International Journal of Biological Chemistry

ISSN 1819-155X

Academic Journals Inc.

www.academicjournals.com

International Journal of Biological Chemistry 3 (2): 84-89, 2009
ISSN 1819-155X

Preliminary Phytochemical Analysis, Elemental Determination and Antibacterial Screening of *Codium decorticatum*-A Marine Green Algae

[1,2]J. Anbu Jeba Sunilson, [1]R. Suraj, [1]K. Anandarajagopal,
[1]G. Rejitha, [1]M. Vignesh and [1]P. Promwichit
[1]School of Pharmacy, Masterskill University College of Health Sciences,
Taman Kemacahaya 11, Jalan Kemacahaya, Cheras, Selangor, Malaysia
[2]Honorary Associate, School of Pharmacy, La Trobe University, Bendigo,
Victoria 3552, Australia

Abstract: In this study, petroleum ether, chloroform and methanol extracts of *Codium decorticatum* showed the presence of amino acids, carbohydrates, saponins, phytosterols, alkaloids and glycosides. The antibacterial activity against Gram-positive bacteria, such as *Streptococcus pneumoniae*, *Staphylococcus aureus* and Gram-negative bacteria, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Pseudomonas aeruginosa* was carried out using cup-plate method. All the extracts showed good zone of inhibition against *S. pneumoniae* and *K. pneumoniae* at the concentrations of 25, 50 and 100 mg mL^{-1} compared with standard drugs, gentamicin and ampicillin (30 μg mL^{-1}). To standardize the algae, elemental analysis was also carried out on *C. decorticatum* powder which revealed the presence of various elements. The present findings show the importance of *C. decorticatum* in producing new compounds having antibacterial activity.

Key words: *Codium decorticatum*, algae, antibacterial activity, elemental analysis, phytochemicals

INTRODUCTION

Resolution to the growing crisis of antibiotic resistance and their side effects are the breakthrough for the search of new antimicrobial compounds from natural resources. Marine species have been used in a wide array of traditional remedies and provided a good source of antimicrobial activity. Algae are rich in phytochemical ingredients such as agar, carragenean and alginate which were utilized in the field of medicine and pharmacy for decades (Taskin *et al*., 2007). Various algae species such as *Codium fragile*, *Enteromorpha compressa*, *Dictyota lineari*, *Padina pavonica*, *Phyllariopsis brevipes*, *Ulva olivascens*, *Sargassum vulgare*, *Codium dwarkense*, *Codium indicum*, *Codium tomentosum* belonging to the family of Chlorophyceae, Codiaceae and Phaeophyceae are potential sources of bioactive compounds (Chiheb *et al*., 2009). The production of microbial inhibitory substances from marine species was noted as early as in 1917 (Nagi *et al*., 2009). Since then, several studies have been carried out to identify novel antimicrobial compounds from marine sources. Extensive reports are available on the antibacterial (Burkholder *et al*., 1960; Berland *et al*., 1972; Sachithananthan and Sivapalan, 1975; Biard *et al*., 1980; Chenieux *et al*., 1980; Bonnie and Cynthia, 1991; Mahasneh *et al*., 1995; Siddhanta *et al*., 1997; Tuney *et al*., 2006; Karabay *et al*., 2007; El-Gendy *et al*., 2008), antiviral (Richards *et al*., 1978), anticoagulant (Yasantha *et al*., 2007) and antitumor (Alejandro and Betina, 1984) activities of marine green algae.

Corresponding Author: Dr. J. Anbu Jeba Sunilson, School of Pharmacy,
Masterskill University College of Health Sciences, Taman Kemacahaya 11, Jalan Kemacahaya,
Batu 9, 43200 Cheras, Selangor, Malaysia

The study was undertaken to investigate the preliminary phytochemical analysis and evaluate antimicrobial activity of *Codium decorticatum* (Woodward) Howe against selected strains of both Gram-positive and Gram-negative bacteria. *Codium decorticatum* is one among the marine green algae belonging to the family Codiaceae (Howe, 1911), commonly distributed in the Indian Ocean (Paul *et al*., 1996), North Carolina, South Carolina, Florida Gulf Coast and Puerto Rico (Craig and Richard, 1991). Very few studies were available pointing out the medicinal properties of *C. decorticatum* except anticoagulant activity (Rogers *et al*., 1990) and homeostasis effect (Marina *et al*., 2007). No extensive and individual scientific reports are available stating the potential antimicrobial property of these algae. On the light of the above information and since marine algae are rich in active constituents showing antimicrobial activity, *C. decorticatum* was selected for this study.

