Antibacterial activity of herbal plant extracts towards the fish pathogens

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Abstract

Fifteen plant extracts were screened against Vibrio harveyi, V. parahaemolyticus and A. hydrophilla using disc method. Acetone extract of Lawsonia inermis Linn. had high antibacterial activity against three tested fish pathogens. Survival rate of 80% was observed when the P. monodon nauplii were exposed to the low concentration of 0.025 mg/ml in L. inermis extract. Due to its less toxic effect to shrimp and potent antibacterial activity the acetone extract of L. inermis could form one of the best options for developing novel antimicrobial compounds for ecofriendly management of disease caused by V. harveyi, Vibrio parahaemolyticus and hydrophila.

INTRODUCTION

Shrimp farmers are loosing millions of dollars annually due to the large scale mortality of shrimp due to diseases caused by antibiotic resistance pathogens. Antibiotics resistance of microorganisms is a global concern (Austin, 1993). Antibiotic resistant V. harveyi caused mass mortality in Penaeus monodon larvae in a hatchery (Karunasager et al., 1994). The frequency of resistance reflects the pattern of antibiotic usage. Plant secondary metabolites show activity in the micro to submicromolar range to Gram-positive species (Gibbons, 2004). Plant extracts decrease the selective pressure for developing antibiotic resistance (Lewis & Ausubel, 2006). Quinine and berberine from plants remain highly effective to fight against human microbial infections. Today plant materials are present in, or have provided the models for 50% of western drugs (Robbers et al., 1996) 5. Hence, in the present study plants containing antibacterial compound against Vibrio harveyi, V. parahaemolyticus and A. hydrophila were screened and their toxicity to shrimp, Penaeus monodon and Artemia were studied.

MATERIAL AND METHODS

Vibrio harveyi, V. parahaemolyticus and A. hydrophila were obtained from the Central Institute for Brackishwater Aquaculture (CIBA), Chennai and maintained on Zobell's marine agar slants (Himedia, Mumbai). The agar-disc diffusion method of Bauer et al. (1966) was followed for testing the sensitivity of the isolates to 7 standard antibiotics against Vibrio harveyi on Mueller Hinton agar supplemented with 2% sodium chloride. The plates were inoculated with 24 h cultures from Zobell marine broth. The discs were then placed on the agar plates and incubated at 30 1o C for 20 h.

The leaves of freshly harvested plants were washed and chopped with a knife to pieces. About 10g of chopped leaves were immersed in 50ml of acetone and kept in a shaker overnight at 100 rpm speed. The extract was filtered using Whatman no:1 filter paper. The filtrate was concentrated by keeping the extract in the oven at 40°C. 100 mg of concentrated extract was dissolved in 1ml of acetone. Briefly paper discs of 5mm diameter were prepared using Whatman filter paper (No:1) and sterilized. 5 l of plant extracts was impregnated on the discs (500 g/disc) and was dried at room temperature. In the same way control disc was also prepared by using acetone. Plants that showed antibacterial activity against Vibrio harveyi, V. parahaemolyticus and A. hydrophila were selected. Disc at different concentration of plant extracts (5 l, 10 l, 20 l, 40 l and 80 l) were prepared and dried at room temperature. In the same way control disc was also prepared by using acetone. Antibacterial activity was measured using disc diffusion method. P. monodon, Artemia nauplii were collected from a hatchery located in the east coast of India. They were maintained in the glass tank with aeration. They were separated into 5 groups and each containing 100 nauplii per bowl. The crude extract was added in each bowl at different concentrations (0.025, 0.05, 0.1, 0.2 and 0.4mg/ml). The extracts were prepared in 2% ethanol. The survival rate of the nauplii was observed after

24h.

RESULTS AND DISCUSSION

Out of fifteen plant extracts screened, L. inermis and T. indica had antibacterial compound against V. harveyi,Vibrio parahaemolyticus and Aeromonas hydrophila. Adhatoda vasica, Azadirachta indica and Nelumbium speciosum showed antibacterial activity against Vibrio parahaemolyticus and Aeromonas hydrophila as seen in Table 1. Plants have broad spectrum antibacterial activity against human pathogens (Silva et al., 1997 and Navarro and Delgado, 1999). V. cholerae was sensitive to ethanol extract from Terminalia macroptera (Silve et al., 1997). Samy and Raja (1996) reported that V. parahaemolyticus and V. damsela were resistant to 16 aqueous plant extracts.

Figure 1

Table 1: Antibacterial activity of plant extracts against

Sl. No.	Plants	Family	Antibacterial activity		
			Vh	Vp	Ah
1	*Anacardium occidentale,* Linn.	Anacardiaceae	-	-	-
2	*Adhatoda vasica Nees*	Acanthaceae	-	+	+
2	*Anona squamosa,* Linn.	Annonaceae	-	-	-
3	*Azadirachta indica, A.* Juss.	Meliaceae	-	+	+
4	*Cardiospermum halicacabum,* Linn.	Sapindaceae	-	-	-
5	*Carica papaya,* Linn.	Caricaceae	-	-	-
6	*Clitoria ternatea,* Linn.	Fabaceae	-	-	-
7	*Coriandrum sativum,* Linn.	Umbelliferaceae	-	-	-
8	*Crataeva religiosa,* Forst.	Capparidaceae	-	-	-
9	*Hibiscus rosasinensis,* Linn.	Malvaceae	-	-	-
10	*Indigofera tinctoria,* Linn.	Fabaceae	-	-	-
11	*Lawsonia inermis,* Linn.	Lythraceae	+	+	+
12	*Murraya koenigii,* Spreng.	Rutaceae	-	-	-
13	*Nelumbium speciosum,* willd.	Nymphaceae	-	+	+
14	*Rosa damascena,* Mill.	Rosaceae	-	-	-
15	*Tamarindus indicus,* Linn.	Caesalpiniaceae	+	+	+

