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ANTIBIOTIC AND HERBAL SENSITIVITY OF SOME *Aeromonas sp.* ISOLATES COLLECTED FROM DISEASED CARP FISHES

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ABSTRACT

Studies were conducted to identify *Aeromonas sp.* isolates collected from disease affected carp fishes (*Cyprinus carpio* and *Labeo rohita*), their antibiotic sensitivity pattern and sensitivity to 121 herb extracts. A total of 19 *Aeromonas sp.* isolates were identified from a total of 84 different isolates through a series of physiological, morphological and biochemical tests. Seven antibiotics *viz.*, streptomycin, chloramphenicol, erythromycin, sulphamethoxazole, cephradine, oxytetracycline and gentamycin were tested by disc diffusion method where all of the *Aeromonas sp.* isolates were found sensitive to streptomycin, erythromycin and gentamycin. But, 78.95% of the isolates were found resistant to oxytetracycline. In addition, 5%, 11%, and 16% isolates exhibited resistance to chloramphenicol, cephradine and sulphamethoxazole, respectively. Twenty one percent of the isolates showed multiple resistance to the antibiotics. A total of 121 herb extracts were evaluated for their sensitivity to the fish pathogenic *Aeromonas sp.* isolates. Among these, 23 herbs were found to possess antimicrobial activity. Leaf extracts of *Eugenia caryophyllus, Spondias pinnata* and *Terminalia chebula* were found to inhibit the growth of all the *Aeromonas sp.* isolates. In this study, multiple antibiotic resistant isolates were also found to be sensitive to several herb extracts. Thus, herbal treatment would promise a greater viable solution for effective treatment of fish disease.

Key Words: *Aeromonas sp.*, Antibiotic sensitivity, Herb extract, Herbal sensitivity

INTRODUCTION

Aeromonads are gram-negative, facultative anaerobic bacteria that cause diseases in terrestrial and aquatic animals and also in human (Janda and Abbott, 1996). *Aeromonas sp.* are considered to be one of the most important bacteria among the etiological agents of bacterial fish diseases (Rahman *et al.*, 2005). They are associated with hemorrhagic septicaemia, tail and fin rot, ulcer disease or red-sore disease in a variety of freshwater and marine fish of the world (Roberts *et al.*, 1989). Diseases caused by *Aeromonas sp.* is the

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major disease problem for commercial carp farming in Bangladesh (Chowdhury, 1998; Rahman *et al*., 2004).

Vaccination, chemotherapy and other prophylactic measures are generally used for prevention and control of fish diseases. During the last two decades, various treatment methods including the use of antibiotics have been adopted with partial success for the treatment of diseased fish (Aoki, 1992). Antibiotics can literally save lives and are effective in treating illnesses caused by bacterial infections. However, they have the potential to cause unwanted side effects to host body. Due to careless and promiscuous use of antibiotics, various pathogenic microbes are gaining resistance to different antibiotics. For these reasons herbal treatments would be an alternate choice for prevention and control of fish diseases.

Medicinal plants are important elements of traditional medicine in virtually all cultures and promise a cheaper source for therapeutics, greater accuracy than chemotherapeutic agents and a viable solution for several problems (Ahmed *et al*., 2009). Many kinds of herbs possess antibacterial and antifungal activity that can be used to control different diseases. But very little attempts have been undertaken for the treatment of fish diseases.

Considering the above importance, present studies have been conducted to identify the *Aeromonas sp.* isolates collected from diseased fish, to investigate the antibiotic sensitivity of the isolates, and to screen sensitivity of the microorganisms to different herb extracts.

MATERIALS AND METHODS

Isolation of Aeromonas sp. from carp fish

Bacteria were isolated from lesions and kidneys of disease infected rohu (*Labeo rohita*) and common carp (*Cyprinus carpio*). These fishes were collected from Pabna and Sylhet districts during November 2009 to February 2010. External hemorrhagic lesions on the skin and underlying muscular tissue were observed in the diseased rohu and hemorrhagic lesions with loss of scale were found in the disease infected common carp.

Isolation of bacteria from the diseased fishes was carried out aseptically on nutrient agar (NA) media. The agar plates were incubated at 25^{o}C for 36-48 hours. Individual colonies were separated from the plates on the basis of color, shape and size and sub-cultured on the relevant media to obtain pure culture. Then suspected Aeromonad isolates were sub-cultured on *Aeromonas*-selective agar media supplemented with ampicillin antibiotic.

