0971-4693/94 Euro 10.00
Allelopathy Journal 40 (2): 133-142 (2017) *International Allelopathy Foundation* 2017
Table: 1, Figs : 3

Phytotoxicity of leaf constituents from bamboo (*Shibataea chinensis* Nakai) on germination and seedling growth of lettuce and cucumber

Y. Kuang†, S.X. Yang†, D.A. Sampietro[1], X.F. Zhang, J. Tan, Q.X. Gao, H.W. Liu, Q.X. Ni* and Y.Z. Zhang*

Zhejiang Provincial Key Laboratory of Chemical Utilization of Forestry Biomass, Key Lab. for Quality Improvement of Agricultural Products of Zhejiang Province, Zhejiang A&F University, Linan, Zhejiang 311300, China

E-Mail: niqinxue@zafu.edu.cn ; yyzzhang2002@zafu.edu.cn, diegoasampietro@gmail.com

(Received in revised form : January 5, 2017)

ABSTRACT

The leaf extracts of bamboo (*Shibataea chinensis* Nakai) and its fractions (Ethyl acetate, *n*-butanol and water) obtained after extraction with petroleum ether, were assayed on seed germination and seedling growth of lettuce (*Lactuca sativa* L. var. ramosa Hort.) and cucumber (*Cucumis sativus* L.). The leaf extract showed the lowest phytotoxic activity, while, the *n*-butanol fraction was most bioactive on germination and seedling growth of both target plants. A bioassay-guided fractionation of the *n*-butanol fraction led to the isolation of five phytotoxic compounds. The chemical structures of these substances were elucidated by nuclear magnetic resonance as apigenin (I), quercetin (II), daucosterol (III), 3, 3', 4', 5-tetrahydroxy-7-methoxyflavone (IV) and *p*-methoxybenzoic acid (V). The Quercetin, apigenin and daucosterol were more phytotoxic to germination (IC_{50}=7.26-7.95 ppm), root (IC_{50}= 2.97-6.33 ppm) and shoot elongation (IC_{50}=4.89-7.74 ppm) of lettuce than glyphosate (IC_{50} values >20 ppm). In cucumber, the *p*-methoxybenzoic acid showed similar phytotoxic activity (IC_{50} = 20 ppm, 6.93 ppm, 10.45 ppm, respectively) to glyphosate (IC_{50}=20 ppm, 7.71 ppm, 10.84 ppm, respectively). Our research suggested that the leaves of *S. chinensis* have potent allelochemicals and their potential as herbicide should be further investigated.

Keywords: Allelochemicals, allelopathic potential, bamboo, cucumber, *Cucumis sativa*, *Lactuca sativa*, leaf extracts, lettuce, phytotoxicity, *Shibataea chinensis*

INTRODUCTION

Bamboo is an evergreen and perennial plant (*Gramineae* family). It is one of the most primitive and diverse taxa in the world, widely used in southeast Asia and the pacific islands as building material, food and medicine (7,16,18). There are about 1250 kinds of bamboos in the world, some of them are short as grasses and others are tall as trees. An interesting and well known phenomenon is that weeds hardly grow in the bamboo forest and bamboo rarely suffers from diseases and insect pests, perhaps due to the allelochemicals present and produced by the bamboo plants. Bamboo leaves are rich in secondary metabolites [flavonoids, phenolic triterpenoids and polysaccharides (4,17,22)], hence, have various biological activities [anti-oxidant, anti-inflammatory, anti-cardiovascular, anti-ulcer, anti-aging, anti-bacterial, anti-viral and anti-cancer activities (1-2,8,12,13-15,19-20)].

*Corresponding authors, [1]LABIFITO, National University of Tucumán, Ayacucho 471, Tucuman, Argentina. †These authors are joint first authors †Contributing equally to the work.

Dwarf bamboos are native plants of eastern Asia with large leaves and dwarf stalk (4). Compared with other bamboo species, they have the advantages of fast growth, lush foliage and easy to harvest.There are few studies about the allelopathic effects of dwarf bamboos. In our previous research, we evaluated the phytotoxic effects of leaves from 14 kinds of dwarf bamboos from China on the germination and seedling growth of *Lactuca sativa* L. var. ramosa Hort. and *Cucumis sativus* L. (5). The leaf extract from *Shibataea chinensis* Nakai showed the highest phytotoxic activity. In this work, several constituents responsible for the phytotoxic activity were isolated and their structures were elucidated.This study aimed to investigate the chemical constituents and allelopathic activity of the extracts obtained from *S. chinensis* Nakai against germination and seeding growth of four test weeds.

