Journal of Chemical Ecology, Vol. 32, No. 1, January 2006 (©2006)
DOI: 10.1007/s10886-006-9354-y

CHEMICAL CONSTITUENTS OF THE AQUATIC PLANT *Schoenoplectus lacustris*: EVALUATION OF PHYTOTOXIC EFFECTS ON THE GREEN ALGA *Selenastrum capricornutum*

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(Received June 25, 2005; revised September 23, 2005; accepted September 28, 2005)

Abstract—Forty-nine secondary metabolites were isolated from aqueous and alcoholic extracts of the aquatic plant *Shoenoplectus lacustris*. All compounds were characterized based on spectroscopic data. Eleven free and glycosylated low-molecular polyphenols, 17 cinnamic acid and dihydrocinnamic acid derivatives, 11 flavonoids, and 10 C_{13} *nor*-isoprenoids were identified. The structure of the new compound, 1-benzoyl-glycerol-2-α-L-arabinopyranoside, was elucidated by 2D NMR experiments (COSY, HSQC, HMBC, NOESY). To evaluate potential phytotoxic effects, all compounds were tested on the green alga *Selenastrum capricornutum*, a unicellular organism commonly used in tests of toxicity as a bioindicator of eutrophic sites. The most active compound was (−)-catechin, showing an inhibition similar to that of the algaecide $CuSO_4$.

Key Words—*Schoenoplectus lacustris*, polyphenols, cinnamic acids, dihydrocinnamic acids, flavonoids, C_{13} *nor*-isoprenoids, (−)-catechin, phytotoxicity, antialgal, *Selenastrum capricornutum*.

INTRODUCTION

In aquatic systems, plants can release allelochemicals into the water, and complex interactions between phytoplankton and vascular plants have been in-

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vestigated. A number of plants possess inhibitory effects on populations of microalgae by releasing phytotoxic substances into the environment (Gross et al., 2003).

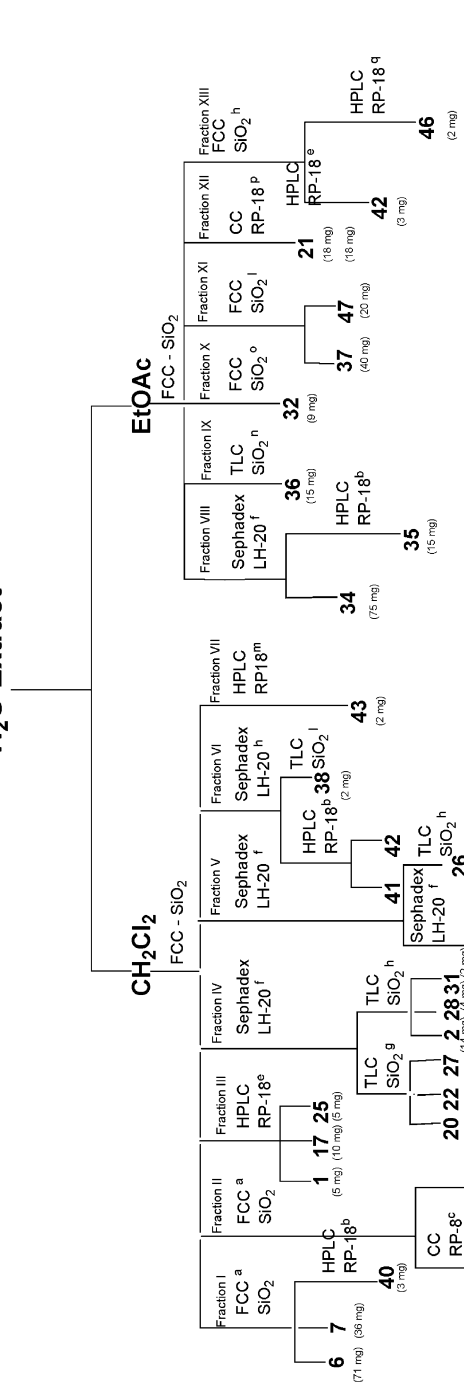
Early studies on interactions between aquatic plants and microalgae were conduced by Schreiter (1928), Hasler and Jones (1949), and Fitzgerald (1969). Later, experiments were designed to identify interactions between hydrophytes and microalgae (Kogan and Chinnova, 1972; Brammer, 1979; Godmaire and Planas, 1983).

Allelochemicals have been isolated from aquatic plants, and the ability of some natural products to inhibit *in vitro* development of microalgae has been reported by our research group in recent years (Cangiano et al., 2001; DellaGreca et al., 2001, 2003a, b).

In this research, we report on a phytochemical study of *Shoenoplectus lacustris* L. (Cyperaceae) and its effects on *Selenastrum capricornutum*, an algal species selected as a bioindicator of eutrophic sites for studies in aquatic environments (OECD, 1984; ISO, 1982). The effects of 49 low-molecular weight secondary metabolites on microalga *S. capricornutum* are reported.

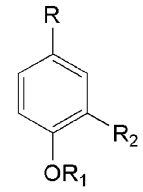
METHODS AND MATERIALS

General Experimental Procedures. NMR spectra were recorded at 500 MHz for ^{1}H and 125 MHz for ^{13}C on a Varian spectrometer (Varian Inc., Palo Alto, CA, USA) in $CDCl_3$ or CD_3OD solutions at 25°C. Optical rotations were measured on a Perkin-Elmer 343 Polarimeter (Norwalk, CT, USA). CD spectra were obtained in MeOH solutions on a Jasco J-715 Spectrophotometer Polarimeter (Jasco, Great Dunmow, UK). Electronic impact mass spectra (EI-MS) were obtained with an HP 6890 instrument equipped with an MS 5973 N detector (SIS Instruments, Ringoes, NJ, USA). The HPLC apparatus (Shimadzu, Kyoto, Japan) consisted of a pump (Shimadzu LC-10AD), a refractive index detector (Shimadzu RID-10A), and a Shimadzu Chromatopac C-R6A recorder. Preparative HPLC was made by using a 250 × 10 mm i.d, 10 μm, RP-18 Luna column (Phenomenex, Torrance, CA, USA) and SiO_2 (MAXSIL, 250 × 10 mm i.d., Phenomenex) columns. Analytical TLC was conducted on Merck Kieselgel 60 F_{254} or RP-8 F_{254} plates with 0.2-mm layer thickness (Merck, Darmstadt, Germany). Preparative TLC was performed on Merck Kieselgel 60 F_{254} plates, with 0.5 or 1 mm film thickness. Flash column chromatography (FCC) was carried out with Merck Kieselgel 60 (230–400 mesh) at medium pressure. Column chromatography (CC) was performed on Merck Kieselgel 60 (70–240 mesh), Baker Bond Phase C18 (0.040–0.063 mm), Fluka (Fluka, Buchs, Switzerland), Reversed phase silica gel 100 C8 (0.040–0.063 mm) or on Sephadex LH-20® (Pharmacia).

