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Efficacy of Thai Traditional Herb Extracts against Fish and Shrimp Pathogenic Bacteria

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Sixteen species of Thai traditional herbs were selected for this study. They were *Andrographis paniculata, Cassia alata, Clinacanthus nutans, Eclipta alba, Momordica charantia, Phyllanthus acidus, P. amarus, P. debilis, P. pulcher, P. reticulatus, P. urinaria, Psidium guajava, Tinospora cordifolia, T. crispa* and white and red strains of *Ocimum sanctum*. Using an agar plate dilution method, they were tested for antibacterial activity against the pathogenic bacteria: *Aeromonas hydrophila*, a *Streptococcus* species and 10 strains of *Vibrio*. Eleven of the tested herbs showed antibacterial activity. Among them, *P. guajava* and *M. charantia* displayed the highest activity against *Vibrio harveyi* and *V. parahaemolyticus*. The minimal inhibitory concentration (MIC) of *P. guajava* against the tested bacteria was found to be 0.625 mg/m*l*, while the MIC of *M. charantia* was 1.25 mg/m*l*.

Key words: herb, antibacterial activity, fish pathogenic bacteria, shrimp pathogenic bacteria

Bacterial disease is a serious problem in aquaculture and various antibiotics are sometimes applied as treatments. Habitual use of antibiotics can lead to problems with bacterial resistance and with unacceptable residues in aquaculture products and environment.

For centuries before the discovery of microorganisms, vegetable drugs were used in many parts of the world as folklore remedies, and claims have thus been made that certain plants have the power to heal wounds and remove inflammation. They have been also claimed to be effective for the treatment of enteric diseases such as cholera.

Disyaboot (1975) conducted experiments to test the antibacterial activity of 63 Thai medicinal plants against *Bacillus subtilis, Escherichia coli, Lactobacillus fermentum, Pseudomonas aeruginosa, Salmonella typhi, Shigella dysenteriae, Staphylococcus aureus*, and *Streptococcus faecalis*. The results showed that 62 species of medicinal plants or herbs could inhibit the growth of these organisms. Therefore, it was of interest to determine whether herbs might be used as an alternative treatments for bacterial diseases in aquatic animals. Since many species of herbs are used as human foods, e.g., holy basil (*Ocimum sanctum*), guava (*Psidium guajava*) and *Momordica charantia*, it is probable that their use would be safe for aquatic animals and for human consumers of aquatic animal products.

The purpose of this study was to determine the efficacy of Thai traditional herbs against fish and shrimp pathogenic bacteria so that they might eventually be developed as alternative controls for bacterial diseases in aquaculture.

Materials and Methods

Preparation of herb extracts

Sixteen species of Thai traditional herbs were selected for this study. They were *Andrographis paniculata, Cassia alata, Clinacanthus nutans, Eclipta alba, Momordica charantia, Phyllanthus acidus, P. amarus, P. debilis, P. pulcher, P. reticulatus*, and *P. urinaria, Psidium guajava, Tinospora cordifolia, T. crispa* and white and red strains of *Ocimum sanctum*. Each plant was dried and extracted by ethanol using a soxhlet apparatus. The crude extract was further prepared as complex granules with polyvinylpyrolidone (PVP) to promote easy dissolution of the herb extracts in antibacterial tests.

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Bacteria

Twelve strains of pathogenic bacteria were used in this study. These included 9 strains of *V. harveyi*, and one strain each of *V. parahaemolyticus*, *Streptococcus* sp. and *Aeromonas hydrophila*. *V. harveyi* and *V. parahaemolyticus* were maintained in trypticase soy agar supplemented with 1.5% NaCl. *Streptococcus* sp. and *Aeromonas hydrophila* were maintained in trypticase soy agar without additional NaCl.

Antibacterial tests

Antibacterial tests was performed by an agar plate dilution method according to Tragen (1983). Serial two-fold dilutions of guava extract were prepared to furnish concentrations from 0.625 to 10 mg/m*l*. One milliliter of each dilution was mixed with 9 m*l* of Muller Hinton Agar supplemented with 2% NaCl and dispensed into agar plates. For the control, polyvinylpyrolidone (10 mg/m*l*) was used instead of herb extract. To prepare the inoculum for the test plates, bacterial strains were precultured at 30°C for 18 h in trysticase soy broth supplemented with 2% NaCl. The preculture broth (0.1 m*l*) was then mixed with 1 m*l* of the same broth before overlayering on the test plates. Antibacterial activity was determined by observation of the growth of bacteria after incubation at 30°C for 24 h.

