

Phycochemical property of *Pithophora oedogonia* (Mont). Wittrock with special reference to its nutraceutical significance

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ABSTRACT

Pithophora oedogonia is a common alga of clear stagnant waters forming dense mats. The alga is generally considered as a nuisance alga and has been extensively studied for its growth requirements and characteristics to suggest control measures. However, its phycochemical qualities are not known. The nutraceutical properties of the alga were evaluated and compared with *Spirulina* which is commonly used in nutraceutical products.

Key words: Phycochemical properties, *Pithophora oedogonia* (Mont). Wittrock, nutraceutical.

INTRODUCTION

Marine algae have been widely used for human consumption. They are consumed as regular part of human diet. In coastal China, since 1850 (Waaland, 1981,) and today they are eaten mainly in the Orient and the Pacific islands. Chapman and Chapman (1980) lists 160 species of sea weeds eaten by humans 25, chlorophyceae, 54, phaeophyceae and 81, rhodophyceae (South and Whittick, 1987). *Porphyra*, *Laminaria*, *Undaria*, *Gigantina* and *Chondrus* are some of the most widely used marine algae as food. Food value, flavour, colour and texture appear to favour the use of algae as food. While the protein content is high in these algae, metabolizable carbohydrates are much less. Nevertheless, these algae are an excellent source of vitamins including, vitamin C at levels equivalent to citrus fruits and vitamins A, D, B₁, B₁₂, E, Riboflavin, Niacin, Pantothenic acid and folic acid. They also serve as trace elements for human nutrition (Yamamoto *et al.*, 1979) and free amino acid (Arasaki *et al.*, 1984; Dave *et al.*, 1993; Thevanathan *et al.*, 1993, Tasneem Fatima *et al.*, 1994; Selvi *et al.*, 1999).

Neutraceutical or health food market is currently flooded with many algae products that

include dried biomass or all extracts produced from *Chlorella* (Lee, 1997; Yamaguchi, 1997;), *Dunaliella* (Avron and Ben-Amotz, 1992) and *Spirulina* (Vonshak, 1997). The nutritional qualities of these algae have led to the development of microalgal biotechnology in the last 2 decades. Considerable attention is now being directed at unicellular algae for the production of oils and fatty acids. The use of algal oils containing long-chain polyunsaturated fatty acids (LCPUFAs) as nutritional supplements is given primary focus (Cohen *et al.*, 1995; Behrens and Kyle, 1996) and algal sources are being identified for the presence of docosahexaenoic acid (DHA) and eicosaapentaenoic acid (EPA). The DHA is a dominant fatty acid in neurological and heart muscle tissues and sperm cells. The fact that humans are not capable of synthesizing DHA de novo implies the dependency on adequate supply of DHA through dietary sources (Emken *et al.*, 1994). The fatty acid is also a conditionally essential nutrient during infancy (Innis, 1994; Makarides *et al.*, 1995) and a number of nutritional and professional organizations including the World Health Organization have recommended the inclusion of supplementary DHA in infant formula. Dinoflagellates are looked upon as a source of DHA for use in infant formulas (Apt and Behrens, 1999). Changes in EPA levels are known to significantly

alter the coronary vascular status of individuals (Salem, 1989) and a number of algae have been proposed for the production of EPA (Cohen, 1990; Sukenik, 1991; Boswell *et al.*, 1992; Molina *et al.*, 1995; Tan and Johns, 1996) and used in nutraceutical products. It has also been suggested that algae can serve as a source of genes involved in LCPUFA synthesis (Apt and Behrens, 1999). Apart from DHA and EPA, Arachidonic acid and has also been shown to be an important component of nutritional supplements and algae serving as a source for the fatty acids (Zhang Cheng Wu *et al.*, 2002).

The fast growing aqua culture industry is also dependent on algae from which the aqua culture animals must obtain all the nutrients and the nutrient properties of the algae are critical for the growth and survival of both larva and adults. Many of the algae are cultured for use in aqua culture industries (Day *et al.*, 1991; Orus *et al.*, 1991; Johnson and An, 1991; Benemamm, 1992; Chaumont, 1993; De Roeck *et al.*, 1993; Barclay *et al.*, 1994; Gladue and Maxey, 1994; Kashiwakura *et al.*, 1994; Renaud *et al.*, 1994; Qiang and Richmond, 1994; Takeyama *et al.*, 1996; Barclay and Zeller, 1996; Bret and Muller-Navarra, 1997; Borowitzka, 1997; Spektorova *et al.*, 1997; Lynch, 1998; Vishwanath Patil *et al.*, 2007). The growing demand for safe natural products by the nutraceutical and aqua cultural industries has signified the importance of algae as a source of these products and the need to screen more algae for the purpose. Very few fresh water algae have been screened for these nutritionally valuable products.