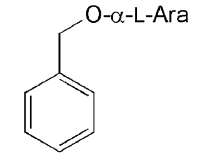


[a]petroleum ether - $CHCl_3$ - EtOAc (35:14:1); [b]MeOH - MeCN - H_2O (2 :1:2); [c]MeOH - MeCN - H_2O (1:2 :17); [d]MeOH - MeCN - H_2O (2:1:2); [e]MeOH - MeCN - H_2O (1:1:3); [f]hexane - $CHCl_3$ -MeOH (1:1:1); [g]petroleum ether - EtOAc (4:1); [h]$CHCl_3$-MeOH (9:1); [i]hexane-Me_2CO (4:1); [l]$CHCl_3$-MeOH (19:1); [m] MeOH - MeCN - H_2O (3:1:1); [n]lower phase of $CHCl_3$-MeOH - EtOH - H_2O (13:9:1:3); [o]lower phase of $CHCl_3$-MeOH- H_2O (13:7:3); [p]MeOH - MeCN - H_2O (1:1:2); [q]MeOH - MeCN - H_2O (1:1:8);

SCHEME 1. Isolation of the metabolites from the aqueous extract of *S. lacustris*.



1	R = CHO	$R_1 = R_2 = H$	
2	R = COOH	$R_1 = R_2 = H$	
3	R = COOH	$R_1 = H$	$R_2 = OH$
4	R = COOMe	$R_1 = R_2 = H$	
5	R = COOMe	$R_1 = Ac$	$R_2 = OMe$
6	R = CH=CH_2	$R_1 = H$	$R_2 = OH$
7	R = CH=CH_2	$R_1 = H$	$R_2 = OMe$
8	R = Et	$R_1 = H$	$R_2 = OH$
9	R = O-β-D-glc	$R_1 = H$	$R_2 = OMe$



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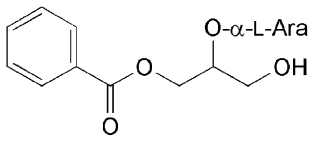

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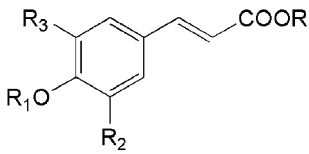
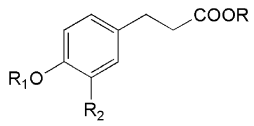


12	$R = R_1 = R_2 = R_3 = H$		
13	R = Me	$R_1 = R_2 = R_3 = H$	
14	$R = R_1$ = Me	$R_2 = R_3 = H$	
15	$R = R_2 = R_3 = H$	R_1 = β-D-glc	
16	R = β-D-glc	$R_1 = R_2 = R_3 = H$	
17	$R = R_1 = R_3 = H$	$R_2 = OH$	
18	$R = R_1 = R_3 = H$	$R_2 = OMe$	
19	R = Me	$R_1 = R_3 = H$	$R_2 = OH$
20	R = Me	$R_1 = R_3 = H$	$R_2 = OMe$
21	$R = R_1 = H$	$R_2 = R_3 = OMe$	
22	R = Me	$R_1 = H$	$R_2 = R_3 = OMe$

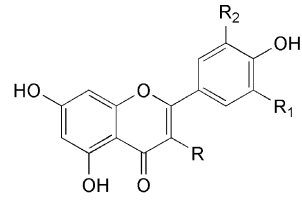
FIG. 1. Chemical constituents of *Schoenoplectus lacustris*.

Plant Material. Plants of *S. lacustris*, collected in the summer of 2002 from the Volturno River (Campania, Italy), were identified by Professor Assunta Esposito at the Second University of Naples (SUN). Voucher specimens (CE 31) were deposited at the Herbarium of the Dipartimento di Scienze della Vita of the SUN.

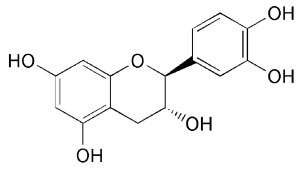
Extraction and Isolation. Fresh plants (12 kg) were extracted in 10% MeOH–H_2O over 3 d, in the dark at 4°C. After removal of the solution, plants



23 R = R_1 = R_2 = H
24 R = R_1 = Me R_2 = H
25 R = Me R_1 = R_2 = H
26 R = R_1 = H R_2 = OMe
27 R = Me R_1 = H R_2 = OMe
28 R = Me R_1 = H R_2 = OH



29 R_2 = H R_1 = OMe
30 R = H R_1 = R_2 = OMe
31 R = H R_1 = R_2 = OH
32 R = R_1 = R_2 = H
33 R = R_1 = OH R_2 = H
34 R = O-β-D-glc R_1 = OH R_2 = H
35 R = O-α-L-rha(1-6)-β-D-glc R_1 = R_2 = H
36 R = O-α-L-rha(1-6)-β-D-glc R_1 = OH R_2 = H



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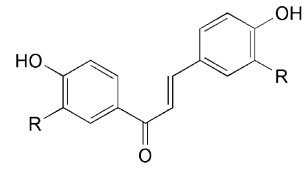
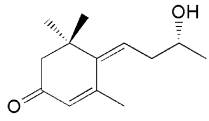


38 R = H
39 R = OMe



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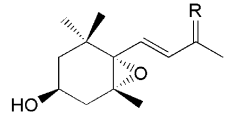
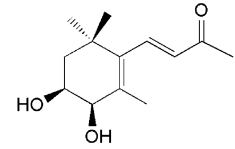


