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Biochemical Composition of Marine Microalgae and Their Potential Antimicrobial Activity

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This study is to investigate biochemical compositions of two species of marine microalgae, *Chlorella ellipsoidea* of Chlorophyta and *Tetraselmis suecica* of Prasinophyta, and to assess their potential antimicrobial activities. Crude protein, lipid and carbohydrate for *C. ellipsoidea* were 43.15%, 12.63% and 13.09%, respectively, and those for *T. suecica* were 44.95%, 4.80% and 24.05%, respectively. The major amino acids of the two microalgae were aspartic acid, glutamic acid, glycine, alanine, valine, leucine, lysine and proline, and no significant difference between the amino acid compositions of both microalgae was observed. The major sugars for both microalgae were glucose, galactose and mannose, and glucose contents showed the highest level, 58.70% for *C. ellipsoidea* and 57.86% for *T. suecica*. The major mineral contents of both microalgae for 100 g were Ca (3,114 mg in *C. ellipoidea* and 3,389 mg in *T. suecica*) and followed by Na (2,881 mg), K (548 mg) and Mg (545 mg) for *C. ellipsoidea* and Na (1,832 mg), Mg (1,510 mg) and K (548 mg) for *T. suecica*. In the content of ATP-related compound, hypoxanthine in *C. ellipsoidea* and IMP in *T. suecica* were absolutely dominant compounds. The highest content of fatty acid in *C. ellipsoidea* was 20:4, 27.15% and that in *T. suecica* was 18:3 (ω-6), 18.10%. In case of physiologically important polyunsaturated fatty acids like eicosapentaenoic acid (20:5) and docosahexaenoic acid (22:6), both microalgae possessed just trace amounts but was rich in arachidonic acid (20:4). Vitamin content in both microalgae was significantly high in choline and inositol. In antimicrobial activity by water- and fat-soluble fraction of the microalgae, hexane extract in the fat-soluble fraction of *C. elliposidea* inhibited the growth of *Bacillus subtilis* by 96% bactericidal activity and tetrachlorocarbon extract of *T. suecica* indicated relatively excellent antimicrobial activity (81% bactericidal activity) against *Escherichia coli*. Hot water extract among water-soluble fraction of both microalgae almost suppressed the growth of *Staphylococcus aureus* by 96% bactericidal activity.

Key words: Marine microalgae, Antimicrobial activity, Biochemical composition

Introduction

Marine microalga is one of the species with the abundance and diversity on the earth and plays an important role in mariculture as food for larval and juvenile molluscs, as well as for the larvae of some crustacean and fish species (Robert et al., 2001; D'Souza and Kelly, 2000; Brown and Jeffrey, 1992). Some species of microalgae have been used for supply of food, energy and biochemical resources. Application of microalgae in those areas is quite depending on the nutritional value which is influenced by their size, shape, digestibility and biochemical composition (Brown et al., 1999; Brown and Miller, 1992; Webb and Chu, 1983).

In the studies about biological composition influencing the nutritional value of microalgae, researchers have studied the levels of proteins (Fuentes et al., 2000; Brown and Jeffrey, 1992) that could be used as healthy food or animal feed, carbohydrates

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(Brown and Jeffrey, 1992; Brown, 1991; Chu et al., 1982) that could be used as stabilizers and emulsifiers in food and bioactive materials having potential medicinal value, and unsaturated fatty acids such as arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid (Carvalho and Malcata, 2000; Viso and Murty, 1993; Dunstan et al., 1992; Shamsudin, 1992; Volkman et al., 1989; Kataoka and Misaki, 1983).

In addtion, pigments (Campo et al., 2000; Brown and Jffrey, 1992) that could be used as natural food dyes and mineral (Fuentes et al., 2000) as well as the other natural bioactive compounds for novel functional food additives, drugs or medicines (Mackeen et al., 2000; Murakami et al., 1988) have been studied.

