International Journal of Biochemistry and Biotechnology ISSN: 2169-3048 Vol. 1 (6), pp.179-182, August, 2012. Available online at http://internationalscholarsjournals.org © International Scholars Journals

Full length Research paper

Efficiency of natural herbal products used as antimicrobial agents against ornamental fish pathogens

Tariq, A.L [1] and Reyaz, A.L [2]

[1]Department of Microbiology Sree Amman and Science College Erode-638003
[2]Department of Biotechnology Periyar University Salem-636011

Received 1 August, 2012; Accepted August, 2012

Water is an essential requirement as fish cannot live without water. The quality of water plays an important role in the aquatic oriental fish culture. The physical and chemical parameters play major role for the survival of fish in the fish tank. The temperature reduced to 21^{0}C while the hydrogen ion concentration came down to 6.3. The total oxygen level found 6.3 and the hardness of water reached to 105.1mg/ml. The water quality was found contaminated with *Escherichia coli* and *Micrococcus* sp. The fish found died due disease caused by *Pseudomonas* sp, *Vibrio* sp and *Staphylococcus* sp. The extracts of mango leaves showed good antibacterial activity against both water bacterial isolates and diseased fish bacterial isolates by showing zone of inhibition against the bacterial isolates. Thus, showing that mango leaves can be used as an antibacterial agent in the ornamental aquatic fish cultures to prevent the disease.

Key words: Fish pathogen, Mango leaves, Herbal products, Antibacterial activity

INTRODUCTION

Ornamental fish culture trend is a new hope in the development of micro enterprises in urban and periurnban areas (Chen et al., 2001; Neely et al., 2002). Most diseases in fishes are due to poor water quality, which lowered the immune system because the presence of microbes such as *Escherichia coli, Salmonella sp, Shigella* sp*, Proteous* sp*, klebsiella* sp*, Streptococcal* sp, *Staphylococcus* sp*, Micrococcus* sp*, Bacillus* sp (Baticados et al., 1990).*Vibrio ichthyodermis* and *Halophilic vibrio* was found to be responsible for disease in cultured *Plecoglossus altivelis* in Japan (Lavilla et al., 1998; Ferguson et al., 2000). Bacterial fin rote is a contiguous disease, which causes hyperplasia of the respiratory epithelium (Mehdi et al., 2012). *Staphylococcus* aureous isolated from the fish and fishery products were enterotoxigenic and produced enterotoxin A, B, C and D either individually or in combination (Akhlaghi and Keshavarzi, 2002). Some microbes are highly virulent, untreatable, causes epizootic ulceratic syndrome in cultured and wild fresh water fish (Chang et al., 2006). Potential pathogens present in the aquatic environments secrete enzymatic chitinase enabling bacteria to erode chitinous membranes in the fish to multiply and invade the vital organs (Colorni et al., 2002). Transmission of micrococcus sp was tested in vitro on healthy fish and manifestation of ulcers took place within 72hrs (Koh et al., 2004). The various fish and fish products harbours pathogenic organisms like *Salmonella* species, *Vibrio cholera* (Mata et al., 2004). *M. indica* and *M. sylvatia* roub showed anti-bacterial, anti-viral, anti-fungal, anti-protozoal activity (Garcia et al., 2003). *Mangifera indica* exhibits a wide range of pharmacological activities including anti-diabetic, anti-HIV, and anti-cancer, anti-inflammatory, anti-oxidant and anti-viral infects (Akinepelum and Onakoya, 2006). Mangiferin protect brain nerve cell from the oxidative damage of skin and widely used as a nutritional medicine (Bagyalakshmi e al., 2009). Mango leaf extract contain a lot of glycosides, such as mangiferin, isomangiferin, neomangiferin, homomangiferin (Farnaz et al., 2011). Stem bark of *M. indica* has been found to possess anti-helminthic and anti allergic properties (Firas and Hassan, 2008). The polyphenols of mangiferin and epigallocatechin-3-gallate

*Corresponding Author's Email: tariqtasin@gmail.com

have potent effects against protozoan and helminthes parasites infect in mammals (Garrido et al., 2004). The in vitro antimicrobial activity of methanolic and aqueous extract of mangiferin inhibits the growth of *Streptococcus aureus and Proteus valgaris* (Martinez et al., 2000). The anti-microbial activities of methanolic extracts of *M.indica* against gram positive and gram negative organisms at concentration of 20mg/ml (Morales et al., 2008).