Identification of Aeromonas sp. isolates

A series of morphological, physiological and biochemical tests were performed to characterize the suspected Aeromonad isolates grown on *Aeromonas*-selective agar media.

A presumptive identification was performed by Gram staining, motility, oxidase activity, catalase production, acid production from glucose, oxidation-fermentation (OF) test and resistance to vibriostatic agent 0/129 tests for determination of biochemical characteristics of the bacteria as described by Rahman *et al.* (2004). The tolerance of the isolates to NaCl concentrations was determined in NA medium containing 0% and 6% NaCl. Growth temperature of the isolates was studied by incubation of the isolates on NA medium at 4, 25 and 37°C for 24-48 hours. Results of the tests were compared with a type strain of *Aeromonas hydrophila* ATCC 7966^T and the bacterial isolates were identified up to genus level following the Manual for the Identification Medical Bacteria (Barrow and Feltham, 1993).

Antibiotic sensitivity test

Sensitivity of the *Aeromonas sp.* isolates to different commercial antibiotics was determined by disc diffusion method as described by Sarker *et al*. (1998). Briefly, individual isolates were cultured into nutrient agar broth and incubated at 25°C for 12 hours. Fifty micro liter of individual broth culture was dropped on the NA plate with micropipette. The broth on the plate was spread aseptically by a sterile 'L' shaped glass rod. Seven commercially prepared antibiotics discs viz., streptomycin (10 µg/disc), chloramphenicol (30 µg/disc), sulphamethoxazole (25 µg/disc), erythromycin (15 µg/disc), oxytetracycline (30 µg/disc) and cephradine (30 µg/disc) manufactured by Oxoid Ltd. and gentamycin (10 µg/disc) manufactured by Becton Disc kinson & company were placed on the surface of the medium with sterile forceps and pressed gently to ensure good contact with the surface of the medium. The plates were then incubated at 25°C for 24 h. After incubation the organism was considered resistant if there was no zone of inhibition surrounding the disc and marked as R. When there was zone of complete inhibition around the disc the organism was considered sensitive. Then the diameter of the discs and the diameter of the zone of inhibitions were measured by measuring scale. The ratio between the diameters was calculated as described by Sarker *et al*. (1998).

Herbal sensitivity of the Aeromonas sp. isolates:

A total of 121 herb extracts from 118 plants were randomly selected in this study to screen their sensitivity to the *Aeromonas sp.* isolates. Most of the herbs were collected from a nursery of Adamdighi, Bogra and different parts of Sylhet district. A list of the plants has been given in Table 2. The fresh parts of plants such as young leaves, bark, bulb, root, flower, rhizome or petiole were collected and washed several times with distilled water. The plant parts were cut into small pieces and paste was made by using mortar-pestle. Approximately 10 µl of individual herb extract was inoculated onto the spread plate culture. The plate was then allowed to incubate at 25°C for overnight. After 12-24 h of incubation, the herb extract was noted for zone of inhibition for each *Aeromonas sp.*

isolates. The diameter of the herb extracts and the diameter of the zone of inhibitions were measured by measuring scale. The ratio between the diameters was calculated.

Table 1. *Aeromonas sp.* isolates with their origin

***Aeromonas sp.* isolates**	**Host (fish name) of isolates**	**Organ of isolation**
C1	Common carp	Hemorrhagic lesions on body surface
C2	Common carp	Hemorrhagic lesions on body surface
C3	Common carp	Hemorrhagic lesions on body surface
C4	Common carp	Hemorrhagic lesions on body surface
C5	Common carp	Hemorrhagic lesions on body surface
C6	Common carp	Hemorrhagic lesions on body surface
C7	Common carp	Hemorrhagic lesions on body surface
C8	Common carp	Kidney
C9	Common carp	Kidney
C10	Common carp	Hemorrhagic lesions on body surface
C11	Common carp	Hemorrhagic lesions on body surface
E 14	Rohu	Ulcerative lesion
E 17	Rohu	Ulcerative lesion
E 20	Rohu	Ulcerative lesion
E 22	Rohu	Ulcerative lesion
C12	Common carp	Kidney
C13	Common carp	Kidney
C14	Common carp	Kidney
C15	Common carp	Kidney