Despite a variety of biological functions of marine microalgae, however, effective application of microalgae is still limited because of their inefficient cultivation and high production cost. Therefore, the most effective utilization of marine microalgae in the economic aspect is to preferentially isolate high valuable biological active compounds and then to use the other useful components remained after such first extraction as a nutritional supplement for animal feed.

In this study, we selected two marine microalgae, *Chlorella ellipsoidea* of Chlorophyta and *Tetraselmis suecica* of Prasinophyta that can be massively cultivated, and investigated biochemical compositions and antimicrobial activity.

Materials and Methods

Materials

Marine microalgae used in this study were *Chlorella ellipsoidea* of Chlorophyta and *Tetraselmis suecica* of Prasinophyta obtained from Korean Microalgae Collection Center of Pukyong National University (Pusan, Korea). The two microalgae were cultured using F/2 culture medium under 20℃, 30 PPT, 6,000 Lux, 24L:D=24:O.

The microalgae cell was harvested at the end of the final growth and freeze-dried for biochemical analyses and antimicrobial activity.

Microorganism tested for antimicrobial acitivity were obtained from Korean Collection of Type (KCTC) and American Type Culture Collection (ATCC).

An amino acid analyzer, Biochrom 20 from Pharmacia Biotech Co. (Sweden), was used for the amino acid composition analysis, and Shimadzu GC 14-A (Japan) equipped with FID was used for fatty acid analysis. In addition, HPLC was used for the analysis of the compound related to nucleic acids using Spectra Physics P-2000 (USA) connected with μBondapak C_{18} (300×3.9 mm). All other reagents used were of the highest grade available commercially.

Biochemical assay

Proximate compositions of the microalgae were determined according to AOAC method (1990). Crude protein was determined by semi-micro Kjeldahl method (nitrogen content×6.25), crude lipid was performed by Soxhlet method, and crude carbohydrate was determined by phenol-sulfuric acid reaction (absorbance at 470 nm, using glucose as the calibration standard). In addition, crude ash was carried out at 550℃ of the dry-type of furnace and mineral analysis was performed using HP-4500 ICP (Inductively coupled plasma, Hewlett Packard, USA) with the crude ash dissolved in 0.5 N HNO_3 solution.

One gram of sample was sonicated in a tube with 5 mL of ice-cold 70% (v/v) ethanol for 10 min, and centrifuged at 13,680 xg for 15 min. The supernatant was filtered though a Whatman No. 41 filter paper, and dried under vacuum. The extract was diluted with 25 mL of 70% ethanol. Ten milliliters of the solution were transferred into a clean tube, and 0.5 g of 5-sulfosalicylic acid was added to it. The mixture was standed for 1 hr at 4℃ in a dark, centrifuged (13,680 xg, 15 min), and dried under vacuum. Two milliliters of 0.2 M lithium citrate loading buffer (pH 2.2) was added to it. An injection volume of 40 μL was then analyzed using automatic amino acid analyzer (LKB 4150).

For the fatty acid analysis, the sample was hydrolyzed with 0.1 N KOH-MeOH, and fatty acid methyl esters were prepared from lipids with BF_3-MeOH. The fatty acid methyl esters were taken up in hexane, and assayed by GC-FID (Shimadzu GC 14-A, Japan) using a capillary column.

For the carbohydrate analysis, the sample (50 mg) was hydrolyzed with 1 N H_2SO_4 at 100℃ for 2 h,

neutrolized to pH 5.0~5.2 with solid barium carbonate and centrifuged (9,500×g, 15 min) to remove the precipitate. The supernatant was concentrated and freeze-dried. The freeze-dried sample was modified to alditol acetate derivative according to Blakeney et al. (1983), and assayed using gas chromatography (HP 58090 series II, USA).

The assay of ATP-related compounds was carried out using HPLC (P-2000, Spectra Physics Co., USA) equipped with μBondapak C_{18} (300×3.9 mm) according to Kitada et al. (1983). Mobile solvent for HPLC analysis was triethylamine-phosphoric acid (pH 7.0), and the flow rate was 0.8 mL/min.