MATERIALS AND METHODS

Plant Material and Sample Preparation

Shibataea chinensis Nakai leaves were collected in September 2014 from the bamboo germplasm Bank in our University and identified by bamboo taxonomist. The leaves were washed, drained and subjected to microwave radiation at 640 W for 1 min to inactivate enzymes and then dried in a vacuum drying oven (60°C for 2 h). The dried bamboo leaves were milled to a powder with a particle size of 40-mesh. One hundred grams powder were extracted thrice with an aqueous solution of 95% ethanol (500 mL) under reflux in a water bath at 80°C for 2 h and then filtered. The filtrates were combined and concentrated under vacuum till dryness. The dry residue was dissolved in 500 ml distilled water and the aqueous solution was sequentially partitioned with petroleum ether, ethyl acetate and *n*-butanol. The partition with each solvent was done thrice, each one with volume of 200 ml. The original crude extract and each fraction were lyophilized with a freeze dryer (HANFILTER, Guangzhou, China) and bioassayed on *L. sativa* and *C. sativus.*

Isolation of Phytotoxic Compounds

Constituents of the most phytotoxic fraction (*n*-butanol,120.0 g) were separated in bioassay guided isolation. The *n*-butanol fraction was subjected to column chromatography in silica gel, which was eluted with a gradient of dichloromethane/MeOH. The obtained fractions were grouped on the basis of their TLC profiles to afford 7 fractions (Fr. 1–7) which were bioassayed. The phytotoxic fractions (Fr. 2 and 3) were further investigated. Fr.2 was chromatographed on a silica gel column developed with dichloromethane/MeOH (7:1, v/v). Their bioactive constituents were separated in Sephadex LH-20 column ($CHCl_3$/ MeOH, 1:1, v/v) to yield compounds **I** (8.2 mg) and **II** (7.9 mg). Constituents of Fr.3 were separated in a C-18 reversed-phase column (MeOH/H_2O= 6:4, v/v) and then on preparative TLC ($CHCl_3$/MeOH, 9:1, v/v) to afford compounds **III** (7.0 mg), **IV** (8.2 mg), and **V** (6.0 mg).

Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker DRX-500 instrument. Chemical shifts were calculated using the solvent residual signal as the internal standard. Analytical TLC was performed on an aluminium sheet covered with silica gel 60 F254. Column chromatography (CC) was carried out on silica gel (90-150 µm; Qingdao Marine Chemical Inc., Qingdao, China), MCI gel CHP 20P (75-150 µm; Mitsubishi Chemical Corp, Tokyo, Japan), Sephadex LH-20 (40-70 µm; Amersham Pharmacia Biotech AB, Uppsala, Sweden) and Lichroprep RP-18 gel (40-63 um; Merck, Marine, Darmstadt, Germany). Fractions were monitored by TLC and spots were visualized by heating silica gel plates sprayed with 10 % H_2SO_4 in ethanol.

Bioassay of Seed Germination and Seedling Growth

Lettuce (*L. sativa*) and cucumber (*C. sativus*) seeds were assayed according to a reported protocol (21). The seeds were imbibed in deionized water for 2 h, soaked in 0.3 % $KMnO_4$ for 15 min and washed with distilled water until they were colourless. The dry residues of leaf extract and their fractions were dissolved in 500 µl of acetone and then diluted with methanol to get 0.63, 1.25, 2.5 and 5 g/L concentrations . Dilutions of the phytotoxic compounds identified in the *n*-butanol fraction of the leaf extract were also prepared at 2.5, 5, 10 and 20 ppm. Fifteen ml were prepared for each dilution of leaf extract, of each fraction or of each pure compound. Petri dishes (9 cm dia) were lined with a layer of filter paper (Whatman #1). The dilutions of leaf extract, of a fraction or of a pure compound were added to the Petri dishes at 3 ml per dish. Methanol and glyphosate were used as blank and positive controls, respectively. After the evaporation of methanol, the seeds of lettuce or cucumber were sown in the Petri dish and irrigated with 3 ml deionized water. Five replicates were prepared for each treatment. The Petri dishes were placed in an illuminated growth chamber (25±1°C, 80±2 % RH and a 12/12 h L/D photoperiod). A seed was considered germinated when 1 cm long radicle protrudes. The germination (%) was calculated after 7 days. Shoot and root lengths of the seedlings were determined 10 d after sowing. The entire experiment was repeated twice. The germination rates (GR) and percent of inhibition of seed germination (%Ig) were calculated as per following equations 1 and 2, respectively. Percentage of inhibition (%I) of shoot and root length were calculated according to equation 3.

