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# *In vitro* antimicrobial activities of *Colocasia esculenta* extract against *Vibrio* spp. – short communication

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This study was revealed antimicrobial property of *Colocasia esculenta* against 5 strains of *Vibrio* spp. The increasing of antibiotic resistance cases among pathogenic bacteria such as *Vibrio* spp. posed a problem to aquaculture industry. Most of the antibiotics were no longer effective in controlling pathogenic bacteria in aquaculture. Therefore, this study was carried out to seek the potential of *C. esculenta* to alternate commercial antibiotic as antimicrobial agent against *Vibrio* spp. Three parts (leaf, stem and legume) of *C. esculenta* were extracted by using methanol and distilled water. The extractions were subjected to antimicrobial test by using disk diffusion method. *Vibrio alginolyticus*, *V. cholera*, *V. harveyi*, *V. parahaemolyticus* and *V. vulnificus* were the bacterial strains that applied in the present study. The results of the present study indicated that only leaf of *C. esculenta* extracted using distilled water shows antimicrobial activity against all the tested bacterial strains whereas other extractions did not possess antimicrobial property against the tested bacteria. This study revealed the huge potential of aqueous extract of *C. esculenta* to alternate commercial antibiotic as antimicrobial agent for aquaculture use.

Key words: *Colocasia esculenta, Vibrio* spp., methanol extract, aqueous extract

## INTRODUCTION

The development of drug resistance in bacterial fish pathogens has been reported from all areas of aquaculture (Storey 2005). Several studies on antibiotic resistance in shrimp have also been carried out. It was also reported that marine bacteria such as *Vibrio harveyi*, *V. alginolyticus*, *V. vulnificus*, *V. cholerae*, and *V. parahaemolyticus* isolated from cultured shrimp are resistant to antibiotics such as erythromycin, kanamycin, penicillin and streptomycin (Roque et al. 2001). The use of oxytetracycline has caused increased bacterial resistance in shrimp farms (Molina-Aja et al. 2002). In addition, increased of bacterial resistance to chloromphenicol has also emerged through indiscriminate use of antibiotics in shrimp hatcheries in Ecuador and the Philippines (Tendencia and de la Pena 2001). Currently, Malaysian government has banned five commercial antibiotics to be used in aquaculture industry; they were chloramphenicol, furazolidone, oxolinic acid, sulphamethoxazole and oxytetracycline (Arthur pers. comm.). Therefore, Malaysian shrimp farmers are restricted by her government to apply those antibiotics in aquaculture practice. Due to the increasing of antibiotic resistance incidences among pathogenic bacteria in Malaysian shrimp farms and most of commonly used antibiotics have been banned by government, Malaysian farmers face big problems in terms of prevention and treatment against these bacteria in aquaculture. Furthermore, application of commercial antibiotics might involve environmental hazard as well as costly (Roque et al. 2001). Vibriosis due to *Vibrio* bacteria is one of the most damaging problems affecting the economic viability and long term sustainability of the marine aquaculture industry. To save crops from disease, farmers are often left with no choice but to resort to treatment with antibiotics, be it legal or illegal. The misuse and overuse of antibiotics over the years has led to prevalent antibiotic resistance not only in *Vibrio* bacteria but also other disease causing bacteria in aquatic animals and human around the world. Besides, antibiotic residue in fish and shrimp products also become a major public health concern, and have resulted in rejection of fish and shrimp products in the European Union, United States of America and Japan. In the present study, to overcome the problems, the antimicrobial property of *Colocasia esculenta* (L) Schott has been investigated to reveal the potential of this plant as an alternative source to commercial antibiotics used in aquaculture.

## MATERIAL AND METHODS

In the present study, five *Vibrio* spp; *Vibrio harveyi*, *V. alginolyticus*, *V. vulnificus*, *V. cholerae*, and *V. parahaemolyticus* which was previously isolated from shrimps were tested for their sensitivity against leaf, stem and legume of *Colocasia esculenta* extracts. The plants were collected from a rural area of Mengabang Telipot in Terengganu, Malaysia. The plant extract was previously reported to be effective against human bacteria such as *Klebsiella pneumoniae* (Nair et al. 2004). They were cleaned and dried in oven at 50 $^{0}$C. The plants were cut into small pieces and 20g of each part of plant were extracted with 150 ml of aqueous and methanol (Ates and Erdougrul 2003). The extraction was filtered using a filter paper (Whatman No. 1, England) and evaporated to dryness in an air current (Daud

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et al. 2005). The extracts were reconstituted to a concentration of 250mg/ml in solvent (methanol or aqueous) (Daud et al. 2005). All the extracts obtained were injected into empty sterile antibiotic disks of 6 mm diameter in amounts of 20 µl (Ates and Erdogrul 2003). Disks injected with 20 µl of solvent served as negative control (Ates and Erdogrul 2003). All the tested bacteria; *V. alginolyticus, V. cholerae, V. harveyi, V. parahaemolyticus* and *V. vulnificus* were inoculated into tryptic soy broth (TSB) (Merck, Germany) for 24 h. Using sterile cotton swabs the bacterial cultures were transferred into a sterile saline solution tube and was mixed until turbidity was equivalent to a 1.0 Mc Farland standard. Then, a sterile swab was inserted into the solution and pressed on the inner part of the tube above the solution level to remove excess fluid. The swab was smeared across the middle of the plate and then the smear was continued in a zig-zag pattern across the plate. The procedure was repeated until every square millimeter of the Mueller Hinton (Oxoid, England) agar was completely covered with thin, even layer of bacteria. The extract disks were placed evenly over the surface of the plate with a sterile forcep. When completed, the plate was labeled and incubated for 24 h. At the end of the incubation period, inhibition zone formed on the agar were measured in mm using a transparent ruler. Then, the results were recorded. In the present study, the extraction of *C. esculenta* was used to investigate its antimicrobial properties against five species of *Vibrio* isolated from shrimps.