MATERIALS AND METHODS

Sample Collection and Extract Preparation

Codium decorticatum were collected by hand picking using snorkeling from Gulf of Mannar, Thoothukudi, Tamilnadu, India in February, 2005 and preserved in ice until further processing. It was authenticated by Dr. R. Santhanam at the Fisheries College and Research Institute in India. A specimen sample was preserved in the Herbarium, Fathima College of Pharmacy in Kadayanallur, Tamilnadu, India for future reference (FCP/MD/CD01/H-002). The algae were washed with tap water to remove epiphytes and salt, further washed with distilled water. Algae were dried under shade for 10 days and crushed in an electric mill until a coarse powder was obtained (Chiheb *et al*., 2009). The powder (500 g) was successively extracted with petroleum ether, chloroform and methanol by Soxhlet extraction (Bose *et al*., 2007). The extracts were concentrated using rotary vaccum evaporator and kept in dessicator until further investigation.

Preliminary Phytochemical Tests

Preliminary phytochemical tests for the identification of amino acids, carbohydrates, saponins, tannins, phytosterols, alkaloids, proteins, glycosides, flavanoids and phenolic compounds were carried out for all the extracts by the methods described by Harborne (1998). The results were reported in Table 1.

Elemental Analysis

The dried powder of *C. decorticatum* was subjected to elemental analysis by applying standard procedures (Serfor *et al*., 1999) for the detection of Nitrogen, Sulphur, Halogen, Thiosulphate, Sulphate, Phosphate, Carbonate, Nitrate, Acetate, Fluoride, Arsenate, Chloride, Calcium, Lead, Magnesium, Barium, Furan, Copper, Sodium and Ammonium. The results were reported in Table 2.

Test Microorganisms

Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi, Pseudomonas aeruginosa* and Gram-positive bacteria such as *Streptococcus pneumoniae*, *Staphylococcus aureus* were used for this study. All the bacterial cultures were procured from microbiology lab, Fathima College of Pharmacy, Kadayanallur, Tamilnadu, India.

Antibacterial Activity

All the extracts were dissolved in Tween 80 at various concentrations of 25, 50 and 100 mg mL^{-1}. The antibacterial activity was performed by cup-plate method (Onkar *et al*., 1995). The respective bacterial culture was poured into the nutrient agar plates for uniform distribution of microorganisms (Idu *et al*., 2007). Using a sterile cork borer, 8 mm wide well was made on each agar plates. Various

concentrations of petroleum ether, chloroform and methanol extracts (25, 50 and 100 mg mL^{-1}) were poured into each wells using a sterile micropipette (Srinivasan *et al*., 2007). Gentamicin and ampicillin (30 μg mL^{-1}) were used as standards. The plates were incubated for 24 h at 37°C. After incubation, the zone of inhibition was measured and the values were shown in Table 3. All the experiments were done in triplicate.

RESULTS

The extracts of *C. decorticatum* varied in their colour and yield. Petroleum ether, chloroform and methanol extracts appeared to be brownish green, yellowish brown and deep brown colour in the day light with yield of 4.2, 9.6 and 15.4 %, respectively. The presence of amino acids, carbohydrates, saponins, phytosterols, alkaloids and glycosides were found in extracts which was shown in Table 1. The powder of *C. decorticatum* showed the presence of nitrogen, sulphur and halogens and revealed the presence of acid radicals such as sulphate, phosphate, carbonate, nitrate, acetate, fluoride, arsenate and chloride. The powder also showed the presence of basic radicals such as calcium, sodium and ammonium which was shown in Table 2.

The antibacterial activity of various extracts exhibited concentration dependant effect against *S. pneumoniae*, *E. coli*, *K. pneumoniae*, *S. typhi* and *Ps. aeruginosa*. All the extracts showed excellent concentration dependent inhibition (25, 50 and 100 mg mL^{-1}) against *K. pneumoniae* and *S. pneumonia*

Table 1: Preliminary phytochemical screening of various extracts of *C. decorticatum*

Constituents	Petroleum ether	Chloroform	Methanol
Amino acids	-	+	+
Carbohydrates	+	+	+
Saponins	+	-	+
Tannins	-	-	-
Phytosterols	+	-	+
Alkaloids	+	+	+
Glycosides	+	-	+
Flavanoids	-	-	-
Phenolic compounds	-	-	-

