Kasetsart J. (Nat. Sci.) 45 : 70 - 77 (2011)

# Effects of Ethanol Tumeric (*Curcuma longa* Linn.) Extract Against Shrimp Pathogenic *Vibrio* spp. and on Growth Performance and Immune Status of White Shrimp (*Litopenaeus vannamei*)

**Ong-ard Lawhavinit[1]*, Pronchai Sincharoenpokai[1] and Patcharee Sunthornandh[2]**


## ABSTRACT

The effects of ethanol turmeric extract against shrimp pathogenic *Vibrio* spp. were investigated. It was found that ethanol turmeric extract inhibited the tested *Vibrio* spp. and the minimum inhibitory concentrations of ethanol turmeric extract on *Vibrio harveyi, V. cholera, V. parahaemolyticus, V. alginolyticus, V. vulnificus* and *V. fluvialis* were 0.47, 0.47, 0.94, 0.47, 3.75 and 0.47 mg/disc, respectively. The effects of ethanol turmeric extract on survival rate, feed conversion ratio and growth rate of white shrimp were examined using different levels of ethanol turmeric extract in feed. The results showed that there were no significant ($P > 0.05$) differences in survival rate and water quality. However, the percentage of average day growth and weight gained were significantly ($P < 0.05$) higher with feed that contained ethanol turmeric extract at 7.5 and 15 g/kg of feed when compared with the control. The efficacy of ethanol turmeric extract as an immunostimulant for white shrimp showed that the total hemocyte count, phenoloxidase activity and bactericidal activity increased as the levels of ethanol turmeric extract in feed increased. The ratio of ethanol turmeric extract that gave the best immunostimulatory effect was 15 g/kg of feed. It was found that the effect of ethanol turmeric extract at a ratio 15 g/kg of feed on the survival rate of white shrimp immerged with *Vibrio harveyii* was significantly ($P < 0.05$) higher than the control group.


**Keywords:** *Curcuma longa* Linn, ethanol turmeric extract, anti-*Vibrio* spp. activity, minimum inhibitory concentrations, immunostimulant.

## INTRODUCTION

The widespread presence of antibiotics has caused major community concerns about the development of antibiotic resistant strains in humans and about food safety. Consequently, this has resulted in a major impact on the efficiency of aquaculture production. White shrimp producers in all parts of the world face increasing legislative and consumer pressure to reduce the use of antibiotics which are chemically related to antibiotics that are used as therapeutic drugs in humans. In recent years, herbal plant supplements have been developed as an innovation and could provide a reliable long term answer for the industry facing the antibiotics ban. Turmeric (*Curcuma longa* Linn.), a plant of the family Zingiberaceae, grown mainly in Thailand, has been used in Thai

1 Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.
2 Department of Microbiology, Faculty of Science, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: fvetonl@ku.ac.th

Received date : 15/06/10 Accepted date : 16/11/10

herbal medicine for the treatment of various diseases. There are several reports indicating a variety of pharmacological activities of turmeric, such as antioxidant (Masuda *et al.,* 2001, 2002; Das and Das, 2002), anti-protozoal (Araujo *et al.,* 1998), anti-microbial (Negi *et al.,* 1999), anti-venom (Ferreria *et al.,* 1992), anti-HIV (Sui *et al.,* 1993), anti-tumor (Ozaki *et al.,* 2000; Kim *et al.,* 2001), anti-inflammatory (Ammon and Wahl, 1991; Surh *et al.,* 2001), hepatoprotective (EL-Ansary *et al.,* 2006), anti-allergic (Yano *et al.,* 2000), anti-ulcer (Rafatullah *et al.,* 1990), anti-dyspeptic (Deitelhoftt *et al.,* 2002) and anti-depressant (Yu *et al.,* 2002). However, to date, there have been no scientific investigations to determine the safety and efficacy of plant-based medicines as feed additives. Therefore, the purpose of this experiment was to investigate the effects of ethanol turmeric extract on *Vibrio* spp. as a bacterial inhibitor, growth promoter and immune response stimulator in white shrimp (*Litopenaeus vannamei*).