Table 2. List of medicinal plants used for herbal sensitivity test

Serial No.	Local name	Botanical name	Parts of plant used	Sample No.
01.	Durbaghas	*Cynodon dactylon*	Leaf	01
02.	Thankuni	*Centella asiatica*	Leaf	02
03.	Chandramallika	*Crysanthemum coronarium*	Leaf	03
04.	Helencha	*Alternanthera philoxeroides*	Leaf	04
05.	Kachu	*Colocasia exculanta*	Leaf	05
06.	Arjun	*Terminalia arjuna*	Leaf	06
07.	Nim	*Azadirachta indica*	Leaf	07
08.	Eucalyptus	*Eucalyptus camaldulensis*	Leaf	08
09.	Ada	*Zingiber officinale*	Rhizome	09
10.	Peaj	*Alium cepa*	Bulb	10

Serial No.	Local name	Botanical name	Parts of plant used	Sample No.
11.	Kadam	*Anthocephalus chinensis*	Leaf	11
12.	Rasun	*Allium sativum*	Bulb	12
13.	Krisnachura	*Delonix regia*	Leaf	14
14.	Pan	*Piper betel*	Leaf	13
15.	Dholkalmi	*Ipomoea fistulosa*	Leaf	15
16.	Amrah	*Spondias pinnata*	Leaf	18
17.	Mehedi	*Lawsonia inermis*	Leaf	19
18.	Arhar	*Cajanus cajan*	Leaf	20
19.	Kagaji Lebu	*Citrus aurantifolia*	Fruit juice	22
20.	Nisinda	*Vitex negundo*	Leaf	23
21.	Karalla	*Momordica charantea*	Leaf	24
22.	Puishak	*Basella alba*	Leaf	25
23.	Patol	*Trichosanthes dioica*	Fruits	26
24.	Bishkatali	*Polygonum tomentosum*	Leaf	28
25.	Lajjabati	*Mimosa pudica*	Leaf	29
26.	Hatishur	*Acalypha hispida*	Leaf	37
27.	Ferngass	*Filicuum decipiens*	Leaf	39
28.	Mehagani	*Swietenia mahagoni*	Leaf	42
29.	Raktakanchan	*Bauhinia purpurea*	Leaf	43
30.	Pepe	*Carica papaya*	Leaf	44
31.	Dhaniya	*Coriandrum sativum*	Leaf	45
32.	Nageswar	*Mesua nagesarium*	Leaf	46
33.	Nayantara	*Catharanthus roseus*	Leaf	47
34.	Nayantara	*Catharanthus roseus*	Stem	48
35.	Nayantara	*Catharanthus roseus*	Root	49
36.	Nayantara	*Catharanthus roseus*	Flower	50
37.	Haritaki	*Terminalia chebula*	Leaf	51
38.	Tulshi	*Ocimum sactum*	Leaf	53/97
39.	Bel	*Aegle marmelos*	Leaf	54/86
40.	Basak	*Adhatoda vasica*	Leaf	60
41.	Amloki	*Phyllanthus embelica*	Fruits	56
42.	Jatropha	*Jatropha curcas*	Leaf	62
43.	Gustavia	*Gustavia insignis*	Leaf	63
45.	Jarul	*Lagerstroemia speciosa*	Leaf	65
46.	Sarpagandha	*Rauwolfia serpentina*	Leaf	68
47.	Labanga	*Eugenia caryophyllus*	Leaf	70
48.	Kusum	*Schleichera oleosa*	Leaf	30

