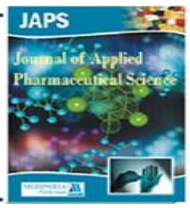
Journal of Applied Pharmaceutical Science 01 (07); 2011: 154-157

Journal of Applied Pharmaceutical Science

Available online at www.japsonline.com



ISSN: 2231-3354
Received on: 09-09-2011
Revised on: 17-09-2011
Accepted on: 24-09-2011

Screening for antimicrobial activity of crude extracts of *Skeletonema costatum*

R. Shanmugapriya, and T.Ramanathan

R. Shanmugapriya, and T.Ramanathan
C.A.S. in Marine Biology, Faculty of Marine Sciences, Annamalai University, Parangipettai 608502, Tamil Nadu, India.

***For Correspondence*:**
Dr.T.Ramanathan,
Asst.Professor, Center of Advanced study in Marine Biology, Faculty of Marine Science, Annamalai University, Porto-Novo 608 502, Tamil Nadu, India. Telephone: 04144-243223 Extn: 213, Mobile: + 91 98941 75200: Fax: 04144-243555

ABSTRACT

Various organic and aqueous extracts of *Skeletonema costatum* were screened for their antibacterial activities. The extracts were tested against different species of human pathogenic bacteria by the agar-solid diffusion method. Water extract of *Skeletonema costatum* showed maximum antimicrobial activity of 19.0 mm against *Klebsiella pneumoniae* and a minimum activity of 9 mm against *Proteus vulgaris*. All the tested microorganisms were resistant to methanol, ethanol and propanol extracts except *Escherichia coli* and *Staphylococcus aureus* which exhibited a least inhibition zone of 6.0 and 7.0 mm respectively in propanol. Acetone extract of *Skeletonema costatum* also showed the highest biological activity of 19.0 mm against *Klebsiella pneumoniae*, moderate activity of 12.0 mm against *Salmonella typhi*, and 11.0 mm against *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. Sequential extract of Spirulina exhibited maximum antimicrobial activity. Inhibition zone of 23.2 mm was observed for *Klebsiella pneumoniae* and 14.0 mm for *Proteus vulgaris*.

***Key words*:** *Skeletonema costatum*; Human pathogenic bacteria; Antimicrobial activity; Phytoplankton; Microorganisms.

INTRODUCTION

Discovering new therapeutic molecules is becoming increasingly important as more and more bacteria become resistant to the usual antibiotics. Traditionally used in Asiatic medicines, algae, since the second half of the 20th century, are screened for their biological activities. Thus, antibacterial effects have been noticed in all the algal classes and notably in diatoms, the major component of the phytoplankton (Burkholder *et al*., 1960; Aubert and Gauthier 1966; Duff *et al*., 1966; Aubert *et al*., 1968a, b; Aubert and Gambarotta 1972; Berland *et al*., 1972; Aubert *et al*., 1979; Gauthier, 1980; Cooper *et al*., 1983; Pesando 1990). Although extremely effective, antibiotics are able to induce resistance in bacteria. For 450 years, bacterial resistance has been the main factor responsible for the increase of morbidity, mortality and health care costs of bacterial infections. The defense mechanism against antibiotics is widely present in bacteria (e.g. *Pseudomonas, Klebsiella, Enterobacter, Acinetobacter, Salmonella, Staphylococcus, Enterococcus and Streptococcus*) and became a world health problem (Clementino, 2005). Several algal species contain natural bioactive compounds that act as potent antimicrobial agents (Ozdemir 2004; Khan 2006).

MATERIALS AND METHODS

Collection of *Skeletonema costatum*

Skeletonema costatum collected from the coastline of parangipettai. About 50 g of powdered *Skeletonema costatum* was taken in a round bottom flask and add ethanol and macerated

for 7 days. During maceration the whole content was warmed 2 times a day at an interval of six hours. At the end of 7th day the extract was filtered through muslin cloth while hot and the extract was concentrated to a semisolid mass and dried in a desiccators. This extract was used for various experimental purposes.

