Effect of dietary willow herb, *Epilobium hirsutum* extract on growth performance, body composition, haematological parameters and *Aeromonas hydrophila* challenge on common carp, *Cyprinus carpio*

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**Abstract**

The present study was conducted to evaluate the use of *Epilobium hirsutum* extract in diet of common carp, *Cyprinus carpio* (20 ± 2 g). Different levels of plant extract (0%, 0.5%, 1, 3% and 3% + 2% multivitamin (2 g of multivitamin per kg diet) were spread on commercial diet. The feed was offered by 8 weeks. Results showed that fish fed experimental diets had no significant difference (\( P > 0.05 \)) in specific growth rates, condition factor, feed conversion ratios and survival compared with control. There were no significant difference (\( P > 0.05 \)) in moisture, crude protein, crude lipid and ash content of common carp fed diets containing various levels of plant extract. The mortality of fish challenged by *Aeromonas hydrophila* have been recorded for 30 days after challenging, results showed that mortality decreased significantly (\( P < 0.05 \)) in fish fed diet containing plant extract compared with the control. The lowest mortality observed in group fed diet containing 3% plant extract. Haematological parameters showed that white blood cells had significantly (\( P < 0.05 \)) increased in infected and uninfected groups compared with the control. Red blood cells, haemoglobin and haematocrit had no significant change (\( P > 0.05 \)) in infected and uninfected groups compared with the control.

**Keywords:** *A. hydrophila* challenge, body composition, common carp, extract, growth performance and willow herb

**Introduction**

Disease outbreaks were recently identified as a major constraint to aquaculture production and trade, with a consequent effect on the industry’s economic development (Yunxia, Jianzhong & Guoliang 2001). The use of disinfectants and antimicrobials has shown limited success in preventing or curing aquatic diseases (Subasinghe 1997).

The organism *Aeromonas hydrophila* is a gram negative facultative anaerobic short bacillus. It is motile and grows wide range of temperature 11.2–40.5 °C (Biradar, Goud, Neogi & Saunya 2007). *Aeromonas hydrophila* is a widespread, opportunistic pathogen, causes high mortality of cultured and feral fish (McDaniel 1979). It is the causative agent of the disease known as ‘haemorrhagic septicemia,’ ‘ulcer disease,’ or ‘red-sore disease’. *Aeromonas hydrophila* is generally found in the gastrointestinal tract of fish is considered an opportunistic pathogen. Most of bacteria, which are termed ‘opportunistic’ usually, do not cause disease unless other factors are involved. *Aeromonas hydrophila* is always capable of producing disease if given the chance. Outbreaks of the disease are usually associated with a change in environmental conditions, such as stress, overcrowding, a sudden change of temperature, transfer of fish, mishandling, poor water quality, high nitrite and carbon dioxide levels (Adanir & Turutoglu 2007).

Motile aeromonad septicemia, caused by various strains of *A. hydrophila*, is one of the most common and challenging disease in freshwater fish (Cipriano, Bullock & Pyle 1984; Choudhury, Pal, Sahu, Kumar, Das & Mukhrejee 2005; Kumari & Sahoo 2005; Yildiz, Bekcan, Karasu Benli & Akan 2005).

Resistance to antimicrobial agents and emergence of multiple drug resistant strains in a wide variety of pathogens pose a serious threat to the management of infectious diseases (Tomlin & Tomasz 1986), rendering the traditional antibiotic and chemotherapeutic...
treatments less successful (Takashima, Aoki & Kitao 1985; Kim, Yoshida & Aoki 1993).

Herbal medicine is a growing field of alternative medicines nowadays. Many active ingredients in manufactured drugs are derived from plant compounds and have a wide range of use. Plants and plant extracts more safe than chemical products whereas natural products is becoming more popular, since drugs of synthetic origin may have a negative impact on the environment and parasite resistance to poisonous chemicals can develop after repeated applications (Magi & Sahk 2003).

Plant-derived phytomedicines have great promise in the treatment of infectious disease and thus represent a vast tapped source, which has the potential to combat pathogen infections in aquaculture.

A potential added bonus to the chemotherapeutic agents isolated from plant origin is the observation that these extracts have also been shown to enhance growth of cultured fish (Abutubul, Golan-Gldhirish, Bavazani & Zilberg 2004; Rao & Chakrabarti 2005; Divyagnanswari, Christy Babita & Dinakaran Michael 2007; Sahu, Das, Mishra, Pradhan & Sarangi 2007).

