SHORT REPORT

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Insecticidal and Repellent Activities of Laurinterol from the Okinawan Red Alga *Laurencia nidifica*

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Abstract: An ethyl acetate (EtOAc) extract of *Laurencia nidifica* was found to have toxic effect against brine shrimp larvae (*Artemia salina*). Bioassay-guided fractionation of the EtOAc extract resulted in the isolation of four known halogenated sesquiterpenes, laurinterol (**1**), isolaurinterol (**2**), aplysin (**3**), and α-bromocuparene (**4**). Their structures were established on the basis of spectral analysis and comparison with literature data. Among isolated compounds, only laurinterol showed strong toxicity against *A. salina*. Further experiments revealed that laurinterol also exhibited repellent activity against the maize weevil *Sitophilus zeamais*, insecticidal activity against the termite *Reticulitermes speratus*, and acetylcholinesterase (AChE) inhibitory effect. This is the first report of insecticidal and repellent activities of laurinterol.

Keywords: Laurinterol; *Laurencia nidifica*; halogenated secondary metabolites; brine shrimp; insecticidal activity; termiticidal activity; *Sitophilus zeamais*.

1. Plant Source

Marine algae are potentially bountiful sources of biologically active secondary metabolites that might be useful as leads in the development of new pharmaceuticals, nutraceuticals, and cosmeceuticals [1-4]. Among the red algae, the genus *Laurencia* is distributed throughout the world's oceans except in the Arctic and Antarctic waters and contains about 140 species, of which more than 20 species occur in Okinawan Sea [5-7]. This genus is considered to be one of the richest producers of halogenated secondary metabolites with diverse and unique structural features depending on the species and localities [7-9]. To date, more than 600 halogenated secondary metabolites, mainly including sesquiterpenes, diterpenes, triterpenes, C_{15}-acetogenins, and indoles, have been isolated from this genus [7,10]. These metabolites produced by *Laurencia* exhibit a variety of biological activities including antibacterial, antifungal, antifeedant, antiviral, anticancer, and antifouling activities [7,10,11], and these findings prompted us to investigate this genus to explore more interesting bioactive compounds having significant biological activities.

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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.

2. Previous Studies

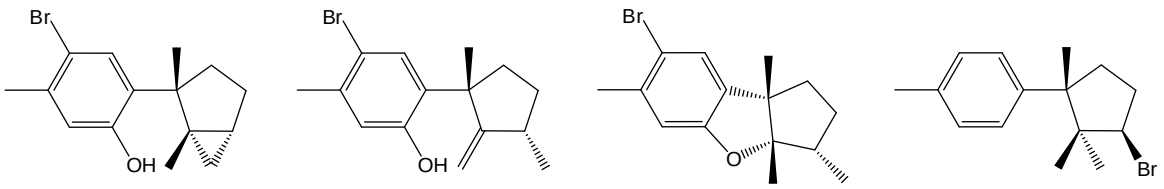
Despite a lot of bioactive halogenated natural products reported from *Laurencia* species, there are only several reports on compounds with insecticidal activity. For examples, insecticidal cyclic ethers, laurepinnacin and isolaurepinnacin were isolated from *L. pinnata* Yamada [12,13]. Deoxyprepacifenol, (*Z*)-laureatin, and (*Z*)-isolaureatin from *L. nipponica* Yamada possessed insecticidal activity against mosquito larvae (*Culex pipiens pallens*) [12,14]. A C_{15}-acetogenin, cyclic enyne (12*E*)-cis-maneonene-E from *L. papillosa* was active against the confused flour beetle larvae (*Tribolium confusum*) and mosquito larvae (*C. pipiens*) [15], while two sesquiterpenes, elatol and obtusol from *L. dendroidea*, and laureatin from *L. nipponica* showed larvicidal activity against the mosquito *Aedes aegypti* [16].

As part of our ongoing investigation to search for biologically active compounds from marine organisms in Okinawa, we primarily studied *L. nidifica* specimens collected off the coasts of Tsuken Island. The EtOAc extract of *L. nidifica* showed toxic activity against brine shrimp larvae (*Artemia salina*) and subsequently it was subjected to bioassay-guided separation to yield four halogenated compounds. In this paper, we describe the isolation of four halogenated secondary metabolites from *L. nidifica*, and insecticidal and repellent activities of the main active component, laurinterol. This study is the first step in a long-term plan dedicated to explore potential bioactive compounds produced by the red algae *Laurencia* spp.