Serial No.	Local name	Botanical name	Parts of plant used	Sample No.
49.	Golmarich	*Piper nigrum*	Leaf	71
50.	Asoke	*Saraca indica*	Leaf	72
51.	Dadmardan	*Cursia alata*	Leaf	73
52.	Kalodutra	*Datura metel*	Leaf	108
53.	Golancha	*Tinospora cordifolia*	Leaf	76
54.	Naglingom	*Cauropita guianensis*	Leaf	77
55.	Shal	*Shorea robusta*	Leaf	78
56.	Bahera	*Terminalia belerica*	Leaf	80
57.	Arshagandha	*Withania somnifera*	Leaf	81
58.	Tentul	*Tamarindus indicus*	Leaf	82
59.	Kalomeg	*Andrographis paniculata*	Leaf	84
60.	Keuri kanta	*Pandanus tectorius*	Leaf	87
61.	Jafran	*Crocus sativus*	Leaf	88
62.	Jayonti	*Sesbania sesban*	Leaf	89
63.	Kanchan	*Bauhinia acuminata*	Leaf	90
64.	Sada dhutra	*Datura suaveolens*	Leaf	91
65.	Garjan	*Dipterocurpus turbinatus*	Leaf	92
66.	Buddha narikel	*Pterygata alata*	Leaf	93
67.	Bakul	*Mimusops elengi*	Leaf	94
68.	Rudrakkhya	*Elaeocarpus siceraria*	Leaf	96
69.	Karamcha	*Carissa carandus*	Leaf	98
70.	Jangli badam	*Sterculia foetida*	Leaf	99
71.	Rangan	*Ixora coccinea*	Leaf	103
72.	Banraj	*Bauhinia racemosa*	Leaf	105
73.	Kastha lohagas	*Aspidistra elatior*	Leaf	106
74.	Baruna	*Crataeva nurvala*	Leaf	107
75.	Casava	*Manihot esculenta*	Leaf	109
76.	Allamanda	*Allamanda schottii*	Leaf	110
77.	Chalmugra	*Hydnocarpus kurzii*	Leaf	115
78.	Golden shower	*Campris radicans*	Leaf	116
79.	Gaab	*Diospyros peregrina*	Leaf	117
80.	Chita	*Plumbago zeylanica*	Leaf	118
81.	Shefali	*Nyctanthes arbortristis*	Leaf	119
82.	Kanthali champa	*Artaborys uncinatus*	Leaf	121
83.	Jiga	*Lannea coromandelica*	Leaf	122
84.	Bamunhatti	*Elerodendrum indicum*	Leaf	126
85.	Bajna	*Zanthoxylum rhetsa*	Leaf	127

Serial No.	Local name	Botanical name	Parts of plant used	Sample No.
86.	Madhobilata	*Hiptage benghalensis*	Leaf	130
87.	Ponarnava	*Trianthema portulacas*	Leaf	128
88.	Keya	*Pandanus kiada*	Leaf	129
89.	Agar	*Aquilaria agallocha*	Leaf	131
90.	Karanja	*Pongamia pinnata*	Leaf	132
91.	Kao	*Garcinia cowa*	Leaf	133
92.	Sarifa	*Annona squamosa*	Leaf	134
93.	Ashphal	*Euphoria longam*	Leaf	138
94.	Bach	*Acorus calamus*	Leaf	139
95.	Pipul	*Mentha piperita*	Fruits	123
96.	Asplenium	*Asplenium nidus*	Leaf	140
97.	Petrisfern	*Pteris cretica*	Leaf	141
98.	Tutghach	*Morus alba*	Leaf	142
99.	Lantana	*Lantana camara*	Leaf	143
100.	Bahari nil	*Indigofera tinctoria*	Leaf	144
102.	Sada pentas	*Pentas lamceolata*	Leaf	145
103.	Aralia	*Polyscias filicifolia*	Leaf	146
104.	Stachytarpheta	*Stachytarpheta indca*	Leaf	147
105.	Thryallis	*Thryallis gluca*	Leaf	148
106.	Urenia	*Urena lobeata*	Leaf	149
107.	Annanta-lata	*Antigonon leptopus*	Leaf	150
108.	Gugli	*Argyreia nervosa*	Leaf	152
109.	Kutala	*Artanema longifoliam*	Leaf	153
110.	Goaji lotgas	*Parmentiera edulis*	Leaf	154
111.	Cassia	*Cassia laevigata*	Leaf	156
112.	Croton	*Croton bonoplandianum*	Leaf	157
113.	Duranta	*Duranta repens*	Leaf	158
114.	Mandar	*Erythrina variegata*	Leaf	169
115.	Pudina	*Mentha spicata*	Leaf	170
116.	Jhanjhani	*Crotalaria saltiana*	Leaf	171
117.	Karipata	*Murraya koenigii*	Leaf	172
118.	Ulatchandal	*Gloriosa superba*	Leaf	100
119.	Kakmachi	*Solanum nigrum*	Leaf	111
120.	Arsol	*Vitex peducularis*	Leaf	114
121.	Amloki	*Phyllanthus emblica*	Leaf	120

RESULTS AND DISCUSSION

Identification of the Isolates

A total of 84 bacterial isolates was isolated from the disease infected carp fishes. Then 25 isolates that exhibited the colony characteristics similar to *Aeromonas sp.* were sub-cultured on *Aeromonas*-selective media where, 19 isolates were grown. These isolates were assessed for their morphological, physiological and biochemical characteristics. All of these isolates were Gram negative, rod shaped, motile with polar flagella, catalase positive, oxidase positive and fermentative bacteria, produced acid from glucose, able to grow at 25^{o}C and 37^{o}C and in absence of NaCl but unable to grow at 4^{o}C and in presence of 6% NaCl and also resistant to vibriostatic agent 0/129 (Table 3). Based on the characteristics these isolates were identified as member of the genus *Aeromonas sp.*