## MATERIALS AND METHODS

### Ethanol turmeric extract

Rhizomes of turmeric (*Curcuma longa* Linn.; Zingiberaceae) were collected from Kanchanaburi province, Thailand. A voucher specimen, BK 63868, was deposited at the Bangkok Herbarium, Department of Agriculture, Bangkok, Thailand. The dry rhizomes of *Curcuma longa* L. (1.15 kg) were extracted with ethanol (6 L) for 8 h by Soxhlet extractor. The solution was evaporated to dryness under vacuum to produce ethanol turmeric extract. The ethanol turmeric extract weighed 230 g, which was 20.0 % of the green weight, and was a dark red gum. All the curcuminoid analysis was carried out by the Natural Products and Organic Syntheses Research Unit (NPOS), Department of Chemistry, Faculty of Science, Kasetsart University, Thailand.

### Bacterial strains and inoculum preparation

*Vibrio harveyi, V. cholera, V. parahaemolyticus, V. alginolyticus, V. vulnificus* and *V. fluvialis* isolated from black tiger shrimp (Lawhavinit *et al.*, 2006) were used in the present study. Each strain of bacteria was cultured on 1.5 % NaCl nutrient agar (Difco) and incubated at 35–37 °C for 18–24 h. The concentrated inocula were adjusted in 4 mL Brain heart infusion to the McFarland standard No. 0.5 (approximate cell density $1.5 \times 10^8$ CFU/mL). Bacterial samples were inoculated on Mueller Hinton agar (MHA; Difco) using the swab plate technique.

### Sensitivity and determination of minimum inhibitory concentrations (MICs)

The Kirby-Bauer method (Bauer *et al.*, 1966) was used for sensitivity testing of the ethanol turmeric extract. Ethanol turmeric extract was dissolved with dimethylsulfoxide (DMSO; Crown Zollerbach, Washington) at 1 g/mL. The minimal microbiocidal concentration of DMSO generally was severalfold higher than the minimal inhibitory concentration (MIC), except for certain species which appeared to be ultrasensitive to this agent, such as *Corynebacterium* sp., *Haemophilus influenzae*, *Pasteurella multocida*, *Herellea* sp., *Mycobacterium tuberculosis* Var. BCG, and *Microsporum audouini* (Basch and Gadebusch, 1968). Therefore, the solvent, pure DMSO, of each extract was used as a negative control and 30 μg per disc of tetracycline (Oxoid) was used as a positive control. Concentrated extracts of turmeric were added at twofold serial dilution with DMSO (0.244 to 1000 ppt). Each solution dilution was applied to sterile filter paper discs (Whatman grade AA discs, size 6 mm in diameter) and placed on the surface of the assay plates, which were then incubated at 37 °C for 24 h. The MIC values were taken as the lowest concentration of extract that completely inhibited bacterial growth or the clear zone of the disc that was bigger than the negative control (pure DMSO) disc.

**Animals and diets**

Four hundred one-month old, postlarva 45, white shrimp with an average weight of 0.25 g were used and divided into four groups, with two replications per group. Each group contained 50 shrimp. Feed was provided at 2.5 % of the body weight, 3 times per day, for 9 w. The 50 % culture water was changed every week. All shrimp received a starter diet containing 32 % protein and 3,100 kcal ME/kg. Four experimental diets were randomly assigned to the groups: 0, 3.75, 7.5 and 15 g ethanol turmeric extract/kilogram of feed.

**Parameter measurement**

Growth rates were recorded up until 9 w. The survival rate, body weight gain, percentage average day growth and feed conversion ratio were calculated weekly for each individual group, based on 10 shrimp per pond. Shrimp were cultured in circular concrete ponds, 1.2 m in diameter, containing saline solution at a concentration of 10 parts per thousand (ppt.). Dissolved oxygen, pH, total ammonia and nitrite contents were checked with a water quality test kit (Advance Parma Company). Mortality, temperature and salinity were monitored daily and recorded. Differences between mean values of the parameters measured were analyzed by one way ANOVA. Significance was tested at $P = 0.05$ and means were separated by Duncan's multiple range test (Steel and Torrie, 1980).

**Efficacy of ethanol turmeric extract as an immunostimulant for white shrimp**

After 9 w, a blood sample of 1 mL from the ventral sinus was collected from each of 15 shrimp from each individual group and mixed into 0.5 mL of 3.8 % sodium citrate. The samples were measured for total hemocyte count, phenoloxidase specific activity (Soderhall and Hall, 1984), bactericidal activity (Tunkjjanukij and Olafsen, 1998) and survival rate of 9-week old shrimp fed with 15 g ethanol turmeric extract/kilogram of feed, after 1-hour immersion in $10^6$ CFU/mL of *Vibrio harveyi*. Differences between mean values of the parameters measured were analyzed by one way ANOVA. Significance was tested at $P = 0.05$ and means were separated by Duncan's multiple range test (Steel and Torrie, 1980).

