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ANTIBACTERIAL ACTIVITY OF *CURCUMA LONGA* ON FISH MICROBIAL PATHOGENS

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Antibacterial properties of turmeric, *Curcuma longa* were tested against virulent pathogenic isolates of *Aeromonas hydrophila*, *Pseudomonas aeruginosa*, *P. fluorescens*, *Edwardsiella tarda*, *Escherichia coli* and *Flavobacterium columnare*. Aqueous and ethanolic extracts of *C. longa* were tried for the antibacterial study. Both the extracts showed moderate to high sensitivity against *A. hydrophila* (10-14 mm), *P. aeruginosa* (12-16 mm), *P. fluorescens* (8-15 mm), *E. coli* (9-15 mm), *E. tarda* (10-12 mm) and *F. columnare* (12-14 mm), respectively, as measured by zone of inhibition test. On silica gel column chromatography, ethanolic extract of *C. longa* were fractionated. Out of 10 fractions collected, 4 fractions showed antibacterial properties against these pathogenic bacteria. Both the aqueous and ethanolic extracts of turmeric can serve as therapeutics for controlling bacterial infection in aquaculture practice.

INTRODUCTION

Turmeric (*Curcuma longa* L. Zingiberaceae) is a native of tropical Asia, specifically India. Sometimes turmeric is also called "Indian saffron" because of its brilliant yellow colour. It has been used as a dye, medicine and flavour agent since 600 B.C. Turmeric is used extensively in the Indian medical systems (Ayurveda, Unani and Siddha) and officially entered in the Ayurvedic Pharmacopoeia of India (API, 1989). In both the Ayurvedic and Siddha systems of medicine, a turmeric paste is used topically as well as internally to treat ulcers and scabies (Charles and Charles, 1992). It also serves as a disinfectant and antiseptic in treatment of wounds and used extensively in traditional Chinese and Japanese medicines (PPRC, 1992; JSHM, 1993). Turmeric or its constituents curcumin are known to have antibacterial, antifungal and anti-inflammatory activities and are used as home medicines in Indian households (Arora *et al.,* 1971; Ghatak and Basu, 1972; Lutomski *et al*., 1974; Banerjee and Nigam, 1978; Satoskar *et al*., 1986).

Diseases due to bacteria become discrimental in the culture practices. Aeromonasis, pseudomonasis, columnaris and edwardsiellosis are the major bacterial diseases caused by *Aeromonas* spp., *Pseudomonas* spp., *Flavobacterium* spp. and *Edwardsiella tarda*, respectively. These are frequently encountered in the pond culture system (Kumar *et al*., 1986; Karunasagar *et al.,* 1989; Kumar and Dey, 1991). Various antibiotics like

oxytetracycline, chloramphenicol, sulfonamides and nitrofurans are routinely used in aquaculture. However, use of these antibiotics cause adverse effect on aquatic ecosystem and fish. The constant use of these drugs and chemicals lead to residual effect and resistivity risk to all livestock including human (Spanggaard *et al.*, 1993; Das *et al.*, 1999). Hence, attention is being given for the use of alternatives in terms of herbal drugs in aquaculture practices. The potential use of certain herbal materials like neem, turmeric, garlic etc. for controlling fish diseases have been evaluated (Dey, 1997; Das *et al.*, 1999). The present study evaluated the fractionated products of turmeric against fish and shrimp pathogenic bacteria as possible substitute to antibiotics used in aquaculture.

MATERIAL AND METHODS

Sample preparation and extraction

The rhizome of turmeric (*Curcuma longa*) was bought from local market, washed and sun-dried. The turmeric made into powder by hammer and grinder, and then sieved to form uniform powder. Ethanolic extract of turmeric was prepared by soaking 50 g of the powder with 200 ml of the solvent for 24 h at ambient temperature. After incubation, it was filtered using Whatman filter paper No 40 and made solvent-free by using a Rotavapor (Büchli rotary evaporator-11). The same procedure was followed for aqueous (aqueous: alcohol, 3: 1) extraction. All the crude extracts were stored in darkness at 4^0C.