MATERIALS AND METHODS

Selection of Tanks

A circular cement tank of size 86cm diameter and 90cm height was selected (Gracia et al., 2003) from fresh water ornamental fish culture unit of Integrated Rural Technology Centre at Erode Tamil Nadu. The selected tank was cleaned with tap water and then sterilized with potassium permanganate solution.

Preparation of Fish Test Tank

The fish tank was prepared by following Garcia et al., (2003). The tank was filled with water up to a height of 30 ± 3 cm and the level was retained throughout the experimental periods. The green mango leaves of weight 100gm/ml was introduced in the tank then ten fishes belongings to two species *Poecilia reticulate* (guppy), C*arassius auratus* (gold fish) were introduced into tank.

Physical and Chemical Parameters

The water samples from the fish tanks were analyzed (Mothana and Lindequist, 2005) at an interval of one day, fifth day, tenth day, fifteenth day, twentieth day for any change in the temperature, hydrogen ion concentration, hardness of water, biological oxygen demand.

Bacterial analysis of water sample

The water sample was taken from the fish tank and serially diluted for plating on nutrient agar (Morales et al., 2008). The dilutions plates of 10^{-4}, 10^{-5} and 10^{-6} were taken and were incubated at 37ºC for 48 hours. The isolated colonies were observed for macroscopic and microscopic morphological examinations then biochemically identified by following Holt et al., (1994).

Isolation of pathogen from disease affected fishes

The fishes died due to disease were taken out from the aquarium tanks by using sterile forceps and placed on a sterile petriplates. The bacteria were isolated (Gosh et al., 2008) by surfacing the fish and fins using sterile cotton wool swab and then swabbed on to a nutrient agar plates.The swabbed nutrient agar plates were incubated at 37ºC for 24 hours. The isolated colonies were identified based on colony morphology and biochemical tests (Holt et al., 1994).

Collection of Mango leaves

Green ripe mango leaves were collected (Chessbrough, 200) from dense mango garden located at erode were washed under tap water, air dried and them homogenized to fine powder.

Preparation of crude extract

The preparation of crude extract was followed (Ncube et al., 2008) by taking 10g of powdered leaf sample soaked in 50ml of ethanol and it was kept in Soxhlet apparatus at 80^0C for 48 hours. This extraction was taken, allowed for evaporation and then concentrated with dimethyl sulfoxide to three different concentration such as 25% (25µg leaf extract + 100µl DMSO), 50% (25µg leaf extract + 100µl DMSO) and 75% (75µg leaf extract + 100µl DMSO) were prepared.

Antibacterial activity of Mango leaves

The antibacterial activity of Mango leaves powder was carried by (Kim et al., 2006) taking sterile paper discs (6mm, Hi-media, Mumbai) loaded with 100µl of 25%, 50% and 75% solution of the crude extracts and were left to dry for 12 hrs at 37ºC in a sterile room. Bacterial suspensions were diluted to match the 0.5 McFarland standard scale 8 (approximately 1.5x10 CFU/ml) and they were further diluted to obtain a final inoculum. The Mueller Hinton agar was poured into petri dishes and allowed for solidification, then inoculated with 100µl of suspension containing 1x10 CFU/ml of bacteria. The discs treated with 25%, 50% and 75% extracts were applied on the medium. The plates were incubated at 37ºC for 24 hrs. After incubation the inhibition zone diameters around each of the disc were measured and recorded.