GR = (number of germinated seeds)/(total number of seeds) Eq 1

%Ig = 100 – GR Eq 2

%I = 1-T/C Eq. 3

Where, T: Shoot or root length in treatment and C: Shoot or root length in blank control. Negative values were obtained from equation 3 when the growth was stimulated instead of inhibited.

Statistical Analysis : The means ± standard deviations (SD) were calculated with five replicates.The minimum inhibitory concentration required for 100% of suppression (MIC) of germination or root or shoot growth was visually determined.

RESULTS AND DISCUSSION

Allelopathic Potential of Leaf Extract and its Fractions

The aqueous leaf extract from *S. chinensis* and its fractions were assayed *in vitro* on seed germination and seedling elongation of lettuce and cucumber. Figure 1 shows that, in the range of concentrations assayed (0.63-5‰), the leaf extract had the lowest inhibitory effect on germination (16.33-35.33% on lettuce; 6.67-21.67% on cucumber), root length (15-48% on lettuce; 5-36% on cucumber) and shoot elongation (13-44% on lettuce; 9-34% on cucumber). A moderate inhibitory activity was observed for the petroleum ether fraction on cucumber germination (25.67-48%), root length (38-65%) and shoot elongation (32-61%) and for the water fraction on lettuce germination (23-63%), root length (37-65%) and shoot elongation (35-62%). The petroleum ether fraction drastically reduced the germination and seedling growth of lettuce (43-100% of germination; 48-100% of root length; 42-100% of shoot elongation). The ethyl acetate fraction also severely inhibited the lettuce (67-100% of germination; 63-100% of root length; 60-100% of shoot elongation) and caused almost a complete suppression of cucumber growth (47-98% of germination; 52-99% of root length; 51-99% of shoot elongation). The *n*-butanol fraction proved most inhibitory and caused inhibition in concentrations of 0.63-5‰ on lettuce (83-100% of germination; 84-100% of root length; 74-100% of shoot elongation) and cucumber (73-100% of germination; 76-100% of root length; 67-100% of shoot elongation). The low inhibitory activity obtained for the aqueous leaf extract and the higher one for its organic fractions are often observed in bioassay guided isolation of allelopathic compounds. This situation could be due to the fact that secondary metabolites, including allelochemicals, usually participate in low amounts in the total dry mass of their plant producers (9). This situation seems to be a natural mechanism to prevent autotoxicity (10). Several allelochemicals exert their actions in natural conditions at concentrations lower than those required for complete growth suppression (11). Thus main source of allelochemicals was expected to be in the *n*-butanol fraction, which causes the highest inhibition in germination and seedling growth in the assayed target plants.

Identification of allelochemicals and their allelopathic potential

Five compounds were isolated from the *n*-butanol fraction by multiple chromatographic procedures and were identified according to their NMR data (Table 1). Compounds **I**, **II** and **IV** were flavonoids identified as apigenin, quercetin and 3,3',4',5-tetrahydroxy-7-methoxyflavone (Fig. 2). Compound **III** was the triterpenoid daucosterol and compound **V** was *p*-methoxybenzoic acid, a simple phenolic acid derivative. These compounds were assayed on cucumber and lettuce germination and seedling growth (Fig. 3). In theconcentrations range of 2.5-20 ppm, their effects were comparable with glyphosate (a broad-spectrum systemic herbicide). Apigenin, quercetin and daucosterol proved most inhibitory to lettuce germination (24-78%, 27-73% and 18-69%, respectively), root length (32-97%, 46-97% and 17-96%, respectively) and shoot elongation (26-95%, 35-94% and 11-90%, respectively). The remaining isolated compounds (3,3',4',5-tetrahydroxy-7-

Table 1. Structural elucidation of phytotoxic compounds isolated from the *n*-butanol fraction of the leaf extract of *S. chinensis*.