Silica gel column chromatography

The active ethanolic extract (0.5 g) was chromatographed over silica gel (100-200 mesh size) column. The elution was carried out successively with methanol/chloroform (5%, 20%, 50%, 70%), 100% methanol and 100% ethanol, and 150 ml of each fraction were collected. The fractions were reduced to 5 ml by distillation and total 10 fractions were collected. These are Fr 1 (1:33 :: MeOH:Chlo), Fr 2 (1: 19 :: MeOH:Chlo), Fr 3 (1:4 :: MeOH:Chlo), Fr 4 (1:4 :: MeOH:Chlo), Fr 5 (1:1 :: MeOH:Chlo), Fr 6 (1:1 :: MeOH:Chlo), Fr 7 (7:3 :: MeOH:Chlo), Fr 8 (7:3 :: MeOH:Chlo), Fr 9 (MeOH) and Fr 10 (MeOH). According to their TLC behaviour, it was observed that the eluents of Fr 3 and Fr 4 are showing similar spots on TLC plates. The eluents of 1:4 :: MeOH:Chlo (Fr 3, Fr 4) and 1:1 :: MeOH:Chlo (Fr 5, Fr 6) were showing similar spots on TLC plate, whereas other chromatographic fractions were different from each other. Finally eight fractions were collected from silica gel column chromatography of crude ethanolic extract of turmeric and the details of these fractions are represented with code numbers and R_f values in Table 1.

$$R_f \text{ value} = \frac{\text{Distance traveled by the compound}}{\text{Distance travelled by the solvent}}$$

Table 1. Fractionated products of *C. longa* showing R_f values

Extract used	Solvent of elution		Code No. of fractions collected	TLC-R_f values of spots	Wt. of fractions (g)
T EtOH	1	MeOH :Chlo (1:33)	TEF1	0.73	0.02
	2	MeOH :Chlo (1:19)	TEF2	0.61, 0.38	0.08
	3	MeOH :Chlo (1:4)	TEF3	0.51	0.07
	4	MeOH :Chlo (1:1)	TEF4	0.4	0.04
	5	MeOH :Chlo (7:3)	TEF5	0.42	0.05
	6	MeOH :Chlo (7:3)	TEF6	0.6, 0.77	0.03
	7	MeOH	TEF7	0.39	0.01
	8	MeOH	TEF8	0.71	0.04

Microorganisms

The pathogenic bacteria namely *Aeromonas hydrophila* (AH1, AH2), *P. aeruginosa* (PA1, PA2, PA3, PA4), *Pseudomonas fluorescens* (PF1, PF2, PF3, PF4), *Edwardsiella tarda*, *Escherichia coli* (O1, O115, O156, O164, O111, O109) and *Flavobacterium columnare* isolated and maintained in the laboratory of the Institute were used in this study.

Antibacterial sensitivity test

Antibacterial activity of the extracts was assessed following disc diffusion method (Acar, 1980). Pure bacterial cultures of *A. hydrophila*, *E. tarda*, *P. aeruginosa*, *P. fluorescens*, *E. coli* and *F. columnare* were first grown separately in nutrient agar (NA) plates for overnight at 37^0C. The cultures were then incubated in 50 ml of BHI broth and incubated in a shaking incubator (100-150 rpm) at 30^0C for 24 h. A concentration of 10^6 CFU/ml was made by the standard dilution technique. An inoculum of approximately 0.1 ml (10^5 CFU) of each test pathogen culture was aseptically transferred to agar plates, which were prepared by pouring 15 ml of sterilized molten nutrient agar uniformly spreaded with the help of a sterilized spreader. Pilot screening of the extracts was carried out by impregnating 20 µl (300 µg) of extract (in respective solvent of extraction) to 6 mm diameter sterile whatman No. 1 filter paper discs and firmly placed over NA plates after evaporation of the solvent under aseptic condition. The plates were incubated for 24 h at 37^0 C. The discs loaded with solvent (20 µl) for dissolution were taken as control after evaporation of the solvent. Triplicate sets were maintained for each microorganism. The zone of inhibition around the disc (average of 3 experiment) were measured and the assay was scored positive (+) if it was <5 mm, double positive (++) if ≥ 5 mm and <10 mm, triple positive (+++) if ≥10 mm and negative (-) if there was no inhibition of microbial growth. The antibacterial activities of turmeric extracts were compared with inhibition

zones around three commercial antibacterial discs i.e. Fluconazole (Fu), Clotrimazole (Cc) and Cephalexin (Cp) (Hi-Media, India) that were used as references.

RESULTS AND DISCUSSION

From the detail screening of crude extracts and fractions of turmeric against different strains of *Pseudomonas*, it was observed that crude ethanolic extracts showed maximum zone of inhibition (14, 15, 16 mm) against *Pseudomonas aeruginosa* (PA1, PA2, PA3) and *P. fluorescens* (PF1, PF2, PF4). From four active chromatographic fractions of crude ethanolic extracts, TEF1 and TEF2 are more active (15,18,20 mm) against *P. aeruginosa* (PA2) and *P. fluorescens* (PF2, PF4). Other eluents except TEF3 showed moderate to high antibacterial activity against all the strains of *Pseudomonas*. Crude aqueous extract (TAq) of turmeric were active against all the strains of *Pseudomonas*. It showed maximum zone size (13 mm) against *P. aeruginosa* (PA2) and *P. fluorescens* (PF3).