41 R = O
42 R = αOH,βH



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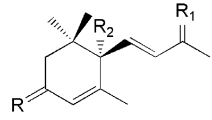
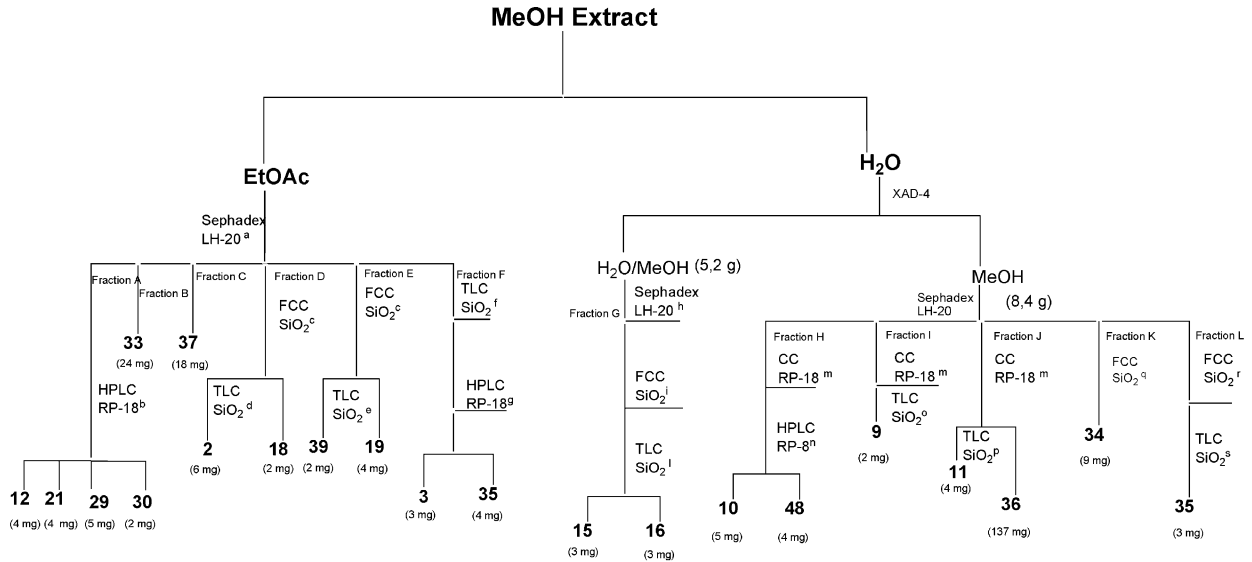


44 R = O R_1 = αOH,βH R_2 = H
45 R = OH,H R_1 = αOH,βH R_2 = H
46 R = O R_1 = αOH,βH R_2 = OH
47 R = OH,H R_1 = αOH,βH R_2 = OH
48 R = OH, H R_1 = αOH,βH R_2 = O-β-D-glc,H
49 R = O R_1 = O R_2 = OH

FIG. 1. Continued.



[a]hexane - $CHCl_3$ - MeOH (1:1:1); [b]MeOH - MeCN - H_2O (10:1:9); [c]$CHCl_3$ - MeOH (9:1); [d]$CHCl_3$ - EtOH (9:1); [e]hexane - $CHCl_3$ - MeOH (4:5:1); [f] lower phase of $CHCl_3$ - MeOH -H_2O (13:7:4); [g]MeOH - MeCN - H_2O (3:4 :13); [h]MeOH - H_2O (1:1); [i]lower phase of $CHCl_3$ - MeOH - H_2O (13:7:4); [l]lower phase of $CHCl_3$-MeOH - EtOH - H_2O (10:7:5:5); [m] MeOH - MeCN - H_2O (1:1:3); [n]MeOH - MeCN - H_2O (1:1:8); [o]lower phase of $CHCl_3$-MeOH -H_2O (13:7:2); [p] lower phase of $CHCl_3$ - MeOH - EtOH - H_2O (14:9:1:3); [q]lower phase of $CHCl_3$ - MeOH - H_2O (12:7:4); [r]lower phase of $CHCl_3$ - MeOH - H_2O (13:7:3); [s]lower phase of $CHCl_3$ - MeOH - H_2O (13:7:3).

SCHEME 2. Isolation of the metabolites from the methanolic extract of *S. lacustris*.

were extracted with MeOH for 5 d. After removal of methanol, the hydro-alcoholic fraction was extracted first with methylene chloride and then with EtOAc. The two fractions were evaporated under reduced pressure to obtain crude residues (Scheme 1). Structures are provided in Figure 1. CH_2Cl_2 extract (13.3 g) was chromatographed on SiO_2 eluting with petroleum ether and EtOAc solutions to give seven fractions I–VII: Fraction I, eluted with petroleum ether, furnished compounds **6**, **7**, and **40**. Fraction II, eluted with petroleum ether–EtOAc (19:1), gave seven compounds: **5**, **13**, **14**, **4**, **8**, **23**, and **24**. Fraction III, eluted with petroleum ether–EtOAc (9:1), furnished three compounds: **1**, **17**, and **25**. Fraction IV, eluted with petroleum ether–EtOAc (4:1), gave six compounds: **2, 20**, **22**, **27**, **28**, and **31**. Fraction V, eluted with petroleum ether–EtOAc (3:1), led to the isolation of **26**, **44**, **49**, and **45**. Fraction VI, eluted with petroleum ether–EtOAc (3:2), furnished compounds **38**, **41**, and **42**. Fraction VII, eluted with petroleum ether–EtOAc (1:1), gave isoprenoid **43**.

EtOAc extract (16.8 g) was chromatographed on SiO_2 eluting with $CHCl_3$ and EtOAc solutions to give six fractions VIII–XIII. Fraction VIII, eluted with $CHCl_3$, led to the isolation of **34** and **35**. Fraction IX, eluted with $CHCl_3$–EtOAc (19:1), gave flavone **36**. Fraction X, eluted with $CHCl_3$–EtOAc (9:1), furnished compound **32**. Fraction XI, eluted with $CHCl_3$–EtOAc (4:1), gave compounds **37** and **47**. Fraction XII, eluted with $CHCl_3$–EtOAc (3:2), led to the isolation of **21**, while fraction XIII, eluted with $CHCl_3$–EtOAc (1:4), gave compounds **42** and **46**.

MeOH extract (50.8 g) was dissolved in water and beaten with EtOAc to obtain an organic fraction and an aqueous one (Scheme 2). Structures are shown in Figure 1. The EtOAc fraction (30.4 g) was chromatographed by CC on Sephadex LH-20, eluting with hexane–$CHCl_3$–MeOH (1:1:1) to give five fractions, A–F. Fraction A furnished metabolites **12**, **21**, **29**, and **30**. Fraction B contained quercetin **33**. Fraction C consisted of compound **37**. Fraction D gave compounds **2** and **18**. Fraction E led to the isolation of **39** and **19**, whereas fraction F gave **3** and **35**.