Epilobium hirsutum (willow herb) is a medical plant is found all over Europe, Asia apart from the tropical islands, Africa and America, Australia, Tasmania and New Zealand. The medical parts of E. hirsutum are the herb and the roots. The chemical compositions of this plant consist of flavonoids (in particular quercetin-3-O-beta-D-glucuronide, and quercitrin), steroids (in particular beta-sitosterol and its ester, including among other beta-sitosterol caproate) and tannins (Gruenwald, Brendler & Jaenicke 1998).

Willow herb is reported to have antiphlogistic and antiexudative effects. A watery infusion revealed a significant inhibitory effect on oedemas in rat paws (Gruenwald et al. 1998). Willow herb is used for a variety of purposes, including the treatment of skin infections and respiratory problems.

Antibacterial effects have also been demonstrated. A suspension of fresh drug in ethanol stunts the growth of the bacteria of Pseudomonas pyocyanaea. Tincture and the extract work antimicrobially against Candida albicans, Staphylococcus albus and Staphylococcus aureus. The dried residue of a maceration, which is fixed on filter paper, showed a weak effect against Bacillus subtilis, Escherichia coli, Mycobacterium smegmatis, Shigella flexneri, Shigella sonnei and S. aureus. In spite of many reports about Epilobium species, there are not sufficiently studies about Epilobium hirsutum in fish (Gruenwald et al. 1998; Battinelli,Tita, Evandi & Mazzanti 2001).

The objectives of the present study were to evaluate the effect of E. hirsutum at different levels into common carp, Cyprinus carpio diets on growth performance, body composition, haematological parameters and Aeromonas hydrophila challenge.

Materials and methods

Fish

The common carp, C. carpio were obtained from Shahid Marjani’s fish propagation and breeding center, Golestan province, Iran. Fish were kept under the same environmental conditions and placed in 420 L circular fiberglass tanks (350 L water volume and 30 fish per tank) for 2 weeks as an acclimation period to the laboratory condition and they fed a commercial diet [Starter food Kutum (SFK)]. The proximate compositions of the commercial diet (wet basis %) contained 8.7% humidity, 32% protein, 10.5% lipid and 11.2% ash.

Plant extract

Epilobium (E. hirsutum) ethanolic extract was obtained from Giah Essence Company, Gorgan, Golestan province, Iran.

In midsummer, E. hirsutum were collected from Grow and Industry Farm of Giah Essence. Aerial parts of this plant were washed, dried at room temperature and ground. One kilogram powdered sample was extracted by percolation with 6 L methanol (40%). The resulting extract was concentrated over a rotary vacuum evaporator and then freeze-dried. Then 6 g concrete was dissolved in 100 mL absolute ethanol.

Multivitamin

Multivitamin was obtained from a veterinary pharmacy. Ingredients of the multivitamin used in this study have been shown in Table 1.

Aeromonas hydrophila

Aeromonas hydrophila (AT118) was obtained from Faculty of Veterinary Medicine, Shiraz University, Iran.

Antibacterial activity of plant extract

Zones of growth inhibition were determined on Mueller–Hinton agar (MHA) surface inoculated to yield a confluent A. hydrophila lawn that was autoclaved for 15 min [121 °C at 1.05 kg cm−2 (15 psi)], poured in sterile condition onto a sterile petri dish (diameter = 10 cm), and cooled to room temperature.
Each petri dish was inoculated with a diluted *A. hydrophila* culture at $1.5 \times 10^8$ colony forming unit (CFU)/mL (McFarland No. 0.5) onto the surface of MHA and distributed evenly with a sterile L-shaped glass rod. Then, sterile paper discs (Whatman No. 1) previously impregnated with herbal extract for 10 min were placed on the MHA medium. Ethanol-impregnated disc and tetracycline-impregnated discs served as positive and negative controls respectively. The experiments were performed in triplicate. After the plates were incubated at room temperature for 24 h, the inhibition zones around the discs where no growth occurred were measured in millimeters (Harikrishnan & Balasundaram 2008).