Two strains of *A. hydrophila* (AH1, AH2) and *F. columnare* showed maximum zone of inhibition to crude ethanolic extract of turmeric (TEtOH). Four partial purified fractions (TEF1, TEF2, TEF3, TEF4) were active against all the selected strains with maximum zone size (11, 13 mm). Aqueous extracts of turmeric showed that highest antibacterial activity against *A. hydrophila* (AH1). *E. tarda* and *F. columnare* also showed maximum zone of inhibition (10 mm) to TAq. The detailed results of the antibacterial activity of turmeric extract against *A. hydrophila* (AH1, AH2), *E. tarda* and *F. columnare* are presented in Table 2.

Six strains of *E. coli* (O1, O115, O156, O164, O111 and O109) were selected for antibacterial activity test. Crude ethanolic extract as well as partial purified fractions were screened against above selected pathogens, which showed maximum activity (14, 15 mm) against serotype O1, O115, O164 and O111. While two strains of *E. coli* (O115 and O109) were resistant to crude aqueous extracts of turmeric, other strains of *E. coli* (O111 and O156) showed maximum zone (13,14 mm) of inhibition to TAq (Table 2; Fig. 1). Rotavapour extracts, fractionated ethanolic extracts of turmeric and conventional antibiotics such as Fluconazole (Fu), Clotrimazole (Cc) and Cephalexin (Cp) produced identical zone of inhibition against all the pathogens tested (Table 3).

In vitro extracts of crude turmeric obtained by different methods was studied against *Aeromonas*, *Vibrio*, *Pseudomonas*, *Staphylococcus* and *Streptococcus* (Hota and Dey, 1997). According to them, 25% concentration of crude extract of turmeric was effective while making the crude extract in different solvents except in case of extraction by Soxhlet apparatus method where a low concentration was found effective. Further, they reported that the crude extracts of turmeric in distilled water, acetone and ethanol obtained from "extraction by heat method" and "extraction by rotation and heat treatment method"

gives comparatively better zone of inhibition than the previous one against *Staphylococcus* and *Aeromonas*. However, the crude extracts of turmeric in ethanol and acetone obtained by the "Soxhlet apparatus method" were found to give higher zone of inhibition against *Staphylococcus* and *Aeromonas* only. In the present experiment, crude extracts of turmeric by rotary evaporation showed antibacterial activity to all the fish and prawn microbial pathogens and *E. coli* showing moderate to high sensitivity as revealed from zone of inhibition. However, *Staphylococcus* and *Streptococcus* were not tested in the present experiment.

Table 2. Antibacterial activity of various crude extracts and different fractions of turmeric against fish pathogens

	TEtOH	TEF1	TEF2	TEF3	TEF4	TAq
Disc potency (µg)	300	200	200	200	200	300
P. aeruginosa						
B1	+ + +	+ + +	+ +	+ + +	+ + +	+ + +
B2	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
B3	+ + +	+ + +	+ +	-	+ + +	+ + +
B4	+ +	+ + +	+ +	+ +	+ + +	+ + +
P. fluorescens						
C1	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
C2	+ + +	+ + +	+ + +	+ + +	+ + +	+ +
C3	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
C4	+ + +	+ + +	+ + +	+ +	+ + +	+ + +
A. hydrophila						
A30	+ + +	+ + +	+ +	+ + +	+ + +	+ + +
A31	+ + +	+ + +	+ + +	+ +	+ +	+ + +
E. tarda	+ + +	+ + +	+ +	+ + +	+ +	+ + +
F. columnare	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
E. coli						
O1	+ + +	+ + +	+ + +	+ + +	+ + +	+ +
O115	+ + +	+ + +	+ + +	+ +	+ + +	-
O156	+ +	+ + +	+ + +	+ + +	+ + +	+ + +
O164	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
O111	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +
O109	+ + +	+ + +	+ + +	+ + +	+ + +	+ +

Bhavani Shankar and Murthy (1979) reported both curcumin and the oil fraction suppress growth of several bacteria like *Streptococcus*, *Staphylococcus* and *Lactobacillus*. The aqueous extract of turmetic rhizomes has anti-bacterial effects against beta lactamase producing microorganism (Kumar *et al.,* 2001).