Preparation of *Skeletonema costatum* extracts

Freshly dried *Skeletonema costatum*, was mixed with acetone, ethanol, methanol, petroleum ether and diethyl ether (150ml solvent/100g of *Skeletonema costatum*) in soxhlet apparatus and extracted for 60 minutes. The extracts were filtered and the solvent was removed using rotary evaporator. Sequential extraction was performed with all solvents in the order, water, acetone, ethanol, methanol and petroleum ether. The extracts were stored in an airtight glass bottles in a refrigerator. For antimicrobial activity, extracts obtained with organic solvents and water extracts were prepared at a concentration of 100 mg/ mL1 (freeze dried material/mL of solvent).

Microorganisms tested

Strains of human pathogenic microorganisms used in this study were as follow: *Klebsiella pneumoniae, Shigella shigae, Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, Proteus vulgaris and Salmonella typhi*. All these strains were colleted from Rajah Muthiah Medical College & Hospital, Annamalai Nagar, Chidambaram, Tamil Nadu, India, and maintained in ultra low temperature freezer (-80oC).

Preparation of 24 hours pure culture

A loop full of each of the microorganisms was suspended in about 10 ml of physiological saline in a Roux bottle. Each of these were streaked on to the appropriate culture slants and incubated at 37 ºC for 24 hours.

Standardization of micro-organisms

Each of the 24 hour old pure culture was suspended in a Roux bottle containing 5 ml of physiological saline. Each suspension of microorganism was standardized to 25% transmittance at 560 nm using an Ultraviolet (UV) visible spectrophotometer.

Antimicrobial activity using disc diffusion method

Antimicrobial activity was checked by disc gel diffusion method. The cultures were grown in nutrient broth and incubated at 37 °C for 24 h. After incubation period is over, the O.D. of the culture was adjusted to 0.1 with sterile nutrient broth. 20 ml molten Mueller-Hinton agar medium was poured into sterile petri plates and allowed to solidify. The discs (8 mm diameter) impregnated with 100 µg of respective extracts/ml were placed on the surface of the petri plates seeded with 0.1 ml of microbial suspension (5×10^5 CFU/ml). Soon afterwards the plates were kept at 10^0C for 30 min. After it normalized to room temperature the plates were incubated at 37 ^{0}C for 24 h. After incubation period the zone of inhibition was measured.

Measurement of zone of inhibition (ZIH)

The zones of inhibition of the tested microorganisms by the extracts were measured using a Fisher-Lilly antibiotic zone reader model 290 (U.S.A). The antimicrobial activities were determined by the ratio of the ZIH diameters of the extracts to that of the standard antibiotic ciprofloxacin 5µg/disc in the same petri dish, where in a higher ratio indicates a more potent extract.

Data and statistical analysis

Data are expressed as mean ± standard deviation (SD) of triplicates. Two ways ANOVA was used to analyze the effect of different solvents on antimicrobial activity. Tukey- Krammer multiple comparison test was used to assess the significance among the extracts.

RESULTS AND DISCUSSION

The results obtained from the present study concerning the antimicrobial activity of *Skeletonema costatum* extracted with different solvents against different species of bacteria are recorded in table 1. It is clear from study that the diameter of the inhibition zone depends mainly on the type of the solvent used and the tested bacteria.

Water extract of *Skeletonema costatum* showed maximum antimicrobial activity of 19.0 mm against *Klebsiella pneumonia* and a minimum activity of 9 mm against *Proteus vulgaris.* All the tested microorganisms were resistant to methanol, ethanol and propanol extracts except *Escherichia coli* and *Staphylococcus aureus* which exhibited a least inhibition zone of 6.0 and 7.0 mm respectively in propanol. Acetone extract of *Spirulina platensis* also gave the highest biological activity of 19.0 mm against *Klebsiella pneumoniae*, moderate activity of 12.0 mm against *Salmonella typhi,* and 11.0 mm againest *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*. Aqueous acetone was better at extracting total phenolics than aqueous methanol.