3. Present Study

3.1. Isolation and identification of halogenated secondary metabolites

The red alga *L. nidifica* was collected in May 2014 from coastal areas of Tsuken Island (Okinawa, Japan) and extracted with MeOH. The MeOH extract was partitioned between EtOAc and H_2O. The EtOAc fraction, which showed the toxicity against brine shrimp larvae, was separated by Si-gel column chromatography to obtain a main active fraction. This active fraction was further subjected to preparative TLC to yield four known compounds consisting of halogenated sesquiterpenes, laurinterol (**1**) [17-20], isolaurinterol (**2**) [18,19,21], aplysin (**3**) [22,23], and α-bromocuparene (**4**) [24] (Figure 1). Laurinterol (**1**) was regarded as the major secondary metabolite of *L. nidifica* in Okinawa [18]. Their structures were determined on the basis of spectroscopic analysis (^{1}H-NMR and MS). All known compounds were identified by comparing their spectral data with those reported in the literature [20,21,23,24].



1 **2** **3** **4**

Figure 1. Structures of compounds **1**-**4**.

3.2. Toxic activity of isolated compounds against brine shrimp

Artemia salina, commonly known as the brine shrimp, has been used as a model for several preliminary evaluations of insecticidal, acaricidal, ecotoxicological, and pharmacological activities [25]. The toxicity assay using larvae of *A. salina* has been considered as one of the useful tools for

detection of residues of insecticides [26]. This assay was used in this study to search for biologically active compounds from *L. nidifica*. The toxic activity of the isolated compounds **1-4** was shown in Table 1. Only laurinterol (**1**) had acute toxicity against *A. salina* with LC_{50} 4.14 μg/mL, whereas the other compounds **2-4** showed no toxicity at 10 μg/mL. A positive compound, natural insecticide rotenone was highly toxic with LC_{50} 0.31 μg/mL.

Table 1. Brine shrimp toxic activity of **1-4**

Compounds	LC_{50} (μg/mL)
Laurinterol (**1**)	4.14 ± 0.19
Isolaurinterol (**2**)	> 10
Aplysin (**3**)	> 10
α-Bromocuparene (**4**)	> 10
Rotenone	0.31 ± 0.01

Data have statistical significance at $p < 0.01$.

3.3. Repellent activity of laurinterol

The maize weevil *Sitophilus zeamais* Motschulsky is a serious pest of a variety of grain crops both in the field and in storage units. Since there is currently no effective control agent for *S. zeamais*, repellency test against them was examined in this study. Repellent activity of major active metabolite, laurinterol and five kinds of insecticides was evaluated by a modified repellency test against adults of *S. zeamais*. Among all tested samples, laurinterol and pyrethrins exhibited strong repellent activity toward *S. zeamais* with ED_{50} values of 12.65 and 1.67 μg/cm^2, respectively. Three natural insecticides, rotenone, nereistoxin, and spinosad were inactive at 104 μg/cm^2 (1 mg/disc). On the other hand, fenitrothion did not show any repellent effect in a wide range of concentrations (1.04-104 μg/cm^2), but killed the insects at 10.4 μg/cm^2.

Table 2. Repellent activity of laurinterol against the maize weevil *S. zeamais*

Compounds	ED_{50} (μg/cm^2)
Laurinterol	12.65 ± 0.30
Rotenone	> 104
Fenitrothion	> 104
Pyrethrins standard	1.67 ± 0.17
Nereistoxin standard	> 104
Spinosad standard	> 104

Data have statistical significance at $p < 0.01$.

3.4. Insecticidal activity of laurinterol

The termite *R. speratus* Kolbe, which is one of the most common species in Japan, is a serious destructive pest, causing great economic losses in wood-frame constructions in Japan [27]. Insecticidal activity was evaluated by a topical application method using adult termites of *R. speratus*. Termiticidal activity of laurinterol and positive controls (commercial insecticides) was shown in Table 3. Even though laurinterol exhibited lower activity than all of the commercial insecticides including rotenone, fenitrothion, and pyrethrins, it showed good activity against termites with LD_{50} 2.2 μg/insect.