The H_2O fraction (20.4 g) was chromatographed on Amberlite XAD-4 and eluted first with H_2O to eliminate sugars and proteins from the extract, then with MeOH–H_2O (1:1) and finally with MeOH. The eluate obtained with MeOH–H_2O (1:1) gave a fraction (G) that furnished compounds **15** and **16**. The eluate obtained with MeOH was chromatographed with H_2O and MeOH solutions to give five fractions H–L. Fraction H, eluted with H_2O–MeOH (17:3), gave compounds **10** and **48**. Fraction I, eluted with H_2O–MeOH (4:1), yielded glucoside **9**. Fraction J, eluted with H_2O–MeOH (4:1), gave compounds **11** and **36**. Fraction K, eluted with H_2O–MeOH (1:1), led to the isolation of glucoside **34**, whereas fraction L, eluted with H_2O–MeOH (1:3), gave rutinoside **35**.

Bioassay. The effects of the extracts and the isolated compounds were assessed on *S. capricornutum*, more recently renamed *Pseudokirchneriella*

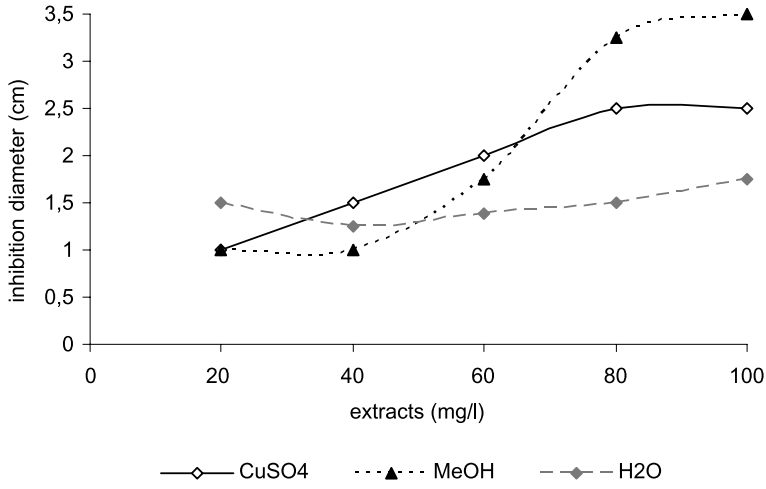


FIG. 2. Antialgal activity of the extracts of *Schoenoplectus lacustris*.

subcapitata. The alga was provided in immobilized algal beads by MicroBio Test (Nazareth, Belgium). The antialgal test was performed by deimmobilizing algae from the matrix with ethylene-bis(oxyethylenenitrilo)tetracetic acid (EGTA). *S. capricornutum* was cultivated on Bold basal medium (BBM) (Nichols, 1973). Cell density was measured with an electronic particle dual threshold counter (Coulter Counter Z2, 100 μm capillary; Instrumentation Laboratory, Miami, FL, USA). When the culture reached exponential growth phase, 1 ml (1.0×10^4 cells/ml) was poured onto a Petri dish with 20 ml of BBM containing 1.5% agar. The plates were incubated in a growth chamber at 25°C under continuous illumination (8000 lx). After 24 hr, a green layer of algae was evident on the agar, and subsequently a known quantity (0.5, 1.0, 2.0, and 3.0 μmol) of each chemical, dissolved in acetone or ethanol, was poured onto paper disks (sterile banks, Difco Bacto Concentration Disks, 3 mm). Each treatment was repeated with four replicates. After evaporation of the solvent, disks were placed on each Petri dish inoculated 24 hr before. Positive controls, for tests on single compounds were prepared by inoculating 0.5, 1.0, 2.0, and 3.0 μmol of algaecide $CuSO_4$ in 10 μl of Milli-Q water on the disk. A solvent control using acetone or ethanol on each disk was also prepared and placed on plates after evaporation (2 hr). The same procedure, with acetone or ethanol as solvent, was used for the extracts, and three independent experiments were performed in four replicates. The plates were incubated in a growth chamber for 96 hr at 25°C under continuous illumination (8000 lx). Chemical inhibition was

calculated by measuring the diameter of the no-growth zone excluding the paper disk (3 mm). The values obtained represent the average of three experiments. Phytotoxicity was compared with that of algaecide $CuSO_4$.

Data Analysis. The statistical significance of differences between groups was determined by Student's *t*-test, calculating mean values for growth inhibition. The significance level was set at $P < 0.05$.

RESULTS AND DISCUSSION

Aliquots of hydroalcoholic and methanolic extracts of *S. lacustris* were dried under reduced pressure, and the residues were tested on *S. capricornutum* to evaluate the antialgal effects. A common algaecide, $CuSO_4$, was used as positive control. This compound shows a median effective concentration (EC_{50}) in standard algal growth inhibition test (ISO 8692) equal to 0.045 mg/l with confidence interval at 95% ranging from 0.032 to 0.070. In this study, high concentrations of $CuSO_4$ were used because no information existed on the activity of the investigated extracts. Our results, reported in Figure 2, showed that the MeOH extract had a no-growth zone 66% greater than that of algaecide $CuSO_4$ at the maximum concentration tested, but became inactive below 40 mg/l. The aqueous extract was less active than the positive control at the highest concentrations but remained active up to 20 mg/l.

The hydroalcoholic fraction was extracted first with methylene chloride and then with EtOAc to obtain crude residues CH_2Cl_2 and EtOAc. The MeOH extract was dissolved in water and shaken with EtOAc to obtain an organic fraction and an aqueous one. Chromatographic processes of the fractions (Schemes 1 and 2) led to the identification of 49 compounds (Figure 1).

