Supporting Information

Rec. Nat. Prod. **11:1 (2017) 63-68**

Insecticidal and repellent activities of laurinterol from the Okinawan red alga *Laurencia nidifica*

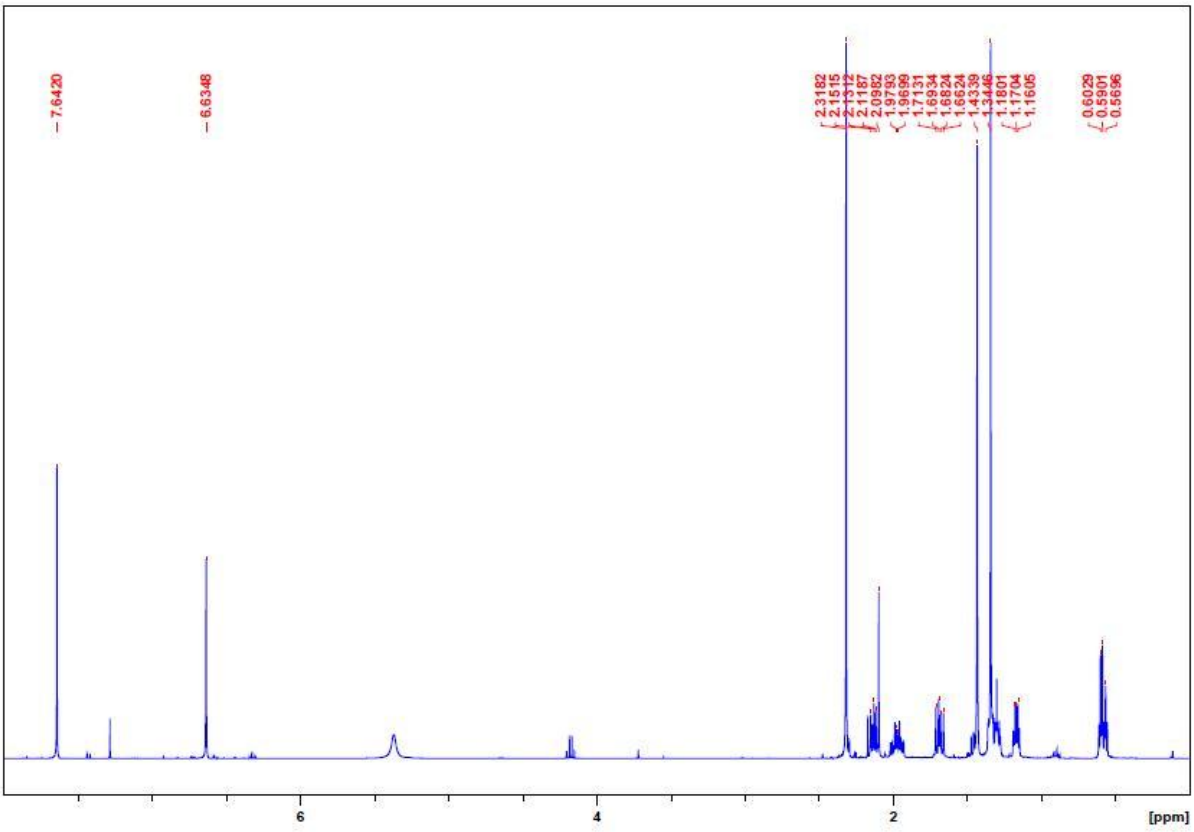
Takahiro Ishii[1*], Takumi Nagamine[1],

Binh Cao Quan Nguyen[2] and Shinkichi Tawata[1]

