*Malaysian Journal of Science* **28** (3): 263 – 268 (2009)

# C-15 Halogenated Acetogenin with Antibacterial Activity against Food Pathogens

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Received on 22nd June 2009, accepted in revised form 3rd November 2009.

**ABSTRACT** As part of our continuous effort in search of bioactive secondary metabolites from marine organisms, we studied a specimen of red algae, *Laurencia nangii* Masuda from Banggi Island, Kudat District, Sabah. One C-15 acetogenin was isolated and identified as *Z*-dihydrorhodophytin (**1**, 15%). This compound showed 100% inhibition against the tested bacteria at 30 μgdisc$^{-1}$. MIC values for *Salmonella enteritidis, Vibrio cholerae* and *Staphalococcus aereus* were 1.25 μgdisc$^{-1}$, 2.25 μgdisc$^{-1}$ and 2.25 μgdisc$^{-1}$, respectively; while *Escherichia coli*, *Salmonella typhii* and *Salmonella thphymunium* were inhibited at MIC value of 7.25 μgdisc$^{-1}$. This study showed that *Z*-dihydrorhodophytin (**1**) has significant antibacterial activity against the tested food pathogens and may have potential to be used as lead pharmaceutical drug candidate in combating "antibiotic resistant bacteria".

**ABSTRAK** Sehubungan dengan usaha kami untuk mengkaji sebatian sekunder bioaktif daripada organisma marin, kami telah menyelidik satu spesimen alga merah, *Laurencia nangii* Masuda dari Pulau Banggi, Kudat, Sabah. Satu sebatian C15-acetogenin telah dipencilkan dan dikenalpasti sebagai *Z*-dihydrorhodophytin (**1**, 15%). Sebatian ini menunjukkan 100% perencatan pada 30 μgdisc$^{-1}$. Nilai MIC bagi *Salmonella enteritidis, Vibrio cholerae* dan *Staphalococcus aereus* adalah 1.25 μgdisc$^{-1}$, 2.25 μgdisc$^{-1}$ dan 2.25 μgdisc$^{-1}$, manakala nilai untuk *Escherichia coli*, *Salmonella typhii* dan *Salmonella thphymunium* adalah 7.25 μgdisc$^{-1}$. Kajian ini menunjukkan bahawa *Z*-dihydrorhodophytin (**1**) dapat merencatkan bakteria yang diuji dengan signifikan dan mempunyai potensi untuk dijadikan sebagai "lead drug" farmaseutikal dalam usaha mengawal "bakteria rintang antibiotik".

(**Keywords:** *Laurencia nangii*, C15-acetogenin, antibiotic resistant bacteria)

## INTRODUCTION

Food-borne diseases are a growing concern in global public health services. It is estimated that as many as 30% of the population in industrialized countries suffer from food-borne illness annually. In 2005, 1.8 million people died of diarrhoeal disease and a great proportion of these cases can be attributed to contamination of food and drinking water [1]. The prevalent disease-causing microbes include Gram-negative bacteria such as *Escherichia coli*, *Salmonella*, *Yersinia*, *Shigella*, *Aeromonas*, *Campylobacter* and *Vibrio*, as well as Gram-positives bacteria such as *Clostridium*, *Staphylococcus*, *Listeria*, *Bacillus*, *Streptococcus* and *Enterococcus* [2]. Due to the rampant emergence of antimicrobial-resistant strains like methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA), there is an urgent need to discover new lead metabolites with potent antibacterial potential [3]. In light of these, there has been a consolidated effort among marine natural products chemists to explore new alternative drugs to combat and control food-borne diseases by incorporating approaches that reduces disease incidence and avoid negative side effects on human health [4]. In addition, there is also a growing awareness among consumers for increased safety, quality and shelf-life of foods with fewer synthetic additives [5]. This has made the exploration of antimicrobial agents from natural sources the only logical approach. The marine red algae genus *Laurencia* (Rhodomelaceae, Ceramiales) are well known source of halogenated secondary metabolites with potent antibacterial, antifungal, antiviral and antitumor properties [6, 7, 8, 9, 10, 11]. In our continuous effort to search for bioactive metabolites from marine organisms [12, 13, 14, 15, 16], we studied a specimen of red algae, *Laurencia nangii* Masuda from Banggi Island, Kudat District, Sabah. We wish to report herein the isolation, antibacterial activities and structure elucidation of *Z*-dihydrorhodophytin (**1**).