Pithophora oedogonia, the 'horse hair' or 'cotton ball' alga is a green alga that belongs to the family Cladophoraceae, order cladophorales of the class chlorophyceae. The alga produces free floating mats of vegetation in static or slow moving bodies of water. Its luxuriant growth in shallow lakes and ponds biomass production in huge quantities, high degree of resistance to copper and many algicides and occurrence as thick large clumps or mats with profusely branched filaments having rigid, coarse cell walls have placed the alga in a prominent position as a filter clogging or nuisance alga of the water systems. Though the alga has been studied

as a nuisance alga, its nutritive and bioactive properties are not exploited. The present investigations explores the possibilities of exploiting this alga in nutraceutical industries. The alga was screened for its mineral, aminoacid, vitamin, sterols and fatty acid content.

MATERIAL AND METHODS

The crude fiber content was estimated following the procedure of AOAC 1999. The ash content was analysed following woodman 1945, Moisture and food energy (Gopalan 1989) and the total lipid content (Bligh and Dyer 1959). The following minerals Sulphate, chloride, Phosphate, Sodium, Boron, Calcium, Magnesium and extractable metals such as Lead, Comium, Iron, Cupper, Cadmium, Nickle and Zinc were determined (APHA 1989). The fatty acid content was analysed using Hewlett Packer GC model No: 5890. Aminoacid analysis was carried out on Beckman 119CL model automatic aminoacid analyzer (Arasaki 1984) átocopherol of the fresh and dry powdered sample was estimated by HPLC (Mallet *et al.* 1993). The vitamin was analysed according to AOAC 1990. Extracted Sterols from dry lipid preparations of the algal mats was separated identified and quantified by GC.

RESULTS

Mineral and biochemical composition of *pithophora oedogonia*

Mineral composition

Dry powdered material of the alga was analysed for its mineral and heavy metal composition (Table 1). The dry algal powder was found to contain the elements sodium, potassium, copper, iron, sulphate, phosphate, chloride, boron, calcium and magnesium. Of these elements, magnesium formed the bulk with 14.666 mg/g dry weight of the material. This is followed by sulphate with 9.9 mg/g and chloride with 9.73 mg/g dry weight of the alga. Potassium was present at a concentration of 6.45 mg/g dry weight. In addition to these heavy metals, lead, zinc, chromium, cadmium and nickel were present in the dry powder at detectable levels. Zinc was present at a concentration of 1.445 ppm, followed by chromium (0.763 ppm), nickel (0.325 ppm) and lead (0.215 ppm) in the decreasing order (Table 1). Cadmium

was present in trace amounts (less than 0.001 ppm). The ash content of the dry powder was 34.75% and acid washable ash content was 16.19%. Total nitrogen level of the dry algal powder was 83.6 – 84.2 mg/g dry weight while the moisture content was 6.79%. Fat content of the dry powder was 1.67% while that of protein was 20.5%. Crude fiber constituted 15.17% while the total carbohydrate content amounted to 21.12% of the dry powdered alga. Hundred grams of the dry material had a calorific value of 181.5.

Fatty acid composition

The fatty acid composition of the alga was estimated per unit lipid *vide* Materials and Methods. Thirteen fatty acids, namely undecanoic acid, lauric acid, tridecanoic acid, myristic acid, pentadecanoic acid, palmitic acid, heptadecanoic acid, stearic acid, arachidic acid, palmitoleic acid, oleic acid, cis-linoleic acid and arachidonic acid were detected in GC in the lipid sample of the alga (Table 2). Palmitoleic acid, oleic acid, cis-linoleic acid and arachidonic acid are the unsaturated fatty acids present in the sample and they constituted nearly 64% of the total fatty acid fraction. cis-linoleic acid and the saturated arachidic acid formed the bulk of total fatty acids. Cis-linoleic acid alone constituted 50% of the total content while arachidic acid took a share of 26%. The rest of the fatty acids were present in quantities less than 6.0 mg/g.