Compounds **1**–**4** were identified as 4-hydroxybenzaldehyde, benzoic acid, protocatecuic acid, and methyl benzoate, respectively. Compound **5** was identified as methyl 4-acetyloxy-3-methoxy-benzoate. Its EI-MS spectrum showed the molecular peak at m/z = 224. The ^{1}H NMR spectrum showed three aromatic protons of a 1,2,4-trisubstituted benzene, two methoxyls at δ = 3.87 and 3.84 and a methyl at δ = 2.20. These data, together with NOE observed between this latter methyl and the aromatic doublet, allowed an acetoxyl group to be localized at the C-4 carbon and a methoxyl at C-3 carbon with a methyl benzoate structure. In compounds **6** and **7**, ^{1}H NMR evidenced the ABX spin system of the vinyl group. The differences in their collected spectral data could be justified by the 3,4-dihydroxystyrene structure for **6** and 4-hydroxy-3-methoxystyrene for **7**. Compound **8** was identified as 1-(3,4-dihydroxyphenyl)ethane, and compound **9** as 4-hydroxy-3-methoxyphenyl-1-*O*-β-D-glucopyranoside. ^{1}H NMR showed three aromatic protons as two doublets at δ = 6.82 (J = 2.1 Hz) and 6.79 (J = 8.2 Hz)

and a double doublet at $\delta = 6.58$. The spectrum also indicated a methyl singlet at $\delta = 3.82$, a doublet at $\delta = 4.75$, and six protons ranging from 3.75 to 3.30 ppm. GC analysis of the compounds, after hydrolysis, reduction, and acetylation, showed the sugar moiety to be glucose. The coupling constant (7.4 Hz) of the anomeric proton indicated a β configuration at C-1′. NOE, between the H-1′ proton at $\delta = 4.75$ and the H-2 and H-6 protons localized the sugar at the C-1 carbon of the aromatic ring. Compound **10** was identified as benzyl-1-*O*-α-L-arabinopyranoside. EI-MS spectrum showed the molecular ion at $m/z = 240$, and ^{1}H NMR revealed signals of five aromatic protons as well as two diasterotopic protons as two doublets ($J = 11.4$ Hz) at $\delta = 4.25$ and 4.67. NMR data of the glycidic moiety revealed the presence of a pentose, identified as arabinose by GC analysis of the alditol acetate derivative. Compound **11** showed a molecular formula $C_{15}H_{20}O_8$, according to EI-MS spectrum, indicating a molecular ion peak at $m/z = 328$. The ^{1}H NMR spectrum revealed three aromatic signals as a doublet integrated for two protons at $\delta = 8.02$, a triplet at $\delta = 7.59$, and a triplet integrated for two protons at $\delta = 7.48$. These data suggested the presence of a benzoyl unit in the molecule. In the aliphatic region of the spectrum, there were also a doublet at $\delta = 4.92$, two double doublets at $\delta = 4.34$ and 4.44, as well as eight protons in the range 4.20–3.40 ppm. ^{13}C NMR showed a carboxyl carbon at $\delta = 167.1$ and four signals in the aromatic range, two of them due to the omotopic C-2′/C-6′ and C-3′/C5′ carbons of the benzoyl group. Eight carbons, five methines, and three methylenes were also evident in the spectrum. In the HMBC experiment, carboxyl carbon showed correlations with the H-2′/H-6′ and H-3′/H-5′ protons. The same carbon was also correlated with the protons at $\delta = 4.34$ and 4.44, which result correlated with the carbon at $\delta = 67.4$ in the HSQC experiment. This carbon was in turn correlated with the proton at $\delta = 4.18$ bonded to the carbon at $\delta = 70.3$, which showed heterocorrelations with the doublet at $\delta = 4.92$, due to an anomeric proton, and with two methylene protons at $\delta = 3.56$ and 3.92 bonded to the carbon at $\delta = 71.2$. The remaining ^{1}H and ^{13}C NMR signals were consistent with the presence of a pentose in the molecule. GC analysis of alditol derivative allowed the sugar moiety to be identified as L-arabinose.

Compounds **12**–**22** were identified as *p*-coumaric acid (**12**), caffeic acid (**17**), ferulic acid (**18**), and synapic acid (**21**), their corresponding methyl esters **13**, **19**, **20**, and **22**, 4-methoxycinnamic acid (**14**), and glucoside *p*-coumaryl-4-*O*-β-D-glucopyranoside (**15**) and glucoside *p*-coumaryl-1-*O*-β-D-glucopyranoside (**16**). Compounds **12**, **17**, **18**, and **21** were reported as allelochemicals by Li et al. (1993). These phenols inhibited lettuce seedling growth and seed germination. Compounds **19** and **20** were isolated from *Cestrum parqui* (D'Abrosca et al., 2004a), and their phytotoxicity assayed on the seeds of lettuce, tomato, and onion. The glucoside of *p*-coumaric acid (compounds **15** and **16**) were isolated from Riesling wine and play an important role as antioxidant components of this white wine (Baderschneider and Winterhalter, 2001).

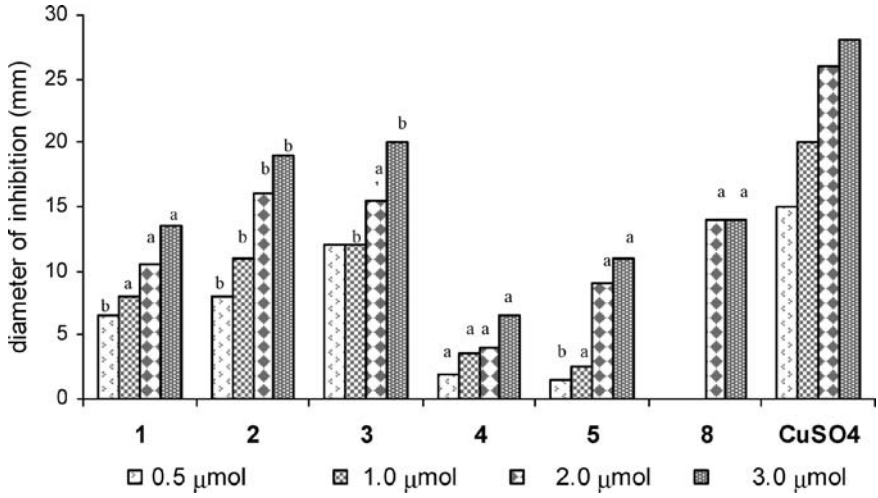


FIG. 3. Antialgal activity of phenols on *Selenastrum capricornutum*. Values are presented as diameter of no-growth zone (mm) for Student's *t*-test. (a) $P < 0.01$, (b) $0.01 < P < 0.05$.

