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Cytokinins from Marine Green Alga, *Caulerpa racemosa* (Kuetz) Taylor

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Cytokinin - like substances were extracted from green seaweed, *Caulerpa racemosa* and purified using an ion exchange column of CM-cellulose. Bioassay of these extracts with dark-germinated cotyledons of cucumber for chlorophyll biosynthesis proved the presence of cytokinin - like substances in the extract of *C. racemosa*. The efficiency of the extract was comparable to that of commercially available kinetin. The study is indicative of strong possibilities of producing cytokinin from unutilized and commonly occurring *C. racemosa* on a commercial scale and at low cost.

Key words: Seaweed, *Caulerpa racemosa*, cytokinin, bioassay, kinetin

A large number of bioactive substances of marine origin such as sesquiterpene, prostaglandins, furans, phenols, sterols, quinones, pigments etc. are derived from sponges, corals and holothurians (Baker & Murphy, 1976). Information on the availability of similar compounds from algae is scanty. Among the chemicals isolated from algal sources, the common ones are sesquiterpenoids from *Dictyopteris divaricata* (Phaeophyceae), (Takoaka & Ando, 1951; Irie *et al.*, 1965) and *Laurencia glandulifera* (Rhodophyceae), (Obata & Fukushi, 1953), friedelin from *Cyanophycean monostroma nitidum* (Tsuda & Sakai, 1960); and sterols and acyl glycerol from Phaeophycean *Colpomenia peregrina* and *Scytosiphon iomentaria* (Stefanov *et al.*, 1996).

Chlorophycean species such as *Ulva, Caulerpa, Codium* etc. are mainly used for edible purposes. Apart from this, many substances like caulerpicin, an indole derivative, and caulerpin, a pyrazine derivative from *Caulerpa racemosa* (Santos & Doty, 1968) and tarakerd from *C. racemosa* and *C. lamoureuxii* (Santos & Doty, 1971) are some of the major examples of bioactive substances obtained from green seaweeds. The extract of the seaweed, *Ascophyllum nodosum* contains cytokinin and the effect of these extracts on some legumes was reported by Reitz & Trumble (1996). The aim of the present study was to extract and isolate plant growth hormones from the easily available and unutilized marine green alga, *C. racemosa* and to compare its activity with the commercial grade kinetin.

Materials and Methods

Fresh *C. racemosa* (1.0 kg), collected from the intertidal region of Rameswaram (Tamilnadu) was ground with 400 ml distilled water and 1.6 l of ethanol was added to the mixture to make the final concentration of ethanol 80% (v/v). The filtered solution was extracted with petroleum ether (100 ml, twice). The photosynthetic pigments were removed from the organic layer and the cytokinin-like substances were isolated and extracted according to Hutton & Van Staden (1981). The pH of the pigment - free extract was adjusted to 2.5 with HCl and allowed to percolate through a column of CM-cellulose at the rate of 20 ml/h. The column

was then washed with 200 ml of 80% ethanol and then with double distilled water. Eluates were discarded. Cytokinins adsorbed by the cellulose were eluted with 100 ml of 5 N ammonium hydroxide solution. The eluate was concentrated to dryness under vacuum. Solutions of known concentrations (1-4 ppm) of cytokinin-like substances extracted from *C. racemosa* were prepared in double distilled water. The activity of cytokinin was assayed by its chlorophyll formation effect (Fletcher *et al.*, 1982) using etiolated cucumber cotyledons (*Cucumis sativus*). The activity of cytokinin extracted from *C. racemosa* was compared with that of commercial grade kinetin (6-furfuryll aminopurine, (UV Min 99%).

Results and Discussion

Cytokinin-like substances obtained from *C. racemosa* were found to induce chlorophyll biosynthesis in the dark - germinated cotyledons of cucumber. The level of chlorophyll formation was proportionate to the concentration of the cytokinin - like substance in the medium (Table 1). The results were comparable to the effect of commercially available kinetin.

The results obtained during the bioassay with the dark - germinated cotyledons of cucumber showed the presence of cytokinin-like substances in the extracts of *C. racemosa*. Seaweed extracts are very effective on crops like vegetables, fruits, flowers, pulses and cereals. These beneficial effects from the application of seaweed extracts have been attributed to the presence of hormones, especially cytokinins (Blunden, 1977; Verkleij, 1992; Crouch & Van Staden, 1993). 1.5 g of dry powder of cytokinin-like substances could be recovered from 1.0 kg (wet weight) of *C. racemosa*. The method employed was very simple, quick and inexpensive. The bioassay proved that this product was as efficient as the commercial grade kinetin in terms of its ability for chlorophyll biosynthesis. This extract may contain other cytokinin-like substances such as zeatin, 2R-ribosyl zeatin, 2G-glucosyl zeatin etc (Hutton & Van Staden, 1982). In India, *C. racemosa* is unexploited and found distributed abundantly throughout the year on the easily accessible coasts of Okha, Dwaraka, Goa, Quilon, Rameswaram and Lakshadweep (Kaliaperumal *et al.*, 1997) and hence this can be considered as a good raw material for the production of cytokinins on a commercial scale at low cost.

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Table 1. Formation of chlorophyll pigments in the dark-germinated cotyledons of cucumber in the presence of cytokinin-like substances and kinetin

Concentration of hormone (ppm)	Initial level of total chlorophyll (µg/g, wet wt)	Final level of total chlorophyll (µg/g, wet wt)	
		Kinetin	Cytokinin
0.0	6.12	148.47	143.63
1.0	6.12	215.32	210.73
2.0	6.12	290.17	294.06
3.0	6.12	373.49	386.85
4.0	6.12	421.32	438.93

References

Baker, J.T. & Murphy, V. (1976) *Handbook of Marine Sciences* Vol.1, p.59 CRC Press, Cleveland

Blunden, G. (1977) In: *Marine Natural Products Chemistry.* (Faulkner, D.L. & Fenical, W.H. eds.) p.337 Plenum, New York

Crouch, J.J. & Van Staden, J. (1993) *Plant Growth Regul.* **13**, 21

Fletcher, R.A., Kallidumbil, V. & Steele, P. (1982) *Plant Physiology,* **69**, 675

Hutton, M.J. & Van Staden, J. (1981) *Ann. Bot.* **47**, 527

Irie, T., Suzuki, M. & Masamune, T. (1965) *Tetrahedron Lett.* **37**, 1053

Kaliaperumal, N., Kalimuthu, S. & Ramalingam, J.R. (1997) *CMFRI Spl. Publn.* No. **62**, p.35

Obata, Y. & Fukushi, S. (1953) *J. Agrl. Chem. Soc. Japan,* **27**, 331

Reitz, S.R. & Trumble, J.T. (1996) *Bot. Mar.* **39**, 33

Santos, G.A. & Doty, M.S. (1968) *Drugs from the sea* (Freudenthal, H.D., ed.) p.173, Mar. Tech. Society, Washington, DC

Santos, G.A. & Doty, M.S. (1971) *Lloydia* **34**, 88

Stefenov, K., Bankova, V., St. Dimitrova-Konaklieve, Aldinova, R. , Dimitrov, K. & Popov, S. (1996) *Bot. Mar.* **39**, 475

Takoaka, M. & Ando, Y. (1951) *J. Chem. Soc. Japan. Pure Chem. Soc.,* **72**, 999

Tsuda, K. & Sakai, K. (1960) *Chem. Parm. Bull.* (Tokyo), **8**, 554

Verkleij, F.N. (1992) *Biological Agriculture Horticulture,* **8**, 309