## RESULTS AND DISCUSSION

**Sensitivity and minimum inhibitory concentration of ethanol turmeric extract**

Figure 1 shows the inhibition zone of *Vibrio cholerae* with turmeric extract, negative control (no inhibition zone), and tetracycline. The ethanol turmeric extract showed inhibitory effects for *Vibrio harveyi, V. cholera, V. parahaemolyticus, V. alginolyticus, V. vulnificus* and *V. fluvialis* (Table 1). Lutomoski *et al*. (1974), Bhavanishankar and Murthy (1986) and Negi *et al*. (1999) reported the antibacterial activity of turmeric oil extracted from spent turmeric oleoresin against *Bacillus cereus, B. coagulans, B. subtilis, Staphylococcus aureus, Esherichia coli* and *Pseudomonas aeruginosa*. However, the present results were similar to Supamattaya *et al*. (2005) who reported ethanol turmeric extract inhibitory effects for *Vibrio* spp.

The MIC values of ethanol turmeric extract against *Vibrio harveyi, V. cholera, V.*

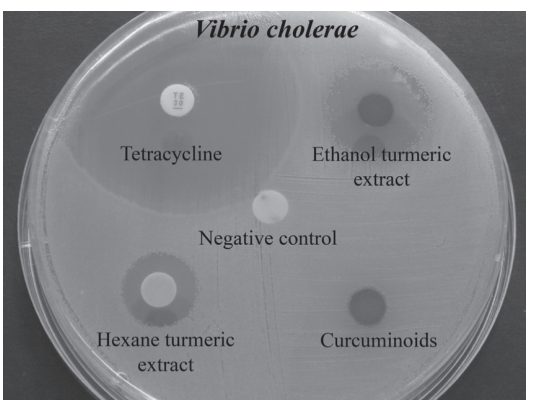


**Figure 1** Inhibition zone of *Vibrio cholerae* with turmeric extract, negative control and tetracycline.

*parahaemolyticus, V. alginolyticus, V. vulnificus* and *V. fluvialis* were 0.47, 0.47, 0.94, 0.47, 3.75 and 0.47, respectively. The results showed that the ethanol turmeric extract could inhibit *Vibrio* spp. in shrimp. This result indicated that the ethanol turmeric extract has high potential for it inhibitory effects and was effective with many species of *Vibrio,* as reported by Lutomoski *et al*. (1974). Therefore, this study provided more information on the inhibitory effect of ethanol turmeric extract and its activity with more species of bacteria.

**Growth performance after using ethanol turmeric extract**

A completely randomized design was used to study four experimental diets; 0, 3.75, 7.5 and 15 g ethanol turmeric extract/kilogram of feed that were randomly assigned to each group. The results showed that the groups fed with ethanol turmeric extracts had no significant ($P > 0.05$) differences in survival rate and feed conversion ratio of white shrimp when compared to the control. However, the body weight gain and percentage average day growth (Table 2) were significantly ($P < 0.05$) different. The survival rate of mixed feed with 15 g/kg ethanol turmeric extract (74 ± 4 %) was tested by the paired t-test and was significantly ($P < 0.05$) different when compared to the control (48 ± 6 %). The results suggested that ethanol turmeric extract in the diet could not improve the feed conversion ratio in white shrimp, but the addition of 15 g/kg ethanol turmeric extract in the feed could improve body weight gain, percentage average day growth and survival rates. This was consistent with the results reported for *Macrobrachium rosenbergii* de Man (Wanprapa, 2004).