Compounds	NMR data	Identity
I (Yellow powder)	^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.98 (1H, s, 5-OH), 7.92 (2H, d, J=8.0 Hz, H-2′, 6′), 6.91 (2H, d, J=8.0 Hz, H-3′, 5′), 6.77 (1H, s, H-3), 6.47 (1H, d, J=2.0 Hz, H-8), 6.18 (1H, d, J=2.0 Hz, H-6); ^{13}C-NMR (125 MHz, DMSO- d_6) δ: 182.2 (C-4), 164.3 (C-2), 103.1 (C-3), 163.9 (C-7), 161.1 (C-5), 157.6 (C-9), 128.6 (C-2′, 6′), 121.5 (C-1′), 116.3 (C-3′, 5′),161.5 (C-4′), 103.9 (C-10), 99.0 (C-6), 94.8(C-8)	Apigenin (29)
II (Yellow powder)	^{1}H-NMR (500 MHz, DMSO- d_6) δ: 12.49 (1H, s, 5-OH), 10.75 (1H, s, 8-OH), 9.58 (1H, s, 3-OH), 9.36 (1H, s, 4′-OH), 9.30 (1H, s, 3′-OH), 7.68 (1H, d, J=2.0 Hz, H-2′), 7.53 (1H, dd, J=8.0, 2.0 Hz, H-6′), 6.88 (1H, d, J=8.0 Hz, H-5′), 6.40 (1H, d, J=2.0 Hz, H-8), 6.19 (1H, d, J=2.0 Hz, H-6); ^{13}C-NMR (125 MHz, DMSO- d_6) δ: 176.0 (C-4), 164.2 (C-7), 160.8 (C-9), 156.1 (C-5), 147.1 (C-4′), 146.7 (C-2), 145.3 (C-3′), 135.7 (C-3), 129.2 (C-1′), 119.9 (C-6′), 115.6 (C-5′), 115.2 (C-2′), 103.0 (C-10), 98.1 (C-6), 93.4 (C-8).	Quercetin (30)
III (White powder)	^{1}H-NMR (500 MHz, DMSO- d_6) δ: 5.36(1H, m, H-6), 5.06 (1H, d, J=7.5 Hz, H-1'), 4.57 (1H, m, H-3), 0.98 (3H, d, J=6.5 Hz, H-21), 0.89 (3H, t, J=7.0 Hz, H-29), 0.87 (3H, d, J=5.5 Hz, H-26), 0.85 (3H, d, J=7.0 Hz, H-27), 0.67(3H, s, H-18). ^{13}C-NMR (125 MHz, DMSO- d_6) δ: 139.4(C-5), 120.6(C-6), 101.2(C-1'), 77.3 (C-3'), 77.2(C-3), 76.8(C-5'), 73.8(C-2'), 70.4(C-4'), 61.5(C-6'), 55.5 (C-17), 54.9(C-14), 48.8 (C-9), 44.7(C-24), 41.1(C-13), 38.6(C-12), 37.8(C-4), δ36.1 (C-1), 35.3(C-10), 35.1(C-20), 32.9 (C-23), 30.9(C-7), 30.8(C-8), 28.8(C-22), 28.2 (C-2), 25.1(C-16), 25.1(C-25), 23.2(C-15), 22.1(C-28), 19.8(C-11), 18.7(C-19), 18.1 (C-27), 17.9(C-26), 17.8(C-21), 10.9(C-18), 10.7(C-29).	Daucosterol (31)
IV (Yellow powder)	^{1}H-NMR (500 MHz, DMSO-d_6) δ: 12.58(1H, s, 5-OH), 9.74 (1H, s, 4'-OH) , 9.48 (1H, s, 3-OH), 9.42 (1H, s,3'-OH), 7.86 (1H, d, J=2.0 Hz, H-2'), 7. 68 (1H, dd, J=8.5, 2.0 Hz, H-6'), 6.96 (1H, d, J= 8.5 Hz, H-5'), 6.82 (1H, d, J= 2.0 Hz, H-8), 6. 48 (1H, d, J=2.0 Hz, H-6), 3.98 (3H, s, 7-OCH3); ^{13}C-NMR (125, DMSO- d_6) δ:176.2 (C-4), 165.0 (C-7), 161.1 (C-9), 160.2 (C-5), 148.7 (C-4'),147.8 (C-2), 145.8 (C-3'), 136.9 (C-3), 122.6 (C-1'), 120.6 (C-6'), 116.9 (C-2'), 115.8 (C-5'), 105.3 (C-10), 98.2 (C-6), 92.9 (C-8), 57.2 (7-OCH_3).	THMF[1] (32)
V (White powder)	^{1}H-NMR (500 MHz, DMSO- d_6) δ: 7.78 (2H, d, J=8.8 Hz, H-2, 6), 6.73 (2H, d, J=8.8 Hz, H-3,5), 3.76 (3H, s, -OMe). ^{13}C-NMR (125 MHz, DMSO-d_6) δ: 168.9 (-COOH), 163.6 (C-4), 132.9 (C-2, 6), 123.3 (C-1), 115.3 (C-3, 5), 55.4 (-OMe).	*p*-MBA[2] (33)

([1]THMF: 3, 3',4',5-tetrahydroxy-7methoxyflavone, [2]*p*-MBA: *p*-methoxybenzoic acid)

methoxy-flavone and *p*-methoxybenzoic acid) were inhibitory to seed germination (16-35%) and root elongation (11-29%). The response was similar to glyphosate (17-32% of germination and 11-27% of root elongation). Contrarily the 3,3',4',5-tetrahydroxy-7- methoxyflavone stimulated the shoot growth at 2.5 ppm (9%) and 5 ppm (13%) and *p*-methoxybenzoic acid at 10 ppm (21%). As observed, apigenin, quercetin and daucosterol were more inhibitory to lettuce seed germination and seedling growth than glyphosate.