The assay of various vitamin was performed according to AOAC method (1990).

Fractionation of marine microalgae

The two marine microalgae were divided into water- and fat-soluble fractions according to Fig. 1. A 70% ethanol extract was fractionated by a 70% ethanol (400 mL) reaction of a sample (20 g) at 80 ±1℃ for 2 h. The 70% ethanol extract was partitioned with hexane, petroleum ether and carbon tetrachloride, successively and their respective fat-soluble fractions were obtained. An aqueous layer was obtained after removing all fat-soluble fractions. A hot aqueous fraction was obtained by homogenizing (12,000 rpm×2 min, 2 times) the sample with 80℃ hot water and centrifuging (9,500×g, 15 min) of the homogenized sample. All fractions obtained were concentrated by the evaporation, and the two aqueous fractions were freeze-dried.

Antibacterial assay

Antibacterial activity of the fractions obtained from the two marine microalgae was examined against 15 strains of bacteria including five gram-negative bacteria (*Escherichia coli* KCTC 1682, *Escherichia coli* O-157 ATCC 11775, *Salmonella typhi* KCTC 2424, *Pseudomonas aeruginosa* KCTC 1750, *Vibrio parahaemolyticus* ATCC 17802), nine gram-positive bacteria (*Streptococcus mutans* KCTC 3065, *Micrococcus luteus* KCTC 10240, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus epidermidis* KCTC 1917, *Bacillus subtilis* KCTC 1028, *Lactobacillus bulgaricus* KCTC 3188, *Lactobacillus casei* KCTC 3189, *Lactobacillus fermentum* KCTC 3112, *Streptococcus faecalis* ATCC 10541) and a yeast (*Candida albicans* KCTC 1940). The assay was carried out by the colony count method on agar plate according to Jeon et al. (2001). The mixture of 0.5 mL of the cultured bacteria solution, 0.5 mL of the autoclaved sample solution (200 mg/mL) and 19 mL of tryptic soy broth medium was incubated at 35±0.2℃ for 20~32 h. After the incubation, the colony formed was counted to indicate bactericidal activity which was calculated by the formular: Bactericidal activity (%)=[(C-T)/C]×100, where C is the colony numbers counted in the control, and T is those in the tested sample plate.