## MATERIALS AND METHODS

### Collection

Sample of *Laurencia nangii* was collected from

Banggi Island, Kudat District, Sabah, Malaysia (07°21.711'N, 117°15.223'E). Latitude and longitude were recorded using GPS 12XL (GARMIN Olathe, KS, USA). A voucher herbarium specimen, BORH 37588 (Figure 1) was deposited in Borneensis Herbarium Collection, Institute for Tropical Biology and Conservation, Universiti Malaysia Sabah.

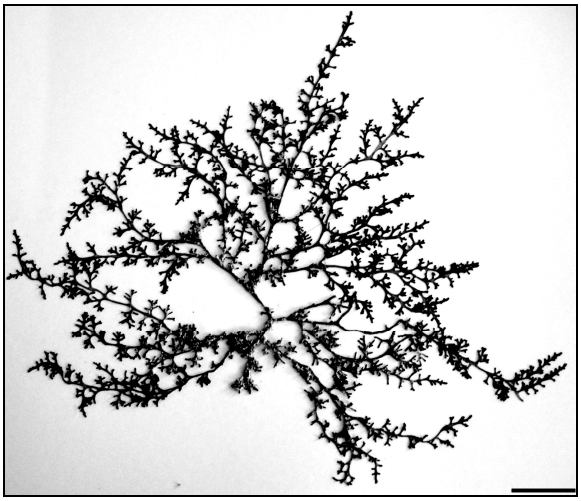

**Figure 1.** Herbarium specimen of *Laurencia nangii* Masuda (BORH 37588) collected from Banggi Island, Kudat District, Sabah. Scale bar = 1 cm.

**Extraction and Isolation**

Partially dried sample (90 g) was soaked in methanol (MeOH) for seven days. The MeOH solution was filtered and concentrated *in vacuo* and partitioned between diethyl ether ($Et_2O$) and distilled water ($dH_2O$) in the ratio of 1:3 (v/v). $Et_2O$ solution was washed with two changes of $dH_2O$, dried over sodium sulphate anhydrous ($Na_2SO_4$) and evaporated in *vacuo* to obtain crude extract. Chemical profiling of the crude extract was done by spotting crude extract on $SiO_2$ gel $F_{254}$ nm Thin Layer Chromatography plates and developed in toluene (100%) and Hexane: EtOAc (3:1) solvent systems. The spots on TLC were visualized by UV light (254 nm) and molybdophosphoric acid. High Performance Liquid Chromatography profile of crude extract was obtained on a Shimadzu HPLC using Preparative pump LC-6AD, UV Detector SPD-20A and oven CTO-20A under isocratic mode of acetonitrile (MeCN) 70% at a flow rate of 2mL $min^{-1}$ in a phenyl hexyl, 10 mm x 250 mm column (Luna Phenomenax), UV detection at 220 nm. Further separation of the active metabolite involved fractionation *via* silica gel (Merck, Kieselgel 60, 0.063-0.2 mm mesh) column chromatography with a step gradient of hexane and ethyl acetate (EtOAc); gradient ratios: 9.5:0.5, 8.0:2.0, 7.0:3.0, 6.0:4.0 and 5.0:5.0. Fraction one was further purified using HPLC under isocratic mode of MeCN 70% at a flow rate of 4 mL $min^{-1}$ in a phenyl hexyl, 10 mm x 250 mm column (Luna Phenomenax), UV detection at 220 nm to give *Z*-dihydrorhodophytin (**1**). Yield of compound is based on the weight of crude extract.