Amino acid composition

The protein fraction of the alga showed the presence of 22 amino acids namely cysteine, ornithine, aspartic acid, glutamic acid, serine, glycine, asparagine, alanine, glutamine, histidine,

Table 1: Mineral composition of *P.oedogonia*

Moisture	6.79 %
Ash	34.75 %
Acid washable ash	16.19 %
Protein	20.5 %
lipid	1.67 %
Crude fiber	15.17%
Total nitrogen	83.6-84.2mg
Sodium	2.24 mg/g
Potassium	6.45 mg/g
Copper	0.006 mg/g
Iron	0.198 mg/g
Sulphate	9.9 mg/g
Chloride	9.73 mg/g
Phosphate	0.1626 mg/g
Boron	0.157 mg/g
Calcium	1.866 mg/g
Magnesium	14.676 mg/g
Heavy metals	
Cadmium	Trace
Lead	0.215ppm
Nickle	0.325ppm
chromium	0.763ppm
Zinc	1.445 ppm

Table 2: Fatty acid composition of *P. oedogonia*

S. No.	No. of carbon	No.of double bonds	Fatty acid	Quantitymg/g	%	Sat/ unsat
1.	11	0	Undecanoic acid	3.1873	2.73	Sat
2.	12	0	Lauric acid	0.2755	0.23	Sat
3.	13	0	Tridecanoic acid	3.7306	3.20	Sat
4.	14	0	Myristic acid	3.1686	2.74	Sat
5.	15	0	Pentadecanoic acid	1.6523	1.41	Sat
6.	16	0	Palmitic acid	3.1361	2.69	Sat
7.	17	0	Heptadecanoic acid	0.9815	0.84	Sat
8.	18	0	Stiaric acid	4.6559	3.99	Sat
9.	20	0	Arachidic acid	31.161	26.7	Sat
10.	16	1	Palmitolic acid	0.5753	0.49	Unsat
11.	18	1	Oleic acid	5.2328	4.49	Unsat
12.	18	2	Cis linoleic acid	58.675	50.38	Unsat
13.	20	4	Arachidonic acid	0.0224	0.019	unsat
			Total	116.455		

lysine, arginine, proline, methionine, leucine, tyrosine, tryptophan, phenylalanine, threonine, valine, isoleucine and γ -amino butyric acid (Table 3). Interestingly the amides asparagine and glutamine were found only in traces in the protein fraction. Aspartic acid constituted 22% of the proteins. The other major components are glutamic acid (9.9%), leucine (7.7%), glycine (8.7%), isoleucine (6.1%) and arginine (6.09%).

The free amino acid pool showed the presence of only 20 amino acids of which tyrosine and tryptophan were present in trace amounts (Table 3). In contrast to that observed for protein amino acids, cysteine is the major component of the free amino acid pool comprising 22.4% of the total amino acid. The lysine constituted 10.6% of the total pool. The other major constituents are asparagine (12.2%) arginine (10.6%) leucine (5.9%) and ornithine (5.7%).

A-tocopherol content in hexane extract of fresh and dry mats of *P.oedogonia*

The hexane extracts of both fresh and dried mats of the alga were analysed for α -tocopherol levels by HPLC. The analysis showed variations in the levels of α -tocopherol between the extract of dry and fresh material. Fresh extract contained 0.073% of α -tocopherol while the same in the extracts of dry mats was 0.09%.

Vitamin composition

Apart from α -tocopherol, vitamin A and vitamin B₂ and other components of vitamin B complex were detected in sufficient levels (Table 4). Nicotinic acid, nicotinamide and pantothenate were found in high levels. Nicotinic acid was present at 0.65 mg/g dry weight of the algal mats, while vitamin B₂ was present at a concentration of 0.04 mg/g dry weight. The levels of vitamin A per gram dry weight of the algal mats was in the range of 200 \pm 17 IU. From the values obtained for vitamin A, β -carotene level was calculated in the tissues. Gram dry weight of the material contain 120.0 μ g carotene.