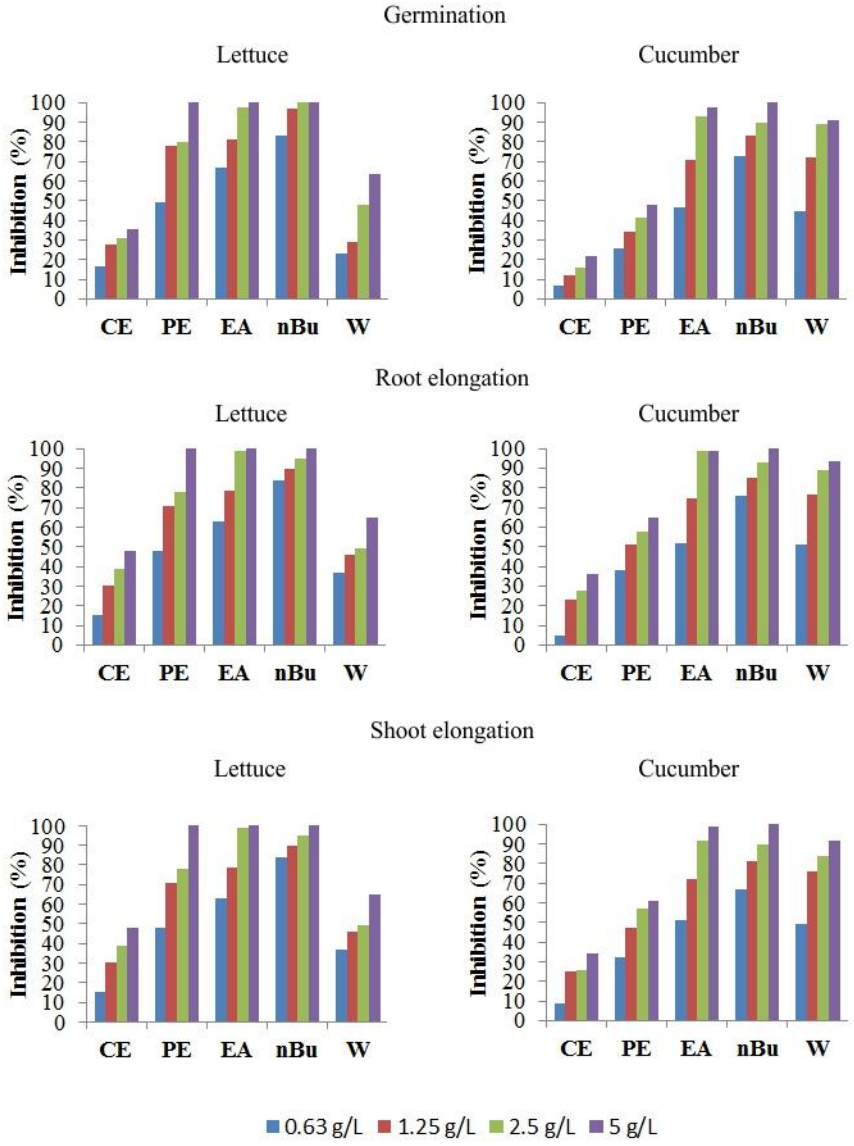

Figure 1. Allelopathic potential of the leaf extract of *S. chinensis* and its fractions on germination and seedling growth of lettuce (*Lactuca sativa* L.) and cucumber (*Cucumis sativus* L.). LE: leaf extract, PE: petroleum ether fraction, EA: ethyl acetate fraction, nBu: *n*-butanol fraction, W: water fraction.