Table 3. Common phenotypic properties of the *Aeromonas sp.* isolates

Traits	Results
Gram stain	-
Shape	Rod
Motility	+
Oxidase	+
Polar flagella	+
Catalase	+
O-F test	Fermentative
Acid production in Glucose	+
Growth in 4^{o}C	-
Growth in 25^{o}C	+
Growth in 37^{o}C	+
Growth in 0% NaCl	+
Growth in 6% NaCl	-
Resistance to Vibriostatic agent (0/129)	+

F = Fermentative, (-) Ve = Negative, (+) Ve = Positive

Antibiotic sensitivity of the Aeromonas sp. isolates

The *Aeromonas sp.* isolates were found to vary in their antibiotic sensitivity pattern to the seven antimicrobial agents tested. All of the isolates showed sensitivity to three antibiotics such as streptomycin, gentamycin and erythromycin. But, most (78.95%) of the isolates were found resistant to oxytetracycline. Moreover, 5%, 11%, and 16% isolates exhibited resistance to chloramphenicol, cephradine and sulphamethoxazole, respectively (Table 4).

Table 4. Antibiotic sensitivity pattern of the *Aeromonas sp.* isolates

Isolates	Antibiotic sensitivity pattern						
	OTC	S	E	C	SXT	CE	GE
C1	R	4.4	4.4	6	4	3.8	3
C2	R	4	4.4	7	4.4	4.4	3
C3	2.2	3.2	2	5	2	4.4	3.2
C4	R	3.4	3.2	6.4	4.4	R	2.6
C5	R	4.4	4.4	6	R	R	3
C6	R	3.6	3.4	7	4.6	4	3
C7	R	3.6	3	6	3	1.6	3
C8	R	3	2.4	R	R	3.6	2.8
C9	R	4	4.4	7	3.6	3.6	3
C10	2.3	4.4	4.4	7	2	4	3
C11	R	4	4.4	7	3.6	4.6	3.6
E 14	R	4.4	4.2	6.6	R	5	2.6
E 17	R	4.4	4.6	6.4	4	3.2	4
E 20	3.2	4.6	4.6	6.8	3.2	4	3
E 22	R	4.4	4.2	7.4	3	3.2	2.4
C12	R	4	4.8	5.6	3.4	4.4	3
C13	3.0	4.2	3	6.2	2	3.6	2.8
C14	R	4	3	6	5	4.4	3
C15	R	3.6	3.4	6.4	2.4	3	3.4

OTC : Oxytetracycline (30 µg/disc), S : Streptomycin (10 µg/disc), E : Erythromycin (15 µg/disc), C : Chloramphenicol (30 µg/disc), SXT : Sulphamethoxazole (25 µg/disc), CE : Cephradine (30 µg/disc), GE : Gentamycin (10 µg/disc), R : Resistant, Numbers indicate the zone ratio

Herbal sensitivity of the Aeromonas sp. isolates

The *Aeromonas sp.* isolates were found to be sensitive to 23 herbs extract out of 121 samples tested. The leaf extract of Labanga, Amrah, and Haritaki inhibited the growth of all of the *Aeromonas sp.* isolates (Table 5). The *Aeromonas sp.* isolates exhibited 84% and 73% sensitivity to the leaf extracts of Eucalyptus and Dholkallami, respectively. The crude herb extract of Arjun, Bahera, Tentul, Jiga and Bamunhatti also inhibited the growth of 50-59 % of the bacterial isolates. These isolates also exhibited 1-49% sensitivity to the herb extracts of Basak, Kanthalichampa, Dadmardan, Shal, Jafran, Garjan, Buddha narikel, Allamonda, Chalmugra, Golden shower, Kalomeg, Sarifa and Amloki.