**Fish rearing**

After 2 weeks of acclimatization period, healthy *C. carpio* (20 ± 2 g) were divided into five groups (control and treatment named E1–E4), and there were three replicates for each treatment arranged randomly. Each replicate contained 30 individuals in circular fibreglass tanks (420 L capacity with 350 L water volume). Fish were fed at the rate of 2% of their body weight per day in the period of experiment for 8 weeks. The daily ration was subdivided into two meals at 10:00 and 18:00 hours. Water temperature was $23 \pm 1 \degree C$. Fish on each tank were weighed monthly and the amounts of given feed were readjusted according to increase in body weight.

**Feeding**

Four experimental groups (Control and E1–E3) were fed with a commercial diet (SFK) contained different level of plant extract (0%, 0.5%, 1% and 3% of diet respectively). The fifth group (E4) was fed with a commercial diet contained plant extract (3% of diet), added 2% multivitamin (2 g of multivitamin per kg diet). Diet of each treatment was poured in dish and the necessary amounts of ethanolic plant extract (and multivitamin for E4 group) were spread in the diet thoroughly (by using of a small perfume glass) half an hour before each feeding time. Feeding was done manually and observed that almost all of diets were eaten by fish immediately after pouring in each tank.

**Growth parameters and feed utilization**

At the end of feeding trial 10 fish each tank were randomly taken and their weights and lengths were measured. Specific growth rates (SGR), condition factor (CF), feed conversion ratios (FCR) and survival rate were calculated as following:

$$SGR = \frac{100}{\text{day}} \left( \frac{\ln \text{final weight}}{\ln \text{initial weight}} \right)$$

$$CF = \left[ \frac{\text{weight} (\text{g})}{\text{length} (\text{cm})^{3/4}} \right] \times 100$$

$$FCR = \frac{\text{feed intake} (\text{g})}{\text{weight gain} (\text{g})}$$

$$\text{Survival} = \frac{100}{\text{initial fish number} - \text{dead fish number}}$$

$$/ \text{initial fish number}$$

**Proximate body composition**

At the end of feeding period, three fish from each tank were sampled for proximate composition analysis 24 h after the last feeding. Sampled fish were anaesthetized by using clove powder (100 ppm). Then chemical compositions of whole body of fish (moisture, protein, lipid and ash) were determined following the Association of Official Analytical Chemists (AOAC) methods. The proximate compositions of whole body of fish were analysed based on the standard methods of the Association of Official Analytical Chemists (AOAC 1995). Moisture was determined by drying in oven (Binder, Tuttingen, Germany), at 105 °C for 24 h. Crude protein was determined by using a Kjeldal system (Gerhardt, type VAP40, Königswinter, Germany). Crude lipid was determined with ether extraction in a Soxhlet extractor (Gerhardt, type SE-416), and ash was determined using a muffle furnace (Nabertherm, Lilienthal, Germany), at 550 °C for 8 h.

**Bacterial challenge test**

After 8 weeks of feeding trial diets, 10 fish (34 ± 2 g) from each tank were transferred into the same other tanks and were challenged by intraperitoneal injection (IP) with pathogenic *A. hydrophila* diluted in

<table>
<thead>
<tr>
<th>Item</th>
<th>Each gram contain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin A</td>
<td>15 000 IU</td>
</tr>
<tr>
<td>Vitamin D3</td>
<td>10 000 IU</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>10 mg</td>
</tr>
<tr>
<td>Vitamin B1</td>
<td>20 mg</td>
</tr>
<tr>
<td>Vitamin B2-SD</td>
<td>3 mg</td>
</tr>
<tr>
<td>Vitamin B6</td>
<td>3 mg</td>
</tr>
<tr>
<td>Vitamin B12</td>
<td>10 mcg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>100 mg</td>
</tr>
<tr>
<td>Vitamin K3</td>
<td>3 mg</td>
</tr>
<tr>
<td>Ca.D-Pantothenate</td>
<td>10 mg</td>
</tr>
<tr>
<td>Nicotinamide</td>
<td>30 mg</td>
</tr>
<tr>
<td>Excipients</td>
<td>q.s</td>
</tr>
</tbody>
</table>

| Table 1 | Ingredients of the used multivitamin in this experiment |

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distilled water (1 mL = 3 x 10^8 CFU). During the challenge test, the fish were not fed. Mortality of the challenged fish was noted up to 30 days. The survived fish after 30 days post-challenge and unchallenged fish were sampled for haematological studies.