Compounds **23**–**28** were identified as phenyl propionic acid derivatives. 3-(4-Hydroxyphenyl)propionic acid (**23**) and 3-(4-hydroxy-3-methoxyphenyl)propionic acid (**26**) were reported from *C. parqui* (D'Abrosca et al., 2004a). Compounds **24**, **25**, **27**, and **28** were characterized as methyl 4-methoxydihydrocinnamate, methyl 4-hydroxydihydrocinnamate, methyl dihydroferulate, and methyl dihydrocaffeate, respectively.

Compounds **29**–**39** were identified as flavonoids. Compound **29** was identified as chrysoeriol, the aglycone of compounds isolated from *Potamogeton* spp. (Boutard et al., 1973; Roberts and Haynes, 1986; Les and Sheridan, 1990). Compound **30** was identified as tricin, an allelopathic flavone released from rice seedlings (Kong et al., 2004). Compounds **31**–**33** were identified as luteolin, kaempferol, and quercetin, respectively. Glycosides **34**–**36** were isolated from *Sambucus nigra* (D'Abrosca et al., 2001). Compound **37** showed spectral data corresponding to catechin (Nahrstedt et al., 1987). It showed an $[\alpha]_D$ value (c, 0.16) −14.8 and a positive Cotton effect $\Delta\varepsilon_{280\ nm}$ +3.2 nm, in accordance with the presence of (−)-catechin (Bais et al., 2003a). Calcones **38** and **39** were identified on the basis of their spectral data. ^{1}H NMR of **38** showed two protons as doublet of a *trans* double bond at δ = 6.27 and 7.59, an α,β unsaturated carbonyl group and four doublets of two aromatic system at δ = 7.44, 7.16, 6.80, and 6.73. EI-MS spectrum confirmed the proposed structure showing the molecular ion at *m*/*z* = 240 and fragments at *m*/*z* = 147 [CO–CH–CH–C_6H_4OH]$^+$, 119 [CH–CH–C_6H_4OH]$^+$, 121 [CO–C_6H_4OH]$^+$, and 93

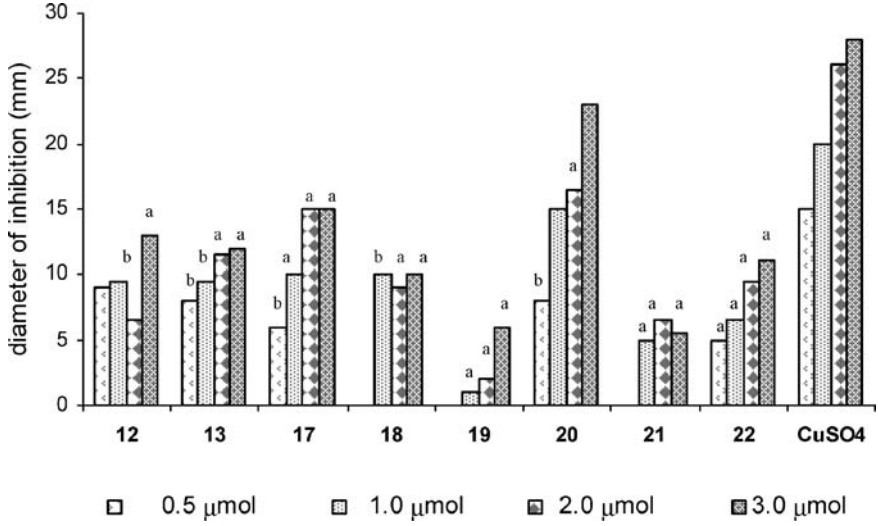


FIG. 4. Antialgal activity of cinnamic acids derivatives on *S. capricornutum*. Values are presented as diameter of no-growth zone (mm) for Student's *t*-test. (a) $P < 0.01$, (b) $0.01 < P < 0.05$.

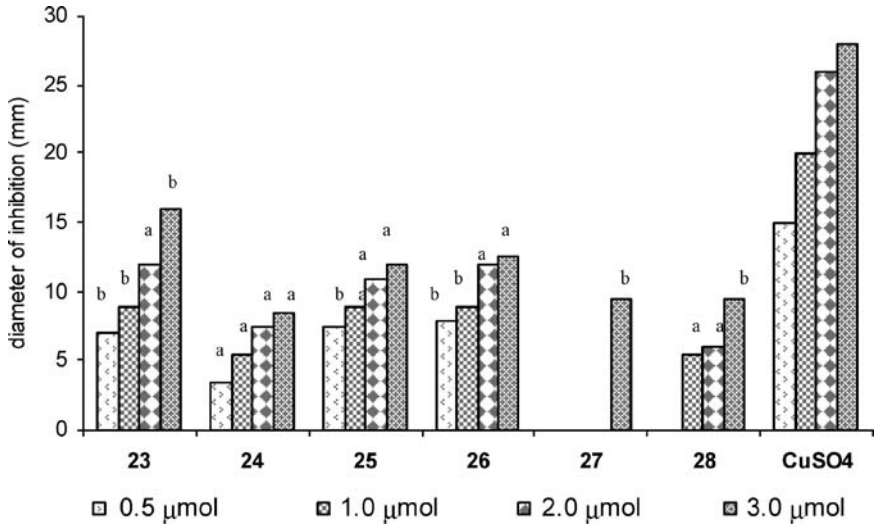


FIG. 5. Antialgal activity of dihydrocinnamic acids derivatives on *S. capricornutum*. Values are presented as diameter of no-growth zone (mm) for Student's *t*-test. (a) $P < 0.01$, (b) $0.01 < P < 0.05$.

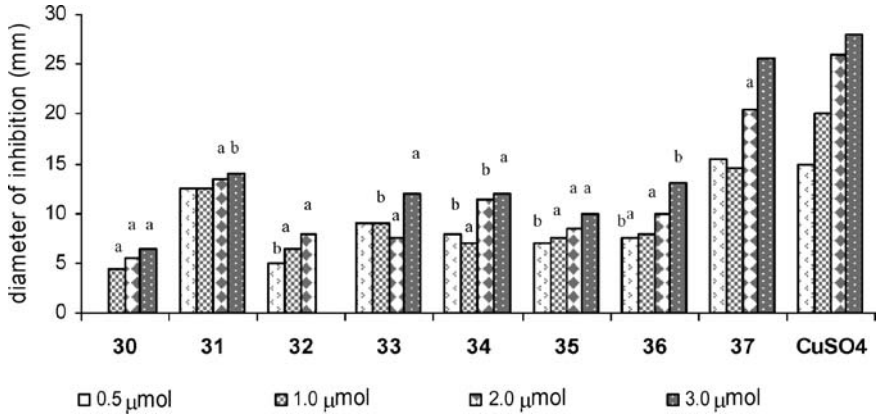


FIG. 6. Antialgal activity of flavonoids on *S. capricornutum*. Values are presented as diameter of no-growth zone (mm) for Student's *t*-test. (a) $P < 0.01$, (b) $0.01 < P < 0.05$.

