

SHORT COMMUNICATION

Antimicrobial Property of 12 Spices and Methanol Extract of Ornamental Sea Anemone (*Radianthus ritteri*) Against Edwardsiellosis Agent and Other Bacteria

¹Lee Seong Wei, ¹Najiah Musa, ¹Wendy Wee, ¹Nadirah Musa and ²Chuah Tse Seng

¹Department of Fishery and Aquaculture, Faculty Agrotechnology and Food Science, Universiti Malaysia Terengganu, 21030, Kuala Terengganu, Terengganu, Malaysia

²Department of Agrotechnology, Faculty Agrotechnology and Food Science, Universiti Malaysia Terengganu, 21030, Kuala Terengganu, Terengganu, Malaysia

Asia contributed 91.7% of the total aquaculture product of the world [1] due to the intensive aquaculture farming. However, the intensive farming aquaculture needs a good management of the health of the aquaculture product. Therefore, antibiotic has been used for many years in controlling infectious disease among the cultured aquatic animal [2]. Antibiotic has been widely used for prophylactic and therapeutic purpose in aquatic health management [3]. Nevertheless, overuse and misuse of antibiotic to control infectious disease among fish farmers contribute to the increasing of antibiotic resistance cases among the fish pathogenic bacteria. Thus, antibiotic become less effective in terms of controlling fish bacteria diseases [3]. In 1997, World Bank reported that the total loss due to the disease outbreak in aquaculture industry was recorded as USD 3 billion [3]. Furthermore, antibiotic residue in aquaculture products for human consumption may threaten public health. Therefore, only the last decade scientists all over the world struggled to find out the alternative sources to the available commercial antibiotic in market from natural resources. Thus, this study was conducted to screen the potential of 12 types of spices and ornamental sea anemone (*Radianthus ritteri*) for aquaculture use as antimicrobial agent. 12 spices namely *Coriandrum sativum* L. (Coriander), *Cinnamomum zeylanicum* L., *Piper nigrum* L., *Elettaria cardamomum* L. (Maton), *Illicium verum* (Hook) f., *Trigonella foenum-graecum* L., *Foeniculum vulgare* Mill., *Papaver somniferum* L., *Cuminum cyminum* L., *Laurus nobilis* L., *Carum Carvi* L. and *Brassica juncea* L. were bought from markets and made into fine powder using electrical blender (Pana Lux, China). Ornamental sea anemone (*Radianthus ritteri*) was

bought from pet shop in Kuala Terengganu, Terengganu. The sample was oven dried at 37°C for 5 days until the sample was totally dehydrated. Aqueous extraction of 12 spices was done according to Yano *et al.* [5] with slight modification. Briefly, the powdered samples were added into sterile water until concentration of dry matter/volume of water was 1%. The extracts were then kept at 4°C until further use. The dried sea anemone (*Radianthus ritteri*) was extracted with 70% of methanol at the ratio of dry matter to methanol 1 : 4 for 5 days. The extract was filtered through Whatman filter paper (No 1.) and oven dried at 37°C. The extract was then concentrated at 100 mg/ml and kept at 4°C until further use. A total of 18 isolates of *Edwardsiella tarda* obtained from diseased freshwater fish and 7 other reference strains; *Escherichia coli* (ATCC 25922), *Citrobacter freundii* (ATCC 8090), *Aeromonas hydrophila* (ATCC 49140), *Pseudomonas aeruginosa* (ATCC 35032), *Streptococcus agalactiae* (ATCC 13813), *Edwardsiella tarda* (ATCC 15947) and *Yersinia enterocolitica* (ATCC 23715) were subjected to the antibiotic sensitivity test against spices extracts and methanol extract of *Radianthus ritteri*. All the isolates were cultured in tryptic soy broth (Oxoid, England) at room temperature for 24 h. The bacterial cell was harvested by centrifuged at 14,000 rpm for 5 min. The concentration of the bacterial cell was adjusted using Bio photometer (Eppendorf, Germany) to 10⁷ CFU using sterile saline water. Hole-diffusion method as described by Daud *et al.* [5] was applied to determine antimicrobial properties of the extracts. After prepared bacterial spread plated on Mueller Hinton agar (Oxoid, England), holes with diameter 6 mm were punched on the agar plates. A total of 20 µl of each extracts was dispersed into the

Table 1: Sensitivity of the present isolates against 12 types of aqueous extracts of spices and methanol extract of *Radianthus ritteri*

Bacteria	1	2	3	4	5	6	7	8	9	10	11	12	13
ET 1	+	-	-	-	-	-	-	-	-	-	-	-	-
ET 2	++	-	-	-	-	-	-	-	-	-	-	-	-
ET 3	+++	-	-	-	-	-	-	-	-	-	-	-	-
ET 4	+++	-	-	-	-	-	-	-	-	-	-	++	-
ET 5	+++	-	-	-	-	-	-	-	-	-	-	-	-
ET 6	++	-	-	-	-	-	-	-	-	-	-	+++	-
ET 7	+++	+	-	-	-	-	-	++	++	+++	-	++	-
ET 8	+++	-	++	+	-	-	-	+	-	-	-	-	-
ET 9	++	-	-	-	-	-	-	-	-	-	-	-	-
ET 10	+++	-	+++	+	-	-	-	+	-	++	-	++	-
ET 11	+++	-	++	-	-	-	-	-	+	+	-	-	-
ET 12	+++	-	-	-	-	-	-	-	-	-	-	-	-
ET 13	++	-	-	-	-	-	-	-	-	-	-	-	-
ET 14	+++	-	-	-	-	-	-	-	-	-	-	-	-
ET 15	+++	-	-	-	-	-	-	-	-	-	-	-	-
ET 16	+++	-	-	-	-	-	-	+	-	-	-	-	-
ET 17	++	-	-	-	-	-	-	-	-	-	-	-	-
ET 18	+	-	-	-	-	-	-	-	-	-	-	+	-
EC	+	-	-	-	-	-	-	-	-	-	-	-	-
AH	++	-	-	-	-	-	-	-	-	-	-	-	-
CF	+	-	-	-	-	-	-	-	-	-	-	-	-
ET	+++	-	-	-	-	-	-	-	-	+	-	-	-
PE	++	-	-	-	-	-	-	-	-	-	-	-	-
STA	++	-	-	-	-	-	-	-	-	-	-	-	-
YE	+++	-	-	-	-	-	-	-	-	-	-	-	-