RESULT

Physical and chemical analysis of water

The maximum temperature was reached to 24.5^0C. There was decrease in the pH value up to 6.3. The total hardness of water was reached to 105.1mg/ml. The dissolved oxygen level reached to 3.5mg/ml. Thus there was not much variation in physical and chemical parameters which are presented in table 1.

Bacterial analysis of water

Bacterial colonies were isolated from fish water tank showed two different colonies. The colony one appeared

Table 1: Physical and chemical parameters observed in the fish tank

Parameters	Intervals of sample taken (days)				
	1	5	10	15	20
Temperature (°C)	24.2	24	23.5	21	24.5
pH	7.8	6.5	6.3	6.4	6.3
Total hardness (mg/l)	85.08	105.1	100.1	105.1	90.09
Dissolved oxygen(mg/l)	6.85	6.25	5.2	4.55	3.5

Table 2: Biochemical tests of colonies obtained from fish water sample and disease affected fish

S/No	Biochemical Test	WS Colny1	WS Colny2	DAF Colony1	DAS Colony2	DAS Colony3
1	Indole production test	+	-	-	+	-
2	Methyl red test	+	-	-	-	+
3	Vogesproskauer test	-	-	-	-	+
4	Citrate utilization test	-	-	+	-	-
5	Triple sugar iron test	A/A	K/A	K/K	A/A	K/K
6	Urease test	+	-	-	-	-
7	Catalase test	+	+	+	+	+
8	H2S Production test	-	-	-	-	-
9	Starch hydrolysis	-	-	-	-	-

Note where= WS--Water sample of fish tank, DAF--Disease effected dead fish,(+)—Positive, (-)- - Negative, K- - Alkaline, A = Acid

white, moist, glistering shape. Under microscopic examinations it appeared gram negative rod shaped bacteria. The colony second looked circular, convex, smooth, yellow colorations. The microscopic examination revealed the gram positive rod shaped bacteria. The biochemical test of colony one and two are presented in table 2 showed the presence of *Escherichia coli* and *Micrococcus* species.

Bacterial analysis of dead fish

In the disease affected dead fish three different colonies were isolated. The colony one showed abundant, thin, white growth with medium turning green in the cultural medium. Under microscopic examination it appeared gram negative rod shaped motile bacilli. The colony morphology of second colony appeared moist, translucent and rounded colonies. The microscopic examinations showed gram negative curved rod shaped active motile bacteria. The colony morphology of third bacterial isolate appeared abundant, opaque, smooth, rounded colonies. Under microscopic examination it appeared gram positive cocci shaped bacteria. The biochemical characters of the three isolated bacteria are presented in the table 2. Thus confirming the presence of *Pseudomonas* sp, *Vibrio* sp and *Staphylococcus* sp respectively.

Microbial Sensitivity Test

After identification of bacterial colonies, they are subjected to microbial sensitivity test by using 25%, 50% and 75% concentration of mango leaves extract. The result shows that all the organisms were sensitive to

Table 3: Antibacterial activity Mango leaf extracts against isolated colonies on water and disease affected fish bacterial pathogens.

Bacterial isolates	Crude extracts of various %	Zone of Inhibition in mm
Escherichia coli	25 50 75	8mm 12mm 16mm
Micrococcus sp	25 50 75	7mm 9mm 14mm
Pseudomonas sp	25 50 75	9mm 13mm 16mm
Vibrio sp	25 50 75	8mm 12mm 16mm
Staphylococcus sp	25 50 75	6mm 8mm 14mm

Where: mm-millimeter

mango leaf extract. The sensitivity test of colonies obtained from water sample and from disease affected fish are shown in table 3.