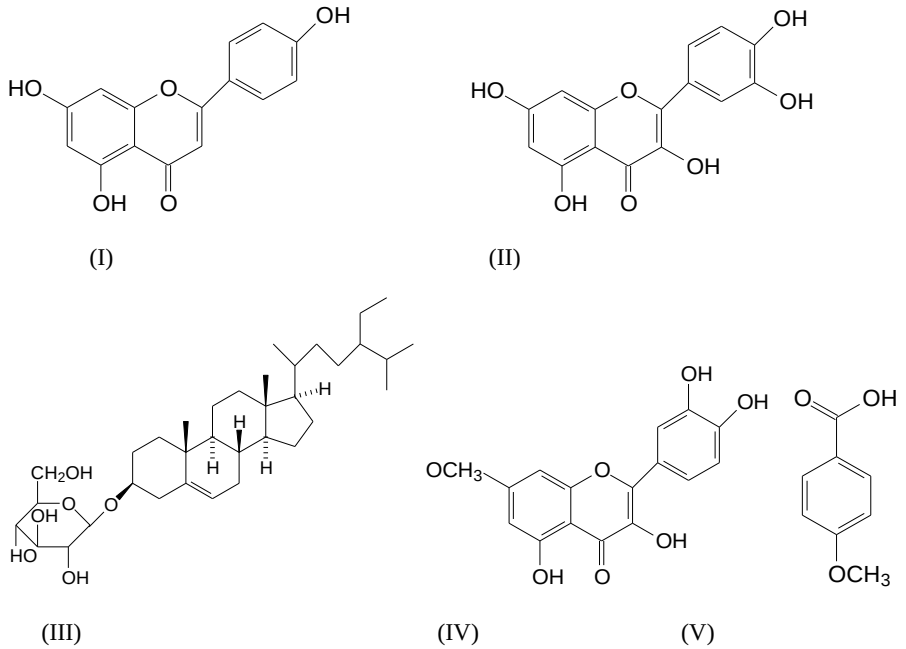

Figure 2. Structures of the identified compounds apigenin (I), quercetin (II), daucosterol (III), 3, 3',4',5-tetrahydroxy-7 methoxyflavone (IV) and *p*-methoxybenzoic acid (V).

Cucumber was less sensitive to apigenin, quercetin and daucosterol than lettuce. These compounds reduced the cucumber seed germination (4-36%, 7-29% and 4-42%, respectively), root elongation (4-67%, 6-47% and 8-56%, respectively) and shoot elongation (3-52%, 4-46%, 5-53%, respectively). The 3,3',4',5-tetrahydroxy-7-methoxyflavone did not affect the cucumber, while the *p*-methoxybenzoic acid and glyphosate had similar inhibitory effects on cucumber seed germination (13-53% and 23-51%, respectively), root elongation (29-78% and 36-72%, respectively) and shoot length (26-67% and 32-65%, respectively). Except the 3,3',4',5-tetrahydroxy-7- methoxyflavone, the inhibitory range of concentrations agree with those of flavonoids (1-10 ppm, i. e. quercetin and apgenin), triterpenoids (0.1-10 ppm, i. e. daucosterol) and simple phenolic acids (10-1000 ppm, i. e. *p*-methoxybenzoic acid). These bioactive concentrations are into the dose rates of 250-4 ppm, used for the second generation of commercial herbicides (glyphosate, triazines, and substituted phenyl ureas). However, these are berrer than third generation low dose herbicides such as the sulfonyl ureas, which are applied at dose of 0.002-0.075 ppm (3).

