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Screening of Seventeen Oak Extracts for the Growth Inhibition of the Cyanobacterium *Microcystis aeruginosa* Kütz. em. Elenkin

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Allelochemicals produced by higher plants can inhibit or stimulate the growth of aquatic microorganisms in an aquatic ecosystem (Pillinger et al. 1994; Everall and Lees 1997; Nakai et al. 2000, 2001; Ebana et al. 2001). These chemicals originate from various tissues or organs of plants, including leaves, flowers, fruits, stems, and roots, and they include mainly phenolic acids, flavonoids, terpenoids, steroids, alkaloids, and organic cyanide (Whittaker and Feeny 1971). Of these, phenolic compounds, including caffeic, gallic, syringic, and tannic acids, have been widely applied as biocides to control phytoplankton (Pillinger et al. 1994; Everall and Lees 1997; Nakai et al. 2000, 2001). In general, tannins are well-known to be effective bactericides and algicides (Hussein-Ayoub and Tankov 1985; Lee and Shin 1991).

The Korean oak tree accounts for approximately 27% of the total national forest (Song et al. 2002). Compared to other higher plants, the oak tree contains relatively high levels of tannic acid, which plays an important role in the control of nuisance phytoplankton such as cyanobacterium *Microcystis aeruginosa* (Rice 1984; Pillinger et al. 1994). The use of phenolic compounds, such as tannins, is currently relevant in Korea due to the increased interest in environmental friendly biological control of nuisance algal bloom in eutrophicated waters.

In the present study, we examined extracts from nine types of Korean oak tree (*Castanopsis cuspidata var. sieboldii, Quercus acuta, Q. acutissima, Q. aliena, Q. dentata, Q. gilva, Q. glauca, Q. salicina,* and *Q. serrata*) to determine which one and what concentration is the most effective at inhibiting the growth of the cyanobacterium *Microcystis aeruginosa*.

MATERIALS AND METHODS

The cyanobacterium *Microcystis aeruginosa* strain UTEX 2388 was obtained from the Culture Collection of Algae at the University of Texas at Austin. This alga was grown in Allen medium (Allen 1968) at 28°C with a 14-h light/10-h

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dark cycle in a shaking incubator and illumination at 100 μmol/m^2/s with cool-white fluorescent lamps. The *Microcystis* was batch-cultured in Allen liquid media under the above conditions for 2 weeks. Every 2 weeks, 10 ml of culture were taken and inoculated into 90 ml of fresh medium for use as a seed culture.

Extracts from nine representative Korean oak trees were examined in this study for their ability to inhibit algal growth. Table 1 summarizes the extracts, the species, and the part of the plant from which they were obtained. In the first experiment, 17 different methanol extracts were made from leaves and/or stems. Each extract was adjusted to 100 mg/l (w/v) and was added to 100 ml of algal culture in a 250-ml triangle flask. Water from the Daechung reservoir was used as the culture medium after filtration through a GF/C filter (No. 1822 047, Whatman, England). Changes in the cell density of *M. aeruginosa* were counted with a Coulter counter (Coulter Z1, Coulter Corp., Miami, FL) and kept at 20°C. The suspensions were used as inoculum within 60 min after preparation.

In the second experiment, we examined the algicidal activity of each plant extract at different concentrations. Five extracts were selected because they inhibited algal growth by more than 90% when used at 100 mg/l in the first experiment. These five extracts were tested at 10, 20, 50 mg/l of final concentration, respectively. Otherwise, the experimental conditions were the same as in the first experiment.

In the third experiment, we examined the algicidal activity of tannic acid (Sigma-Aldrich Chemical Co., St. Louis, MO, USA) at different concentrations (1, 5 and 10 μM; 1.7, 8.5 and 17 mg/L, respectively), a major phenolic compound in the extracts. Otherwise the conditions for the analysis of algal growth inhibition were as described above.