DISCUSSION

Disease and water quality are the two important problems affecting aquaculture, to overcome these problems a proper and effective methods are needed (Chen et al., 2001). This study was aimed at finding some remedy for these problems. The invivo study reveals that addition of mango leaves to aquaculture tank reduced the bacterial load in water (Gracia et al., 2003). The quality of water was an important factor in aquarium tank, poor water quality lead to disease (Mehdi et al., 2012). All the water quality parameters were maintained in a tolerable level in test fish tank there was not much variation. The temperature reached to 24.5^0C. The total hardness of water was reached to 105.1mg/ml. The dissolved oxygen level reached to 3.5mg/ml (Farnaz et al., 2012). After adding mango leaves to fish test tank, within 5 days there was a notable decrease in pH value to 6.3 (Martinez et al., 2000). The isolated bacteria from water and disease affected fishes were also identified *Escherichia coli*, *Micrococcus* sp, *Pseudomonas* sp, and *Staphylococcus* (Lilly et al., 1998). In vitro study showed that mango leaf extract possessed antibacterial activity against water and fish pathogen at 25%, 50%, 75% concentration with increase in the sensitivity (Parekh and Chanda, 2007). The phytochemical analysis for *Mangifera indica* extract revealed the presence of polyphenols, alkaloids, saponins, tannins these compounds were biologically active (Garrido et al., 2004; Kiyota, 2006). The polyphenol mangiferin shows antibacterial, viral, fungal activity (Akinpelum and Onakoya, 2006). Tannins also inhibit the cell protein synthesis (Bagyalakshmi et al., 2009). Polyphenols are an antioxidant agent that protects the tissue against oxidative stress (Firas and Hassan, 2008). During infection of bacteria, inflammatory response occurs may be attenuated by polyphenols (Mothana and Lindequist, 2005). Development of antibiotic resistance among bacterial population from aquatic environment has been reported previously (Colorni et al., 2002). According to world health organization the medicinal plants would be the best to obtain secondary plant metabolite (Morales et al., 2008). Plants with previous pharmacological applications have been extensively investigated as some of medicinal agents. The extracts of such plants have to be taken and antimicrobial activity studied (Ncube et al., 2008).

CONCLUSION

The mango leaves were showed good antibacterial activity against *Escherichia coli*, *Micrococcus* sp, *Pseudomonas* sp, *Vibrio* sp and *Staphylococcus* sp. Thus paves the new way of treatment against fish pathogens. There was no water contamination after the addition of mango leaves thus suggested that these mango leaves can be used to prevent the water contamination and

control the fish pathogen in the aquatic water.

REFERENCES

Akhlaghi M, Keshavarzi M (2002). Occurrence of *Streptococcosis* in rainbow trout fish farms in Fars Province. Iran. Vet. Res. J. 3(2):183-189.

Akinpelum DA, Onakoya TM (2006). Antimicrobial activities of medicinal plants used in folklore remedies in south-western. Afr. J. Biotech. 5(11): 1078-1081.

Bagyalakshmi B, Sridhar D, Ponmurugan P (2009). Antimicrobial activity of important Indian medicinal plants against Pyogenic infection. J.Phytol. 1(6): 391-396.

Baticados MCL, Lavilla Pitago CR, Cruz-Lacierda ER, Delapena LD, Suneaz NA (1990). Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *Vibrio spendillus* isolated from diseased Penaeusmonodon larvae and rearing water. Dis Aquatt Org. 9:133-139.

Chang PH, Lin CW, Lee YC (2002). *Lactococcus garvieae* infection of cultured rainbow trout, *Oncorhynchus mykiss*, in Taiwan and associated biophysical characteristics and histopathology. Bull. Eur. Ass. Fish. Pathol. 22: 319-327.

Chen FR, Liu PC, Lee KK (2001). Lethal attribute of some protease secreted by *Vibrio alginolyticus* strains in *Kurama prawn penaeusjaponicus*. Nature forsch. 55: 94-99.

Chessbrough M (2000). Medicinal laboratory manual for tropical countries, Lianacre Houses. Volume II: Microbiology. Chap. 44:289-311. Butterworth-Heinemann Ltd., Linacre House, Jordan. Hill, Oxford.

Colorni A, Diamant A, Eldar A, Kvitt H, Zlotkin A (2002). *Streptococcus iniae* infections in Red Sea cage cultured and wild fishes. Dis. Aquat. Org. 49:165–170.