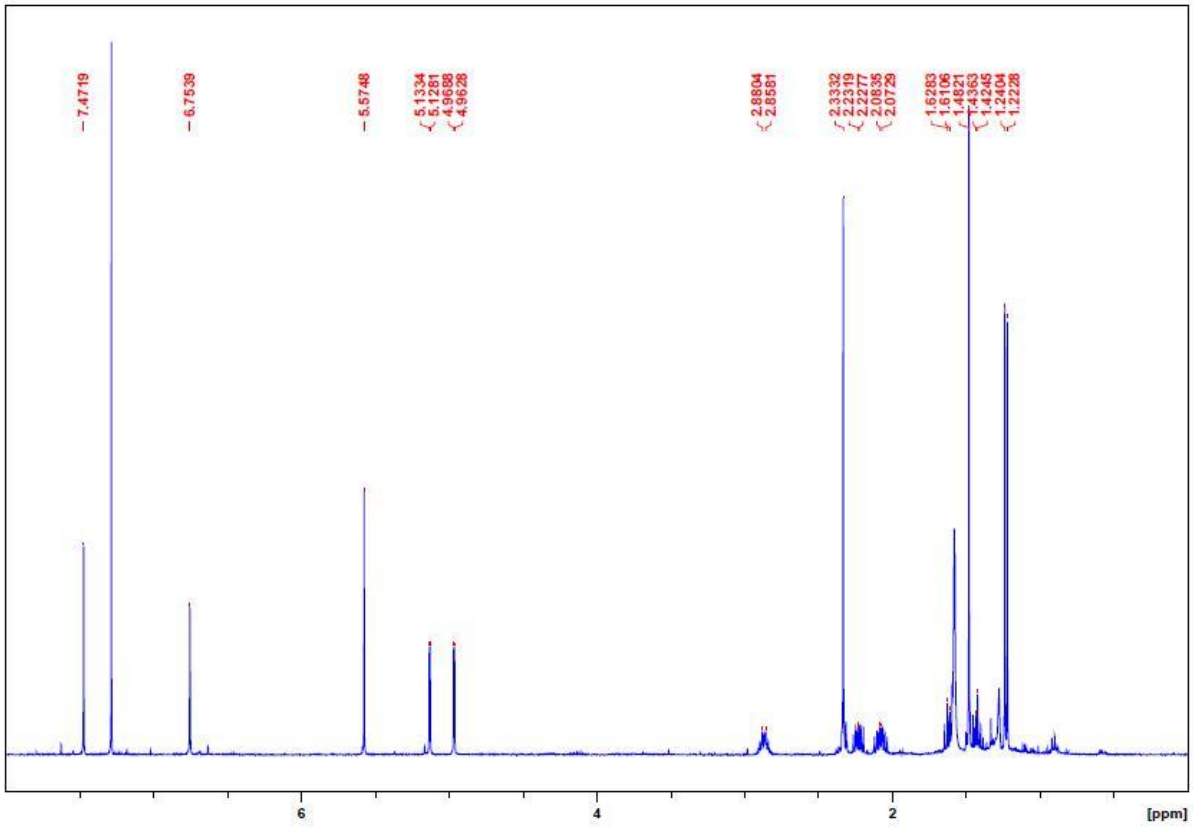
[1]*Department of Bioscience and Biotechnology, Faculty of Agriculture, University of the Ryukyus, Senbaru 1, Nishihara, Okinawa 903-0213, Japan*

[2]*Department of Bioscience and Biotechnology, The United Graduate School of Agricultural Sciences, Kagoshima University, Korimoto 1-21-24, Kagoshima 890-8580, Japan*