While diethyl ether and petroleum ether were the best organic solvents for extracting the antibacterial agents from *Skeletonema costatum* in the present evaluation. Statistically the effects of the two diethyl ether and petroleum ether were insignificant. Comparatively diethyl ether showed a marked activity against *Klebsiella pneumoniae* followed by *Shigella shigae* exhibiting 20.4mmand 20.1mm of inhibition zone. The antibacterial properties of *Skeletonema costatum* as previously described in the literature (Duff *et al*., 1966; Aubert *et al*., 1968a; Aubert and Gambarotta 1972; Kogure *et al*., 1979). Bactericidal activity of unsaturated and saturated long chain fatty acids have been reported by Nieman (1954), Galbraith and Miller (1973a, b, c). They have shown that fatty acids of chain length more than 10 carbon atoms induced lysis of bacterial protoplasts. Many authors have found antibacterial activities of microalgae due to fatty acids (Aubert *et al*., 1968a,b; Berland *et al*., 1972; Gauthier 1980; Cooper *et al*., 1983; Pesando 1985). More recently an auto inhibitor (a fatty acid named 15-hydroxyeicosapentaenoic acid) has also been identified in *Skeletonema costatum* (Imada *et al*., 1992).

Table1: Antimicrobial effect of various extracts of *Skeletonema costatum*. R-Resistance

S.No	Microorganism	Water	Methanol	Ethanol	Propanol	Acetone	Petroleum ether	Diethyl ether	Sequential extract
1	*Klebsiella pneumoniae*	19	R	R	R	19	20	20.4	23.2
2	*Shigella shigae*	16	R	R	R	17	22	20.1	21.1
3	*Pseudomonas aeruginosa*	11	R	R	R	11	11.3	11.9	16.2
4	*Escherichia coli*	11	R	R	6	11	12.4	14.1	18.1
5	*Staphylococcus aureus*	11	R	R	7	11	13	14.5	18.3
6	*Proteus vulgaris*	9	R	R	R	9	11	10	14
7	*Salmonella typhi*	12	R	R	R	12	10	15.3	20.1

Antimicrobially active lipids and active fatty acids are present in a high concentration in this alga (Lampe 1998). It was hypothesised that lipids kill microorganisms by leading to disruption of the cellular membrane (Bergsson 2005) as well as bacteria, fungi and yeasts because they can penetrate the extensive meshwork of peptidoglycan in the cell wall without visible changes and reach the bacterial membrane leading to its disintegration. The external leaflet of the outer membrane of *Entero bacteriacae*, such as *E. coli* that lives in the rectum, an environment rich in hydrophobic compounds, is almost entirely composed of lipopolysaccharides and proteins. These bacteria have a hydrophilic surface because of the side chains of lipopolysaccharides, and thus hydrophobic molecules, like lipids, have difficulty in entering the bilayer (Bergsson 2002). This could have contributed to the comparatively less susceptibility of *E. Coli* than other organisms. Sequential extract of *Skeletonema costatum* exhibited maximum antimicrobial activity. Inhibition zone of 23.2 mm observed for *Klebsiella pneumoniae* and 14.0 mm for *Proteus vulgaris*. The enhanced antimicrobial activity expressed in sequential extraction might be due to the fact that both hydrophobic and hydrophilic bioactive compounds were extracted. When extraction was done with any one solvent bioactive compounds soluble in the respective solvent only be extracted.

CONCLUSION

In conclusion, the present study suggest that *Skeletonema costatum* has antibactericidal activity against pathogenic bacteria. An improved knowledge of the composition, analysis, and properties of *Skeletonema costatum* with respect to antimicrobial compounds would assist in efforts for the pharmaceutical application.

ACKNOWLEDGEMENTS

The authors would like to thank Prof.Dr.T.Balasubramanian, Dean& Director, Faculty of Marine Science, Annamalai University, Tamil Nadu for providing all facilities during the study period.

REFERENCES

Aubert M and Gauthier M. Note sur 1 active antibacterienne d une diatomee marine: Chaetoceros teres. Rev. Intern. Oceanogr. Med 1966; IV: 33-37

Aubert M, Aubert J and Gauthier M. Pouvoir autoepurateur de l eau de mer et substances antibiotiques produites par les organisms marins. Rev. Intern. Oceanogr. Med.1968a; X: 137-207.

Aubert J, Pesando D and Thouvenot H. Action antibiotique d extraits planktoniques vis a vis de germs anaerobies. Rev. Intern. Oceanogr. Med. 1968b; X: 259-265.

Aubert J and Gambarotta JP. Etude de I action antibacterienne d especes phytoplanktoniques marine vis a vis de germes anerobies. Rev. Intern. Oceanogr. Med. 1972; XXV : 39-47.