The algicidal activity was calculated using the modified equation of Suzuki et al. (1998):

$$\text{Algicidal activity (\%)} = [(\text{control} - \text{treatment})/\text{control}] \times 100$$

The differences in cell densities between treated and control cultures were analyzed by ANOVA, and data were compared using linear contrasts (SPSS Inc., 1989-2003). A *P*-value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

Figure 1 shows the effects of 100 mg/l of various the tree extracts on the growth of the cyanobacterium *M. aeruginosa*. Of 17 different methanol extracts, 5 (QAT-L, QAT-S, QAS-L, QGI-S, and QSA-L; see Table 1 for abbreviations) decreased the cell density of *M. aeruginosa* by over 90% for 7 days. Another 7 extracts (CC-L, CC-S, QAI-LS, QAS-S, QGL-L, QSA-S, and QSE-S) decreased the growth by approximately 50% compared to the control during the same period. Thus, the genus *Quercus*, which has a relatively high level of tannin (Ridge et al. 1999), effectively inhibited the growth of *M. aeruginosa*, although the compositions of the extracts differ according to the type of plant, tissue

sampled, and season (Feeny and Bostock 1968; Bianco and Savolainen 1997; Kamalaka et al. 2004).

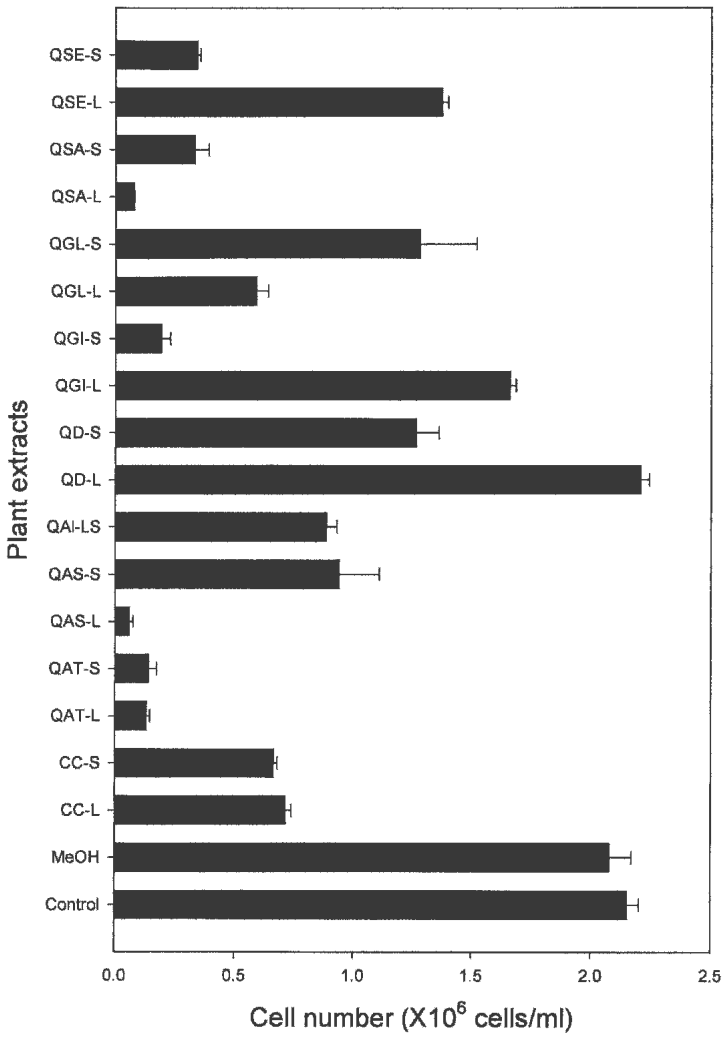

Figure 1. Effects of plant extracts (100 mg/l) on the growth of *M. aeruginosa* in Daechung Reservoir. Results indicate the means ± SEM (n=3). MeOH, methanol; CC, *Castanopsis cuspidata* var. *sieboldii*; QAT, *Quercus acuta*; QAS, *Quercus acutissima*; QAI, *Quercus aliena*; QD, *Quercus dentata*; QGI, *Quercus gilva*; QGL, *Quercus glauca*; QSA, *Quercus salicina*; QSE-S, *Quercus serrata*; L, leaf extract; S, stem extract; LS, leaf and stem extract.

Table 1. Methanol extracts from *Quercus* and *Castanopsis* in Korean Oak.