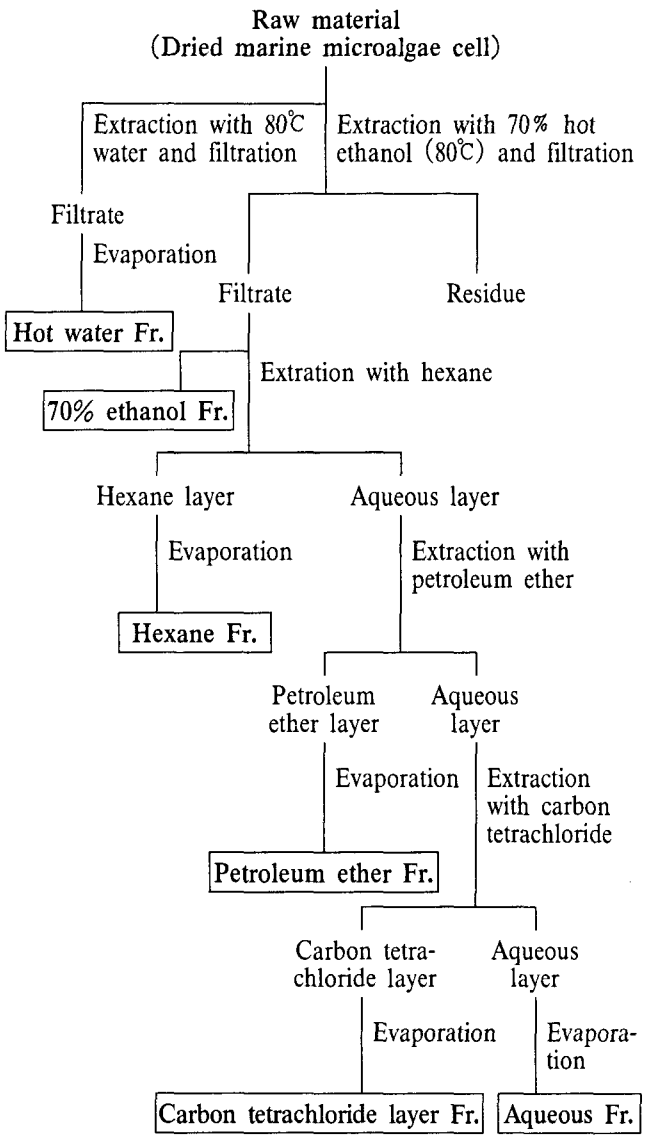

Fig. 1. Flow diagram for extraction of water- and fat-soluble fraction from the the dried marine microalgae cell.

Results and discussion

Biochemical composition

In order to evaluate the usefulness of the microalgae, *Chlorella ellipsoidea* and *Tetraselmis suecica* as a functional material, the biochemical composition was preferentially analysed in terms of proximate chemical composition, sugar composition of the polysaccharides, mineral composition, and vitamin composition.

Proximate compositions of the two marine microalgae were shown in Table 1. Moisture contents of the two dried microalgae, *C. ellipsoidea* and *T. suecica* were 4.04% and 3.36%, respectively. Crude protein, lipid and carbohydrate content for *C. ellipsoidea* were 43.15%, 12.63% and 13.09%, respectively, while those for *T. suecica* were 44.95%, 4.85% and 24.05%, respectively. The two microalgae showed almost same content in crude proteins, but quite differences in crude lipids and carbohydrates. In the case of crude ash, both microalgae had 16.16% and 11.41%, respectively. Crude protein was the most major component in approximate compositions of both microalgae. Brown and Jeffrey (1992) reported that there were significant differences in the chemical compositions between 4 Chlorophyte species and 6 Prasinophyte species examined and the content ranges were varied from 5.5% to 25.6% for protein, from 5.9% to 16.7% for carbohydrate, and from 8.5% to 18.4% for lipid. The two microalgae tested in this study were thought to be valuable materials in the aspect of the nutrient due to their high level of protein content.

Table 1. Proximate compositions of *C. ellipsoidea* and *T. suecica*

(g/100 g-dry weight)

	Chlorella ellipsoidea	*Tetraselmis suecica*
Moisture	4.04	3.36
Crude protein	43.15	44.95
Crude lipid	12.63	4.85
Crude ash	16.16	11.41
Crude carbohydrate	13.09	24.05

The amino acid compositions of the two microalgae were presented in Table 2. There were almost no differences between the amino acid compositions of the two microalgae. The major amino acids were aspartic acid, glutamic acid, glycine, alanine, valine, leucine, lysine and proline, and the minor ones were cystine, methionine, histidine and tyrosine. In the content of hydroxyproline, however, *T. suecica* only had just a trace amount. On the other hand, there were β-aminoisobutyric acid (<1.0%), γ-aminobutyric acid (<2.0%), and ornithine (<2.0%) as non-protein amino acids in the two microalgae. In the case of taurine which has been known well as a biological active amino acid, *T. suecica* only contained 1.25%. Brown and Jeffrey (1992) reported that generally aspartic acid and glutamic acid were high content amino acids ranging from 7.1% to 12.4% and cysteine, methionine, histidine and hydroxyproline were low content amino acids. Brown et al. (1993) proved that all of *Isochrysis* sp., *Pavlova lutheri* and *Nannochloropsis oculata* con-

Table 2. Amino acid compositions of *C. ellipsoidea* and *T. suecica*

% (A.A./100 g-A.A.)