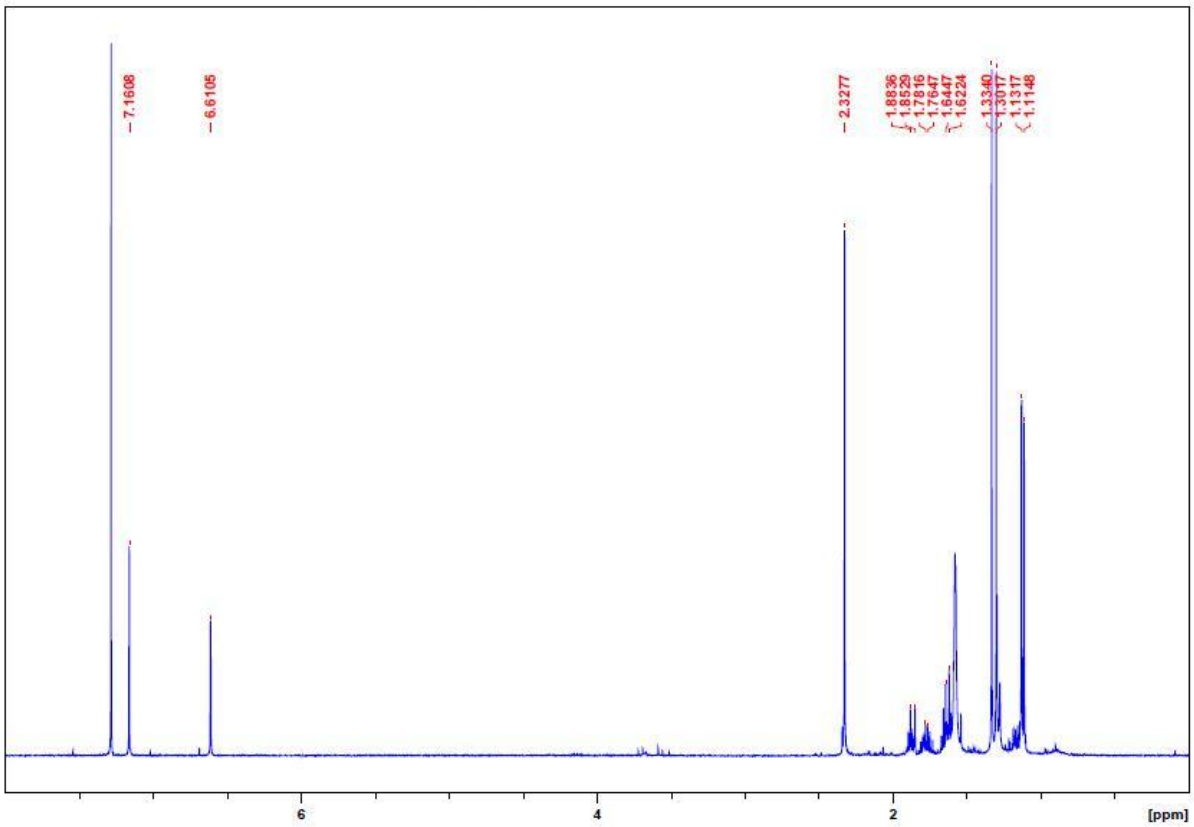
Table of Contents **Page**



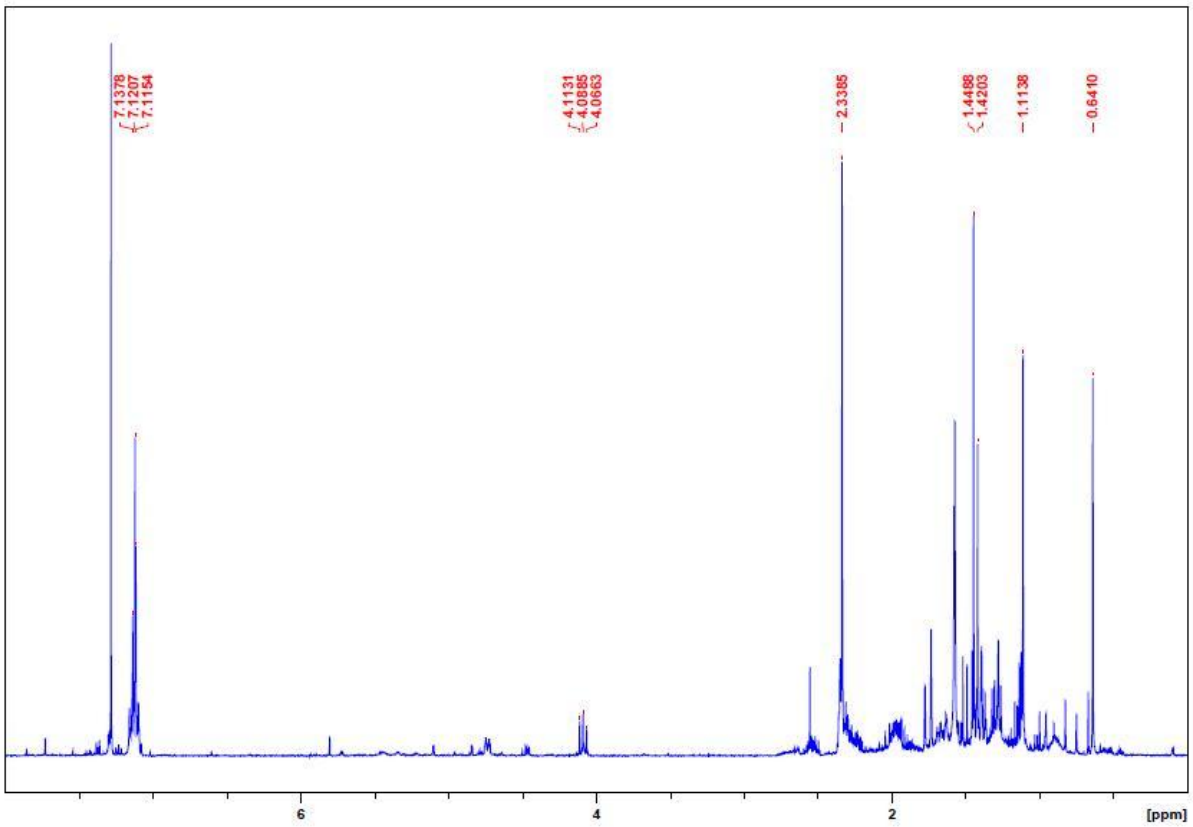
S1: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of laurinterol (**1**)