**Read phonetically**

**Blood sampling**

Ten fish from each infected (challenged) treatment and 10 fish from each uninfected (unchallenged) treatment were anaesthetized with clove powder (100 ppm). Blood was sampled individually by peduncle severance and immediately used for haematological examination.

**Haematology**

Red blood cells (RBC) and white blood cells (WBC) counts were counted manually, using Neubaur haemocytometer (Paul Marienfeld Gmbh, Lauda, Koenigshofen, Germany) after diluting blood samples by adding Daice solution [Made based on laboratory methods of fish pathology, Roberts (1989)]. Haemoglobin concentration (Hb: g/dL) was measured spectrophotometrically (Libra S12 biochrom, Cambridge, England) at 540 nm with cyanomethemoglobin method. Haematocrit (Ht: %) was measured with microcentrifuge method (IEC MB centrifuge, Needham, MA, USA), using standard heparinized microhaematocrit capillary tubes (75 mm at 3000 g for 5 min) (Harikrishnan, Balasundaram & Heo 2010).

**Statistical analysis**

Statistical analysis was performed by one way ANOVA. Difference means were tested at the 5% probability level using Duncan test. All the statistical analyses were carried out by using SPSS program version 16. Values are expressed as mean ± standard deviation.

**Results**

**Zone of inhibition**

Ethanolic plant extract were tested against *A. hydrophila*. The inhibitory zone diameters obtained by the disc diffusion test have been shown in Table 2. Inhibitory zone diameter of plant extract was equal to tetracycline.

**Growth parameters and feed utilization**

The survival rates (per cent), SGR, CF and FCR of *C. carpio* fed diet containing different levels of plant extract have been shown in Table 3. Between treatments survival, SGR, CF and FCR value had no significant difference (*P* > 0.05). All diets were accepted by the fish and survival of fish fed the experimental diets for 8 weeks was 100%.

**Proximate body composition**

The proximate chemical compositions of whole body of common carp fed diets containing different levels of plant extract have been shown in Table 4. Results in Table 4 indicated that there was no significantly difference between treatments (*P* > 0.05). Therefore, body compositions were not affected by different levels of plant extract.

**Bacterial challenge studies**

The survival rate of *C. carpio* fed with experimental diet after challenging with *A. hydrophila* have been shown in Table 5. In the period of 4 days post-challenge, survivals had no significant difference (*P* > 0.05) between groups (Control and E1–E4 treatments). In the period of 30 days post-challenge, survivals were significantly different between groups (*P* < 0.05). At the end of 30 days post-challenge, the survival percentages were found highest (96 ± 6%) in the group E3 and lowest (75 ± 5%) in control group (Fig. 1).

**Haematology**

**Infected groups**

The blood parameters of infected fish fed diets containing different levels of plant extract have been shown in Table 6. The RBC count decreased significantly (*P* < 0.05) in the E2 group.

The WBC count increased significantly (*P* < 0.05) between treatments when compared with control. In contrast, there were no significant differences in

### Table 2  Bacterial inhibition zone (mm) of plant extract, tetracycline and methanol on *Aeromonas hydrophila*

<table>
<thead>
<tr>
<th>Items</th>
<th>Zone of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epilobium hirsutum</td>
<td>7 ± 1^a</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>7 ± 1^a</td>
</tr>
<tr>
<td>Methanol (96%)</td>
<td>1 ± 0^b</td>
</tr>
</tbody>
</table>

Disc diffusion test. Values are expressed as mean ± SD. Mean the same letter is not significantly different (*P* < 0.05).
Table 3  SGR, CF, FCR and survival of *Cyprinus carpio* fed diets containing different levels of *Epilobium hirsutum* extract

<table>
<thead>
<tr>
<th>Items</th>
<th>Plant extract</th>
<th>Control</th>
<th>0.5%</th>
<th>1%</th>
<th>3%</th>
<th>3% + 2% MV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGR</td>
<td></td>
<td>0.66 ± 0.14</td>
<td>0.64 ± 0.1</td>
<td>0.68 ± 0.03</td>
<td>0.64 ± 0.03</td>
<td>0.58 ± 0.09</td>
</tr>
<tr>
<td>CF</td>
<td></td>
<td>1.26 ± 0.07</td>
<td>1.27 ± 0.01</td>
<td>1.25 ± 0.01</td>
<td>1.28 ± 0.00</td>
<td>1.27 ± 0.00</td>
</tr>
<tr>
<td>FCR</td>
<td></td>
<td>2.73 ± 0.81</td>
<td>2.52 ± 0.52</td>
<td>2.58 ± 0.2</td>
<td>2.72 ± 0.19</td>
<td>3.08 ± 0.59</td>
</tr>
<tr>
<td>Survival</td>
<td></td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD.