+: Present, -: Absent

Table 2: Analysis of elements and radicals in *C. decorticatum* powder

Constituents	*Codium decorticatum* powder
Nitrogen	+
Sulphur	+
Halogen	+
Thiosulphate	-
Sulphate	+
Phosphate	+
Carbonate	+
Nitrate	+
Acetate	+
Fluoride	+
Arsenate	-
Chloride	+
Calcium	+
Lead	-
Magnesium	-
Barium	-
Furan	-
Copper	-
Sodium	+
Ammonium	+

+: Present, -: Absent

Table 3: Antibacterial activity of various extracts (mg mL^{-1}) of *C. decorticatum*

	Diameter of zone of inhibition (mm)									
	Petroleum ether			Chloroform			Methanol			
Organisms tested	25	50	100	25	50	100	25	50	100	Standard antibiotic*
Escherichia coli	0	15	21	0	0	0	0	16	24	28 (G)
Klebsiella pneumoniae	16	18	22	12	18	20	15	24	26	34 (G)
Salmonella typhi	9	12	14	0	0	0	12	17	20	24 (G)
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0	0	26 (G)
Streptococcus pneumoniae	9	14	16	10	13	19	16	20	22	33 (A)
Staphylococcus aureus	0	0	0	0	0	0	0	0	0	18 (A)

*G: Gentamicin (30 μg mL^{-1}), A: Ampicillin (30 μg mL^{-1})

which was well compared with standard drugs, gentamicin and ampicillin (30 μg mL^{-1}). The petroleum ether and methanol extracts at higher concentrations showed remarkable antibacterial activity against *E. coli*, where as chloroform extract was devoid of activity even at higher concentrations. Petroleum ether and methanol extracts inhibited *S. typhi* on all the selected concentrations except chloroform extract which showed deficient inhibition. *Ps. aeruginosa* and *S. aureus* were not susceptible to the extracts and all the values were shown in Table 3.

DISCUSSION

Marine genus synthesizes active constituents which are used in traditional and complementary medicine. Different varieties of marine algae were reported to contain active ingredients that can cure diseases. Nowadays, higher percentage of population prefer to use remedies of natural origin for curing illness as these claimed to produce less side effects (Tyagi and Bohra *et al*., 2002). The present study was focused on *C. decorticatum* for the presence of phytochemical substances, powder elemental analysis and antibacterial activity against Gram-positive and Gram-negative bacteria.

Algae are eukaryotic organisms inhabited in salty sea water and is recognized to synthesize several bioactive (Micheal *et al*., 2002) compounds which show antimicrobial property. In addition, other substances identified as antimicrobial agents were chlorellin derivatives, acrylic microbial acid, halogenated aliphatic compounds, terpenes, sulphur containing heterocyclic compounds and phenolic inhibitors (Espeche *et al*., 1984).

The activity of the algae against both Gram-positive and Gram-negative bacteria may be indicative of the presence of broad spectrum antibiotic compounds or simply the content of pharmacological active constituents like alkaloids, saponins, glycosides, tannins etc. (Omulokoli *et al*., 1997; Phang *et al*., 1994; Milgate and Roberts, 1995). Phytochemical screening of *C. decorticatum* revealed the presence of carbohydrates and alkaloids in all the extracts where as saponins, phytosterols and glycosides were found only in petroleum ether and methanol extracts. In addition, chloroform and methanol extracts also showed the presence of amino acids. The antifungal, antiviral and antibacterial activities of saponins are well documented (Lacaille-Dubois and Wagner, 1996; Milgate and Roberts, 1995) which clearly explains the activity of petroleum ether and methanol extracts against *E. coli* (Cowan, 1999).

Alkaloids are commonly found to have antimicrobial properties (Omulokoli *et al*., 1997) against both Gram-positive and Gram-negative bacteria (Cowan, 1999). Presence of alkaloids in all the extracts and hence exerting a remarkable antibacterial activity against Gram-positive (*S. pnemoniae*) and Gram-negative (*K. pneumoniae*) bacteria fall in line with the above findings. An earlier study reported the antibacterial activity of methanol extract of six marine macroalgae including *C. decorticatum* which inhibited the growth of *S. aureus* and *Bacillus subtilis* (Juan *et al*., 2006). The present study differs from the previous study since the antibacterial activity was evaluated using petroleum ether, chloroform and methanol extracts of *C. decorticatum*. Apart from this, *C. decorticatum* being studied

individually for the first time was comparatively a new concept. In conclusion, the preliminary phytochemical screening of *C. decorticatum* indicates the presence of chemical constituents playing an indispensable role in antibacterial activity. On evaluating the antibacterial property of *C. decorticatum*, the algae proved to be a potent antibacterial agent. The findings of this study also paves the way for further research to identify the specific active compounds that is responsible for its claimed antibacterial activity.