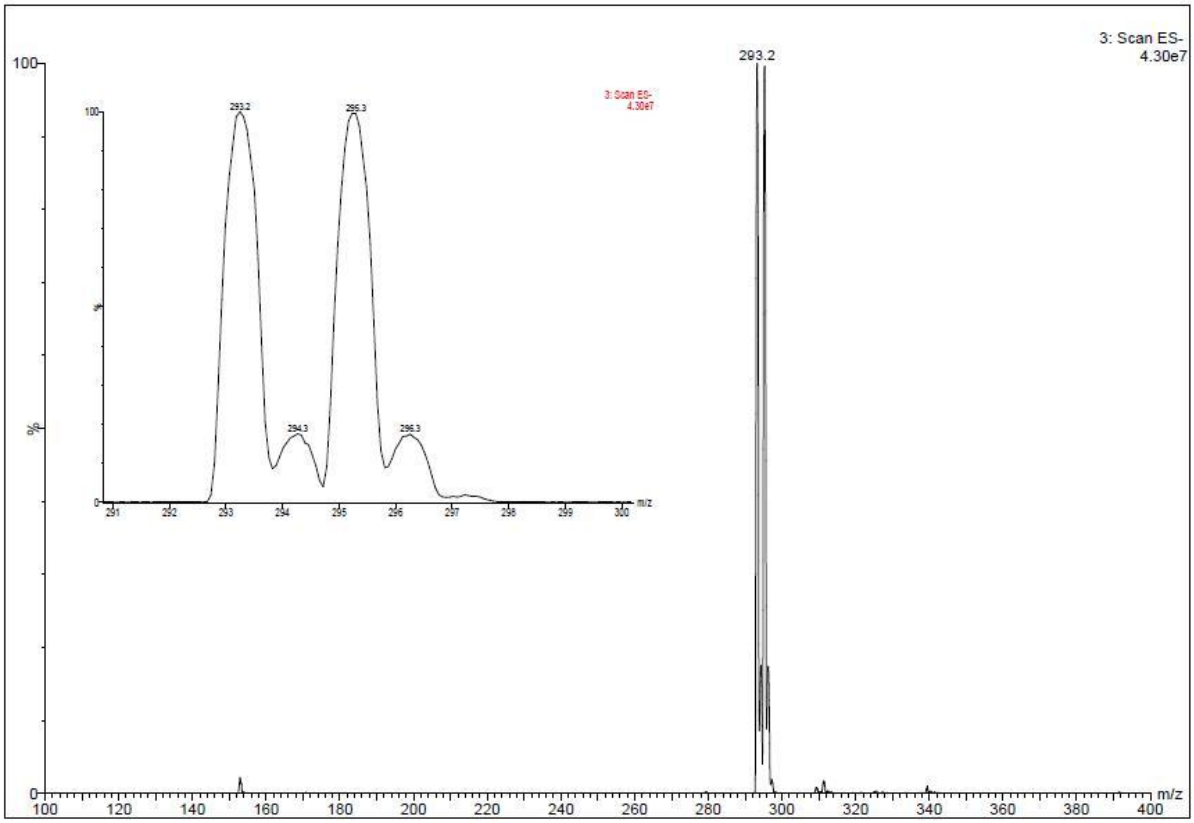
S2: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of isolaurinterol (**2**)