Table 5. Sensitivity of the *Aeromonas sp.* isolates to herb extracts

Name of Herbs	Sensitivity of herb extracts to *Aeromonas sp.* isolates																		
	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	E14	E17	E20	E22	C12	C13	C14	C15
Adhatoda vasica	-	1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.5
Eugenia caryophyllus	1.69	1.7	1.58	1.6	1.7	1.92	1.46	1.5	1.46	1.6	1.8	1.9	1.5	1.6	1.3	1.53	2	1.33	1..25
Cursia alata	-	1.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shorea robusta	-	-	-	-	-	-	1.5	-	-	2.3	1.8	1.4	1.2	1.5	1.2	-	-	0.75	-
Terminalia belerica	-	-	1.5	-	-	-	1.5	31.5	-	1.3	-	0.8	1.4	1.5	1.6	-	1.88	-	1.24
Tamarindus indicus	1.88	-	-	1.53	-	1.5	-	-	-	1.5	1.5	-	-	1.4	1.4	1.5	1.6	-	1.88
Andrographis paniculata	-	-	-	-	-	-	-	-	-	-	-	-	1.2	-	-	-	-	-	-
Crocus sativus	1.5	-	-	1.5	-	-	1.5	-	-	-	-	1.5	-	1.6	-	1.5	1.7	-	-
Dipterocurpus turbinatus	1.4	-	-	-	1. 4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pterygata alata	-	-	-	-	-	1.5	1.47	2.14	-	1.8	1.8	-	-	1.8	1.8	-	1.83	-	1.33
Allamanda schottii	-	-	-	-	1.58	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydnocarpus kurzii	-	1.5	-	-	1.4	-	-	-	-	-	-	-	-	-	-	-	1.7	-	-
Campris radicans	-	1.4	1.51	-	1.71	-	1.5	1.5	-	-	-	-	1.3	1.4	1.4	-	1.64	-	-
Artaborys uncinatus	1.58	1.7	1.7	-	-	-	-	-	1.2	-	-	1.6	-	-	-	-	-	-	-
Lannea coromandelica	1.2	1.2	-	1.3	-	1.5	1.5	-	1.7	-	-	1.5	1.7	-	1.5	-	1.2	-	1.4
Elerodendrum indicum	-	1.4	-	-	-	-	1.3	1.42	1.2	-	-	1.8	1.3	1.4	2.5	1.29	-	-	1.7
Terminalia arjuna	1.3	-	1.2	ND	-	1.45	-	-	ND	1.6	-	-	1.3	-	-	1.47	1.38	1.33	1.5
Eucalyptus camaldulensis	1.5	1.6	1.4	1.38	-	1.4	-	-	1.4	2.3	1.6	1.8	1.3	1.4	1.6	1.4	1.4	1.4	1.8
Ipomoea fistulosa	1.3	-	ND	2	2.35	2.2	ND	-	1.8	2.2	1.5	1.3	ND	1.6	1.7	1.2	1.4	ND	1.33
Spondias pinnata	1.5	1.6	1.6	2.9	2.6	1.56	1.6	1.8	1.6	1.7	1.7	1.4	1.3	1.7	1.8	1.3	1.5	1.7	1.25
Terminalia chebula	1.7	1.6	1.4	1.4	1.8	1.6	1.5	1.7	3.6	1.5	1.8	1.6	1.5	1.3	2.7	1.4	1.6	1.7	1.64
Annoa squamosa	-	-	-	-	-	-	1.3	1.86	-	1.3	1.7	ND	1.6	ND	1.8	1.3	1.36	1.33	1.5
Phyllanthus emblica	-	-	1.67	-	-	-	-	2	1.4	-	-	1.5	-	-	-	-	-	-	1.43

- : not sensitive; ND : Not done

In the present study, a total of 84 bacterial isolates were recovered from the hemorrhagic lesions and kidneys of the diseased carp fishes. Then a total of 25 isolates that exhibited colony characteristics similar to *Aeromonas sp.* were sub-cultured on *Aeromonas*-selective media supplemented with ampicillin antibiotic. Among these isolates, 19 isolates showed characteristic growth of *Aeromonas sp.* on the selective media. In order to verify their identity, several morphological, physiological and biochemical tests were performed and the results were compared with that of a type strain *Aeromonas hydrophila* ATCC 7966^T. Based on their characteristics all of the 19 isolates were confirmed to belong to the genus *Aeromonas sp.* All of theses isolates were reported as pathogenic to carp fish (Hossain, 2010).