Table 3. LD_{50} values of laurinterol against termites by topical application

Compounds	LD_{50} (μg/insect)
Laurinterol	2.20 ± 0.34^{b}
Rotenone	0.11 ± 0.01^{a}
Fenitrothion	0.025 ± 0.0^{a}
Pyrethrins standard	0.013 ± 0.002^{a}

[a,b] Mean values with different letters in the same column are significantly different at $p < 0.01$.

3.5. AChE inhibitory activity of laurinterol

The *in vitro* inhibitory effect of laurinterol on acetylcholinesterase (AChE) activity was examined to explore the mode-of-action. AChE inhibitory activity of laurinterol and three kinds of commercial insecticides was shown in Table 4. In topical application test, laurinterol had a lower inhibitory effect than all of the commercial insecticides including rotenone, fenitrothion, and pyrethrins. However, laurinterol showed the strongest AChE inhibition compared to other tested compounds, and was more potent than a known AChE inhibitor, fenitrothion.

Table 4. Inhibition of AChE activity of laurinterol

Compounds	IC_{50} (μg/mL)
Laurinterol	46.85 ± 0.05^{a}
Rotenone	> 100
Fenitrothion	79.12 ± 3.47^{b}
Pyrethrins standard	88.73 ± 0.73^{b}

[a,b] Mean values with different letters in the same column are significantly different at $p < 0.01$.

3.6. Discussion

The EtOAc extract of the red alga *L. nidifica* showed toxic activity against larvae of *A. salina*. Laurinterol, a cyclolaurane-type halogenated sesquiterpene identified as a major secondary metabolite in this extract, exhibited repellent activity against the maize weevils *S. zeamais*, insecticidal activity against the termites *R. speratus*, and AChE inhibitory activity as well as toxicity against *A. salina*. The red alga *L. nidifica* has been reported to produce a variety of halogenated secondary metabolites (more than 30 compounds) such as nidificene, nidifidiene, laurinterol, isolaurinterol, and laurenidificin [18,28-30]. However, there are only a few reports on the biological activities of *L. nidifica* [18,28,29]. In addition, a literature review showed that laurinterol exhibited significant antibacterial activity against pathogenic bacteria, cytotoxicity against human tumor cell lines, antifouling activity, Na/K-ATPase inhibitory activity, and antioxidant activity [20,31-35]. To our knowledge, this is the first report of toxicity on *A. salina*, repellent activity against *S. zeamais*, insecticidal activity against *R. speratus*, and AChE inhibitory activity of laurinterol.

Stored-product insects and termites are one of the major pests in the world. Control of these insects is primarily dependent on excessive use of synthetic insecticides and fumigants, which has led to some serious problems such as toxic residues, disturbances in the environment, pest resistance, and lethal effects on non-target organisms [36]. Hence, researchers are nowadays focused on finding available alternative insecticides which will be effective, selective, biodegradable, and environmentally benign. The development of environmentally-friendly natural pesticides would help to decrease the negative impacts of synthetic agents; however, there are not many examples of commercially available insecticides derived from marine organisms. Therefore, in order to improve

the functionality of laurinterol and overcome the above-mentioned problems, chemical conversion of promising compounds based on laurinterol and evaluation of structure-activity relationship (SAR) are needed for further research.

In conclusion, the present study reports the repellent, insecticidal and AChE inhibitory activities of laurinterol from *L. nidifica* for the first time. These findings suggest that the red algal genus *Laurencia* may be a good source of bioactive natural products with insecticidal activity, and laurinterol have potential for future development of new repellents and/or insecticides for controlling the pests like stored-product insects and termites. Results of this study could be helpful in further research on the isolation of natural products with insecticidal activity from marine organisms in Okinawa.

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Supporting Information

Supporting information accompanies with this paper on http://www.acgpubs.org/RNP

References

[1] H. M. D. Wang, C.C. Chen, P. Huynh and J. S. Chang (2015). Exploring the potential of using algae in cosmetics, *Bioresour. Technol.* **184**, 355-362.