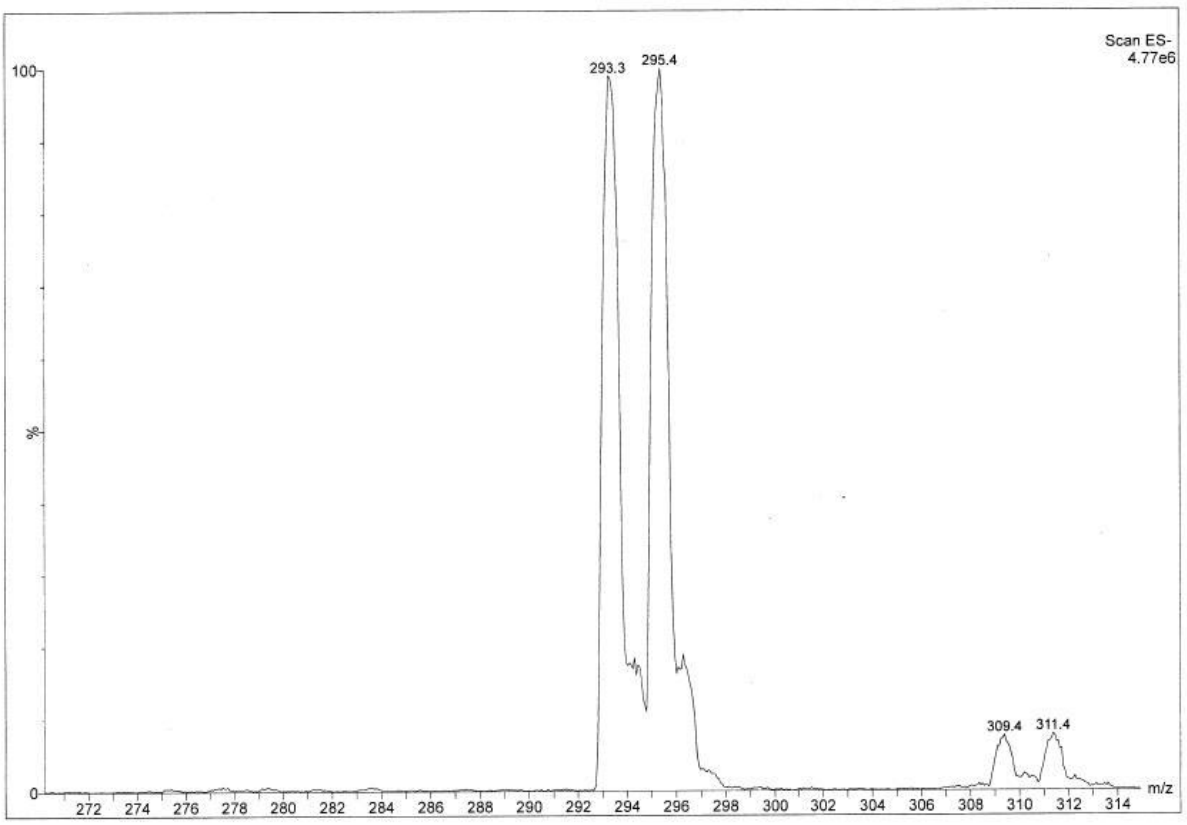
S3: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of aplysin (**3**)



S4: ^{1}H-NMR (400 MHz, $CDCl_3$) spectrum of α-bromocuparene (**4**)



S5: ESI-MS (negative) spectrum of laurinterol (**1**)



S6: ESI-MS (negative) spectrum of isolaurinterol (**2**)

S7: ANOVA experiments

Table S7-1. Anova analysis for brine shrimp toxic activity of **1-4**.

Source of Variation	SS	df	MS	F	P-value
Treat	80814.449	4	20203.61	912.8307	<.0001***
Rep	21.63156032	2	10.81578	0.488674	0.63062
Error	177.0633994	8	22.13292		
Total	81013.14396	14			

Table S7-2. Anova analysis for repellent activity of laurinterol against the maize weevil *S. zeamais*

Source of Variation	SS	df	MS	F	P-value
Treat	474148.3079	5	94829.66	4.613998	0.01921*
Rep	28.85367778	2	14.42684	0.000702	0.999298
Error	205526.008	10	20552.6		
Total	679703.1696	17			

Table S7-3. Anova analysis for LD_{50} values of laurinterol against termites by topical application

Source of Variation	SS	df	MS	F	P-value
Treat	10.443115	3	3.481038	40.96	0.000217***
Rep	0.180453	2	0.090227	1.061612	0.402966
Error	0.5099406	6	0.08499		
Total	11.133509	11			

Table S7-4. Anova analysis for inhibition of AChE activity of laurinterol.