Farnaz M, Shahzad H, Tahira M, Abdul H, Safia A, Humayun R, Pervaiz AS and Usmanghani K (2011). Screening for antimicrobial activity of thirty-three medicinal plants used in the traditional system of medicine in Pakistan. Journal of Medicinal Plants Researc. 5(14): 3052-3060.

Ferguson HW, John S, Roach VS, Willoughby CJ, Parker S, Ryan R (2000). Caribbean reef fish mortality associated with *Streptococcus iniae*. Vet. Rec. 147: 662-664.

Firas AA, Hassan FA (2008). Antibacterial and antifungal activities of different parts of Tribulus terrestris L. growing in Iraq. J. Zhejiang Univ. Sci. Biol. 9(2): 154-159.

Garcia D, Escalante M, Delgado R, Ubeira FM, Sanmartin ML, Leiro J (2003). Antihelminthic and anti allergic activity of *Mangifera indica* Lp Hytother Res.17:1203-1208.

Garrido G, Gonzalez D, Lemus Y, Garcia D, Lodeiro L, Quintero G, (2004). *In vivo* and *in vitro* anti-inflammatory activity of *Mangifera indica* L. extract (VIMANG;). Pharmacol Res. 50:143-149.

Ghosh A, Das BK, Roy A, Mandal B, Chanda G. (2008). Antimicrobial activity of some medicinal plants extracts. Journal of Nat. Med. 62(2):259-262.

Holt J, Krieg N, Stanely P and William S (1994). Enterobacteriaceae. In: Bergey's manual of Determinative Bacteriology, 9th edn. Baltmore: Williams and Walkin Co., pp. 87-175.

Kim HG, Cho JH, Jeong EY, Lim JH, Lee SH and Lee HS (2006). Growth inhibitory activity of active compound isolated from *Terminalia chebula* fruits against intestinal bacteria. Journal of Food Production. 69(9): 2205-2209.

Kiyota H. (2006). Synthesis of naturally derived bioactive compounds of agricultural interest. Biosci Biotechnol Biochem.70(2):317-24.

Koh TH, Kurup A, Chen J (2004). Streptococcus iniae discitis in Singapore. Emerg. Infect. Dis. 10: 1694–1696.

Lavilla PCR, leno EM, Panner MG (1998). Mortalities of pond cultured juvenile shrimp *Penaeusmonodon* associated with dominance of luminescent vibrio in the rearing environment. Aquaculture. 164:337-349.

Martinez G, Delgado R, Perez G, Garrido G, Nunez Selles AJ, Leon OS (2000). Evaluation of the in vitro antioxidant activity of *Mangifera indica* L. extract (Vimang). Phytother Res., 14:424-227.

Mata AI, Blanco MM, Dominguez L, Fernandez-Garayzabal J, Gibello l (2004). Development of a PCR assay for *Streptococcus iniae* based on the lactate oxidase (lctO) gene with potential diagnostic value. Vet. Microbiol. 101(2): 109-116.

Mehdi R, Sasan Z, Arman A, Mohammad SF, Firouz F (2012). Experimental evaluation of pathogenicity of *Streptococcus iniae* in Silver Shark and Rainbow Shark. Afr. J. Microbiol. Res., 6(14):3560-3563.

Morales G, Paredes A, Sierra P, Loyola LA (2008). Antimicrobial Activity of Three Baccharis Species Used in the Traditional Medicine of Northern Chile, Molecules. 13: 790-794.

Mothana RA, Lindequist U (2005). Antimicrobial activity of some medicinal plants of the island Soqotra. J. Ethnopharma. 96(1-2):177-181.

Ncube, NS, Afolayan AJ, Okah AI (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. Afr J. Biotechnol.,7 (12):1797-1806.

Neely MN, Pfeifer JD, Caparon M (2002). *Streptococcus zebrafish* model of bacterial pathogenesis. Infect. Immun. 70: 3904-3914.

Parekh J, Chanda S (2007). Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. Afr. J. Biomed. Res. 10:175-181.