[2] H. Kharkwal, D. D. Joshi, P. Panthari, M. K. Pant and A. C. Kharkwal (2012). Algae as future drugs, *Asian J. Pharm. Clin. Res.* **5**, 1-4.

[3] S. K. Kim, Y. D. Ravichandran, S. B. Khan and Y. T. Kim (2008). Prospective of the cosmeceuticals derived from marine organisms, *Biotechnol. Bioprocess Eng.* **13**, 511-523.

[4] A. J. Smit (2004). Medicinal and pharmaceutical uses of seaweed natural products: A review, *J. Appl. Phycol.* **16**, 245-262.

[5] M. Masuda, T. Abe, T. Suzuki and M. Suzuki (1996). Morphological and chemotaxonomic studies on *Laurencia composita* and *L. okamurae* (Ceramiales, Rhodophyta), *Phycologia* **35**, 550-562.

[6] T. Ishii (2015). Halogenated compounds from the Okinawan red algal genus *Laurencia*, *J. Soc. Trop. Res. Technologists* **30**, 5-14.

[7] M. Suzuki and C. S. Vairappan (2005). Halogenated secondary metabolites from Japanese species of the red algal genus *Laurencia* (Rhodomelaceae, Ceramiales), *Curr. Top. Phytochemistry* **7**, 1-34.

[8] T. Abe, M. Masuda, T. Suzuki and M. Suzuki (1999). Chemical races in the red alga *Laurencia nipponica* (Rhodomelaceae, Ceramiales), *Phycol. Res.* **47**, 87-95.

[9] M. Masuda, T. Abe, S. Sato, T. Suzuki and M. Suzuki (1997). Diversity of halogenated secondary metabolites in the red alga *Laurencia nipponica* (Rhodomelaceae, Ceramiales), *J. Phycol.* **33**, 196-208.

[10] B. G. Wang, J. B. Gloer, N. Y. Ji and J. C. Zhao (2013). Halogenated organic molecules of Rhodomelaceae origin: chemistry and biology, *Chem. Rev.* **113**, 3632-3685.

[11] M. T. Cabrita, C. Vale and A. P. Rauter (2010). Halogenated compounds from marine algae, *Mar. Drugs* **8**, 2301-2317.

[12] K. A. El Sayed, D. C. Dunbar, T. L. Perry, S. P. Wilkins and M. T. Hamann (1997). Marine natural products as prototype insecticidal agents, *J. Agric. Food Chem.* **45**, 2735-2739.

[13] A. Fukuzawa and T. Masamune (1981). Laurepinnacin and isolaurepinnacin; new acetylenic cyclic ethers from the marine red alga *Laurencia pinnata* Yamada, *Tetrahedron Lett.* **22**, 4081-4084.

[14] K. Watanabe, K. Umeda and M. Miyakado (1989). Isolation and identification of three insecticdal principles from the red alga *Laurencia nipponica* Yamada, *Agric. Biol. Chem.* **53**, 2513-2515.

[15] Z. S. Abou-Elnaga, W. M. Alarif and S. S. Al-lihaibi (2011). New larvicidal acetogenin from the red alga *Laurencia papillosa*, *Clean Soil Air Water* **39**, 787-794.

[16] O. Salvador-Neto, S. A. Gomes, A. R. Soares, F. L. S. Machado, R. I. Samuels, R. Nunes da Fonseca, J. Souza-Menezes, J. L. C. Moraes, E. Campos, F. B. Mury and J. R. Silva (2016). Larvicidal potential of

the halogenated sesquiterpene (+)-obtusol, isolated from the alga *Laurencia dendroidea* J. Agardh (Ceramiales: Rhodomelaceae), against the Dengue vector mosquito *Aedes aegypti* (Linnaeus) (Diptera: Culicidae), *Mar. Drugs* **14**, 20.

[17] T. Irie, M. Suzuki, E. Kurosawa and T. Masamune (1966). Laurinterol and debromolaurinterol, constituents from *Laurencia intermedia*, *Tetrahedron Lett.* **7**, 1837-1840.

[18] C. S. Vairappan, M. Suzuki, T. Abe and M. Masuda (2001). Halogenated metabolites with antibacterial activity from the Okinawan *Laurencia* species, *Phytochemisty* **58**, 517-523.