ACKNOWLEDGMENT

The authors are thankful to the management, Fathima College of Pharmacy, Kadayanallur, Tamilnadu, India and Dr. R. Santhanam, M.Sc., Ph.D., Dean, Fisheries College and Research Institute, Thoothukudi, Tamilnadu, India for providing facilities and technical assistance. Also the authors are grateful to Dato' Prof. Dr. Ishak Bin Tambi Kechik, Vice-Chancellor and Dato' Edmund Santhara, CEO, Masterskill University College of Health Sciences, Malaysia, for their funding, continuous encouragement and support.

REFERENCES

Alejandro, M.S. and P. Betina, 1984. Antitumor evaluation of marine algae in Argentina. Hydrobiologia, 116-117: 529-533.

Berland, B.R., D.J. Bonin, A.L. Cornu, S.Y. Maestrini and J.P. Marino, 1972. The antibacterial substances of the marine alga *Stichochrysis immobilis* (Chrysophyta). J. Phycol., 8: 383-392.

Biard, J.F., J.F. Verbist, J. Le Boterff, G. Ragas and M. Lecocq, 1980. Seaweeds of French Atlantic coast with antibacterial and antifungal compounds. Planta Med., 40: 136-151.

Bonnie, L. and B. Cynthia, 1991. Antibiotic production by marine algae isolated from the New York/New Jersey coast. Bull. Environ. Contam. Toxicol., 46: 329-335.

Bose, A., S. Sumanta, G. Jayanta Kumar, G. Tirtha, D. Gouri Kumar and S. Sudam, 2007. Analgesic, anti-inflammatory and antipyretic activities of the ethanolic extract and its fractions of *Cleome rutidosperma*. Fitoterapia, 78: 515-520.

Burkholder, P.R., L.M. Burkholder and L.R. Almodovar, 1960. Antibiotic activity of some marine algae of puerto Rico. Bot. Mar., 2: 149-156.

Chenieux, J.C., J.F. Verbist, J.F. Biard and E. Clement, 1980. Seaweeds of French Atlantic coast with antimitotic compounds. Planta Med. Suppl., 40: 152-162.

Chiheb, I., H. Riadi, L.J. Martinez, J.F. Dominguez Selgar, V.A. Gomez, H. Bouziane and M. Kadiri, 2009. Screening of antibacterial activity in marine green and brown macroalgae from the coast of Morocco. Afr. J. Biotechnol., 8: 1258-1262.

Cowan, M.M., 1999. Plant products as antimicrobial agents. Clin. Microbiol. Rev., 12: 564-582.

Craig, W.S. and B.S. Richard, 1991. Seaweeds of the South Eastern United States. Duke University Press, USA., ISBN-10: 0822311011, pp: 84.

El-Gendy, M.M., U.W. Hawas and M. Jaspars, 2008. Novel bioactive metabolites from a marine derived bacterium *Nocardia* sp. ALAA 2000. J. Antibiot., 61: 379-786.

Espeche, M.E., E.R. Fraile and A.M.S. Mayer, 1984. Screening of Argentine marine algae from antimicrobial activity. Hydrobiologia, 117: 525-528.

Harborne, J.B., 1998. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. 3rd Edn., Chapman and Hall, London, ISBN: 0-412-57270-2, pp: 302.

Howe, M.A., 1911. Phycological studies. V. Some marine algae of lower California. Mexico Bull. Torrey Bot. Club, 38: 489-514.

Idu, M., S.E. Omonigho and C.L. Igeleke, 2007. Preliminary investigation on phytochemistry and antimicrobial activity of *Senna alata* L. Flower. Pak. J. Biol. Sci., 10: 806-809.

Juan, L.M., Z.O. Cantillo Ciau, I.S. Molina and G.J. Mena Rejon, 2006. Screening of antibacterial and antifungal activities of six marine macroalgae from coasts of yucatan peninsula. Pharma. Biol., 44: 632-635.