Source of Variation	SS	df	MS	F	P-value
Treat	189319.14	3	63106.38	424.73	2.25E-07***
Rep	267.92459	2	133.9623	0.901609	0.454603
Error	891.48804	6	148.5813		
Total	190478.55	11			

S9. Materials and Methods

9.1. General

^{1}H NMR (400 MHz) spectra were recorded with a Bruker AVANCE III Nanobay spectrometer. ESIMS spectra were obtained with a Waters Quattro micro API spectrometer. Preparative TLC was performed with silica gel plates (Merck, Kieselgel 60 F_{254}). Silica gel (Merck, Kieselgel 60, 70–230 mesh) was used for column chromatography. Analytical TLC was performed on Merck Kieselgel 60 F_{254}. Spots were visualized by UV light and/or spraying with a 5% phosphomolybdic acid-ethanol solution followed by heating at 100°C.

9.2. Algal Material

The red alga *Laurencia nidifica* was collected off the coasts of Tsuken Island, Okinawa, on May 29, 2014. Voucher specimen was deposited in the Faculty of Agriculture, University of the Ryukyus. Algal material was kept in MeOH until workup.

9.3. Extraction and Isolation

The dried alga (167.8 g) was cut into small pieces, homogenized in MeOH and then soaked in MeOH (1 L) at room temperature for 7 days. After filtration, the crude extract was evaporated under reduced pressure and then partitioned between EtOAc and H_2O. The EtOAc solution was washed with H_2O, dried (anhydrous Na_2SO_4) and evaporated to leave a dark green oil (9.36 g). A portion (2 g) of the extract was fractionated by Si-gel column chromatography with a step gradient (*n*-hexane and EtOAc). A portion (330 mg) of the fraction (1.03 g) eluted with *n*-hexane/EtOAc (8:2) was further separated by preparative TLC with *n*-hexane/toluene (1:1) containing 0.2% acetic acid to yield compounds **1** (100 mg, 0.87%), **2** (8.5 mg, 0.074%), **3** (4.8 mg, 0.042%), and **4** (9.7 mg, 0.085%). Yield was calculated based on the weight of the dried alga.

9.4. Chemicals

Acetylthiocholine iodide, 5,5'-dithiobis-(2-nitrobenzoic acid), and rotenone were obtained from Sigma-Aldrich (Tokyo, Japan). Fenitrothion was obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Standards of pyrethrins, spinosad, and nereistoxin oxalate were purchased from Wako Pure Chemical Industries (Osaka, Japan).

9.5. Brine shrimp toxicity assay

The toxic activities against brine shrimp larvae (*Artemia salina*) were evaluated by a modified method of Solis *et al.* using 96-well micro-plates [1]. Dried eggs of brine shrimp (Tetra Brine Shrimp Eggs, Tetra Japan Co., Ltd.) (200 mg) were hatched in 200 ml artificial seawater supplemented with 16 mg dried yeast with suitable aeration at 28°C before testing. After 48 h incubation, active nauplii were transferred with a Pasteur pipette into a glass Petri dish containing fresh artificial supplemented seawater. Samples were dissolved in DMSO and then diluted in artificial seawater (the final concentration of DMSO is 1% v/v). 100 µl of sample solution was added into each well containing 5

larvae in artificial seawater (100 µl) and then incubated at 20°C for 24 h. The number of dead larvae in each well was counted using magnifying lens (5x or 10x). Methanol (50 µl) was then added to each well and after 1 h the total number of larvae in each well was counted. DMSO was used for negative control and rotenone was used as positive control. All samples and controls were repeated in 8 wells and tested in triplicate.