Amino acids	*Chlorella ellipsoidea*	*Tetraselmis suecica*
Phosphoserine	0.12	0.34
Taurine	–	1.25
Aspartic acid	5.89	5.76
Threonine	4.65	4.09
Serine	3.12	2.93
Glutamic acid	11.62	12.10
Proline	4.63	6.34
Glycine	6.79	6.71
Alanine	7.80	9.33
α-Aminobutyric acid	0.10	–
Valine	6.75	6.19
Cystine	0.42	0.31
Methionine	1.57	1.76
Cystathionine	0.04	0.06
Isoleucine	4.84	4.38
Leucine	9.67	8.84
Tyrosine	2.61	2.45
Phenylalnine	5.04	5.49
β-Aminoisobutyric acid	0.56	0.69
γ-Aminobutyric acid	1.85	0.19
Ornithine	1.48	0.87
Lysine	7.82	6.62
1-Methylhistidine	0.05	0.02
Histidine	1.86	1.59
3-Methylhistidine	0.89	0.02
Carnosine	0.00	0.16
Arginine	4.54	3.79
Hydroxyproline	–	0.04
Proline	5.32	7.69
Total	100.00	100.00

tained glutamic acid with the highest content of amino acids in the amino acid composition of Chlorophyceae and Prasinophyceae. From the fact that the proteins from marine microalgae such as Chlorophyte and Prasinophyte have high contents of glycine, proline, aspartic acid and glutamic acid which are savory in the taste, the proteins or their protein hydrolysates may be very available for the use as a food additive or a feed.

In the sugar composition of *C. ellipsoidea*, glucose (58.70%), galactose (20.23%), and mannose (15.75%) were major sugars, and the total content of the three sugars reached to 94.68%. A similar trend has been observed for *T. suecica* which contained glucose, galactose and mannose at 57.86%, 29.39% and 9.72% of total sugars. The minor sugars were rhamnose, ribose and fucose in *C. ellipsoidea* and xylose and ribose in *T. suecica*. Their total sugar contents were about 5.0% or less (Table 3).

Table 3. Sugar compositions of *C. ellipsoidea* and *T. suecica* (%)

Sugars	*Chlorella ellipsoidea*	*Tetraselmis suecica*
Rhamnose	2.46	–
Fucose	0.67	–
Ribose	2.20	1.25
Arabinose	–	–
Xylose	–	1.79
Mannose	15.75	9.72
Glucose	58.70	57.86
Galactose	20.23	29.39
Total	100.00	100.00

Both microalgae tested in this study showed the highest proportion of glucose. Brown and Jeffrey (1992) reported that the range of glucose content were from 19% to 88% for most marine microalgae tested. On the other hand, Whyte (1987) reported that Chlorella contained the least glucose (19.3%) in its polysaccharide and possessed approximately equivalent similar proportions of galactose (20.4%) and mannose (18.4%). This means that glucose, galactose and mannose in Chlorella are very similar in the proportion of the three sugars. These differences between our results and Whyte's ones may be due to a difference in strain specificity or culture conditions. The two marine microalgae contained glucose-rich polysaccharide, which should be effectively digested by amylase in the digestive organs of molluscs and crustaceans (Brown and Jeffrey, 1992; Kristensen, 1972).

The major minerals of both microalgae were Ca (3,114 mg in 100 g of dried *C. ellipoidea* and 3,389 mg in 100 g of dried *T. suecica*) and followed by Na (2,881 mg), K (548 mg) and Mg (545 mg) for *C. ellipoidea* and Na (1,832 mg), Mg (1,510 mg) and K (548 mg) for *T. suecica* (Table 4). Calcium content was over 3,000 mg in both of the microalgae, but in the case of iron content *T. suecica* was approximate twice higher than *C. ellipsoidea*.