The mean values ± SD of temperature, salinity, pH, dissolved oxygen, total ammonia and nitrite were 26.93 ± 0.27°C, 10.68 ± 0.33 ppt, 7.07 ± 0.07, 7.03 ± 0.05 ppm, 0.18 ± 0.07 ppm and 0.05 ± 0.02 ppm, respectively. There was no

**Table 1** Results of inhibitory effects, (mean ± SD mm; n = 3) as measured by the diameter of the inhibition zone of ethanol turmeric extract and tetracycline against *Vibrio* spp.

| Bacteria | Ethanol turmeric extract | Tetracycline |
|---|---|---|
| *Vibrio harveyi* | 23.33 ± 0.09 | 47.17 ± 0.12 |
| *Vibrio cholerae* | 21.37 ± 0.49 | 43.00 ± 0.12 |
| *Vibrio parahaemolyticus* | 21.50 ± 0.29 | 40.93 ± 0.47 |
| *Vibrio alginolyticus* | 22.37 ± 0.26 | 42.77 ± 0.39 |
| *Vibrio vulnificus* | 22.23 ± 0.12 | 40.91 ± 0.36 |
| *Vibrio fluvialis* | 24.17 ± 0.03 | 43.67 ± 0.43 |

**Table 2** Percentage survival rate, weight gain, percentage average day growth (ADG) and feed conversion ratio (FCR) in white shrimp, after feeding with ethanol turmeric extract for 9 w. (Data shown are mean ± SD; n=10).

| Extract (g/kg) | %Survival rate | Weight gain (g) | ADG (g) | FCR |
|---|---|---|---|---|
| 0 | 48± 6 | 0.81±0.04$^{a}$ | 1.29±0.07$^{a}$ | 1.19±0.08 |
| 3.75 | 57±15 | 1.33±0.11$^{ab}$ | 2.11±0.17$^{ab}$ | 0.97±0.07 |
| 7.50 | 59± 1 | 1.54±0.12$^{b}$ | 2.44±0.19$^{b}$ | 1.06±0.07 |
| 15.00 | 74± 4 | 1.78±0.24$^{b}$ | 2.83±0.37$^{b}$ | 1.16±0.20 |

Means within the same column with the same superscript or no superscript are not significantly different ($P > 0.05$).

statistical difference between the water quality of the control and the treatment ponds during the period of white shrimp culture. Therefore, the water was suitable for shrimp culture, in agreement with the report by Lawhavinit *et al*. (2006). However, the presence of ammonia at 5.24 mg/L is a serious cause of a reduction in phenoloxidase and causes mortality in shrimp (Lui and Chen, 2004), but in the present study the level was well below this and thus, the water quality was safe for shrimp.

**Efficacy of ethanol turmeric extract as an immunostimulant for white shrimp**

After 9 w, 15 shrimp from each group were measured for total hemocyte count, phenoloxidase specific activity and bactericidal activity. The percentage survival rate of shrimp that had been fed for 9 w with 15 g/kg of ethanol turmeric extract was determined after immersion for 1 h in $10^6$ CFU/mL of *Vibrio harveyi*. The results showed that there was no significant ($P > 0.05$) difference in the total hemocyte counts among the groups fed with ethanol turmeric extracts when compared with the control. However, the phenoloxidase activity (Table 3) was significantly ($P < 0.05$) different for the 7.5 and 15 g/kg concentrations of ethanol turmeric extract. Soderhall and Hall (1984) reported phenoloxidase activity was very important for crustacean immunity. From the present study, the highest phenoloxidase activity was shown with 15 g/kg. The blood plasma of treated shrimp could inhibit *Vibrio harveyi, V. cholera, V. parahaemolyticus, V. alginolyticus, V. vulnificus* and *V. fluvialis*. The results showed that the group fed 15 g/kg also had the highest bactericidal activity with different species and concentrations of ethanol turmeric extracts (Table 4). The survival rate of the group fed 15 g/kg ethanol turmeric extract after

**Table 3** Total hemocyte count ($10^6$ cell/mL) and prophenol oxidase activity (unit/min/mg protein) in white shrimp after feeding with ethanol turmeric extract for 9 w. (Data shown are mean ± SD; n=15).

| Extract (g/kg) | Total hemocytes | Prophenol oxidase activity |
|---|---|---|
| 0 | 9.0± 0.4 | 315.0±25.0[a] |
| 3.75 | 10.0±12.0 | 324.0±26.0[a] |
| 7.50 | 10.0± 0.4 | 388.5±10.5[ab] |
| 15.00 | 14.3± 1.7 | 412.5±14.5[b] |

Means within the same column with the same superscript or no superscript are not significantly different ($P > 0.05$).