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

Lacaille-Dubois, M.A. and H. Wagner, 1996. A review of the biological and pharmacological activities of saponins. Phytomedicine, 2: 363-386.

Mahasneh, I., M. Jamal, M. Kashashneh and M. Zibdeh, 1995. Antibiotic activity of marine algae against multiantibiotic resistant bacteria. Microbios, 83: 23-26.

Marina, C., Q. Irene, I.V. Maria, K. Luciana, D. Agustina de, M.E. Jose and S.C. Alberto, 2007. Polysaccharides from the green seaweeds *Codium fragile* and *C. vermilara* with controversial effects on hemostasis. Int. J. Biol. Macromol., 41: 641-649.

Micheal, T.M., M.M. John and P. Jack, 2002. Brock Microbilogy of Microorganism. 10th Edn., Prentice Hall, New Jersey, ISBN-10: 0130662712, pp: 1104.

Milgate, J. and D.C.K. Robert, 1995. The nutritional and biological significance of saponins. Nutr. Res., 15: 1223-1249.

Nagi, A.A.H., I.M. Nurmas, N.S. Mariana, M. Habsah, S.V. Charles and S. Zamberi, 2009. Antibacterial activity in Marine algae *Eucheuma denticulatun* against *Staphylococcus aureus* and *Streptococcus pyogenes*. Res. J. Biol. Sci., 4: 519-524.

Omulokoli, E., B. Khan and S.C. Chhabra, 1997. Antiplasmodial activity of four Kenyan medicinal plants. Ethnopharmacology, 56: 133-137.

Onkar, D., Dhingra and B.S. James, 1995. Basic Plant Pathology Methods. 2nd Edn., CRC Press, India, ISBN: 0873716388, pp: 287-305.

Paul, C.S., W.B. Philip and L.M. Richard, 1996. Catalogue of the Benthic Marine Algae of the Indian Ocean. University of California Press, California, pp: 852.

Phang, S.M., Y.K. Lee, M.A. Borowitzka and B.A. Whitton, 1994. Algal Biotechnology in Asia Pacific Region. University of Malaya, Kualalumpur, pp: 75-81.

Richards, J.T., E.R. Kern, L.A. Glasgow, J.C. Overall, E.F. Deign and M.T. Melvin, 1978. Antiviral activity of extracts from marine algae. Antimicrobial Agents Chemother., 14: 24-30.

Rogers, D.J., K.M. Jurd, G. Blunden, S. Paoletti and F. Zanetti, 1990. Anticoagulant activity of a proteoglycan in extracts of *Codium fragile* ssp.atlanticum. J. Appled Phycol., 2: 357-361.

Sachithananthan, K. and A. Sivapalan, 1975. Antibacterial properties of some marine algae of Sri Lanka. Bull. Fish. Res. Station, Srilanka, 26: 5-9.

Serfor, A.Y., B.J.B. Nyarko, E.K. Osae, D. Carboo and F. Seku, 1999. Elemental analysis of some green and brown seaweeds from the coastal belt of Ghana. J. Radioanal. Nucl. Chem., 242: 193-197.

Siddhanta, A.K., K.H. Mody, B.K. Ramavat, V.D. Chauhan and H.S. Garg *et al*., 1997. Bioactivity of marine organisms: Part VIII-Screening of some marine flora of Western coast of India. Indian J. Exp. Biol., 35: 638-643.

Srinivasan, K., N. Devarajan, C. Mohanasundari, V. Chinthambi and N. Nandakumar, 2007. Antibacterial, preliminary phytochemical and pharmacognostical screening on the leaves of *Vicoa indica* (L)DC. Iran. J. Pharmacol. Therapeutics, 6: 109-113.

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

Tuney, I., B.H. Cadirci, D. Nal and A. Sukatar, 2006. Antimicrobial activities of the extracts of marine algae from the coast of Urla (Izmir, Turkey). Turk. J. Biol., 30: 171-175.

Tyagi, N. and A. Bohra, 2002. Screening of phytochemicals of fruit plant and antibacterial potentiala against *Pseudomonas aeruginosa*. Biochem. Cell. Arch., 2: 21-24.

Yasantha, A., L. Ki-Wan, K. Se-Kwon and J. You-Jin, 2007. Anticoagulant activity of marine green and brown algae collected from Jeju Island in Korea. Bioresour. Technol., 98: 1711-1716.