## In vitro Antigenotoxicity of Ulva rigida C. Agardh (Chlorophyceae) Extract against Induction of Chromosome Aberration, Sister Chromatid Exchange and Micronuclei by Mutagenic Agent MMC<sup>1</sup>

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**Objective** To determine the *in vitro* possible clastogenic and cytotoxic activities of *Ulva rigida* crude extracts (URE), and identify their antigenotoxic and protective effects on chemotherapeutic agent mitomycine-C (MMC). **Methods** Anti-clastogenic and anti-genotoxic activities of *Ulva rigida* crude extracts (URE) were studied using chromosome aberration (CA), sister chromatid exchange (SCE), and micronuclei (MN) tests in human lymphocytes cultured *in vitro*. **Results** The chromosome aberration, sister chromatid exchange or micronuclei tests showed that URE at concentrations of 10, 20, and 40 µg/mL had no clastogenic activity in human lymphocyte cell culture. Three doses of URE significantly decreased the number of chromosomal aberrations and the frequencies of SCE and MN when compared with the culture treated with MMC (P < 0.0001). **Conclusion** Although URE itself is not a clastogenic or cytotoxic substance, it possesses strong antigenotoxic, anti-clastogenic, and protective effects on MMC *in vitro*.

Key words: Ulva rigida; Anticlastogenicity; Antigenotoxicity; Chromosomal aberration; Sister chromatid exchange; Micronuclei; Mitomycin-C

## REFERENCES

- Michanek G (1979). Seaweed resources for pharmaceutical uses. In: Hoppe H A, Levring T, Tanaka Y, and de Gruyter W (Eds.). Marine algae in pharmaceutical science (pp. 203-234). New York: Berlin.
- Fujimot K, Ohmura H, Kaneda T (1985). Screening antioxygenic compounds in marine algae and bromophenols as effective principles in a red alga *Polysiphonia urceolata*. *Bull Jpn Soc Sci Fish* **51**, 1139-1143.
- Potterat O (1997). Antioxidants and free radical scavengers of natural origin. *Curr Org Chem* 1, 415-440.
- Kloareg B, Quatrano R S (1988). Structure of the cell walls of marine algae and ecophysiological function of the matrix polysaccharides. Oceanogr. *Mar Biol Annu Rev* 26, 259-315.
- Lindequist U, Schweder T (2001). Marine biotechnology. In: Rehm H J, Reed G (Eds.) Biotechnology 10, Wiley-VCH, Weinheim, 441-484.
- Mayer A M S, Hamann M T (2002). Marine pharmacology in 1999: compounds with antibacterial, anticoagulant, antifungal, anthelmintic, anti-inflammatory, antiplatelet, antiprptozoal and antiviral activities affecting the cardiovascular, endocrine, immune and nervous systems, and other miscellaneous mechanisms of action. *Comp Biochem and Physiol* Part C 132, 315-319.
- 7. Newmann D J, Cragg G M, Snader K M (2003). Natrural

products as source of new drugs over the period 1981-2002. J Nat Prod 66, 1022-1037.

- Faulkner D J (2000a). Highlights of marine natural products chemistry (1972–1999). *Natural Product Reports* 17, 1-6.
- 9. Faulkner D J (2000b). Marine pharmacology. Antonie Van Leeuwenhoek 77(2), 35-145.
- Witvrouw M, De Clercq E (1997). Sulfated polysaccharides extracted from sea algae as potential antiviral drugs. *Gen Pharmac* 29/4, 497-511.
- Vairappan C S (2003). Potent antibacterial activity of halogenated metabolites from Malaysian red algae, *Laurencia* majuscule (*Rhodomelaceae*, *Ceramiales*). Biomol Eng 20, 255-259.
- 12.Ely R, Supriya T, Naik C G (2004). Antimicrobial activity of marine organisms collected off the coast of South East India. J Exp Marine Biol and Ecol 309, 121-127.
- Bansemir A, Blume M, Schröder S, Lindequist U (2006). Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture* 252, 79-84.
- 14. Furusawa E, Furusawa S (1985). Anticancer activity of a natural product, viva-natural, extracted from Undaria pinnantifida on intraperitoneally implanted Lewis lung carcinoma. Oncology 42, 364-369.
- 15. Wu L C, Ho J A, Shieh M C, *et al.* (2005). Antioxidant and antiproliferative activities of *Spirulina* and *Chlorella* water extracts. *J Agricul Food Chem* **53**, 4207-4212.
- 16. Deslandes E, Pondaven P, Auperin T, et al. (2000). Preliminary study of the *in vitro* antiproliferative effect of a hydroethanolic

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extract from the subtropical seaweed *Turbinaria ornata* (Turner J. Argadh) on a human non-small-cell bronchopulmonary carcinoma cell line (NSCLC-N6). *J Appl Phycol* **12**, 257-262.