**Table 4** Percentage bactericidal activity in shrimp blood after feeding with ethanol turmeric extract for 9 w. (Data shown are mean ± SD % inhibition; n=3).

| *Vibrio* sp. | Ethanol turmeric extract | | | |
|---|---|---|---|---|
| | 0 g/kg | 3.75 g/kg | 7.50 g/kg | 15.00 g/kg |
| *Vibrio harveyi* | 0 | 9.84±0.02 | 18.03±0.01 | 78.69±0.01 |
| *V. cholerae* | 0 | 58.17±0.03 | 80.39±0.04 | 88.89±0.03 |
| *V. parahaemolyticus* | 0 | 27.33±0.05 | 40.00±0.01 | 66.67±0.02 |
| *V. alginolyticus* | 0 | 30.43±0.02 | 69.57±0.02 | 85.22±0.23 |
| *V. vulnificus* | 0 | 61.86±0.03 | 80.72±0.02 | 84.95±0.41 |
| *V. fluvialis* | 0 | 42.42±0.02 | 100 | 100 |

All mean values in rows are significantly different ($P < 0.05$).

immersion for 1 h in $1\times10^6$ CFU/mL of *Vibrio harveyi* was significantly ($P < 0.05$) different compared with the control (Table 5), which indicated that the ethanol turmeric extract played an importance role in white shrimp immunity, as was also reported for *Penaeus monodon* after feeding with ethanol turmeric extract (Vanichkul *et al*., 2007).

## CONCLUSION

Turmeric (*Curcuma longa* Linn.), a plant of the family Zingiberaceae, grown mainly in Thailand, has been used in Thai herbal medicine for the treatment of various diseases. Several reports have indicated a variety of pharmacological activities of turmeric. Thus, in this study, the efficiencies of ethanol turmeric extract were evaluated by investigating its inhibition of 6 *Vibrio* spp. isolated from shrimp by an in vitro study, and the minimum inhibitory concentrations of these ethanol turmeric extracts. The results showed that the ethanol turmeric extract from *Curcuma longa*, which contained 20.0 % by dry weight of curcumin showed inhibitory effects against the six species of *Vibrio*: *Vibrio harveyi, V. cholera, V. parahaemolyticus, V. alginolyticus, V. vulnificus* and *Vibrio fluvialis*. The minimum inhibitory concentrations of ethanol turmeric extract on *Vibrio harveyi, V. cholera, V. parahaemolyticus, V. alginolyticus, V. vulnificus* and *V. fluvialis* were 0.47, 0.47, 0.94, 0.47, 3.75 and 0.47 mg/disc, respectively. The effects of ethanol turmeric extraction on the percentage survival and growth rate of white shrimp were examined using different feeding levels of ethanol turmeric extract. The results showed that there was no significant ($P > 0.05$) difference in the percentage survival rate and water quality. However, the percentage of average daily growth and weight gained were significantly ($P < 0.05$) higher with feed that contained turmeric at 7.5 and 15 g/kg feed when compared with the control. The efficacy of ethanol turmeric extract as an immunostimulant for white shrimp was indicated by increases in the total hemocyte count, phenoloxidase activity and bactericidal activity as the level of ethanol turmeric extract in the feed increased. The highest amount of ethanol turmeric extract in feed was 15 g/kg feed. It was found that the effect of ethanol turmeric extract at the rate of 15 g/kg feed on the survival rate of white shrimp immerged with *Vibrio harveyi* was significantly ($P < 0.05$) higher than the control group. The results indicated that ethanol turmeric extract has high potential for its inhibitory effects with some *Vibrio* spp. of shrimp. Therefore, this study provided more information on the inhibitory effects of ethanol turmeric extract, which can be used in the study of potentially useful sources of antimicrobial compounds.

## ACKNOWLEDGEMENTS

The authors would like to thank the Kasetsart University Research and Development Institute (KURDI) for financial support.

**Table 5** Survival rate (%) after feeding with 15 g/kg ethanol turmeric extract for 9 w of white shrimp after immersion for 1 h in $10^6$ CFU/mL of *Vibrio harveyi*. (Data shown are mean ± SD; n=20).

| Treatment | Number of dead shrimp (piece) | % Survival rate |
|---|---|---|
| 0 g/kg | 13 | $35.0\pm10.0^a$ |
| 15 g/kg | 8 | $62.5\pm 7.5^b$ |

Means within the same column with the different superscripts are significantly ($P < 0.05$) different.

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