Sensitivity of the *Aeromonas sp.* isolates to seven commercial antibiotics was determined by disc diffusion method. In the present study, it was found that all of the isolates were sensitive to streptomycin, gentamycin and erythromycin. But, 78.95% of the isolates were found resistant to oxytetracycline. In addition, 5%, 11%, and 16% isolates also exhibited resistance to chloramphenicol, cephradine and sulphamethoxazole respectively. Twenty one percent of the isolates were found resistant to more than one antibiotics tested. Sarker *et al*. (1998) reported multiple antibiotic resistance of fish pathogenic *Aeromonas hydrophila* isolates which were highly resistant (60%) to oxytetracycline. Banu (1996) also reported high percentage of oxytetracycline resistance among *Aeromonas* spp. isolates collected from farmed fish and water. Guz and Kozinska (2004) examined susceptibility of 22 antimicrobial agents to fish pathogenic *Aeromonas sp.* isolates where the isolates were sensitive to trimethoprim-sulphamides, oxolinic acid, flumequine, chloramphenicol, norfloxacin, linkomycin, pefloxacin, but, exhibited 100% resistance to ampicillin and penicillin.

Different types of antibiotics such as oxytetracycline, ampicillin, sulphamethoxazole, erythromycin etc. are frequently used in different countries including Bangladesh for the treatment of fish, poultry, dairy and human diseases. Since, microorganisms have the ability to acquire resistance against different antibiotics, different types of microorganisms including *Aeromonas sp.* are showing high frequencies of resistance against these antibiotics. Several reports also suggested that microorganisms have already attained resistance against third or fourth generation antibiotics. Thus, it is very difficult to choose any one type of antibiotic as a therapeutic agent for fish for a long time. Moreover, antibiotic residues may persist in sediments for a long time and prolonged use of synthetic antibiotics reveals the threats to consumer, non target organisms and the environment. For these reasons sustainable and environment friendly alternate therapeutic agents should be developed for the treatment of fish diseases. Medicinal herbs would be an alternate and effective therapeutic agent for fish disease.

In the present study, a total of 121 herb extract samples prepared from 118 plants were examined to find out their sensitivity to the *Aeromonas sp.* isolates associated with disease of carp fishes. Among these, 23 herb extracts showed antimicrobial activity against the *Aeromonas sp.* isolates. Herb extracts of three medicinal plants *viz*., Labanga, Amrah, and

Haritaki found to possess potential antimicrobial activity that inhibited the growth of all of the *Aeromonas sp.* isolates. The leaf extracts of Eucalyptus and Dholkallami were also found highly sensitive (84% and 73%, respectively) for the *Aeromonas sp.* isolates. Moreover, leaf extracts of Arjun, Bahera, Tentul, Jiga and Bamunhatti were observed to contain moderate to high antibacterial activity for the *Aeromonas sp.* isolates tested. Other herb species that were found to inhibit the growth of the bacteria were Basak, Kanthalichampa, Dadmardan, Shal, Jafran, Garjan, Buddha narikel, Allamonda, Chalmugra, Golden shower, Kalomeg, Sarifa and Amloki. Muniruzzaman and Chowdhury (2004) tested antibacterial activity of 26 medicinal plants of Bangladesh to different fish pathogenic bacteria and reported that *A. Arabica*, *A. cepa*, *A. sativum*, *C. gigantia*, *M. charantia, P. hydropiper*, *P. guajava* and *T. indica* had high inhibitory response and moderate inhibitory response for *A. indica*, *B. alba*, *C. Zedoaria*, *C. longla*, *C. indica*, *H. indicum*, *H. asiatica*, and *P. betle* against *Aeromonas hydrophilia*. In this study, although moderate inhibitory activity was observed for Arjun (*T. indica*) but, no antibacterial activity was observed for *A. cepa*, *A. sativum*, *P. guajava*, *A. indica*, *B. alba*, *C. Zedoaria*, *C. longla*, *C. indica*, *H. indicum*, *H. asiatica*, and *P. betle* for the *Aeromonas sp.* isolates.

In the present study, a large number of plant extracts were examined for evaluation of their antibacterial activity against fish pathogenic *Aeromonas sp.* isolates but, low or no antibacterial activity was observed for the previously reported plants possessing antibacterial activity. However, it is the first report where more than 100 herbs was screened for their antimicrobial activity against fish pathogenic *Aeromonas sp.* Isolates. It is also a first report about the antimicrobial activity of leaf extracts of three medicinal plants *viz*., Labanga, Amrah and Haritaki that inhibited the growth of all of the *Aeromonas sp.* isolates including the multiple antibiotic resistant isolates. Recently, Foysal (2010) also found antimicrobial activity of leaf extracts of Labanga, Amrah and Haritaki for fish pathogenic multiple antibiotic resistant *Pseudomonas fluorescence* isolates. Crude leaf extracts of these medicinal plants could be used for the treatment of fish diseases. Although, leaf extracts of Eucalyptus and Dholkallami were also found to possess high antibacterial activity against *Aeromonas sp.* isolates but, their toxic effect on fish need to be evaluated. Further studies are needed to find out the active antimicrobial ingredients of the medicinal plants.