Results

The results shown in Table 1 indicate that the extract of *P. guajava* had the highest activity against *A. hydrophila* (MIC 0.63 mg/m*l*) among 16 tested herbs. Also for *A. hydrophila*, the MIC of *M. charantia* was 1.25 mg/m*l* and that of 3 other herbs was 2.5 mg/m*l* (i.e., *E. alba*, *P. reticulatus* and *P. urinaria*).

Streptococus sp. was unaffected by any herbal extract in the range of 0.62–1.25 mg/m*l*. However, *M. charantia* showed antimicrobial activity at 2.5 mg/m*l*, *A. paniculata* and *P. guajava* at 5 mg/m*l*, and *E. alba* and *P. reticulatus* at 10 mg/m*l*.

With respect to activity against *V. harveyi* (Table 2), *M. charantia* was the most effective herb (i.e., 1.25 mg/m*l* for 7 bacterial strains and 2.5 mg/m*l* to 2 strains)., *P. guajava* inhibited 4 strains at 2.5 mg/m*l* and 5 strains at 5 mg/m*l*. MICs for the other 5 herbs were between 5 to 10 mg/m*l*.

Five herbs showed activity against *V. parahaemolyticus* at 5 mg/m*l* or a lower concentration. The MIC of *M. charantia* and *P. guajava* was 1.25 mg/m*l*, and that of *A. paniculata*, *E. alba* and *P. reticulatus* was 5 mg/m*l*.

The percentage of tested fish and shrimp pathogenic bacterial strains inhibited by extracts from the 16 herbs tested is shown in Table 3. Overall, *M. charantia* was the most effective herb since it inhibited 75% of the

Table 1. MIC of 16 species of Thai traditional herbs against *Aeromonas hydrophila*

Herb species	Concentration of herb (mg/m*l*)						
	0	0.31	0.63	1.25	2.5	5	10
Andrographis paniculata	–	–	–	–	+	+	+
Cassia alata	–	–	–	–	–	–	–
Clinacanthus nutans	–	–	–	–	–	–	–
Eclipta alba	–	–	–	–	+	+	+
Momordica charantia	–	–	–	+	+	+	+
Ocimum sanctum (red)	–	–	–	–	–	–	–
O. sanctum (White)	–	–	–	–	–	–	–
Phyllanthus acidus	–	–	–	–	–	–	–
P. amarus	–	–	–	–	–	–	–
P. debilis	–	–	–	–	–	–	+
P. pulcher	–	–	–	–	–	–	–
P. reticulatus	–	–	–	–	+	+	+
P. urinaria	–	–	–	–	+	+	+
Psidium guajava	–	–	+	+	+	+	+
Tinospora cordifolia	–	–	–	–	–	–	+
T. crispa	–	–	–	–	–	–	–

Table 2. MIC of 16 species of Thai traditional herbs against 9 strains of *Vibrio harveyi*

Herb species	Concentration of herb (mg/ml)						
	0	0.31	0.63	1.25	2.5	5	10
Andrographis paniculata	-	-	-	-	-	+(1)	+(7)
Cassia alata	-	-	-	-	-	-	-
Clinacanthus nutans	-	-	-	-	-	-	-
Eclipta alba	-	-	-	-	-	+(2)	+(9)
Momordica charantia	-	-	-	+(7)	+(9)	+(9)	+(9)
Ocimum sanctum (red)	-	-	-	-	-	-	-
O. sanctum (White)	-	-	-	-	-	-	-
Phyllanthus acidus	-	-	-	-	-	-	-
P. amarus	-	-	-	-	-	-	-
P. debilis	-	-	-	-	-	+(2)	+(9)
P. pulcher	-	-	-	-	-	-	-
P. reticulatus	-	-	-	-	-	+(2)	+(9)
P. urinaria	-	-	-	-	-	+(1)	+(9)
Psidium guajava	-	-	-	+(1)	+(4)	+(9)	+(9)
Tinospora cordifolia	-	-	-	-	-	-	-
T. crispa	-	-	-	-	-	-	-

( ) = Number of sensitive strains

Table 3. Percentage of fish and shrimp pathogenic bacterial strains tested that were inhibited by different concentrations of extracts from 16 Thai traditional herbs.