Aubert M, Aubert J and Gauthier M.. Antibiotic substances from marine flora. In: Hoppe, H.A., Levring T., Tanaka, Y. (Eds.), Marine algae in Pharmaceutical Science. 1979; pp. 275-291

Berland BR, Bonin DJ, Cornu AL, Maestrini SY and Marino JP. The antibacterial substances of the marine alga Stichochrysis immobilis (Chrysophyta). J. Phycol. 1972; 8: 383-392.

Bergsson G. *Antimicrobial polypeptides and lipids as a part of innate defence mechanism of fish and human fetus.* Karoliska Institute, Stockholm(2005).

Bergsson G, and Steingrimsson O and Thormar H. Bactericidal effects of fatty acids and monoglycerides on *Helicobacter pylori***.** Int. J. Antimicrob.Agents. 2002; 20: 258-262 .

Burkholder PR, Burkholder LM and Almodovar LR. Antibiotic activity of some marine algae of Puerto Rico. Bot. Mar 1960; 2:149-156.

Cooper S, Battat A, Marot P and Sylvester M. Production of antibacterial activities by two bacillariophyceae grown in dialysis culture. Can. J. Microbiol.1983; 29: 338-341.

Deivy Clementino de Lima, PaulaAlvarezAbreu, Cýcero Carlos de Freitas, Dilvani Oliveira Santos, RodrigoOliveira Borges, Tereza Cristina dos Santos, LúcioMendes Cabral, CarlosR.

Rodrigues andHelena Carla Castro: Snake Venom: Any Clue for Antibiotics and

CAM? AlternatMed. 2005; 2: 39-47.

Duff DCB, Bruce DL and Anita NJ. The antibacterial activity of marine planktonic algae. Can. J.Microbiol. 1966; 12: 877-884.

Galbraith H and Miller TB. Effect of metal cations and PH on the antibacterial activity and uptake of long chain fatty acids. J. Appl. Bact. 1973a; 36: 635-646.

Galbraith H and Miller TB. Physiochemical effects of long chain fatty acids on bacterial cells and their protoplasts. J. Appl. Bact. 1973b; 36: 647-658.

Galbraith H and Miller TB. Effect of long chain fatty acids on bacterial respiration and amino acid uptake. J. Appl. Bact. 1973c; 36: 659-675.

Gauthier MJ. Note ser la frequence de la production d antibiotiques lipidiques chez les algues planktoniques. Rev. Intern. Oceanogr. Med. 1980 ;LVIII: 41- 44.

Imada N, Kobayashi K, Isdmura K, Saito H, Imura S and Tahara Oshima Y. Isolation and Identification of an auto inhibitor produced by *Skeletonema costatum*. Nippon. Suisan. Gakk. 1992; 58: 1687-1692.

Khan M, Shobha CJ, Mohan JI, Rao UM, Prayag AN and Kutala KV. *Spirulina attenuates* cyclosporine-induced nephrotoxicity in rats. J.Appl. Toxicol. 2006; 26: 444- 451.

Kogure K, Simidu U and Taga N. Effect of *Skeletonema costatum* (Grev.) Cleve on the growth of marine bacteria. J. Exp. Biol. Ecol. 1979; 36: 201-215.

Lampe MF, Ballweber LM, Isaacs CE, Patton DL, and Stamm WE. Killing of *Chlamydia trachomatis* by Novel Antimicrobial Lipids Adapted from Compounds in Human Breast Milk. Antimicrob.AgentsChemoth.1998; 45:1239-1244.

Nieman C. Influence of trace amounts of fatty acids on the growth of microorganisms. Bacteriol. Rev. 1954; 18: 147-163.

Ozdemir G, Karabay NU, Dalay MC and Pazarbasi B. Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. Phytother Res. 2004; 18(9): 754-757.

Pesando D. Antibacterial and antifungal activities of marine algae. In: Akatsuka, I. (Ed.), Introduction to Applied Phycology. 1990; pp.3-26.

Pesando D. Etude des proprieties antifongiques et antibacteriennes d' algues marinesa. Isolement, etude chimique et activite d'un polysaccharide et d'un glycolipide extraits de deux diatomees: *Chaetoceros lauderi* (Ralfs) et Asterionella japonica (Cleve). These de Doctorat. Universite Pierre et Marie Curie, Paris VI, 1985 ; 136 pp.