Table 3: Amino acid composition of *P. oedogonia*

S. No.	Amino Acid	Protein Amino acid		Free Pool	
		mg/g protein	% Total	mg/g fresh wt.	% Total
1.	Cystine	10.2	1.48	126.8	22.4
2.	Ornithine	-		31.7	5.62
3.	Aspartate	150.3	21.9	18.2	3.22
4.	Glutamic acid	66.3	9.6	15.4	2.73
5.	Serine	34.1	4.9	12.7	2.25
6.	Glycine	58.4	8.5	11.8	2.09
7.	Asparagine	Trace		69.2	12.27
8.	Alanine	34.7	5.05	21.4	3.79
9.	Glutamine	Trace		15.8	2.8
10.	Histidine	10.3	1.5	28.6	5.07
11.	Lysine	28.6	4.16	60.0	10.64
12.	Arginine	41.8	6.09	60.0	10.64
13.	Proline	21.0	3.0	12.0	2.12
14.	Methionine	10.0	1.4	9.2	1.63
15.	Leucine	53.5	7.7	33.6	5.95
16.	Tyrosine	22.0	3.2	Trace	
17.	Tryptophane	Trace		Trace	
18.	Phenylalanine	38.4	5.59	19	3.36
19.	Threonine	36.7	5.34	9.2	1.63
20.	Valine	27.4	3.99	-	
21.	Isoleucine	42.3	6.16	-	
22.	Amino butyric acid	-		9.2	1.63
	Total amino acid	686		563.8	

Table 4: Vitamin composition of *P. oedogonia*

S. No	Vitamin	Quantity mg/g
1.	Nicotinic acid	0.658
2.	Nicotin amide	0.219
3.	Ca- pantothenate	0.278
4.	Vitamin B6	0.083
5.	Folic acid	0.029
6.	Vitamin B2	0.04
7.	Vitamin A	200 1U
	B-carotene	120 µg

Sterol composition

Gas chromatographic analysis of the sterol showed the presence of four peaks with retention time of 33.42, 35.22, 39.16 and 41.26 respectively under the analytical conditions described elsewhere. Of the four sterol peaks, two with retention time 35.22 and 39.16 were identified as stigma sterol and b-sitosterol respectively. The other two sterols

were not identified and were present in quantities comparable to that of stigma sterol using the same standard. Stigma sterol was present at a concentration of 0.067 mg/g lipid while b-sitosterol was present at a concentration of 0.586 mg/g lipid. The latter was the major sterol component of *Pithophora oedogonia*.

DISCUSSION

Biochemical composition especially proteins, carbohydrates, lipids, amino acids, fatty acids and the composition of minerals and vitamins are vital in determining the nutritional status of the alga and its usefulness as a nutritional substitute. Analysis of the dry powdered material for its mineral and heavy metal composition revealed the presence of heavy metals in the range of 0.001-0.763 ppm. Zinc was present at 1.445 ppm level. The moisture content was 6.79% and ash 34.75%. Protein and lipid constituted 20.5%, 1.67 % respectively. The concentrations of other minerals are given in

Table 5: A comparison of the Chemical composition of *Pithophora oedogonia* and *Spirulina*

Chemical	<i>P.oedogonia</i>	* <i>Spirulina</i>
Moisture	6.79 %	7.0%
Ash	34.75 %	9.0%
Acid washable ash	16.19 %	-
Protein	20.5 %	71.0%
Lipid	1.67 %	7.0%
Crude fiber	15.17%	0.9%
Total nitrogen	83.6-84.2mg	13.35%
Sodium	2.24 mg/g	412mg/kg
Potassium	6.45 mg/g	15400mg/kg
Copper	0.006 mg/g	-
Iron	0.198 mg/g	580mg/kg
Sulphate	9.9 mg/g	-
Chloride	9.73 mg/g	4400mg/kg
Phosphate	0.1626 mg/g	-
Boron	0.157 mg/g	-
Calcium	1.866 mg/g	1315mg/kg
Magnesium	14.676 mg/g	1915mg/kg
Heavy metals		
Cadmium	Trace	>0.10ppm
Lead	0.215ppm	0.40ppm
Nickle	0.325ppm	-
Chromium	0.763ppm	-
Zinc	1.445 ppm	39mg/kg

*Source MCRC Hand book 'The wonder gift of nature'

Table 6: A comparison of the amino acids of *Pithophora oedogonia* and *Spirulina**