CONCLUSION

Medicinal plants are important elements of traditional medicine in virtually all cultures and promise a cheaper source for therapeutics. Herb extracts are natural product, available, cheap and safe for the environment. The present study revealed that some medicinal plants possess potential antimicrobial activity against fish pathogenic, antibiotic resistant Aeromonads. Valuable drug could be developed from some herbs for the treatment of *Aeromonas*-associated fish diseases.

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REFERENCES

Ahmed, I. E., Noor, E. I. D., Mahmud, R. A. 2009. Application of some medicinal Plants to eliminate *Trichodina sp.* in tilapia (*Oreochromis niloticus*). *Rep. and Opi.*, 1(6): 1-5.

Aoki, T. 1992. Chemotherapy and drug resistance in fish farms in Japan. ***In**: Proceedings of the first symposium on diseases in Asian aquaculture*. M. Shariff, R. P. Subasinghe, J. R. Arthur (eds.). Fish Health Section, Asian Fisheries Society, Manila, Philippines. pp. 519-529.

Banu, G. R. 1996. Studies on the bacteria *Aeromonas* spp. in farmed fish and water in Mymensingh. M. S. Thesis, Department of Fisheries Biology and Limnology. Bangladesh Agricultural University, Mymensingh, Bangladesh. 95 pp.

Barrow, G. I. and Feltham, R. K. M. 1993. Cowan and Steel's Manual for the Identification of Medical Bacteria. Third edn. Cambridge University press. P. 331

Chowdhury, M. B. R. 1998. Involvement of Aeromonads and Pseudomonads in diseases of farmed fish in Bangladesh. *Fish Pathol*., 33: 247-254.

Foysal, M. J. 2010. *Identification, Pathogenicity, Antibiotic and Herbal Sensitivity of Pseudomonas fluorescens Isolated from Diseased Fish*. B. Sc. Thesis, Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh. 94 pp.

Guz , L., and Kozinska, A. 2004. antibiotic susceptibility of *Aeromonas hydrophila* and *A. sobria* isolated from farmed carp (*Cyprinus carpio* L.) *Bul. Vet. Ins. Pulawy*., 48: 391-395.

Hossain, M. N. 2010. *Identification, Pathogenicity, Antibiotic & Herbal Sensitivity of Aeromonas Spp. Isolated from Diseased Carp Fish*. B. Sc. Thesis. Department of Genetic Engineering and Biotechnology, Shahjalal University of Science and Technology, Sylhet, Bangladesh. 80 pp.

Janda, J. M. and. Abbott, S. L. 1996. Human Pathogens. ***In**: The genus Aeromonas*. B. Austin, M. Altwegg, P.J. Gosling, S. Joseph (eds.). John Wiley & Sons, Chichester, England. 151-173.

Muniruzzaman, M. and Chowdhury, M. B. R. 2004. Sensitivity of fish pathogenic bacteria to various medicinal herbs. *Bang. J. Vet. Med*., 2 (1): 75-82

Rahman, M. M., Somseri, T., Ezura, Y., and Tajima, K. 2004. Distribution of *Aeromonas* spp. emphasizing on a newly identified species *Aeromonas sp.* T8 isolated from EUS-affected fish and aquatic animals in Southeast Asia. *J. Biol. Sci*., 7 (2): 258-268.

Rahman, M. M., Somseri, T, Tanaka, R., Sawabe, T. and Tajima, K. 2005. PCR-RFLP analysis of *Aeromonas* isolates collected from diseased fish and aquatic animals. *Fish Pathol*., 40(4): 151-159.

Roberts, R. J., Wotten, R., Macrae, I, Miller, S. D. and Struthers, W. 1989. Ulcerative Disease Survey, Bangladesh. Final report to the Government of Bangladesh and the Overseas Development Administration, UK, Institute of Aquaculture, University of Starling, Scottland. 104 pp.

Sarker, M. A., Chowdhury, M. B. R., Rahman, M. M. and Kashem, M. A. 1998. Pathogenicity and Antibiotic Resistance of Some *Aeromonad hydrophila* isolates. *Bangladesh J. Fish*., 21(2): 61-64.