$[C_6H_4OH]^+$. ^{1}H NMR of compound **39** showed two doublets of the *trans* double bond at $\delta = 7.32$ and 6.33, two methoxyl groups as a singlet at $\delta = 3.88$, and two 1,2,4-trisubstituted aromatic rings at $\delta = 7.58$ (d, $J = 2.0$ Hz), 7.48 (dd, $J = 8.5$ and 2.0 Hz), 7.11 (d, $J = 2.0$ Hz), 6.97 (dd, $J = 8.5$ and 2.0 Hz), 6.76 (d, $J = 8.5$ Hz), and 6.74 (d, $J = 8.5$ Hz). EI-MS spectrum showed the molecular ion at $m/z = 300$ and fragments at $m/z = 177$ $[CO–CH–CH–C_6H_3(OCH_3)OH]^+$, 149 $[CH–CH–C_6H_3(OCH_3)OH]^+$, 151 $[CO–C_6H_3(OCH_3)OH]^+$, and 123 $[C_6H_3(OCH_3)OH]^+$.

Compounds **40**–**49** were identified as C_{13} *nor*-isoprenoids. Compounds **40**–**42** and **44**–**45** were isolated and characterized from *C. parqui* (D'Abrosca et al., 2004b), whereas compounds **46**, **47**, and **49** were isolated from *Chenopodium album* (DellaGreca et al., 2004). Compound **43** was identified as the aglycone of some glucoside isolated from *Acanthus ebracteatus* (Kanchanapoom et al., 2001).

All compounds were tested on the green alga *S. capricornutum* to assay their antialgal effect. This organism is commonly present in aquatic systems and has been selected as bioindicator of the eutrophic sites. The test was conducted on agar using a modified antibiotic assay method. The phytotoxicity results are summarized in Figures 3–6, where only the active compounds are reported.

The results of the active low-molecular phenols **1**–**11**, which are reported in Figure 2, show a correspondence between dose and activity. The antialgal effect for compounds **1**–**3** increased with their polarity. The most active was protocatechuic acid **3**. The activity should be correlated with the hydrophilicity and/or acidity: the antialgal effect of the methyl ester of **1** (compound **4**) was

about 50% of the activity of the corresponding acid. Compound **5**, which has all its hydroxyl groups derivatized, was less phytotoxic in respect to the more polar compounds **1**–**3**. Compound **8** only showed a moderate antialgal effect for the highest doses used (2 and 3 μmol).

Figure 3 reports the results of the cinnamic acid derivatives. The most active compound was methyl ferulate **20**. For ferulic and synapic acids, esterification of the carboxyl group increased activity (**18** vs. **20** and **21** vs. **22**). *p*-Coumaric acid and its methyl derivative **13** showed a similar toxicity, whereas methyl caffeate was over 50% less active than caffeic acid (**19** vs. **17**). The most active of the dihydrocinnamic acid derivatives (Figure 4) was 3-(4-hydroxyphenyl)propionic acid (**23**); the introduction of another hydroxylated function in the molecule reduced activity (cf. **26** and **28**). Methylation also made the molecule less active with the carboxymethyl group in compound **25**, and with the carboxy and methoxy groups in compound **24**. Hydrogenation of the double bond of cinnamic acid increased correspondence between dose and activity and had a slow effect on toxicity (**12** vs. **23** and **18** vs. **26**). Hydrogenation of methyl ester **20** reduced the activity considerably, whereas methyl dihydroferulate **27** was less phytotoxic in respect to methyl ferulate **19**.

Results of bioassay with the flavonoids are reported in Figure 5. The presence of the catechol ring made the compound especially active (**31**, **33**, **34**, **36**, **37**). Glycosylation had little effect on activity, and the presence of the hydrophobic groups (methoxyls) decreased activity. The most active compound was (−)-catechin **37**, showing a no-growth zone similar to that of algaecide $CuSO_4$. C_{13} *nor*-isoprenoids were inactive at all quantities tested. Only **42**, **44**, and **45** metabolites showed slight no-growth zones of 3.0-, 4.2-, and 2.5-mm diameters, respectively, with 3.0 μmol of chemicals.

Among the constituents of *S. lacustris*, (−)-catechin **37** was the most active compound. The no-growth zone showed diameters similar to those of $CuSO_4$, with an anomalous brown coloration probably as result of a photoxidation reaction that caused blocking of photosystem activity. The production of the reactive species (i.e., singlet oxygen) and the absence of photoprotection could cause the degradation of the photosynthetic pigments. The toxicity of (−)-catechin seemed to inhibit the photorespiration phenomenon. These results were in accordance with Bais et al. (2003b), showing that allelopathic effects of the weed *Centaurea maculosa* are attributable to a racemic mixture of (±)-catechin. They demonstrated that phytotoxic activity was due to (−)-catechin, whereas (+)-catechin had an antibiotic activity (Bais et al., 2002).

The natural abundance of *S. lacustris* along our rivers could optimize its use. In fact, the chemical characterization of the algal inhibitory component of the plant could provide a reliable method for controlling algal growth in eutrophic sites. Among the isolated metabolites, low-molecular phenolic compounds are toxic to algae and show a superior inhibitory activity on algal growth.

REFERENCES

BADERSCHNEIDER, B. and WINTERHALTER, P. 2001. Isolation and characterization of novel benzoates, cinnamates, flavonoids, and lignans from Riesling wine and screening for antioxidant activity. *J. Agric. Food Chem.* 49:2788–2798.

BAIS, H. P., WARKER, T. S., STERMITZ, F. R., HUFBAUER, R. A., and VIVANCO, J. M. 2002. Enantiomeric-dependent phytotoxic and antimicrobial activity of (±)-catechin. A rhizosecreted racemic mixture from spotted knapweed. *Plant Physiol.* 128:1173–1179.