*MV, multivitamin.

Table 4  Proximate chemical analysis (% wet basis) of whole body of common carp fed diets containing different level of plant extract

<table>
<thead>
<tr>
<th>Items</th>
<th>Plant extract</th>
<th>Control</th>
<th>0.5%</th>
<th>1%</th>
<th>3%</th>
<th>3% + 2% MV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td></td>
<td>78.91 ± 0.19</td>
<td>78.66 ± 0.43</td>
<td>78.5 ± 0.19</td>
<td>78.36 ± 0.18</td>
<td>78.95 ± 0.2</td>
</tr>
<tr>
<td>Protein</td>
<td></td>
<td>16.9 ± 0.44</td>
<td>17.15 ± 0.09</td>
<td>17.47 ± 0.27</td>
<td>17.34 ± 0.48</td>
<td>17.41 ± 0.36</td>
</tr>
<tr>
<td>Lipid</td>
<td></td>
<td>2.46 ± 0.46</td>
<td>2.06 ± 0.25</td>
<td>2.22 ± 0.12</td>
<td>2.48 ± 0.09</td>
<td>1.99 ± 0.09</td>
</tr>
<tr>
<td>Ash</td>
<td></td>
<td>0.07 ± 0.00</td>
<td>0.07 ± 0.00</td>
<td>0.07 ± 0.00</td>
<td>0.06 ± 0.00</td>
<td>0.07 ± 0.00</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD.

*MV, multivitamin.

Table 5  Survival rate of *Cyprinus carpio* fed diets containing different levels of plant extract at the end of 4 days and 30 days post challenge

<table>
<thead>
<tr>
<th>Items</th>
<th>Plant extract</th>
<th>Control</th>
<th>0.5%</th>
<th>1%</th>
<th>3%</th>
<th>3% + 2% MV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survival rate (%) 4 days post challenge</td>
<td></td>
<td>90 ± 10</td>
<td>92 ± 6</td>
<td>96 ± 6</td>
<td>96 ± 6</td>
<td>92 ± 6</td>
</tr>
<tr>
<td>Survival rate (%) 30 days post challenge</td>
<td></td>
<td>75 ± 5b</td>
<td>77 ± 10b</td>
<td>79 ± 1b</td>
<td>96 ± 6a</td>
<td>90 ± 11ab</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Mean the same letter in the same row is not significantly different (p < 0.05).

*MV, multivitamin.

Table 6  Hematological parameters of infected fish fed diet containing different levels of plant extract

<table>
<thead>
<tr>
<th>Items</th>
<th>Plant extract</th>
<th>Control</th>
<th>0.5%</th>
<th>1%</th>
<th>3%</th>
<th>3% + 2% MV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td></td>
<td>8.88 ± 1</td>
<td>9.35 ± 2.34</td>
<td>9.2 ± 0.33</td>
<td>8.89 ± 1.08</td>
<td>8.86 ± 1.03</td>
</tr>
<tr>
<td>Ht</td>
<td></td>
<td>41.5 ± 0.57ab</td>
<td>38.11 ± 3.88a</td>
<td>37.6 ± 3.13b</td>
<td>45.11 ± 4.31a</td>
<td>44.4 ± 6.42a</td>
</tr>
<tr>
<td>RBC</td>
<td></td>
<td>2.48 ± 0.2a</td>
<td>2.41 ± 0.17a</td>
<td>2.02 ± 0.61b</td>
<td>2.3 ± 0.32ab</td>
<td>2.5 ± 0.26a</td>
</tr>
<tr>
<td>WBC</td>
<td></td>
<td>1.91 ± 0.47b</td>
<td>2.28 ± 0.29ab</td>
<td>3.19 ± 0.34a</td>
<td>2.85 ± 0.78a</td>
<td>2.67 ± 0.84a</td>
</tr>
<tr>
<td>Lymphocyte</td>
<td></td>
<td>82.09 ± 4.48</td>
<td>82.75 ± 5.57</td>
<td>86.3 ± 3.19</td>
<td>85.09 ± 5.46</td>
<td>83.33 ± 7.39</td>
</tr>
<tr>
<td>Neutrophil</td>
<td></td>
<td>0.83 ± 0.29</td>
<td>0.77 ± 0.18</td>
<td>0.81 ± 0.21</td>
<td>0.7 ± 0.00</td>
<td>0.7 ± 0.00</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Mean the same letter in the same row is not significantly different (p < 0.05).