Figure 2

Table 2: Antibacterial activity of plant acetone extracts against

Concentration (µl)	Zone of inhibition (mm)	
	L. ineimis	*T. indica*
5	7 .0 ± 0	6.0±0
10	7.33 ± 0.47	6.67 ± 0.47
20	7.66 ± 0.47	7.33 ± 0.47
40	8.33 ± 0.47	10.33 ± 1.88
80	10.33 ± 0.47	12.33 ± 2.05

Each value is mean ± SD of three values

Figure 3

Table 3: Antibacterial activity of plant extracts against

Plant	Concentration (µl)					Rank
	5	10	20	40	80	
A. indica	8 .0± 0	8.33 ± 0.47	9.33 ± 1.25	10 ± 0.82	10.33 ± 1.25	III
A. vasica	-	6.0 ± 0	6.33 ± 0.47	6.67 ± 0.47	7.33 ± 0.47	V
L. inermis	8.66 ± 0.47	9.67 ± 0.47	10 ± 0.82	10.33 ± 0.94	11.0 ± 0.82	I
N. speciosum	6.0 ± 0	6.66 ± 0.47	7.0 ± 0	7.33 ± 0.47	7.67 ± 0.47	IV
T. indicus	0.66 ± 0.47	7.33 ± 0.47	7.67 ± 0.47	8.33 ± 0.47	10.67 ± 0.47	II

Each value is mean ± SD of three values

Figure 4

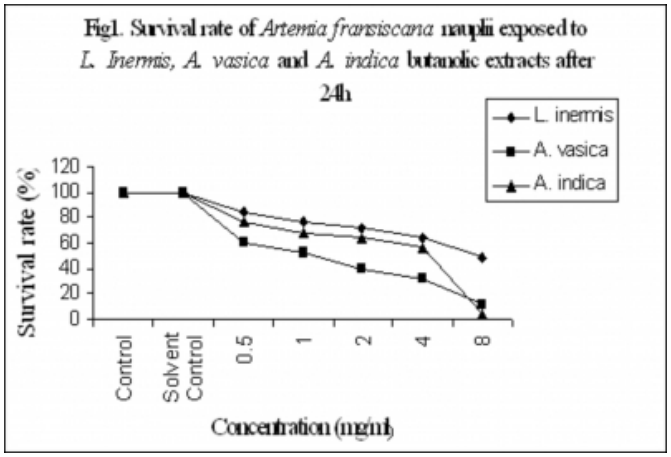
Table 4: Antibacterial activity of herbal plant extracts against

Plants	Concentration (µl)						Rank
	Control	5	1	2	4	8	
A. vasica	-	10±1.0	11.66±0.6	12.0±0.0	13.33±1.2	13.67±0.6	II
A. indica	-	8.33±0.6	8.66±0.6	10.0±0.0	11.0±1.0	12.66±0.6	III
L. inermis	-	10.67±2.1	12.0±2.2	13.67±2.1	14.0±1.0	15.33±1.2	I
N. speciosum	-	-	6.33±0.6	7.3±0.6	8.0±0.0	8.67±0.6	V
T. indicus	-	7.67±2.3	9.67±2.3	10.33±1.2	11.0±1.4	11.33±1.2	IV

Each value is the mean standard deviation of minimum three observations.

L. inermis showed high antibacterial activity against V. harveyi (Table 2). V. parahaemolyticus was sensitive to Lawsonia inermis with 11 mm inhibition zone as in Table 3. L. inermis extracts had high antibacterial activity against A. hydrophilla with antibacterial activity of 15.3mm at 80 l as in Table 4. Earlier reports showed the presence of bioactive compounds in L. inermis. The ethyl acetate extract of L. inermis showed broad spectrum of antibacterial activity (Ali et al., 2001). Tuberculostatic activity of the L. inermis was reported by Sharma (1990). Survival rate of 80% was observed at the concentrations of 0.025 mg/ml. of L. inermis extract. L. inermis extract was less toxic to P. monodon nauplii. The maximum survival rate of 48% was observed when the Artemia nauplii were exposed to L. inermis extract at 16 mg/ml after 24h(Fig.1).

Figure 5



Spray-dried preparation of Tetraselmis suecica inhibited shrimp pathogenic strains of Vibrio sp., V. alginolyticus, V. anguillarum, V. parahaemolyticus and V. vulnificus (Austin and Day,1990). Veterinary pathogens have been inhibited by herbal medicines (Ernst, 1998). Aloe vera and A. spicata were used for chicken health management (Marizvikuru et al., 2005). V. anguillarum was inhibited by the dichloromethane extracts of Asparagopsis armata, Ceramium rubrum, Drachiella minuta, Falkenbergia rufolanosa, Gracilaria cornea and Halopitys incurvus (Bansemir et al., 2006). The pathogens A. hydrophila and V. alginolyticus were inhibited by H. scoparia, L. acaciae, and P. harmala (7-20.5 mm) (Bansemir et al., 2006). A. hydrophila, P. fluorescens and Myxobacteria spp. exhibited maximum sensitivity to Aquaneem in terms of percentage reduction of bacterial cell population in comparison to E. coli (Das et al., 1999). Due to its less toxic effect to shrimp and potent antibacterial activity the acetone extract of L. inermis could form one of the best options for developing novel antimicrobial compounds for ecofriendly management of disease caused by V. harveyi, Vibrio parahaemolyticus and Aeromonas hydrophila.

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