**Spectroscopy Measurement**

$^{1}$H-NMR (600 MHz) and $^{13}$C-NMR (150 MHz) spectra were obtained in deuterated chloroform with tetramethylsilane as internal standard (coupling constant, *J* in Hz) on a JEOL ECA 600 spectrometer, IR spectrum was measured on a Thermo Nicolet Avatar FTIR spectrophotometer, LREIMS and HREIMS were recorded on a JEOL JMS-A500 spectrometer. Optical rotation was measured on a JASCO DIP-140 polarimeter, while melting point was measured on a micro-melting point apparatus (Fisher Scientific) and uncorrected. Compound **1** was identified by comparison of spectral data with those reported in the literature.

**Antibacterial Assay**

Antibacterial bioassay was carried out by paper disc diffusion method using six strains of foodborne pathogenic bacteria obtained from clinical pathological laboratory in Queen Elizabeth Hospital, Kota Kinabalu, Sabah, Malaysia. These bacteria were: *Escherichia coli* (E.c) (NIHJ C-1), *Salmonella typhii* (S.ty) (NIHJ C-2), *Salmonella enteritidis* (S.e) (NIHJ C-3), *Salmonella thphymunium* (S.t) (SR0875-1), *Vibrio cholerae* (V.c) (SR0875-2) and *Staphalococcus aereus* (S.a) (SR0875-3). One loopful of bacteria was pre-cultured in nutrient broth (Oxoid, England) for 24 hours. The turbidity of culture was adjusted to optical density of McFarland 0.5 [17, 18]. Then, 0.1ml of the precultured bacterial suspension was seeded on nutrient agar (Oxoid, England) plates. Paper discs (Whatman, 6 mm) impregnated with 30 µg $disc^{-1}$ of compound **1** were placed on the seeded agar plates. The diameters of inhibition zone were measured after 24 hours incubation at 28°C. Potency of the compound was compared against four types of commercially available antibiotics (Biomérieux, France); Cefuroxime (CXM30), Tetracyclin (TE30), Minocycline (MI30) and Vancomycin (VA30). Concentration used was 30 µg$disc^{-1}$, as recommended by the Clinical and Laboratory Standard Institute (CLSI). Minimum inhibition concentration (MIC) of compound **1** was determined using disc diffusion method as described above with the initial concentration of 10µg$disc^{-1}$, and reduced to 7.25µg$disc^{-1}$, 5.00µg$disc^{-1}$, 2.25µg$disc^{-1}$ and 1.25µg$disc^{-1}$ by serial dilutions.

## RESULTS

Partially dried specimen of *Laurencia nangii* (90 g) was extracted in methanol and gave 1100 mg of greenish paste-like crude extract. Figure 2 shows the retention time of the compound in its crude extract when analyzed *via* High Performance Liquid Chromatography (HPLC) under isocratic mode of MeCN 70% at flow rate of 2 mL $min^{-1}$ via Phenyl Hexyl Luna column.

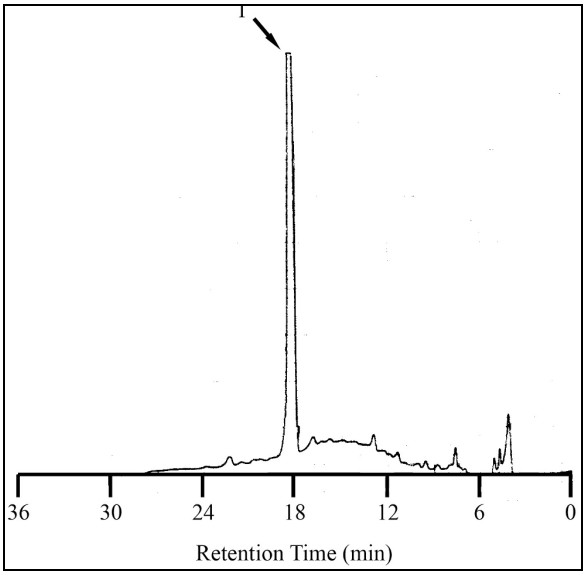


**Figure 2.** Relative retention time of Z-dihydrorhodophytin (**1**) in crude methanol extracts of *L. nangii* (**1**: 18.1 min).