[19] T. Irie, M. Suzuki, E. Kurosawa and T. Masamune (1970). Laurinterol, debromolaurinterol and isolaurinterol, constituents of *Laurencia intermedia* Yamada, *Tetrahedron* **26**, 3271-3277.

[20] S. Tsukamoto, Y. Yamashita and T. Ohta (2005). New cytotoxic and antibacterial compounds isolated from the sea hare, *Aplysia kurodai*, *Mar. Drugs* **3**, 22-28.

[21] S. C. Mao and Y. W. Guo (2010). Sesquiterpenes from Chinese red alga *Laurencia okamurai*, *Chin. J. Nat. Med.* **8**, 321-325.

[22] S. Yamamura and Y. Hirata (1963). Structures of aplysin and aplysinol, naturally occurring bromo-compounds, *Tetrahedron* **19**, 1485-1496.

[23] T. Irie, M. Suzuki and Y. Hayakawa (1969). Isolation of aplysin, debromoaplysin, and aplysinol from *Laurencia okamurai* Yamada, *Bull. Chem. Soc. Jpn.* **42**, 843-844.

[24] T. Suzuki, M. Suzuki and E. Kurosawa (1975). α-Bromocuparene and α-isobromocuparene, new bromo compounds from *Laurencia* species, *Tetrahedron Lett.* **16**, 3057-3058.

[25] C. Vidotto, D. B. da Silva, R. Patussi, L. F. G. Brandão, J. D. Tibúrcio, S. N. Alves and J. M. de Siqueira (2013). Brine shrimp lethality test as a biological model for preliminary selection of pediculicidal components from natural source, *Biosci. J.* **29**, 255-263.

[26] J. H. Stevenson (1961). Biological assay of insecticides using the brine shrimp, *Artemia salina* (L.), *J. Sci. Food Agric.* **12**, 650-652.

[27] K. Tsunoda (2005). Improved management of termites to protect Japanese homes, *Proceedings of the 5th Conference of Urban Pests* 33-37.

[28] S. M. Waraszkiewicz and K. L. Erickson (1974). Halogenated sesquiterpenoids from the Hawaiian marine alga *Laurencia nidifica*: nidificene and nidifidiene, *Tetrahedron Lett.* **23**, 2003-2006.

[29] J. Kimura, N. Kamada and Y. Tsujimoto (1999). Fourteen chamigrane derivatives from a red alga, *Laurencia nidifica*, *Bull. Chem. Soc. Jpn.* **72**, 289-292.

[30] X. Liu, X. M. Li, C. S. Li, N. Y. Ji and B. G. Wang (2010). Laurenidificin, a new brominated C_{15}-acetogenin from the marine red alga *Laurencia nidifica*, *Chin. Chem. Lett.* **21**, 1213-1215.

[31] C. S. Vairappan, T. Kawamoto, H. Miwa and M. Suzuki (2004). Potent antibacterial activity of halogenated compounds against antibiotic-resistant bacteria, *Planta Med.* **70**, 1087-1090.

[32] M. Kladi, H. Xenaki, C. Vagias, P. Papazafiri and V. Roussis (2006). New cytotoxic sesquiterpenes from the red algae *Laurencia obtusa* and *Laurencia microcladia*, *Tetrahedron* **62**, 182-189.

[33] G. Ryu and O. S. Yoon (2003). Studies on marine natural antifoulant laurinterol, *Kor. J. Env. Hlt.* **29**, 1-8.

[34] Y. Okamoto, N. Nitanda, M. Ojika and Y. Sakagami (2001). Aplysiallene, a new bromoallene as an Na, K-ATPase inhibitor from the sea hare, *Aplysia kurodai*, *Biosci. Biotechnol. Biochem.* **65**, 474-476.

[35] Kuwano K, S. Matsuka, S. Kono, M. Ninomiya, J. Onishi and N. Saga (1998). Growth and the content of laurinterol and debromolaurinterol in *Laurencia okamurae* (Ceramiales, Rhodophyta), *J. Appl. Phycol.* **10**, 9-14.

[36] B. C. Gerwick and T. C. Sparks (2014). Natural products for pest control: an analysis of their role, value and future, *Pest Manag. Sci.* **70**, 1169-1185.