9.6. Repellency test against maize weevil

The repellent activities against the maize weevils *Sitophilus zeamais* were evaluated using the filter paper impregnation method [2,3] with slight modifications. The insects were purchased from Sumika Technoservice Corporation (Hyogo, Japan) and reared on whole brown rice in the laboratory at 25°C. Filter papers (Advantec Toyo No.1, 35 mm diameter) were folded in wave shape. 100 µl of appropriate concentration of each sample was applied to folded filter papers. EtOAc or MeOH or EtOH was used for the control in all experiments. The treated and untreated papers were air dried at room temperature for 10 minutes to evaporate the solvent completely. After drying, the treated and control papers were placed individually in a glass Petri dish (45 mm diameter) and then several grains of brown rice, which are attractants, were put under each filter paper. Both prepared Petri dishes were set in a plastic container (H6 x W14 x D9 cm). 10 unsexed adult beetles, which were feed-deprived for 48 h prior to the experiments, were released at the center of the plastic container. The plastic container was covered with a lid and kept in darkness at 25°C. Three replicates were carried out for each treatment and the number of *S. zeamais* settled in each glass Petri dish were counted and recorded at 24 and 48 h after treatment. Five kinds of insecticides were used as positive controls.

9.7. Topical assay against termite

An acute toxicity bioassay was performed by topical application to adult worker termites of *Reticulitermes speratus* Kolbe, as previously described [4]. Termites were collected from field colonies in University of the Ryukyus. Tested samples were prepared and diluted in acetone to obtain desired doses for topical application. Aliquots (0.5 µL) of diluted samples were applied topically to the back of worker termites. Controls were treated with 0.5 µL acetone only. Termites were transferred into Petri dishes (45 mm diameter) containing a filter paper (42 mm diameter) and kept in an incubator at 23°C. A few drops of distilled water were supplied daily to the bottom edge of each filter paper to maintain moisture during the experiment. Three replicates with 20 termites each were used per treatment. Rotenone, fenitrothion, and pyrethrins standards were used as positive controls. Mortality of insects was evaluated after 48 h treatment.

9.8. Acetylcholinesterase (AChE) inhibition assay

Whole bodies of termites (*R. speratus*) (1 g) were homogenized in 50 mL of 0.1 M phosphate buffer (pH 8.0), and the homogenate was centrifuged at 12,000 rpm for 20 min. The supernatant was used as the enzyme source. All procedural steps for preparing the crude enzyme were performed at 4°C [5]. The AChE activity was measured using the modified Ellman's method [6]. 25 µL of sample was transferred into each well of a 96-well micro-plate containing 120 µL of 0.1 M phosphate buffer (pH 8.0), and 30 µL of the enzyme solution was added to each well. The mixture was pre-incubated

for 10 min at 25°C and then 50 µL of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) and 25 µL of acetylthiocholine iodide (ATCI) were added. Finally, the micro-plate was further incubated for 20 min at 25°C and then the absorbance was measured at 412 nm. The AChE inhibition assay was performed using three replicates of each treatment and was repeated three times.

9.9. Statistical analysis

Statistical analyses were performed using statistical analysis system (SAS) software, version 9.1.3 (SAS Institute Inc., Cary, NC, USA). Significance was assessed by one-way ANOVA analysis, and means were separated using Duncan's test at $p < 0.01$.

References

[1] P. N. Solis, C. W. Wright, M. M. Anderson, M. P. Gupta and J. D. Phillipson (1993). A microwell cytotoxicity assay using *Artemia salina* (brine shrimp), *Planta Med.* **59**, 250-252.

[2] L. Wei, R. Hua, M. Li, Y. Huang, S. Li, Y. He and Z. Shen (2014). Chemical composition and biological activity of star anise *Illicium verum* extracts against maize weevil, *Sitophilus zeamais* adults, *J. Insect Sci.* **14**, 80.

[3] T. Ishii, H. Matsuzawa and C. S. Vairappan (2010). Repellent activity of common spices against the rice weevil, *Sitophilus zeamais* Motsch (Coleoptera, Curculionidae), *J. Trop. Biol. Conserv.* **7**, 75-80.

[4] B. C. Q. Nguyen, J. Chompoo and S. Tawata (2015). Insecticidal and nematicidal activities of novel mimosine derivatives, *Molecules* **20**, 16741-16756.

[5] P. Siramon, Y. Ohtani and H. Ichiura (2009). Biological performance of *Eucalyptus camaldulensis* leaf oils from Thailand against the subterranean termite *Coptotermes formosanus* Shiraki, *J. Wood Sci.* **55**, 41-46.

[6] H. J. Yeom, J. S. Kang, G. H. Kim and I. K. Park (2012). Insecticidal and acetylcholine esterase inhibition activity of Apiaceae plant essential oils and their constituents against adults of German cockroach (*Blattella germanica*), *J. Agric. Food Chem.* **60**, 7194-7203.