The extract was then fractioned *via* Si gel column chromatography with step gradient of hexane and ethyl acetate. Fraction eluted with hexane: EtOAc (9.5:0.5) was further separated with reverse phase HPLC to yield compound **1** (15.0%). The isolated pure compound was subjected to $^{1}$H-NMR, $^{13}$C-NMR, IR, LREIMS, HREIMS, optical rotation and melting point measurements. The H$^{1}$-NMR data showed the presence of methyl protons at δ1.08, four sets of methylene protons at δ1.91/2.00, δ2.25/2.55, δ2.58/2.73 and δ2.79/2.87, four sets of methane protons at δ4.02, δ4.05, δ4.11 and δ4.31, one acetylenic proton at δ3.14. Presence of four olefinic protons was also detected at δ5.55, δ5.77, δ5.85, δ6.06. The presence of a 1-buten-3-ynyl moiety was evident from NMR spectra. The coupling constant value of 11.0 Hz for H-3 and H-4 as well as chemical shift value (δ 3.14) of the acetylenic proton indicated the geometry of the double bond at C-3 to be *Z* configuration. Based on these information and HMBC data, the compound was determined as *Z*-dihydrorhodophytin (**1**) (Figure 3), previously reported from *Laurencia pinnatifida* [19]. Detailed spectroscopic data of *Z*-dihydrorhodophytin (**1**) is as shown below:

*Z*-dihydrorhodophytin (**1**)
Mp 36-38 °C; $[\alpha]_D$ +69.45° (*c*, 0.5, $CHCl_3$); IR (KBr) $\nu_{max}$ $cm^{-1}$ 3280, 3026, 2924, 2361, 2336, 1733, 1436, 1362, 1321, 1280, 1248, 1195, 1162, 1109, 1072, 800, 763, 710, 659, 624; $^{1}$H-NMR ($CDCl_3$, 600 MHz) δ 1.08 (3H, t, *J*=7.6 Hz; $H_3$-15), 1.91 (1H, dq, *J*=15.2, 7.6 Hz; H-14), 2.00 (1H, m; H-14), 2.25 (1H, dd, *J*=10.8, 6.8 Hz; H-11), 2.56 (1H, m; H-11), 2.56 (1H, m; H-8), 2.73 (1H, ddd, *J*=14.5, 7.6, 6.9 Hz; H-8), 2.79 (1H, ddd, *J*=14.4, 8.3, 5.5 Hz; H-5), 2.87 (1H, ddd, *J*=14.4, 6.9, 5.5 Hz; H-5), 3.14 (1H, s; H-1), 4.02 (1H, ddd, *J*=14.7, 4.8, 4.1 Hz; H-13), 4.05 (1H, dd, *J*=8.3, 3.5 Hz; H-7), 4.11 (1H, brd, *J*=10.8 Hz; H-12), 4.31 (1H, ddd, *J*=8.3, 5.5, 5.5 Hz; H-6), 5.55 (1H, d, *J*=11.0 Hz; H-3), 5.77 (1H, dd, *J*=10.4, 7.6 Hz; H-9), 5.85 (1H, ddd, *J*=10.4, 6.9, 3.5 Hz; H-10), 6.06 (1H, ddd, *J*=11.0, 8.3, 6.9 Hz; H-4); $^{13}$C-NMR ($CDCl_3$, 150 MHz) δ 141.8 (C4), 130.7 (C10), 127.9 (C9), 111.7 (C3), 83.2 (C1), 80.8 (C2), 79.7 (C12), 74.5 (C6), 64.9 (C7), 62.6 (C13), 35.9 (C5), 34.5 (C8), 32.1 (C11), 29.7 (C14), 13.2 (C15); LR-EIMS m/z: 330.0386 (100.0%), 332.0366 (97.3%), 332.0357 (32.0%), 334.0336 (31.1%), 331.0420 (16.2%), 333.0399 (15.8%), 333.0390 (5.2%), 335.0370 (5.0%), 332.0453 (1.2%), 334.0433 (1.2%); HR-EIMS m/z: 331.6757. Calculated for $C_{15}H_{20}{}^{79}Br^{35}ClO$ 330.0386.