Table 4. Mineral contents of *C. ellipsoidea* and *T. suecica* (mg/100 g)

Minerals	*Chlorella ellipsoidea*	*Tetraselmis suecica*
Na	2,881.62	1,831.92
Mg	545.26	1,509.85
Al	185.80	316.36
K	548.05	548.16
Ca	3,114.09	3,389.24
Mn	10.52	21.41
Fe	274.23	496.77
Cu	1.33	3.09
Zn	7.83	16.64

The contents of ATP-related compounds for *C. ellipsoidea* and *T. suecica* were shown in Table 5. Hypoxanthine was an absolutely dominant compound in *C. ellipsoidea*, accounting for 9.48 μmol/g sample of the total ATP-related content (12.44 μmol/g). In *T. suecica* IMP was the first major compound, which possessed 14.68 μmol/g of the total 23.90 μmol/g, while hypoxanthine was a secondary highest content (5.21 μmol/g). A small amount of ATP, ADP and AMP were found in both microalgae. *T. suecica* was two-fold higher than *C.*

Table 5. Contents of ATP-related compounds in *C. ellipsoidea* and *T. suecica* (μmol/g)

ATP-related compound	*Chlorella ellipsoidea*	*Tetraselmis suecica*
ATP	0.79	1.15
ADP	0.42	0.27
AMP	0.57	2.59
IMP	1.18	14.68
Hypoxanthine	9.48	5.21
Total	12.44	23.90

ellipsoidea in the total content of ATP-related compounds.

From the above results, it considered that the taste of *T. suecica* would be excellent because *T. suecica* has a considerable amount of IMP, comparing to the other ATP-related compounds, which has been known as a good taste component among the ATP-related compounds (Kuninaka, 1960; Yamaguchi et al., 1968). In addition, it also indicates a strong synergistic effect with glutamic acid in the taste (Hayashi et al., 1981). As showed in the result of amino acid composition, the content of glutamic acid was the highest in the amino acid composition of the crude protein from *T. suecica*. This result implies that *T. suecica* with high contents of glutamic acid and IMP appears to be available as feed for fish growth because of its good taste for fish cultivated in mariculture and inducing the diet stimulation of fish.

In the fatty acid composition shown in Table 6, the content of saturated fatty acids, monoenoic fatty acids and polyenoic fatty acids for *C. ellipsoidea* were 28.69%, 35.27% and 36.04%, respectively, and for *T. suecica* were 22.03%, 39.46% and 38.51%, respectively. The major fatty acids in *C. ellipsoidea* were 20.73% of 16:0, 20.40% of 16:1, and 27.15% of 20:4, and in *T. suecica* were 18.10% of 18:3 (ω-6), 17.92% of 16:0, 14.12% of 16:1, and 13.57% of 18:1 (ω-9).

Both microalgae showed a low content of polyunsaturated fatty acids such as eicosapentaenoic acid (EPA, 20:5, ω-3) and docosahexaenoic acid (DHA, 22:6, ω-3) which are essential fatty acids and are needed in the maricultural industries of many marine invertebrates and fish. However, arachidonic acid (20:4), which physiologically plays an important role as a precursor of prostaglandin, was rich in *C. ellipsoidea*.

As shown in Table 7, the content of the water-soluble vitamins in the both microalgae was 6 times higher than that of fat-soluble vitamins. Among the water-soluble vitamins, the contents of choline, inositol, and vitamin C were 380 mg, 221 mg, and 58 mg in 100 g dried *C. ellipsoidea* and 323 mg, 108 mg, and 42 mg in *T. suecica*, respectively. In addition, the content of vitamin B complexes except choline and inositol in both microalgae was almost trace (approximately 0.07~5.70 mg/100 g). Most previous studies in physiological aspects have focused on the importance of ω-3 polyunsaturated fatty acids like EPA and DHA. However, vitamin content of microalgae may be also equally important because it is necessary for the normal growth and nourishment of the body.

