed, *Laminaria religiosa*. Hydrobiologia., 116/117: 534–536.

Athukorala, Y., Ki-Wan Lee, Se-Kwon, K. and You-Jin, J. 2006. Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. Bioresour. Technol., 98(9): 1711–1716.

Farias, WRL., Valente, AP., Pereira, MS. and Mourào, PAS.2000. Structure and Anticoagulant Activity of Sulfated Galactans. J. Biol. Chem., 275(38): 29299–29307.

Hellio, C., Marechal, JP., Véron, B., Bremer, G., Clare, AS. and Le Gal, Y.2004. Seasonal variation of antifouling activities of marine algae from the Brittany coast (France). Mar. Biotechnol., 6: 67–82.

Hong-Yu Luo, Bin Wang, Chun-Guang Yu, You-le Qu, Chuan-ling Su.2010. Evaluation

of antioxidant activities of five selected brown seaweeds from China. Journal of Medicinal Plants Research., 4(18): 2557–2565.

Kolanjinathan, K. and Stella, D.2009. Antibacterial activity of marine macro algae against human pathogens. Recent Research in Science and Technology., 1(1): 020– 022.

Freile-Pelegrı´n, Y. and Morales, JL.2004. Antibacterial activity in marine algae from the coast of Yucatan, Mexico. Botanica Marina., 47: 140–146.

Schaeffer, DJ. and Krylov, VS.2000. Anti-HIV activity of extracts and compounds from algae and cyanobacteria. Ecotoxicol Environ Saf ., 45: 208–227.

Luescher-Mattli, M.2003. Algae, a possible source for new drugs in the treatment of HIV and other viral diseases. Cur Med Chem., 2: 219–225.

Wang KJ., Huang WS., Yang M., Chen HY., Bo J., Li SJ., et al.2007. A malespecific expression gene, encodes a novel anionic antimicrobial peptide, scygonadin, in Scylla serrata. Mol Immunol., 44: 19611968.

Valdebenito, H., Bittner, M., Sammes, PG., Silva, M. and Watson, WH.1982. A compound with antimicrobial activity isolated from the red seaweed *Laurencia chilensis*. Phytochem., 21: 1456–1457.

Xu, N., Fan, X., Yan, X., Li, X., Niu, R., Tseng, CK.2003. Antibacterial bromophenols from the marine red alga Rhodomela confervoides. Phytochem 62: 1221–1224.

Freile-Pelegrın, Y. and Morales, JL.2004. Antibacterial activity in marine algae from the coast of Yucatan. Mexico. Bot Mar., 47: 140–146.

Wijnands, LM. 2008. *Bacillus cereus* associated food borne disease: quantitative aspects of exposure assessment and hazard characterization. PhD Thesis, Wageningen University, Wageningen, Netherlands .pp. 1–175.

Jawetz, E., Mellnick, JL. and Adelberg, EA.1995. Review of Medical Microbiol, 20th Edition. Applellation Lange Norwalk, Connecticut., pp. 139–218.

Perez, C., Pauli, M. and Bazevque, P.1990. An antibiotic assay by the agar well diffusion method. *Acta Biologiae et Medicine Experimentalis*., 15: 113–115.

Hornsey, IS. and Hide, D.1985. The production of antimicrobial compoundsby British Marine algae & Variation of antimicrobialactivity withalgal generation. Br Phycol J,. 20.

Devi, KP., Suganthy, N., Kesika, P. and Pandian, SK.2008. Bioprotective properties of seaweeds: *in vitro* evaluation of antioxidant activity and antimicrobial activity against food borne bacteria in relation to polyphenolic content. Altern Med., 8: 38.

Haliki, A., Denizci, A.A. and Cetingul, V. 2005.An investigation on antifungal activities of some marine algae (Phaeophyta, Rhodophyta). EU J Fish Aquat Sci., 22: 13-15.

Tuney, I., Cadirci, B.H., Unal, D. and Sukatar, A.2006. Antimicrobial activities of the extracts of marine algae from the coast of Urla (‹zmir, Turkey). Turk J Biol., 30: 1-5.

Tuney, I., Cadirci, B.H., Unal, D. and Sukatar, A.2007. In antimicrobial activities of crude extracts of marıne algae from the coast of Izmir (Turkey). Fres Environ Bull., 16: 428-434.

Ozdemir, G., Horzum, Z., Sukatar, A. and Karabay-Yavasoglu, NU.2006. Antimicrobial activities of volatile components and various extracts of *Dictyopteris membranaceae* and *Cystoseira barbata* from the Coast of Izmir, Turkey. Pharm Biol., 44: 183-188.

Karabay-Yavasoglu, NU., Sukatar, A., Ozdemir, G. and Horzum, Z.2007. Antimicrobial activity of volatile components and various extracts of the red alga *Jania rubens*. Phytother Res., 21: 153-156.

Nair, R., Chabhadiya, R. and Chanda, S.2007. Marine algae: screening for a potent antibacterial agent. J Herb Pharmacother., 7: 7386.

Plazaa, M., Santoyob, S., Jaimeb, L., Garcia-Blairsy Reinac, G., Herrerob, M., Senoransb, FJ., et al.2010. Screening for bioactive compounds from algae. J. Phar. Bio.Analysis., 51: 450–455.

Rapini, R.P., Bolognia, JL. and Jorizzo, JL.2007. Dermatology: 2-Volume Set. St. Louis: Mosby. ISBN 1-4160-2999-0.

****