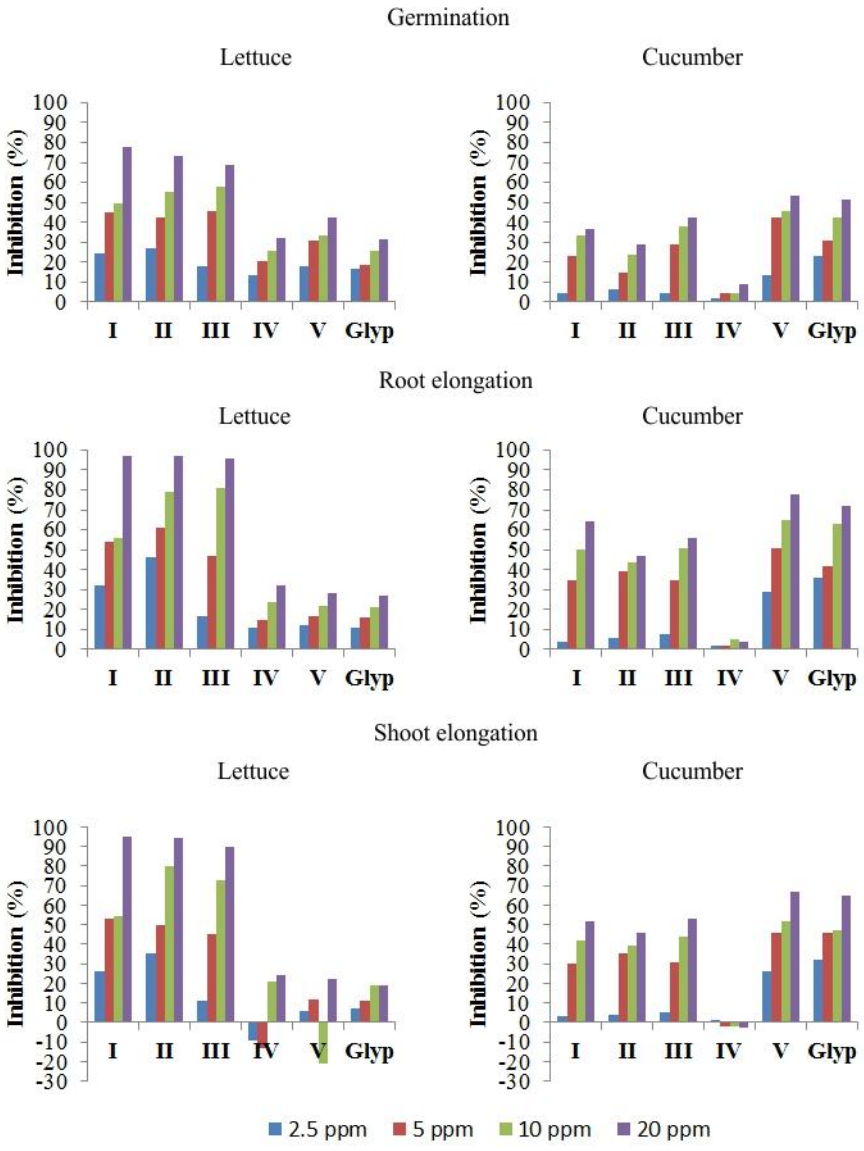

Figure 3. Allelopathic potential of the identified compounds isolated from the *n*-butanol fraction of leaf extract of *S. chinensis* on germination, and seedling growth of lettuce (*Lactuca sativa*) and cucumber (*Cucumis sativus*). **I**: apigenin ,**II**: quercetin, **III**: daucosterol, **IV**: 3, 3',4',5-tetrahydroxy-7 methoxyflavone, **V**: *p*-methoxybenzoic acid.

This preliminary research suggests that the leaves of *S. chinensis* contains several potential allelochemicals. The selective phytotoxicity observed for some fractions and the

identified compounds on the target plant species suggest that some allelochemicals of *S. sinensis* are active on specific plant species and reinforce the need to check the effect of these fractions and compounds on receptor plants usually growing with the donor plant investigated. The ecological involvement of the identified molecules on *S. chinensis* allelopathy (i.e. mechanisms of allelochemical release and presence of the allelochemicals in the environment) and the potential use of the leaf fractions or its constituents as herbicides should be further investigated.

ACKNOWLEDGEMENTS

This work was supported by the National Natural Science Foundation of China (Grant No. 31401497), Program for key Science and Technology Team of Zhejiang Province (2013TD17), the Zhejiang Provincial Natural Science Foundation of China (LQ15C020001, LY13C180001) and the Research Foundation of State General Administration of The People's Republic of China for Quality Supervision and Inspection and Quarantine (No. 2015IK180).