Symbol	Scientific name	Part	Sampling date
CC-L	*Castanopsis cuspidata* var. *sieboldii*	Leaf	2/13/2001
CC-S	*Castanopsis cuspidata* var. *sieboldii*	Stem	2/13/2001
QAT-L	*Quercus acuta*	Leaf	2/13/2001
QAT-S	*Quercus acuta*	Stem	2/13/2001
QAS-L	*Quercus acutissima*	Leaf	5/9/2001
QAS-S	*Quercus acutissima*	Stem	5/9/2001
QAl-LS	*Quercus aliena*	Leaf, Stem	4/19/2001
QD-L	*Quercus dentata*	Leaf	8/30/2001
QD-S	*Quercus dentata*	Stem	8/30/2001
QGI-L	*Quercus gilva*	Leaf	3/3/2001
QGI-S	*Quercus gilva*	Stem	3/3/2001
QGL-L	*Quercus glauca*	Leaf	2/13/2001
QGL-S	*Quercus glauca*	Stem	2/13/2001
QSA-L	*Quercus salicina*	Leaf	3/3/2001
QSA-S	*Quercus salicina*	Stem	3/3/2001
QSE-L	*Quercus serrata*	Leaf	7/4/2001
QSE-S	*Quercus serrata*	Stem	7/4/2001

Table 2. Algicidal activities of plant extracts at different concentrations on the growth of *M. aeruginosa*.

Plant extract	50 mg/l (final conc.)		20 mg/l		10 mg/l	
	Cell number (10^6 cells/ml)[a]	%[b]	Cell number (10^6 cells/ml)[a]	%[b]	Cell number (10^6 cells/ml)[a]	%[b]
Control	1.30±0.09	100.00	3.58±0.59	100.00	1.30±0.09	100.00
QSA-L	0.03±0.00**	2.00	1.84±0.29*	51.00	0.24±0.02*	19.00
QAT-S	0.48±0.01*	37.00	1.49±0.51*	42.00	2.23±0.15	171.00
QGI-S	0.28±0.00*	22.00	1.33±0.23*	37.00	1.35±0.09	104.00
QAT-L	0.12±0.00**	9.00	0.93±0.06*	26.00	0.98±0.03*	75.00
QAS-L	0.12±0.00**	9.00	0.79±0.00*	22.00	2.62±0.17	201.00

[a]: Values represent the means ± SEM (n = 3).
[b]: Algicidal activities of each extract
*: $P < 0.05$; **: $P < 0.01$

Table 2 shows the effects of various concentrations of the five most potent extracts (QAT-L, QAT-S, QAS-L, QGI-S, and QSA-L) on the growth of *M. aeruginosa*. At 50 mg/l, three of the extracts (QAT-L, QAS-L, and QSA-L) inhibited algal growth by more than 90%, while the other two, QAT-S and QGI-S, inhibited growth by 78% and 63%, respectively. At 20 mg/l, all five extracts inhibited the growth of *M. aeruginosa* by approximately 50%. At 10 mg/L, only two extracts, QSA-L (81%) and QAT-L (25%), inhibited the growth of *M. aeruginosa*, and the other three had little effect or stimulated algal growth. Thus, the minimum level of *Quercus* extract needed for effective inhibition of algal growth is 20 mg/l.

Figure 2 shows the anti-algal effect of various concentrations of tannic acid on the growth of *M. aeruginosa* after 2 and 5 days. Concentrations less than 10 μM

(17 mg/L) did not inhibit algal growth. At 1 and 5 μM (1.7 and 8.5 mg/l), tannic acid enhanced algal growth by as much as 150%. For comparison, the growth of another cyanobacterium, *Anabaena* sp., was effectively inhibited by 10 mg/l of tannin (Rice 1984), and similar concentrations of tannin inhibit the growth of the green algae *Chlorella vulagris* (Pillinger et al. 1994).

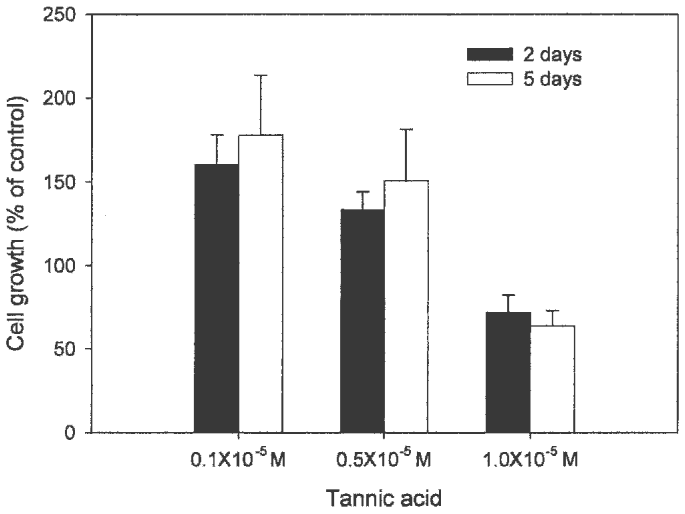

Figure 2. Effects of various concentrations of tannic acid on the growth of *M. aeruginosa*. Initial concentrations of tannic acid were 1, 5, and 10 μM (1.7, 8.5, and 17 mg/l, respectively). Natural water was obtained from the Daechung Reservoir. Results indicate the means ± SEM (n=3).