Antibacterial activity of *Z*-dihydrorhodophytin (**1**) was determined by testing it against food-borne human pathogenic bacteria obtained from clinical patients. Results of paper disc diffusion assay of the isolated compound were compared to the commercially available antibiotics as shown in Table 1. *Z*-dihydrorhodophytin (**1**) showed 100% inhibition against the tested bacteria with comparable inhibitory zones to the commercially available antibiotics. The Minimum Inhibitory Concentration (MIC) of *Z*-dihydrorhodophytin is shown in Figure 4. All the test bacteria were inhibited using a minimum concentration of 10 μgdisc$^{-1}$, further reduction in concentration gave selective inhibition as shown in Figure 3. Bacteria such as *Salmonella enteritidis*, *Vibrio cholerae* and *Staphalococcus aereus* were most sensitive with MIC values of 1.25 μgdisc$^{-1}$, 2.25 μgdisc$^{-1}$ and 2.25μgdisc$^{-1}$, respectively. The other three bacteria exhibited a MIC value of 7.25μgdisc$^{-1}$.

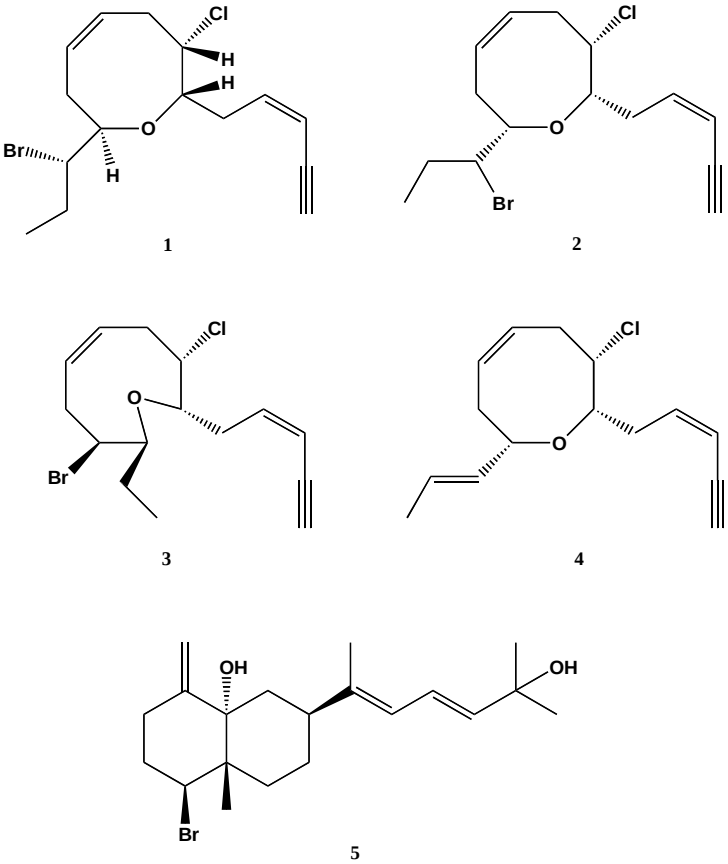


**Figure 3.** Chemical structures of *Z*-dihydrorhodophytin (**1**), *cis*-pinnatifidenyne (**2**), obtusenyne (**3**), 3Z-laurenyne (**4**) and aplysiadiol (**5**).