BAIS, H. P., WALKER, T. S., KENNAN, A. J., STERMITZ, F. R., and VIVANCO, J. M. 2003a. Structure-dependent phytotoxicity of catechins and other flavonoids: Flavonoid conversion by cell-free protein extracts of *Centaurea maculosa* (spotted knapweed) roots. *J. Agric. Food Chem.* 51:897–901.

BAIS, H. P., VEPACHEDU, R., GILROY, S., CALLAWAY, R. M., and VIVANCO, J. M. 2003b. Allelopathy and exotic plant invasion: From molecules and genes to species interactions. *Science* 301:1377–1380.

BOUTARD, B., BOUILLANT, M. L., CHOPIN, J., and LEBRETON, P. 1973. Flavonoid chemotaxonomy of fluviales. *Biochem. Syst.* 1:133–140.

BRAMMER, E. S. 1979. Exclusion of phytoplankton in the proximity of dominant water-soldier (*Stratiotes aloides*). *Freshw. Biol.* 9:233–249.

CANGIANO, T., DELLAGRECA, M., FIORENTINO, A., ISIDORI, M., MONACO, P., and ZARRELLI, A. 2001. Lactone diterpenes from the aquatic plant *Potamogeton natans*. *Phytochemistry* 56:469–473.

D'ABROSCA, B., DELLAGRECA, M., FIORENTINO, A., MONACO, P., PREVITERA, L., SIMONET, A. M., and ZARRELLI, A. 2001. Potential allelochemicals from *Sambucus nigra*. *Phytochemistry* 58:1073–1081.

D'ABROSCA, B., DELLAGRECA, M., FIORENTINO, A., MONACO, P., and ZARRELLI, A. 2004a. Low molecular weight phenols from the bioactive aqueous fraction of *Cestrum parqui*. *J. Agric. Food Chem.* 52:4101–4108.

D'ABROSCA, B., DELLAGRECA, M., FIORENTINO, A., MONACO, P., ORIANO, P., and TEMUSSI, F. 2004b. Structure elucidation and phytotoxicity of C13 *nor*-isoprenoids from *Cestrum parqui*. *Phytochemistry* 65:497–505.

DELLAGRECA, M., FIORENTINO, A., MONACO, P., and ZARRELLI, A. 2001. Two new polyhydroxylated sterols from *Ruppia maritima*. *Nat. Prod. Lett.* 15:111–118.

DELLAGRECA, M., FIORENTINO, A., MONACO, P., PREVITERA, L., TEMUSSI, F., and ZARRELLI, A. 2003a. New dimeric phenanthrenoids from the rhizomes of *Juncus acutus*. Structure determination and antialgal activity. *Tetrahedron* 59:2317–2324.

DELLAGRECA, M., FIORENTINO, A., and ISIDORI, M. 2003b. Bioactive compounds from Potamogetonaceae on aquatic organisms, pp. 35–56, in F. A. Macias, J. C. G. Galindo, J. M. G. Molinillo and H. G. Cuttler (eds.). Recent Advances in Allelopathy Vol II—A Science for the Future. Cadiz.

DELLAGRECA, M., DI MARINO, C., ZARRELLI, A., and D'ABROSCA, B. 2004. Isolation and phytotoxicity of apocarotenoids from *Chenopodium album*. *J. Nat. Prod.* 67:1492–1495.

FITZGERALD, G. P., 1969. Some factors in the competition or antagonism among bacteria, algae and aquatic weeds. *J. Phycol*. 351–359.

GODMAIRE, H. AND PLANAS, D. 1983. p. 227. *in* R. G. Watzel (ed.). Periphyton of Freshwater Ecosystems, W. Junk Publishers, The Hague.

GROSS, E. M., ERHARD, D., AND IVANYI, E. 2003. Allelopathic activity of *Ceratophyllum demersum* L. and *Najas marina* ssp. intermedia (Wolfgang) Casper. *Hydrobiologia* 506–509:583–589.

HASLER, A. D. and JONES, E. 1949. Demonstration of the antagonistic action of large aquatic plants on algae and rotifers. *Ecology* 30:359–365.

ISO. 1982. Water quality—algal growth inhibition test. ISO/DIS 8692. International Organization for Standardization, Geneva, Switzerland.

KANCHANAPOOM, T., KASAI, R., PICHEANSOONTHON, C., and YAMASAKI, K. 2001. Megastigmane, aliphatic alcohol and benzoxazinoid glycosides from *Acanthus ebracteatus*. *Phytochemistry* 58:811–817.

KOGAN, S. I. and CHINNOVA, G. A. 1972. On the relations between *Ceratophyllum demersus* L. and some blue algae. *Hydrobiol. Zh.* 8:21–27.

KONG, C., WENJU, L., XU, X., HU, F., WANG, P., and JIANG, Y. 2004. Release and activity of allelochemicals from allelopathic rice seedlings. *J. Agric. Food Chem.* 52:2861–2865.

LES, D. H. and SHERIDAN, D. J. 1990. Biochemical heterophylly and flavonoid evolution in North American *Potamogeton* (Potamogetonaceae). *Am. J. Bot.* 77:453–465.

LI, H.-H., INOUE, M., NISHIMURA, H., MIZUTANI, JU., and TSUZUKI, E. 1993. Interactions of *trans*-cinnamic acid, its related phenolic allochemicals, and abscissic acid in seedling growth and seed germination of lettuce. *J. Chem. Ecol.* 19:1775–1787.

NAHRSTEDT, A., PROKSCH, P., and CONN, E. E. 1987. Dhurrin, (−)-catechin, flavonol glycosides and flavones from *Chamaebatia foliolosa*. *Phytochemistry* 26:1546–1547.

NICHOLS, H. W. 1973. Culture methods and growth measurements, pp. 174–187, *in* J. R. Stein (ed.). Handbook of Phycological Methods. Cambridge University Press, Cambridge.

Organization for Economic Cooperation and Development. 1984. Algal growth inhibition test. OECD guideline 201. Paris, France.

ROBERTS, M. L. and HAYNES, R. R. 1986. Flavonoid systematics of Potamogeton subsections Perfoliati and Praelongi (Potamogetonaceae). *Nord. J. Bot.* 6:291–294.

SCHREITER, T. 1928. Untersuchungen über den Einfluss einer Helodea-wukerung auf das Netzplankton des Hirschberger Grossteikes in Böhmen in den Jahren 1921 bis 1925 incl. Sborník vyzkumnych ústavu zemedelskych rcs. V. Praze.