The inhibition of algal growth by tannin has been mainly attributed to effects on respiration, calcium and potassium uptake, photosynthesis, and membrane permeability (Duke 1986). The application of tannin to control blooms of cyanobacteria such as *M. aeruginosa* has two major advantages: 1) tannins are easily dissociated by natural 'microorganisms'(mainly bacteria), and 2) tannins do not affect the growth of other aquatic microorganisms (Street 1979; Gary et al. 1983). In addition, tannin extracts are particularly effective against *M. aeruginosa*, possibly because of synergistic action by multiple phenolic compounds.

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REFERENCES

Allen MM (1968) Simple conditions for the growth of unicellular blue-green algae on plates. J Phycol 4: 1-4.

Bianco MA and Savolainen H (1997) Phenolic acids as indicators of wood tannins. Sci Total Environ 203: 79-82.

Duke SO (1986) Naturally occuring chemical compounds as herbicides. Rev Weed Sci 2: 17-44.

Ebana K, Yan W, Dilday RH, Namai H, Okuno K (2001) Variation in the allelopathic effect of rice with water soluble extracts. Agron J 93: 12-16.

Everall NC, Lees DR (1997) The identification and significance of chemicals released from decomposing barley straw during reservoir algal control. Wat Res 31: 614-620.

Feeny PP, Bostock H (1968) Seasonal changes in the tannin content of oak leaves. Phytochem 7: 871-880.

Gary FL, Crawford RL, Kirk TK (1983) Degradation of phenolic compounds and ring cleavage of catechol by *Phanerochate chrysosporium*. Appl Environ Microbiol 46: 191-197.

Hussein-Ayoub SM, Yankov LK (1985) Algicidal properties of tannins. Fitoterapia 56: 227-229.

Kamalaka A, Canbolat O, Ozaya O, Aktas S (2004) Nutritive value of oak (*Quercus* spp.) leaves. Small Rumin Res 53: 161-165.

Lee BW, Shin DH (1991) Screening of natural antimicrobial plant extract on food spoilage microorganisms. Korean J Food Sci Technol 23: 200-204.

Nakai S, Inoue Y, Hosomi M, Murakami A (2000) *Myriophyllum spicatum*-released allelopathic polyphenols inhibiting growth of blue-green algae *Microcystis aeruginosa*. Wat Res 34: 3026-3032.

Nakai S, Inoue Y, Hosomi M (2001) Algal growth inhibition effects and inducement modes by plant-producing phenols. Wat Res 35: 1855-1859.

Pillinger JM, Cooper JA, Ridge I (1994) Role of phenolic compounds in the antialgal activity of barley straw. J Chem Ecol 20: 1557-1569.

Rice EL (1984) Allelopathy. 2nd edn. Academic Press Inc., Orlando, Florida.

Ridge I, Walters J and Street M (1999) Algal growth control by terrestrial leaf litter: a realistic tool? Hydrobiologia 395/396: 173-180.

Song JH, Kim YJ, Yi JS (2002) Optimum growth range and variation in foliar trichome among natural populations of *Quercus variabilis* in Korea. J Korean For Soc 91: 687-693. (in Korean)

SPSS Inc., 1989-2003. SPSS ver 12.0

Street M (1979) The importance of invertebrates and straw. Game Conservancy Ann Rew 11: 34-38.

Suzuki Y, Takabayashi T, Kawaguchi T, Matsunaga K (1998) Isolation of an allelopathic substance from the crustose coralline algae, *Lithophyllum* spp., and its effect on the brown alga, *Laminaria religiosa* Miyabe (Phaeophyta). J Exp Mar Biol Ecol 225: 69-77.

Whittaker RH, Feeny PP (1971) Allelochemicals: Chemical reactions between species. Science 171: 757-770.