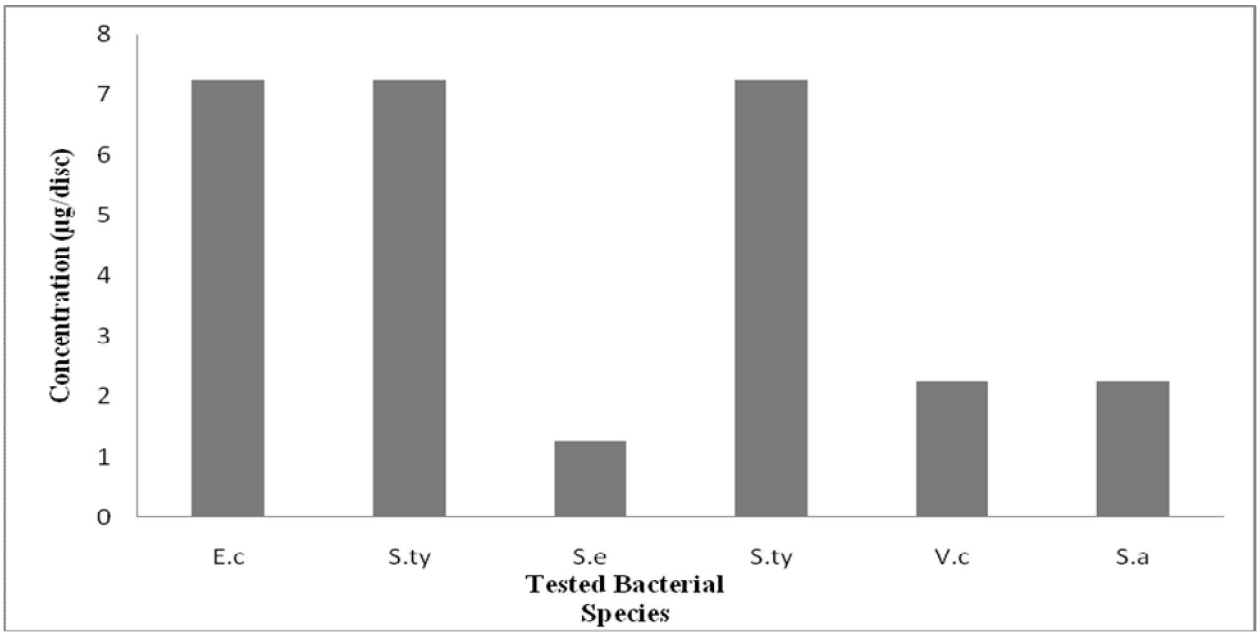


**Figure 4.** MIC values for *Z*-dihydrorhodophytin (**1**) against the tested bacteria. (E.c ~ *Escherichia coli* (NIHJ C-1), S.ty~*Salmonella typhii* (NIHJ C-2), S.e~*Salmonella enteritidis* (NIHJ C-3), S.t ~*Salmonella thphymunium* (SR0875-1), V.c~*Vibrio cholerae* (SR0875-2), S.a~*Staphalococcus aereus* (SR0875-3)).

**Table 1.** Comparative antibacterial activity of *Z*-dihydrorhodophyta (**1**) and four types of commercially available antibiotics against six strains of food-borne pathogenic bacteria. (CXM30~Cefuroxime, TE30~Tetracyclin, MI30~Minocycline, VA30~Vancomycin).

| **TEST BACTERIA** | **TEST COMPOUNDS** | | | | |
|---|---|---|---|---|---|
| | (**1**) | CXM30 | TE30 | MI30 | VA30 |
| *Escherichia coli* (NIHJ C-1) | 18 | 7 | 25 | 28 | 32 |
| *Salmonella typhii* (NIHJ C-2) | 17 | 9 | 18 | 19 | 22 |
| *Salmonella enteritidis* (NIHJ C-3) | 11 | 8 | 20 | 21 | 19 |
| *Salmonella thphymunium* (SR0875-1) | 16 | 10 | 18 | 18 | 18 |
| *Vibrio cholerae* (SR0875-2) | 14 | 8 | 21 | 21 | 30 |
| *Staphalococcus aereus* (SR0875-3) | 12 | 10 | 19 | 17 | 28 |

Inhibition zone diameter: mm, -: no inhibition. Compound concentration: 30µg $disc^{-1}$ (CLSI levels)

## DISCUSSIONS

Presence of halogenated secondary metabolites in red algae genus *Laurencia* is a well-documented fact, and its chemical diversity varies with species and geographical location where it thrives. However, there has only been one documented report on the presence of halogenated metabolites in *L. nangii*, its composition was identified as *cis*-pinnatifidenyne (**2**), obtusenyne (**3**), 3*Z*-laurenyne (**4**) and aplysiadiol (**5**) (Figure 3) [20]. However, the isolated metabolites were not tested for any biological potential. Hence, presence finding is an important discovery in terms of its chemical diversity as well as its biological activity. The isolated metabolite, *Z*-dihydrorhodophytin (**1**), was present in a significantly high percentage as compared to the common amount of halogenated metabolites synthesized by any *Laurencia* species. Hence, making it possible to harvest a large amount of *Z*-dihydrorhodophytin (1) from this species.