Table 6. Fatty acid compositions of total lipid in *C. ellipsoidea* and *T. suecica*

(Area %)

Fatty acids	*Chlorella ellipsoidea*	*Tetraselmis suecica*
11:0	0.19	–
12:0	0.23	–
13:0	0.31	–
14:0	3.30	0.97
15:0	1.05	0.86
16:0	20.73	17.92
17:0	0.45	0.20
18:0	0.98	0.82
20:0	0.18	–
22:0	0.56	0.32
23:0	0.27	0.38
24:0	0.44	0.56
14:1	0.44	0.57
15:1	0.17	0.24
16:1	20.40	14.12
17:1	1.07	3.82
18:1 (n-9)	8.04	13.57
18:1 (n-7)	4.24	6.45
20:1 (n-9)	0.11	0.19
20:1 (n-7)	0.15	–
22:1	0.65	0.50
18:2 (n-5)	1.75	2.06
18:2 (n-5) trans	0.57	1.23
18:3 (n-6)	1.32	18.10
18:3 (n-3)	0.20	3.06
20:3	3.98	0.95
20:4	27.15	10.65
20:5	0.49	1.67
22:2	0.20	0.24
22:6	0.38	0.55
Saturated acids	28.69	22.03
Monoenoic acids	35.27	39.46
Polyenoic acids	36.04	38.51

Isolation of water- and fat-soluble fraction, and their antimicrobial activity

Microalgae were divided into water-soluble fractions (hot and cold water fraction) and fat soluble fractions (70% ethanol, hexane, petroleum ether and carbon tetrachloride fraction) according to the flow diagram of Fig. 1, and the yield of each frac-

Table 7. Contents of vitamin in *C. ellipsoidea* and *T. suecica* (mg/100 g)

Vitamins	*Chlorella ellipsoidea*	*Tetraselmis suecica*
Water-solubles		
Vitamin B_1	1.85	1.50
B_2	5.70	3.04
B_6	0.912	0.539
B_{12}	0.077	0.098
Niacin	22.3	19.8
Pantothenic avid	2.85	2.61
Folic acid	0.049	0.026
Biotin	0.21	0.18
Choline	380	323
Inositol	221	108
Vitamin C	58	42
Fat-solubles		
Vitamin D_2	86	58
Vitamin E	12	5
Vitamin K_1	1.0	0.7

tion was presented in Table 8. The total content of all the fractions extracted from *C. ellipsoidea* was 77.8% and almost two-fold higher than that of *T. suecica* (40.4%). The two 70% ethanol fractions from *C. ellipsoidea* and *T. suecica* showed the highest yields, 38.1% and 18.6%, respectively. At the last fractionation for the separation of aqueous layer from organic layer, the aqueous fraction obtained from *C. ellipsoidea* was almost 3.5-fold higher than that from *T. suecica*.

The fractions extracted from the two microalgae were screened for antimicrobial activity against five gram-negative bacteria (*E. coli*, *E. coli* O-157, *S. typhi*, *P. aeroginosa* and *V. parahaemolyticus*), nine gram-positive bacteria (*Str. mutans*, *M. luteus*, *St. aureus*, *St. epidermidis*, *B. subtilis*, *L. bulgaricus*, *L. casei*, *L. fermentum* and *St. faecalis*), and a yeast (*C. albicans*). The aqueous extract (0.5%) of the water-soluble fractions from *C. ellipsoidea* inhibited the growth of *E. coli* by 85% and hot water fraction showed 96% bactericidal activity against *St. aureus*. The hexane extract of fat-soluble fractions inhibited the growth of *B. subtillis* at 96% inhibitory rate. All the fractions extracted from *C. ellipsoidea* failed to significantly inhibit the growth of most bacteria tested and showed only 30~60% bactericidal activities. None of the fractions from *C. ellipsoidea* possessed antimicrobial activities against lactic acid bacteria such as *L. bulgaricus*, *L. casei*, *L. fermentum* and *St. faecalis* (Table 9). On the other hand, the antimicrobial activities by all the fractions from *T. suecica* showed a similar active trend to those from *C. ellipsoidea*. The growth of *E. coli* by the aqueous extract, however, expressed 53% bactericidal activity unlike the case of *C. elliposidea*, whereas the carbon tetrachloride extract had 81% inhibitory rate (Table 10). Like that from *C. ellipsoidea*, hot water extract from *T. suecica* showed the strongest antimicrobial activity against *St. aureus*.