*MV, multivitamin.
lymphocyte, monocyte and neutrophil count between treatments and control ($P > 0.05$). The Hb concentration of all groups did not show any significant difference ($P > 0.05$). The Ht percentage had significant difference between treatments ($P < 0.05$). The highest Ht percentage was in E2 treatment.

**Uninfected groups**

The blood parameters of uninfected fish fed diets different levels of plant extract have been shown in Table 7.

In the uninfected groups the RBC, Hb and Ht levels did not have significant difference between treatments when compared with the control ($P > 0.05$).

The WBC count increased significantly ($P < 0.05$) between treatments when compared with the control. There was no significant difference in lymphocyte, monocyte and neutrophil count between treatments and control, too ($P > 0.05$).

**Discussion**

During recent decades many substances have shown to enhance the immunity of fish and the route of administration of them have differential effects on the immune system (Chritybapita, Divyagnaneswari & Dinakaran Michael 2007). Hence, the present work focuses on the administration of *E. hirsutum* extract-spread on the diet, to *C. carpio*, a fish species of growing global interest in aquaculture.

In the present study, *E. hirsutum* was screened for its inhibitory activity against *A. hidrophila*. The result indicated that ethanolic extract of *E. hirsutum* inhibited the growth of pathogen in agar plates. This result is consensus with earlier studies of Harikrishnan and Balasundaram (2008) who reported that the extracts of *Curcuma longa*, *Ocimum sanctum* and *Azadirachta indica* showed inhibitory effect on growth of *A. hidrophila*. The highest antibacterial effect of the ethanolic extract of *E. hirsutum* may be due to its high content of flavonoids, tannins and steroids. In fact these compounds are known for their strong antimicrobial activity (Cushnie, Hamilthon & Lamb 2003; Martini, Katerere & Eloj 2004; Rahman, Rana, Zaman, Uddin, Uddin & Akter 2010). Many biological activities and antibacterial promoting effects have been reported for plant tannins and flavonoids (Haslam 1989; Scalbert 1991; Chung, Wong, Wei, Huang & Lin 1998). Tannins are polyphenols that are obtained from various parts of different plants (Gajendiran & Mahadevan 1990). Tannin can be toxic to bacteria, filamentous fungi and yeast (Harborne 1973). The mechanism of antibacterial action of flavonoids remains largely unknown. However, results of recent studies suggested that inhibition of nucleic acid synthesis may be the primary cause of the antibacterial character of at least some of these compounds (Ulanowska, Tkaczyk, Konopa & Wegrzyn 2006).

**Table 7** Hematological parameters of uninfected fish fed diet containing different levels of plant extract

<table>
<thead>
<tr>
<th>Items</th>
<th>Plant extract</th>
<th>Control</th>
<th>0.5%</th>
<th>1%</th>
<th>3%</th>
<th>3% + 2%MV*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb</td>
<td>9.78 ± 1.55</td>
<td>9.42 ± 1.22</td>
<td>10.29 ± 2.17</td>
<td>9.29 ± 0.43</td>
<td>8.86 ± 1.05</td>
<td></td>
</tr>
<tr>
<td>Ht</td>
<td>48 ± 6.44</td>
<td>47.22 ± 6.18</td>
<td>52.9 ± 8.59</td>
<td>46.11 ± 3.33</td>
<td>49 ± 7.36</td>
<td></td>
</tr>
<tr>
<td>RBC</td>
<td>2.28 ± 0.35</td>
<td>2.28 ± 0.42</td>
<td>2.35 ± 0.29</td>
<td>2.36 ± 0.38</td>
<td>2.42 ± 0.39</td>
<td></td>
</tr>
<tr>
<td>WBC</td>
<td>2.93 ± 0.78*</td>
<td>4.15 ± 0.92*</td>
<td>4.28 ± 0.54*</td>
<td>4.63 ± 0.62*</td>
<td>4.21 ± 0.69*</td>
<td></td>
</tr>
<tr>
<td>Lymphocyte</td>
<td>86.28 ± 4.11</td>
<td>84.88 ± 5.46</td>
<td>88.55 ± 4.28</td>
<td>86.66 ± 4.33</td>
<td>88.67 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>Monocyte</td>
<td>13.57 ± 4.19</td>
<td>15.11 ± 5.46</td>
<td>11.11 ± 4.13</td>
<td>13.22 ± 4.4</td>
<td>10.37 ± 3.96</td>
<td></td>
</tr>
<tr>
<td>Neutrophil</td>
<td>0.78 ± 0.19</td>
<td>0.7 ± 0.00</td>
<td>0.7 ± 0.00</td>
<td>0.76 ± 0.17</td>
<td>1.02 ± 0.47</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD. Mean the same letter in the same row is not significantly different ($P < 0.05$).