## RESULTS AND DISCUSSION

Table 1 showed diameter of inhibition zone of five *Vibrio* spp. against extraction of *C. esculenta* by using aqueous and methanol as solvents. All five tested *Vibrio* spp. were sensitive to all extraction of *C. esculenta* leaf using aqueous as solvent. However, all isolates in the present study were resistant to stem and legume extractions using aqueous solvent as well as leaf and legume extractions using methanol solvent. The largest inhibition zone observed in present study was 12 mm, followed by 11, 9 and 8 mm.

Excessive application of antibiotics in aquaculture has raised up several human health issues. For instance, the need for consumer protection from hazardous usage and issues surrounding consumer acceptance of the use of chemicals in the production of fish and shellfish destined for human consumption. Environmental concerns, such as the effects of chemicals from antibiotics on water as well as sediment and the generation of multi drug resistant strains of bacteria have serious impacts on aquaculture industry (Lee et al. 2003). For instance, Ansary et al. (1992) reported that *Aeromonas hydrophila* isolated from various fish species and several geographical locations in Malaysia were found to posscess multiple resistance, most commonly to ampicillin and carbenicillin. Hence, development of new antimicrobial from natural sources, could lead to reduction of use of therapeutants, and synthetic chemicals in aquaculture practice. Thus, new antimicrobial from nature needs to be established. In the present study, all tested *Vibrio* spp were sensitive to *C. esculenta* leaf extraction by using aqueous solvent. *Vibrio harveyi* recorded the largest inhibition zone of diameter (12mm) to *C. esculenta* leaf extract. A study by Nair et al. (2004) showed *Klebsiella pneumoniae* was sensitive (6 mm) to *C. esculenta* leaf extract by using methanol as solvent. In the study of Voravuthikunchai et al. (2004), among 58 crude aqueous and ethanolic medicinal plants extracts, only 14 extracts (24.14%) of 8 plant species were demonstrated to have antimicrobial activity against *Escherichia coli*. The inhibition zones ranged from 7 to 17 mm and the concentration of plants extracts that was applied in that study was 2.5 mg/disk. Another study of Mothana and Lindequist (2005) reported medicinal plants extracted using chloroform, methanol and water solvents were effective to mainly Gram positive bacteria. However, none of the extracts showed antimicrobial activity against Gram negative bacteria. The dosage of medicinal plants extracts applied in that study was 4 mg/disk and the inhibition zones were ranged from 8 to 28 mm. In contrast by using commercial antibiotics, Roque et al. (2001) reported that the largest (mean 29.5 mm) and the smallest (mean 11.4 mm) inhibition zones were observed among 144 isolates of *Vibrio* spp. isolated from shrimp against enrofloxacin at 5 µg/disk and ampicillin at 10 µg/disk, respectively. In another study, Molina-Aja et al. (2002) found that *Vibrio* strain isolated from shrimp demonstrated smaller inhibition zones using 4 types of antibiotics such as ampicillin, amikacin, carbenicilin and cephalotin against *Vibrio* spp. Their results showed that the mean of the inhibition zones of *Vibrio* spp. in that study were 7.00, 7.00, 9.85, and 10.12 mm against ampicillin (10 µg/disk), amikacin (30 µg/disk), carbenicillin (100 µg/disk) and cephalothin (30 µg/disk), respectively. Dosage of *C. esculenta* leaf aqueous extracts applied in the present study was 5 mg/disk and has effect on the growth of tested *Vibrio* spp. with the inhibition zones ranged from 6 to 12 mm.

## CONCLUSION

From these results, it can be suggested that *C. esculenta* leaf extracts has a great potential as an alternative to antimicrobial compounds against shrimp pathogenic bacteria. On top of that, *Vibrio* spp. showed sensitiveness to the extraction of *C. esculenta*, thus, this plant could probably be used in prevention of vibriosis outbreak in shrimp farmings.

**Table 1: Inhibition zone diameter of 5 *Vibrio* spp against *Colocasia esculenta* extraction using methanol and aqueous solvents**

| Vibrio spp. | Inhibition Zone Diameter Around Test Disc (mm) | | | | | |
|---|---|---|---|---|---|---|
| | Leaf | | Stem | | Legume | |
| | M | A | M | A | M | A |
| Vibrio alginolyticus | - | 9 | - | - | - | - |
| Vibrio cholera | - | 11 | - | - | - | - |
| Vibrio harveyi | - | 12 | - | - | - | - |
| Vibrio parahaemolyticus | - | 9 | - | - | - | - |
| Vibrio vulnificus | - | 8 | - | - | - | - |

Keys: M = Methanol A = Aqueous

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