REFERENCES

1. Kim, M.J., Byun, M.W. and Jang, M.S. (1996). Physiological and antibacterial activity of bamboo (*Sasa coreana* Nakai) leaves.*Journal of the Korean Society of Food and Nutrition* **25**: 135-142.
2. Kim, E.Y., Jung, E.Y., Lim, H.S. and Heo, Y.R. (2007). The effects of the *Sasa Borealis* leaves extract on plasma adiponectin, resistin, C-reactive protein, and homocysteine levels in high fat diet-induced obese C57/BL6J mice. *Korean Journal of Nutrition* **40**: 303-311.
3. Macías, F. (1995). Allelopathy in the search for natural herbicide models. In: *Allelopathy: Organisms, Processes and Applications.* (Eds., Inderjit, K.M. Dakshini and F. Einhellig). *ACS Symposium Series*, vol. **585:** 310-329. American Chemical Society, Washington, DC.
4. Mao, J., Yin, J., Ge, Q., Jiang, Z. and Gong, J. (2013). *In vitro* antioxidant activities of polysaccharides extracted from Moso Bamboo-leaf. *International Journal of Biological Macromolecules* **55**: 1-5.
5. Ni, Q., Liu, Y., Gong, L., Lin, X., Fang, W. and Zhang, Y. (2011). Active components in six kinds of ground bamboo leaves and their anti-oxidant activities. *Chinese Traditional and Herbal Drugs* **42**: 2317-2321.
6. Otani, K., Yanaura, S., Yuda, Y., Kawaoto, H. and Kajita, T. (1990). Histochemical studies on the antiulcer effects of bamboo grass in rats. *International Journal of Tissue Reactions***12**: 319-32.
7. Peng, P. and She, D. (2014). Isolation, structural characterization and potential applications of hemicelluloses from bamboo: A review. *Carbohydrate Polymers***112**: 701-720.
8. Sakai, S., Saito, G., Sugayama, J., Kamasuka, T., Takada, S. and Takano, T. (1963). On the anticancer action of bamboo extract. *Journal of Antibiotics* **16**: 387-391.
9. Sampietro, D.A., Soberón, J.R., Sgariglia, M.A., Quiroga, E.N. and Vattuone, M.A. (2007). Allelopathic plants. 17. Sugarcane (*Saccharum officinarum* L.). *Allelopathy Journal* **20**: 243-250.
10. Sampietro, D.A., Sgariglia, M.A., Soberón, J.R., Quiroga, E.N. and Vattuone, M.A. (2007). Effects of sugarcane straw allelochemicals on growth and physiology of crops and weeds. *Allelopathy Journal* **19**: 351-360.
11. Sampietro, D.A. and Vattuone, M.A. (2006). Plant growth inhibitors isolated from sugarcane (*Saccharum officinarum*) straw. *Journal of Plant Physiology* **163**: 837-846.
12. Shibata, M., Yamatake, Y., Sakamoto, M., Kanarmori, M. and Takagi, K. (1975). Phamacological studies on bamboo grass (1). Acute toxicity and anti-inflammatory and antiulcerogenic activities of water-soluble fraction (Folin) extracted form *Sasa albomarginata* Makino et Shibata. *Yakurigaku Zasshi* **75**: 481-490.

13. Shibata, M., Kubo, K. and Onoda, M. (1976). Pharmacological studies on bamboo grass (2). Central depressant and antitoxic actions of a water-soluble fraction extracted from *Sasa albomarginata* Makino et Shibatai. *Yakurigaku Zasshi* **72**: 531-541.
14. Shibata, M., Kubo, K. and Onoda, M. (1978). Pharmacological studies on bamboo grass. III. Effects on cardiovascular and isolated organs of water-soluble fraction extracted from *Sasa albomarginata* Makino et Shibata (Bambusaceae) (author's transl). *Yakugaku Zasshi* **98**: 1436-1440.
15. Shibata, M., Fujii, M. and Yamaguchi, R. (1979). Pharmacological studies on bamboo grass: Toxicological and pharmacological effects of the extract obtained from *Sasa albomarginata* Makino et Shibata. *Yakugaku Zasshi* **99**: 663-668.
16. Teng, J., Xiang, T., Huang, Z.,Wu, J., Jiang, P., Meng, C., Li, Y. and Fuhrmannc, J.J. (2016). Spatial distribution and variability of carbon storage in different sympodial bamboo species in China. *Journal of Environmental Management* **168**: 46-52.
17. Xie, J., Lin, Y., Shi, X., Zhu, X., Su, W. and Wang, P (2013). Mechanochemical-assisted extraction of flavonoids from bamboo (*Phyllostachys edulis*) leaves. *Industrial Crops and Products* **43**: 276-282.
18. Wang, P., Maliang, H., Wang, C. and Ma, J. (2015). Bamboo charcoal by-products as sources of new insecticide and acaricide.*Industrial Crops and Products* **77**: 575-581.
19.Wu, D., Chen, J., Lu, B., Xiong, L., He, Y. and Zhang, Y. (2012). Application of near infrared spectroscopy for the rapid determination of antioxidant activity of bamboo leaf extract. *Food Chemistry* **135**: 2147-2156.
20. Zhang, Y. and Tang, L. (1997). Experimental studies on anti-aging effects of the leaf-extract of *Ph.nigra* Var.henonis. *Journal of Bamboo Research* **14**: 62-66.
21. Zhang, Q., Wang, S.Q., Tang, H.Y., Li, X.J., Zhang, L., Xiao, J., Gao, Y., Zhang, A.L. and Gao, J.M. (2013). Potential allelopathic indole diketopiperazines produced by the plant endophytic *Aspergillus fumigatus* using the one strain−many compounds method. *Journal of Agricultural and Food Chemistry* **61**: 11447-11452.
22. Zulkafli, Z.D., Wang, H., Miyashita, F., Utsumi, N. and Tamura, K. (2014). Cosolvent-modified supercritical carbon dioxide extraction of phenolic compounds from bamboo leaves (*Sasa palmata*). *Journal of Supercritical Fluids* **94**: 123-129.