More interestingly, upon screening against human pathogenic bacteria, it became apparent that *Z*-dihydrorhodophytin has potent antibacterial potential. Detailed analysis of data presented in Table 1 revealed that *Z*-dihydrorhodophytin (**1**) showed better activity compared to cefuroxime. Commercial cefuroxime is a broad-spectrum third generation cephalosporin antibiotics with a typical β-lactam mode of action. Since, the tested microbes were obtained from clinical specimens, it is possible that they have acquired significant amount of resistant against conventional antibiotics with β-lactam mode of action. The other two antibiotics, tetracycline and minocycline are broad-spectrum polyketide antibiotics that inhibit protein synthesis by inhibiting the binding of aminoacyl-tRNA to the mRNA-ribosome complex. These two antibiotics showed significant inhibition against the proliferation of the tested food-borne pathogens. Antibacterial activity of *Z*-dihydrorhodophytin was not as good as vancomycin, hence, vancomycin maintained itself as the "last resort" drug against bacterial infection. Based on this study, it could be speculated that the test bacteria could have acquired slight resistant towards antibiotic having the β-lactam mode of action. Based on this investigation, it was apparent that *Z*-dihydrorhodophytin has better antibacterial potential than cefuroxime and no toxic activities against normal and tumor cell lines (Tan, 2009). Hence, it could be considered for topical application once the pharmacokinetics is done.

## CONCLUSIONS

This study showed that *Z*-dihydrorhodophytin (**1**) inhibited the tested bacteria at notably low concentrations. Presence of strong antibacterial activity of *Z*-dihydrorhodophytin (**1**) promises new hope in our desperate attempt to discover potent drugs. Therefore, halogenated metabolites derived from marine red algal genus *Laurencia* may have some potential to be applied as effective drugs for controlling multi resistant bacteria.

**ACKNOWLEDGEMENTS**

We are grateful to the Prof. Dr. Ridzwan and his team for the support and assistance during field survey. This study was funded under a research grant by the International Foundation for Science (IFS) and Committee for the Prohibition of Chemical Weapon, Hague, The Netherlands (IFS).