Table 3. Antibacterial sensitivity of different antibiotics against fish bacterial pathogens

Bacteria	*A. hydrophila*		*P. aeruginosa*				*P. fluorescens*				*E. tarda*	*F. columnare*
Antibiotics	AH1	AH2	PA1	PA2	PA3	PA4	PF1	PF2	PF3	PF4		
Fluconazole (Fu), 10 mcg	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +
Clotrimazole (Cc), 10 mcg	+ + +	+ + +	+ + +	+ + +	+ + +	+ +	+ + +	+ + +	+ + +	+ + +	+ +	+ + +
Cephalexin (Cp), 30 mcg	+ + +	+ + +	+ +	+ + +	+ +	+ +	+ + +	+ +	+ +	+ + +	+ + +	+ + +

Note: Zone size = 0 = -, 0- 5=+, 5-10 = + + and 10-20 = + + +

The chloroform and methanol fractions (within 50%) were active against the bacterial pathogen tested. Singh *et al*. (2002) reported the antibacterial activity of turmeric rhizomes extract on pathogenic bacteria *S. aureus*, *S. epidermidis*, *E. coli*, *P. aeruginosa* and *S. typhimurium*. Further they reported highest inhibition in gram positive *S. aureus* as compared to standard antibiotic ampicillin, doxycycline, erythromycin and gentamycin. Since the antibacterial activity of fractionated ethanolic extract has yet not been worked out in the fish microbial pathogens, the result obtained in the present experiment could not be correlated with the work carried out by the earlier workers.

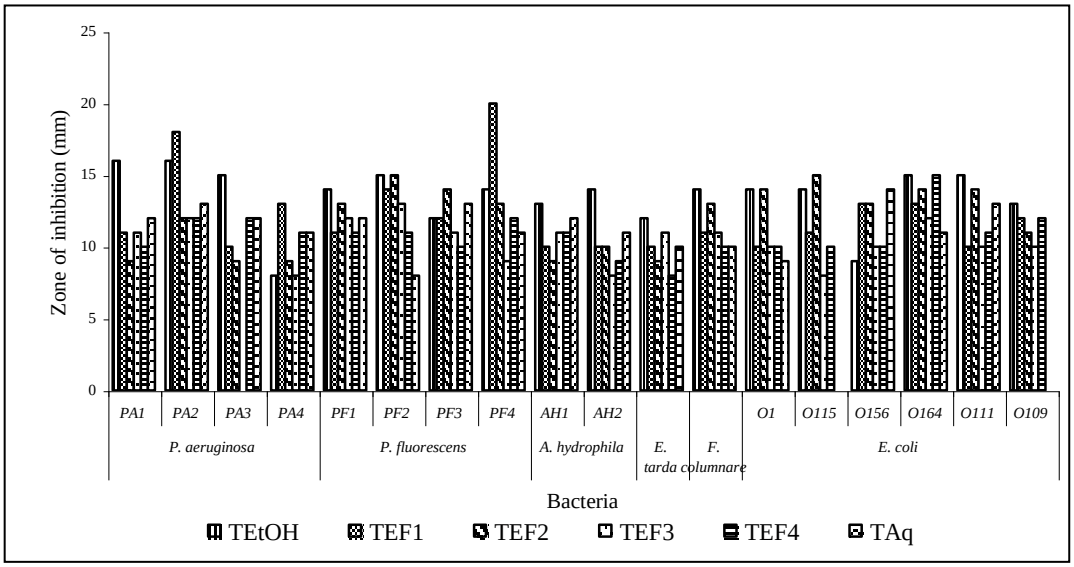

Fig. 1. Antibacterial activity of different crude and fractionated ethanolic extracts of turmeric on fish microbial pathogens

These microorganisms are regarded as important disease-causing agents in freshwater fishes and hence the study assumes remarkable significance in fish health management. The result revealed that a dose of 300 µg were effective for producing moderate to high zone of sensitivity and suppresses the growth of *A. hydrophila*, *P. aeruginosa*, *P. fluorescens*, *E. tarda*, *F. columnare* and *E. coli* mostly due to its bactericidal effect. It was noticed that turmeric also stimulates the immune system of *Labeo rohita* (Sahu, 2004). It is revealed that turmeric whether in crude (aqueous or ethanolic or raw) or fractionated act *in vitro* and *in vivo* for controlling microbial fish pathogens including *E. coli*. Thus, turmeric can be safely recommended in culture system for controlling the fish diseases caused by the bacterial pathogens.