Herb species	Concentration of herb (mg/ml)						
	0	0.31	0.63	1.25	2.5	5	10
Andrographis paniculata	0	0	0	0	8.3	33.3	83.3
Cassia alata	0	0	0	0	0	0	8.3
Clinacanthus nutans	0	0	0	0	0	0	0
Eclipta alba	0	0	0	0	8.3	33.3	100
Momordica charantia	0	0	0	75	100	100	100
Ocimum sanctum (red)	0	0	0	0	0	0	0
O. sanctum (White)	0	0	0	0	0	0	0
Phyllanthus acidus	0	0	0	0	0	0	0
P. amarus	0	0	0	0	0	0	8.3
P. debilis	0	0	0	0	0	16.7	91.7
P. pulcher	0	0	0	0	0	0	8.3
P. reticulatus	0	0	0	0	8.3	33.3	100
P. urinaria	0	0	0	0	8.3	16.7	91.7
Psidium guajava	0	0	8.3	25	50	100	100
Tinospora cordifolia	0	0	0	0	0	0	8.3
T. crispa	0	0	0	0	0	0	0

tested strains at 1.25 mg/m*l* and 100% at 2.5 mg/m*l*. The second most effective herb was *P. guajava* in that 17% of the tested bacterial strains were inhibited at 0.63 mg/m*l* and 25, 50 and 100% were inhibited at 1.25, 2.5 and 5.0 mg/m*l*, respectively. *A. paniculata*, *E. alba*, *P. reticulatus* and *P. urinaria* had lower (i.e., MIC levels from 2.5 to 10 mg/m*l*) and similar inhibitory activities against the studied pathogens. At 2.5 mg/m*l* they inhibited only 8.3% of the tested strains but at 10 mg/m*l* they inhibited 83.3% or more strains. *P. debilis* inhibited 16.7 and 91.7% of the bacterial pathogens at 5 and 10 mg/m*l*, respectively. *C. alata*, *P. amarus*, *P. pulcher* and *T. cordifolia* had the least antibacterial activity since their extracts could inhibit only 8.3% (i.e., 1 strain of bacteria) at 10 mg/m*l*. By contrast, *C. nutans*, *O. sanctum* (red and white strains), *P. acidus* and *T. crispa* had no inhibitory effect on the tested bacterial pathogens.

Discussion

A. paniculata is one of the most famous medicinal herbs for treatment of bacterial infections among Thai and Chinese people. It is also used in China for the treatment of enteritis in freshwater fish (Rath, 1990). In addition, ethanol extracts of this herb have been reported to be effective against α-*Streptococcus* group (Laopaksa *et al.*, 1988). However, in this study *A. paniculata* extracts could not inhibit most of the tested bacterial strains even at the highest concentration of 10 mg/m*l*, indicating that *A. paniculata* is not effective against fish and shrimp bacterial pathogens.

The antibacterial activity of extracts from the leaves of *C. alata* was quite low as it could inhibit only one strain of *Streptococcus* sp. A similar result was reported by Avirutnant and Pongpan (1983) who found that alcohol extracts of *C. alata* were effective against *B. subtilis* but not against *S. aureus*, *E. coli* and *S. typhi*.

All of the tested bacteria (100%) were inhibited by extracts from *E. alba* at 10 mg/m*l*. Concentrations of 2.5 and 5 mg/m*l* inhibited 1 (8.3%) and 10 (83.3%) of the tested strains, respectively. This herb was reported to be effective for treatment of dysentery in humans (Chulasiri *et al.*, 1985) and also for protection of *Macrobrachium rosenbergii* from infections with *Aeromonas hydrophila* (Dung, 1990).

The essential oil of holy basil (*O. sanctum*) had been shown to have antifungal and antibacterial activities (Dey and Choudhuri, 1981). However, in this study, extracts from leaves of both the red and white varieties had no inhibitory effect on any of the tested bacteria.

Many species of *Phyllanthus* have been widely used in herbal medicine systems (Unander *et al.*, 1990). Our results demonstrated that extracts from 2 species of *Phyllanthus*, *P. debilis* and *P. reticulatus*, had antibacterial activity. On the other hand, Avirutnant and Pongpan (1983) found that water or ethanol extracts from the roots of *P. reticulatus* had no activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Shigella dysenteriae*, or *Salmonella typhosa*. In our experiments, extracts from leaves of this plant did inhibit all the tested bacteria. This suggests that antibacterial activity of this herb resides in the leaves.

M. charantia and *P. guajava* were found to be the most effective herbs against fish and shrimp pathogenic bacteria in the present study. It was found by Sindermsuk *et al.* (1989) that *P. guajava* could inhibit *V. cholerae* and *V. parahaemolyticus*, the causative agents of diarrhea in humans. However, *M. charantia* has been reported to possess higher antibacterial activity than *P. guajava* against *B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. dysenteriae* and *S. typhi* (Disyaboot, 1975). In this study, *M. charantia* extract inhibited 70% of the tested bacteria at 1.25 mg/m*l* while it inhibited 100% at 2.5 mg/m*l*. By contrast, *P. guajava* inhibited 50% of the tested bacterial strains at 5 mg/m*l*.

Anyway, *M. charantia* and *P. guajava* seem to be the most promising herbs for the control of fish and shrimp bacterial diseases, although further studies on the *in vivo* efficiency of these herbs are needed.

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