- Byers T, Perry G (1992). Dietary carotenes, vitamin C and vitamin E as protective antioxidants in human cancers. *Annu Rev Nutr* 12, 139-159.
- 18.Qi H, Zhang Q, Zhao T, et al. (2006). In vitro antioxidant activity of acetylated and benzoylated derivatives of polysaccharide extracted from Ulva pertusa (Chlorophyta). Bioorganic and Medicin. Chem Letters 16, 2441-2445.
- 19. Jin D Q, Lim C S, Sung J Y, et al. (2006). Ulva conglobata, a marine alga, has neuroprotective and anti-inflammatory effects in murine hippocampal and microglial cells. *Neuroscience Letters* 402, 154-158.
- 20.Hellio C, Bourgougnon N, Le Gal Y (2000). Phenoloxidase (EC 1.14.18.1) from the byssus gland *Mytilus edulis*: purification, partial characterization and application for screening products with potential antifouling activities. *Biofouling* 16, 235-244.
- 21.Khan N H, nur-E Kamal M S A, Rahman M (1988). Antibacterial activity of Euphorbia thymifolia Linn. *Indian J Med Res* 87, 395-397.
- Fenech M (2000). The *in vitro* micronucleus technique. *Mutat* Res 455, 81-95.
- 23.Benn P A, Perle M A (1992). Chromosome staining and banding techniques, in: D. E. Rooney, B.H. Czepulkowsky (Eds.), Human Cytogenetics: A practical Approach 1-II, 57-83, IRL press, Oxford.
- 24. Parry P, Wolff S (1974). Giemsa method for differential staining of sister chromatids, *Nature* **251**, 156-158.
- 25.1 P C S (1985). Guide to short term tests for detecting mutagenic and carcinogenic chemicals, World Health Organization, Geneva.
- 26.Carrano A V, Natarajan A T (1988). Considerations for population monitoring using cytogenetics techniques, ICPEMC Publication No.14, International Commission for Protection Against environmental mutagens and carcinogens. *Mutat Res* 204, 379-406.
- 27.Bonassi S, Fenech M, Lando C, *et al.* (2001). Human micronucleus project: international database comparison for results with the cytokinesis block micronucleus assay in human lymphocytes: effect of laboratory protocol, scoring criteria, and host factors on the frequency of micronuclei. *Environ Mol Mutagen* 37, 31-45.
- 28. Manoharan K, Banerjee M R (1985). β-Carotene reduces sister

chromatid exchange induce chemical carcinogens in mouse mammary cells in organ culture, *Cell Biol Int Rep* **9**, 783-789.

- 29. Waters M D, Brady A L, Stack H F, *et al.* (1990). Antimutagenic profiles for some model compounds. *Mutat Re* **238**, 57-85.
- 30.Cragg G M, Newman D J, Weiss R B (1997). Coral reefs, forests and thermal vents: the worldwide exploration of nature for novel antitumor agents. *Seminars in Oncology* 24, 156-163.
- 31.Koyabagi S, Tanigawa N, Nakgawa H, et al. (2003). Oversulfation of fucoidan enhances its anti-angiogenic and antitumor activities. *Biochem Pharmacol* 65, 173-179.
- Funahashi H, Imai T, Mase T, et al. (2001) Seaweed prevents breast cancer? Jpn J Cancer Res 92(5), 483-487.
- 33.Poersch A, Dos Santos F V, Medeiros-Maciel M A, et al. (2007). Protective effect of DCTN (trans-dehydrocrotonin) against induction of micronuclei and apoptosis by different mutagenic agents in vitro. Mutat Res 629, 14-23.
- 34. Ishihara K, Murata M, Kaneniwa M, *et al.* (2000). Purification of stearidonic acid (18:4(n-3) and hexadecatetraenoic acid (16:4(n-3)) from algal fatty acid with lipase and medium pressure liquid chromatography. *Biosci Biotechnol Biochem* **64**(11), 2454-2457.
- 35.Kolb N, Vallorani L, Milanovic N, et al. (2004). Evaluation of marine algae wakame (Undaria pinnatifida) and kombu (Laminaria digitata japonica) as food supplements. Food Technol Biotechnol 42, 57-61
- 36.Kim J M, Araki S, Kim D J, *et al.* (1998). Chemopreventive effects of carotenoids and curcumins on mouse colon carcinogenesis after 1, 2-dimethylhydrazine initiation. *Carcinogenesis* **19**(1), 81-85.
- 37.Kotake-Nara E, Kushiro M, Zhang H, et al. (2001). Carotenoids affect proliferation of human prostate cancer cells. J Nutr 131(12), 3303-3306.
- 38.Kikunaga S, Miyata Y, Ishibashi G, et al. (1999). The bioavailability of magnesium from Wakame (Undaria pinnatifida) and Hijiki (Hijikia fusiforme) and the effect of alginic acid on magnesium utilization of rats. Plant Foods Hum Nutr 53(3), 265-274.
- 39. Bartle W R, Madorin P, Ferland G (2001). Seaweed, vitamin K, and warfarin. Am J Health Syst Pharm 58(23), 2300.
- 40. Aaronson S (2000). Important Vegetable Supplements. Chapter C, Part II. Staple Foods: 231-249. In: The Cambridge World History of Food. Cambridge University Press.

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