REFERENCES

Acar, J. F., 1980. The disc susceptibility test. *In*: Antibiotics in Laboratory Medicine, (Ed. V.Lorian,). Williams and Wilkins, Baltimore and London, pp. 24-25.

Arora, R. B., N. Basu, V. Kapoor and, A. P. Jain, 1971. Anti-inflammatory studies on *Curcuma longa* (turmeric). *Indian J. Medi. Res.*, **59**: 1289-1295.

API, 1989. Ayurvedic Pharmacopoeia of India API, 1989. Government of India-Ministry of Health and Family Welfare-Department of Health, New Delhi, pp. 45-46.

Banerjee, A. and S. S. Nigam, 1978. Antibacterial efficacy of the essential oil of *Curcuma longa. J. Medi. Res.*, **68**: 864-866.

Bhavani Sankar, T. N. and S. Murthy, 1979. Effect of turmeric (*Curcuma longa*) fractions on the growth of some intestinal pathogenic bacteria *in vitro*. *Indian J. Exp. Biol.*, **17**: 1363-1366.

Charles, V. and S. X., Charles, 1992. The use and efficacy of *Azadirachta indica* (Neem) and *Curcuma longa* (Turmeric) in scabies. A pilot study. *Trop. Geogr. Med.*, **44**: 178-181.

Das, B. K., S. C., Mukherjee, B. B. Sahu and G. Murjani, 1999. Neem (*Azadirachta indica*) extracts as antibacterial agent against fish pathogenic bacteria. *Indian J. Exp. Biol.*, **37**: 1097-1100.

Dey, R. K., 1997. On the use of herbal material for managing diseases & health conditions of fish during sustainable aquaculture practices. National Seminar on Fish Biology, Santiniketan, India, Abstract No. FEN. 6.

Ghatak, N. and N. Basu, 1972. Sodium curcuminate as an effective anti-inflammatory agent. *Indian J. Exp. Biol.*, **10**: 235-236.

Hota, S. K. and R. D. Dey, 1997. Studies on the *in vitro* effect of turmeric as some fish pathogenic bacteria, *Fish. Chimes,* **17**(7): 9-10.

Karunasagar, I., G. M., Rosalin, I. Karunasagar and R. Gopalrao, 1989. *Aeromonas hydrophila* septicaemia of Indian major carps in commercial fish farms of West Godavari district, Andhra Pradesh. *Curr. Sci.*, **58**: 1044-1055.

Kumar, D. and R. K. Dey, 1991. Fish diseases in India. *In:* Aquaculture Productivity (Eds: V. R. P. Sinha and H. C. Srivastava). Oxford & IBH Publishing Co., New Delhi, pp. 345-566.

Kumar, D., J. Farkas and V. R. P. Sinha, 1986. Bacteria from diseased freshwater fish from India, *Aqua. Hung.* **5**: 113-118.

Kumar, S., U. Narain, S. Tripathi and K. Mishra, 2001. Synthesis of curcumin bioconjugates and study of their antibacterial activities against beta-lactamase-producing microorganisms. *Bioconjug. Chem*., **12**: 464-469.

Lutomski, J., B. Kedzia and W. Debaska, 1974. Effect of an alcoholic extract and active ingradients from C*urcuma longa* on bacteria and fungi. *Planta Med*., **26**: 9-19.

PPRC, 1992. Pharmacopoeia of the People's Republic of China. (English Edition Ed. *Tu*). Beijing: Guangdong Science and Technology Press Beijing, pp. 202-203.

Sahu, S., 2004. Antibacterial activity of plant extracts on fish microbial pathogens M. F. Sc. Dissertation, CIFA, Bhubaneswar, India, 237 pp.

Satoskar, R. R., S. J. Shah and S. G. Shenoy, 1986. Evaluation of anti-inflammatory property of curcumin (diferuloyl methane) in patients with post-operative inflammation. *Int. J. Clin. Pharmacol. Ther. Toxicol*., **24**: 651–654.

Singh, R., R., Chandra, M. Bose and P. M. Luthra, 2002. Antibacterial activity of C*urcuma longa* rhizome extract on pathogenic bacteria. *Cur. Sci.,* **83**: 737-740.

Spanggaard, B., F. Jorgensen, L. Gram and H. H. Huss, 1993. Antibiotic resistance in bacteria isolated from three freshwater fish farms and an unpolluted stream in Denmark. *Aquaculture*, **115**: 195-207.

JSHM, 1993. The Japanese Standards for Herbal Medicines. Yakuji Nippo, Tokyo Ltd., 279 pp.