S. No.	Amino acid	<i>P. oedogonia</i> Protein amino acid	<i>P. oedogonia</i> amino acid free pool	<i>Spirulina</i>
1.	Cystine	1.4 8 %	22.49	3.46 %
2.	Ornithine	5.62		
3.	Aspartate	21.9 %	3.22	6.43 %
4.	Glutamic acid	9.66 %	2.73	8.94 %
5.	Serine	4.9 %	2.25	4.0 %
6.	Glycine	8.5 %	2.09	3.46 %
7.	Asparagine		12.27	
8.	Alanine	5.05 %	3.79	5.82 %
9.	Glutamine		2.8	
10.	Histidine	1.5 %	5.07	1.08 %
11.	Lysine	4.16 %	10.64	4.0 %
12.	Arginine	6.09 %	10.64	5.98 %
13.	Proline	3.0 %	2.12	2.97 %
14.	Methionine	1.4 %	1.63	2.17 %
15.	Leucine	7.7 %	5.95	5.8 %
16.	Tyrosine	3.2 %		
17.	Tryptophane			1.13 %
18.	Phenylalanine	5.5 %	3.36	3.95 %
19.	Threonine	5.34 %	1.63	4.17 %
20.	Valine	3.9 9 %		6.0 %
21.	Isoleucine	6.16 %		4.13 %
			1.63	

* Source MCRC Hand book 'The wonder gift of nature'

Table 7: A comparison of the Fatty acids of *Pithophora oedogonia* and *Spirulina**

S.No	Fatty acid	<i>P.oedogonia</i> mg/Kg	<i>Spirulina</i> mg/Kg
1.	Lauric acid	275.5	229
2.	Myristic acid	3186	664
3.	Palmitic acid	3136	21,141
4.	Palmitolic acid	575.3	2565
5.	Heptadecanoic acid	981.5	142
6.	Stearic acid	4655.9	353
7.	Oleic acid	5232	3,009
8.	Cis- linoleic acid	58675	13784
9.	Undecanoic acid	3187	-
10.	Tridecanoic acid	3730	-
11.	Pentadecanoic acid	1652	-
12.	Arachidonic acid	22.4	-
13.	Arachidic acid	31161	-

* Source MCRC Hand book 'The wonder gift of nature'

** value for *P. oedogonia* are per kg extractable lipid from algal mats Value for *spirulina* are for per unit dry weight of the alga

Table 1. A comparison of the data obtained for *Pithophora oedogonia* with that reported for *Spirulina* is given in Table 5. The comparison shows that protein content of *Pithophora oedogonia* is relatively much less as compared to *Spirulina*. Nevertheless, the crude fiber content, low levels of sodium and high levels of potassium and iron favour *Pithophora oedogonia* also as a nutraceutical product. In recent years, dried biomass or cell extracts produced from *Chlorella* (Lee, 1997; Yamaguchi, 1997) *Dunaliella* (Avron and Ben Amotz, 1992), and *Spirulina* (Vonshak, 1997) have dominated the nutraceutical market. In the search for sources of cheap proteins, minerals and essential amino acids, filamentous algae have not received due attention like unicellular forms mainly because of the rigid cellulosic cell walls. A thick cellulosic cell wall of untreated algae is indigestible by nonruminants. However, with advancements made in post harvest processing technology in recent years, a rigid cellulosic cell wall may not pose a big problem for algae like *Pithophora oedogonia*. Fiber content is very high in this alga and only the protein content is not to the level reported for unicellular forms. Through genetic manipulations and selection techniques, it might be possible to increase the protein content of the alga in future. However, it is only a theoretical possibility at present.

The amino acid analysis showed the presence of 20 amino acids. Cystine, asparagine, lysine, arginine and leucine are found to occur in huge quantities (Table 3). Levels of these amino acids are as high as compared to that reported for *Spirulina* and other SCP algae. In *Pithophora oedogonia* lysine content is 10.64% in free amino acid pool and 4.16% in protein fractions as against 4% in the protein of *Spirulina* (Table 6). Thus the amino acid composition is relatively better in *Pithophora oedogonia* as compared to *Spirulina*. Many amino acids such as leucine, lysine, aspartate, glutamate, glycine, phenylalanine, threonine, and arginine are present in *Pithophora oedogonia* in high levels as compared to *Spirulina* (Seshadri, MCRC hand book, Becker and Venkataraman 1982; Venkataraman, 1983). Total lipid content of *Pithophora oedogonia* is 1.67% and it contained the fatty acids undecanoic acid, lauric acid, tridecanoic acid, myristic acid, pentadecanoic acid, palmitic acid, heptadecanoic acid, stearic acid, arachidic acid,