**REFERENCES**

1. World Health Organization (2007). Food Safety and Foodborne Illness. World Health Organization Fact sheet 237. Available from: www.who.int/mediacentre/factsheets/fs237/en. [Cited 3 March, 2009].
2. Heyndrickx, M., Rijpens, N. and Herman, L. (2001). Molecular detection and typing of foodborne bacterial pathogens: a review. In: *Applied Microbiology – Focus on Biotechnology*. (Eds. Durieux, A. and Simon, J. P.). Volume 2. Kluwer Academic Publishers, Boston, pp. 193-238.
3. Sosa, A. (2006). The threat of antibiotic-resistance bacteria and the development of new antibiotics. In: *Antimicrobial Resistance in Bacteria.* (ed. Amábile-Cuevas, C. F.): Horizon Bioscience, UK, pp. 7-24.
4. Nedorostova, L., Kloucek, P., Kokoska, L., Stolcova, M. and Pulkrabek, J. (2009). Antimicrobial properties of selected essential oils in vapour phase against foodborne bacteria. *Food Control* **20**: 157-160.
5. Bautista-Banos, S., Hernandez-Lauzardo, A. N., Velazquez-del Valle, M. G., Hernandez-Lopez, M., Barka, E. A. and Bosquez-Molina, E. (2006). Chitosan as a potential natural compound to control pre and postharvest diseases of horticultural commodities. *Crop Protection* **25**: 108-118.
6. Caccamese, S., Azzolina, R., Duesler, E. N., Paul, I. C. and Rinehart, K. L. (1980). Laurencienyne, a new acetylenic cyclic ether from the marine red alga *Laurencia obtusa*. *Tetrahedron Lett.* **21**: 2299-2302.
7. König, G. M. and Wright, A. D. (1997). Sesquiterpene content of antibacterial dichloromethane extract of the marine alga *Laurencia obtusa*. *Planta Medica* **63**: 186-187.
8. Vairappan, C. S., Kawamoto, T., Miwa, H. and Suzuki, M. (2004). Potent antibacterial activity of halogenated compounds against antibiotic-resistant bacteria. *Planta Medica* **70**: 1087-1089.
9. Sakemi, S., Higa, T., Jefford, C. W. and Bernardinelli, G. (1986). Venustatriol. A new, anti-viral, triterpene tetracyclic ether from *Laurencia venusta*. *Tetrahedron Lett.* **27**: 4287-4290.
10. Suzuki, T., Suzuki, M., Furusaki, A., Matsumoto, T., Kato, A., Imanaka, Y. and Kurosawa, E. (1985). Teurilene and thyrsiferyl 23-acetate, *meso* and remarkably cytotoxic compounds from the marine red alga *Laurencia obtusa* (Hudson) Lamouroux. *Tetrahedron Lett.* **26**: 1329-1332.
11. Kladi, M., Xenaki, H., Vagias, C., Papazafiri, P. and Roussis, V. (2006). New cytotoxic sesquiterpenes from the red algae *Laurencia obtusa* and *Laurencia microcladia*. *Tetrahedron* **62**: 182-189.
12. Vairappan, C. S., Daitoh, M., Suzuki, M., Abe, T. and Masuda, M. (2001). Antibacterial halogenated metabolites from the Malaysian *Laurencia* species. *Phytochemistry* **58**: 291-297.
13. Vairappan, C. S. (2003). Potent antibacterial activity of halogenated metabolites from Malaysian red algae, *Laurencia majuscula* (Rhodomelaceae, Ceramiales). *Biomol. Eng.* **20**: 255-259.
14. Vairappan, C. S., Ang, M. Y., Ong, C. Y. and Phang, S. M. (2004). Biologically active polybrominated indoles in the red alga *Laurencia similis* from the coastal waters of Sabah (Rhodomelaceae, Ceramiales). *Malaysian J. Sci.* **23**: 119-126.
15. Vairappan, C. S. and Tan, K. L. (2005). Halogenated secondary metabolites from sea hare *Aplysia dactylomela*. *Malaysian J. Sci.* **24**: 17-22.
16. Vairappan, C. S., Anangdan, S. P. and Tan, K. L. (2007). Additional halogenated secondary metabolites from the sea hare *Aplysia dactylomela*. *Malaysian J. Sci.* **26**: 57-64.
17. Sonnenwirth, C. A., Jerett, L. (1980). *Gradwohl's Clinical Laboratory Methods and Diagnosis*, 8th ed. The C.V. Mosby Company, St. Louis, pp. 1959-1970.
18. Hindler, J. A., González, A. H. and Drake, T. A. (1990). Stability of viable-bacterium counts in liquid media used for preparation of inocula and subsequent impact on antimicrobial susceptibility test results. *J. Clin. Microbiol.* **28**: 1271-1275.
19. Norte, M., Fernandez, J. J., Cataldo, F. and Gonzalez, A. G. (1989). *E*-dihydrorhodophytin, a $C_{15}$ acetogenin from the red alga *Laurencia pinnatifida*. *Phytochemistry* **28**: 647-649.
20. Masuda, M., Abe, T., Kogame, K., Kawaguchi, S., Phang, S. M., Daitoh, M., Sakai, T., Takahashi, Y. and Suzuki, M. (2002). Taxonomic notes on marine algae from Malaysia. VIII. Three species of *Laurencia* (Rhodophyceae). *Bot. Mar.* **45**: 571-579.