Keywords: 1: Sea Anemone (*Radianthus ritteri*), 2: *Coriandrum sativum*, 3: *Cinnamomum zeylanicum*, 4: *Piper nigrum*, 5: *Elettaria cardamomum*, 6: *Illicium verum*, 7: *Trigonella foenum-graecum*, 8: *Foeniculum vulgare*, 9: *Papaver somniferum*, 10: *Cuminum cyminum*, 11: *Laurus nobilis*, 12: *Carum carvi*, 13: *Brassica junicea*

prepared holes. The plates were then incubated at room temperature for 24 h. After 24 h, the forming inhibition zones were measured. All the tests were run in 3 replicates. In the present study, not a single aqueous extracts of spice was able to inhibit the growth of all tested bacteria. However, it was interesting to note that only methanol extract of *Radianthus ritteri* was found capable to inhibit the growth of all the present isolates. *Elettaria cardamomum*, *Illicium verum*, *Trigonella foenum-graecum*, *Laurus nobilis* and *Brassica junicea* showed no antimicrobial property activities against the present isolates. Extract of *Coriandrum sativum* could only inhibit the growth of ET 7 whereas both extract of *Piper nigrum* and *Papaver somniferum* were only able to inhibit 2 out of 25 isolates. Both extracts of *Cinnamomum zeylanicum* and *Cuminum cyminum* possess antimicrobial property against 3 present isolates. While, extract of *Foeniculum vulgare* could inhibit the growth of 4 present isolates. Extract of *Carum carvi* could inhibit the bacterial

growth of 5 out of 25 isolates in the present study. To our knowledge, this is the first reported antimicrobial property of *R. ritteri* against pathogenic bacteria isolated from aquaculture sites and other reference strains. So far, a study of isolation and characterization of cytolysins from *R. ritteri* was conducted by the study of Kam *et al.* [6]. Cytolysin is a substance that produced by an organism for immunity or served as venom. However, further study should be conducted to analyse the existence compound in the methanol extract of *R. ritteri*. Many studies of antimicrobial property of spices were conducted. For instance the study of Yano *et al.*[5] showed that both extracts of *C. sativum* and *C. cyminum* could inhibit the growth of *V. parahaemolyticus* and *Escherichia coli*; food borne pathogens. However, there was no study reported on the extract of spices against fish pathogenic bacteria. In the present study, although extract of *C. carvi* can only inhibit the growth of 5 out of 25 isolates fish bacteria but this extract showed the most active inhibitory

properties against bacterial isolates among the tested spices. In the near future, other method of spice extraction should be conducted to reveal the antimicrobial property of spice.

Diameter Zone: +=10-15mm, ++ = 16-20 mm, +++ => 20 mm

ET 1-18 = *Edwardsiella tarda* isolated from diseased freshwater fish, EC = *Escherichia coli* (ATCC 25922), CF = *Citrobacter freundii* (ATCC 8090), AH = *Aeromonas hydrophila* (ATCC 49140), PE = *Pseudomonas aeruginosa* (ATCC 35032), STA = *Streptococcus agalactiae* (ATCC 13813), ET = *Edwardsiella tarda* (ATCC 15947) and YE = *Yersinia enterocolitica* (ATCC 23715)

REFERENCES

1. Sarter, S., H.N.K. Nguyen, L.T. Hung, J. Lazard and D. Montet, 2007. Antibiotic resistance in Gram-negative bacteria isolated from farmed catfish. *Food Control*, 18: 1391-1396.
2. Bansemir, A., Blume Maja, S. Schroder and U. Lindequist, 2006. Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture*, 252: 79-84.
3. Defoirdt, T., N. Boon, P. Sorgeloos, W. Verstraete and B. Peter, 2007. Alternatives to antibiotics to control bacterial infections: luminescent vibriosis in aquaculture as an example. *Trends in Biotechnology*, 25 (10).
4. Daud, A., A. Gallo and A. Sanchez Riera, 2005. Antimicrobial properties of *Phrygilanthus acutifolius*. *Journal of Ethnopharmacology*, 99: 193-197.
5. Yano, Y., M. Satomi and H. Oikawa, 2006. Antimicrobial effect of spices and herbs on *Vibrio parahaemolyticus*. *Int. Journal of Food Microbiol.*, 111 (1): 6-11.
6. Kam, W.K., H.E. Khoo, M.C.M. Chung and P. Gopalakrishnakone, 1994. Purification and characterization of cytolytins from the sea anemone. *Radianthus Ritteri*. *Toxicon*, 32 (5): 531.