*MV, multivitamin.

**Figure 1** Survival rate of *Cyprinus carpio* fed diets containing different levels of plant extract at the end of 4 days and 30 days post-challenge. Mean the same letter is not significantly different ($P < 0.05$).
Survival of the fish was not significantly affected by the experimental diets. This result is in agreement with the studies of Cho, Lee, Park, Ji, Lee, Bae and Oh (2007), who reported that green tea had no effect on survival of olive flounder, Paralichthys olivaceus. Ji, Jeong, Im, Lee, Yoo and Takii (2007) observed that medicinal herbs had no effect on survival of Japanese flounder.

In our experiment, E. hirsutum had no significant effect on FCR, SGR and CF in fish fed diet containing plant extract compared with the control. Yu, Li, Lin, Wen and Ma (2008) reported that FCR in white shrimp (L. vannamei) fed diet containing bacillus and medical herb had no significant difference compared with the control. Ji et al. (2007) found that Japanese flounder fed diet containing medical herbs had no significant difference in CF between groups. Some of plant extract improve SGR (Ji et al. 2007; Yu et al. 2008; Dada & Ikuerowo 2009), however, in this study, SGR had no significant difference between groups fed diet containing different levels of E. hirsutum.

Moisture, crude protein, crude lipid and ash in fish body did not be affected by different levels of plant extract. These results are in agreement with those obtained by Abd. Zaher, Mostafa, Hassan Ahmad, Mousallamy and Samir (2009) who found that fish body composition of Nile tilapia fingerlings did not be affected by different levels of fenugreek seeds. Similarly, red sea breams fed diets containing medicinal herb showed no significant difference in their body compositions (Ji et al. 2007).

The mortality rate of fish challenged A. hydrophil for 30 days was high in fish fed control diet compared with the other groups. The lowest mortality was in E3 treatment, therefore it was considered that we would be able to use the diet containing 3% E. hirsutum extract in common carp against A. hydrophil.

Harikrishnan et al. (2010) observed that herbal supplementation diets increase immune system of goldfish against A. hydrophil. Abd. Zaher et al. (2009) reported that mortality not observed in Nile tilapia fed diet containing different level of fenugreek and challenged A. hydrophil.

Haematological indices are an index and a reflection of the effects of dietary treatments on the animal in terms of the type, quality and amounts of the feed ingested and were available for the animal to meet its physiological, biochemical and metabolic necessities (Ewuola, Folarayan, Gbore, Adebumi Akanji, Ogunlade & Adeneye 2004).

WBC affords protection against infectious agent caused by microbial and chemical factors. In these study, the WBC level of infected and uninfected groups significantly increased (P < 0.05) when compared with the control. This may explain the efficacy of E. hirsutum in terms of the health status that lowered the mortality rate in fish fed diets containing plant extract during the post-challenge test period. These observations are in agreement with the findings of Gopakannan and Arul (2006) who reported that there was an increase in the WBC count after feeding the common carp with immunostimulants like chitin. Sahu Das, Pradhan, Mohapatra, Mishra & Sarangi (2007) reported that WBC count were similar, red sea breams fed diets containing medicinal plants as adjuvant therapy added to fish food to prevent diseases. Further studies are needed to evaluate cost benefits.

This work provides a new perspective for use of medicinal plants as adjuvant therapy added to fish food to prevent diseases. Further studies are needed to evaluate cost-benefits.

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feed additive on immunity and resistance to *Aeromonas hydrophila* in *Labeo rohita* fingerlings. *Fish & Shellfish Immunology* 23, 109–118.