palmitoleic acid, oleic acid, cis-linoleic acid and arachidonic acid (Table 2). Available information on the fatty acid composition of *Spirulina* shows the presence of only lauric, myristic, palmitic, palmitoleic, oleic, heptadecanoic, stearic and cis-linoleic acids only in its dry powder preparations (Table 7). It may be noted that the unsaturated fatty acids are present in high levels in *Pithophora oedogonia* as the data presented in this thesis for these fatty acids is per unit lipid as compared to that of *Spirulina*.

In recent years, considerable attention has been directed at algae for the production of oils and fatty acids and the use of algal oils containing long chain polyunsaturated fatty acids (LCPUFA) as nutritional supplements (Cohen *et al.*, 1995; Behrens and Kyle, 1996; Apt and Behrens, 1999). The presence of arachidonic acid, a polyunsaturated fatty acid (20:4) in *Pithophora oedogonia* is an interesting and significant finding in this context. This fatty acid is a precursor of all eicosonoides and is involved in many vital physiological processes of human beings. Fatty acid and sterol composition is critical and distinct for an algal group and alga as well. Sterol distribution shows greater promise as chemotaxonomic markers (Volkman, 1986; Mansour *et al.*, 1999). Sterols induce a wide range of responses in plant and animal tissues. Gas chromatographic analysis showed the presence of stigmaterol and β -sitosterol in *Pithophora oedogonia*. β -sitosterol is present at a concentration of 0.5861 mg/g while stigmaterol was at 0.067 mg/g. Dry powdered material of the alga contained nicotinic acid (0.65mg/g), nicotinamide (0.219 mg/g), Ca-pantothenate (0.278 mg/g), Vitamin-A 200 IU, Vitamin B₂ (0.49 mg/g), Vitamin B₆ (0.83 mg/g) and folic acid (0.029 mg/g). Vitamin E as α -tocopherol was 0.073% in the n-hexane extract of fresh mats and 0.09% in dry mats of the alga.

High crude fiber content, high levels of iron, ash, calcium and phosphate, low levels of heavy metals, presence of long chain poly unsaturated fatty acids constituting more than 50% of total fatty acid content, presence of arachidonic acid which is the precursor of all eicosonoides, occurrence of essential amino acids in large quantities, presence of vitamins in levels comparable to that reported

for *Spirulina* especially of vitamin E, and the high antioxidant potential of the alga suggest that *Pithophora oedogonia* could serve as a better substitute, if not the best for many available nutraceutical products. Lesser protein as compared to other products and the rigid cellulosic cell wall may be disadvantageous for the alga to be commercially exploited for nutraceutical purposes. However, proper post harvest processing technology, genetic manipulation and selection techniques might help in overcoming these disadvantages. Biochemical composition especially proteins, carbohydrates, lipids, amino acids, fatty acids and the composition of minerals and vitamins are vital in determining the nutritional status of the alga and its usefulness as a nutritional substitute. Analysis of the dry powdered material for its mineral and heavy metal composition revealed the presence of heavy metals in the range of 0.001-0.763 ppm. Zinc was present at 1.445 ppm level. The moisture content was 6.79% and ash 34.75%. Protein and lipid constituted 20.5%, 1.67 % respectively. The concentrations of other minerals are given in Table 1. A comparison of the data obtained for *Pithophora oedogonia* with that reported for *Spirulina* is given in Table 5. The comparison shows that protein content of *Pithophora oedogonia* is relatively much less as compared to *Spirulina*. Nevertheless, the crude fiber content, low levels of sodium and high levels of potassium and iron favour

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Easy availability of the alga, simple growth requirements and fast growth under enriched conditions are added advantage for exploitation of the alga commercially as a nutraceutical substitute. The findings presented in this paper would certainly form a basis to view *Pithophora oedogonia*, the 'nuisance alga' as a potential source of biologically active natural products for nutraceutical application.

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