Table 8. Yields of each fraction extracted from microalgae, *C. ellipsoidea* and *T. suecica*

Fraction	Yields (%, w/w of dried sample)	
	Chlorella ellipsoidea	*Tetraselmis suecica*
70% EtOH	38.1	18.6
Hexane	12.3	6.3
Petroleum ether	0.7	0.9
Carbon tetrachloride	4.3	4.8
Aqueous	17.7	5.2
Hot water	4.7	4.6
Total	77.8	40.4

Only a few researches have been carried out for the screening of antimicrobial or antifugal activity by microalgae. Murakami et al. (1988) found 15 species of microalgae with strong bactericidal activities by the means of the antimicrobial activity test and the inhibition test of development of fertilized echinoderm eggs, and reported that most active compounds existed exclusively in the fat-soluble fractions. In addition to Murakami's study, most studies for the screening of antimicrobial activity have only reported the activity by the fat-soluble fractions of microalgae (Kita and Fukujo, 1988). In our study, the antimicrobial activity was observed not only in fat-soluble fraction, but also in water-soluble fraction. The hot water extracts of both microalgae with a high antimicrobial activity against *St. aureus* could be widely used, because it is a major pathogenic bacterium causing food poisoning and pimples.

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Table 9. Antimicrobial activity[1] against various microorganisms by water- and fat-soluble fractions from *C. ellipsoidea*

Strain	Antimicrobial activity[2]					
	70% EtOH	Hexane	Petroleum ether	Carbon tetrachloride	Aqueous	Hot water
Gram(−) bacterium						
E. coli	65	<10	33	−[3]	85	43
E. coli O-157	47	−	−	−	56	29
S. typhi	−	−	−	−	−	−
P. aerginosa	−	−	−	−	−	−
V. parahaemolyticus	<10	−	−	<10	55	−
Gram(+) bacterium						
Str. mutans	48	37	28	−	−	−
M. luteus	−	−	−	−	−	−
St. aureus	−	−	−	65	−	96
St. epidermidis	−	−	−	−	−	−
B. subtilis	58	96	58	−	−	−
L. bulgaricus	−	−	−	−	−	−
L. casei	−	−	−	−	−	−
L. fermentum	−	−	−	−	−	−
St. faecalis	−	−	−	−	−	−
Yeast						
C. albicans	44	41	37	32	62	43

[1]Experiments for antimicrobial activity were carried out at 30℃ for 2 days for yeast and 37℃ for 20~32 h for the others through treatment of 0.05% sample concentration by colony count method.
[2]Antimicrobial activity was expressed as percentage against colony forming units of control.
[3]No activity.

Table 10. Antimicrobial activity[1] against various microorganisms by water- and fat-soluble fractions from *T. suecica*

Strain	Antimicrobial activity[2]					
	70% EtOH	Hexane	Petroleum ether	Carbon tetrachloride	Aqueous	Hot water
Gram(−) bacterium						
E. coli	−[3]	47	37	81	53	46
E. coli O-157	−	23	44	76	65	31
S. typhi	−	−	−	−	23	−
P. aerginosa	−	−	−	−	−	−
V. parahaemolyticus	−	35	−	<10	16	35
Gram(+) bacterium						
Str. mutans	28	<10	<10	−	−	−
M. luteus	−	−	−	−	−	−
St. aureus	−	−	49	−	−	96
St. epidermidis	−	−	−	−	−	−
B. subtilis	−	40	−	−	−	47
L. bulgaricus	−	−	−	−	−	−
L. casei	−	−	−	−	−	−
L. fermentum	−	−	−	−	−	−
St. faecalis	−	−	−	−	−	−
Yeast						
C. albicans	−	−	24	30	<10	−

[1]Experiments for antimicrobial activity were carried out at 30℃ for 2 days for yeast and 37℃ for 20~32 h for the others through treatment of 0.05% sample concentration by colony count method.
[2]Antimicrobial activity was expressed as percentage against colony forming units of control.
[3]No activity.

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