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Original Article

Effects of dietary administration of yarrow extract on growth performance and blood biochemical parameters of rainbow trout (*Oncorhynchus mykiss*)

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Abstract: The present study was conducted to investigate the clinical effects and possible side effects of yarrow extract (*Achillea millefolium* L.) as feed additive on biochemical blood parameters and growth performance of rainbow trout (*Oncorhynchus mykiss*). Fishes were treated with 0 (control), 0.1, 0.5 and 1% of yarrow extract for 30 days. Plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), creatine kinase (CK), peroxidase activity, total complement and lysozyme activity, glucose, total protein, triglyceride and cholesterol were measured after 15 and 30 days of yarrow treatment. There were no significant changes in the lysozyme activity and glucose levels. Total protein and globulin levels were significantly higher in the fish fed with diets enriched with 1% yarrow extract on day 30. Triglyceride and cholesterol levels was significantly decreased in the fish fed with diets containing 0.5% and 1% yarrow extract on day 30 (P<0.05). LDH, CK and peroxidase activities in the fish fed with diets having 1% yarrow extract were significantly decreased at the end of the experiment (P<0.05). In contrast, a significant increase in AST, ALP and total complement activity was observed in the fish fed with 1% yarrow extract diet, on day 15 (P<0.05). The weight gain and specific growth rate increased and food conversion ratio decreased in in the fish fed 1% yarrow extract on day 30. Condition factor in the fish fed with yarrow extract was significantly higher than control group on 30 day. In conclusion, on the basis of these results, oral administration of yarrow extract up to 0.5% have not side effect on blood biochemical and clinical parameters of fishes. However, oral administration of 1% of yarrow extract caused cytotoxicity and modifications in blood biochemical parameters of fish.

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Introduction

Compared with chemical drugs, the medicinal plants have effective agents and other compounds which accelerate gastrointestinal absorption process, improve the therapeutic effects and reduce side effects and drug toxicity (Platel et al., 2002). Although the results reported by laboratory animals evidence beneficial effects in the application of medicinal plants in treatment and prevention of disease in fish (Sivaram et al., 2004; Rao et al., 2006; Divyagnaneswari et al., 2007), the contradictory effects of some medicinal plants has decelerated using them for fish in a commercial scale. The lack of knowledge about the biological effects of compounds in medicinal plants' extracts on the individual characteristics of different fish species are the main problems in application of these compounds (Banaee, 2010). Therefore, study of the effects of herbal derivatives as a drug on the health of fish may increase our knowledge which can favour the use of certain compound for disease treatment (Banaee et al., 2011).

Yarrow (*Achillea* sp.) has an important role in medicinal plants derived from its antioxidant (Yakhkeshi et al., 2012), analgesic, anti-bacterial (Mazandarani et al., 2007), anti-parasitic (Khalili et

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Table 1. Phytochemical compounds isolated from the dried yarrow extract adopted from Saeidnia et al. (2011) and Ebrahimi et al. (2012).

Flavonoid	Essential oil	
Cyaroside	Tricyclene	n-heptanol
Cosmosiin	α-pinene	3-octanone
Luteolin	β-ocimene	δ-3-carene
Apigenin	γ-terpinene	α-terpinene
Centaureidin	Terpinolene	*p*-cymene
Quercetin	Linalool	Pinocarvone
3´-methoxy luteolin	Trans-thujone	Borneol
Luteolin 7-O-glucoside	Allo-ocimene	α-copaene
5-hydroxy 3´,4´,6´,7´-tetra methoxy	Terpin-1-ol	Geranyl acetate
flavone	Camphor	Longifolene
Salvigenin	(Z)-tagetone	α-gurjunene
Galangin	Isoborneol	α-guaiene
Eupatilin	α-terpineol	*n*-pentadecane
	α-himachalene	α-cadinene
	γ-gurjunene	γ-eudesmol
	β-chamigrene	α-eudesmol
	γ-muurolene	Bornyl acetate
	β-selinene	Neryl acetate
	Viridiflorene	α-cyclogeraniol
	Cis-pinocarveol	β-calacorene
	Isobornylformate	(Z)-cubenol
	α-terpinyl acetate	β-bisabolol
	Caryophyllene alcohol	Phytol
	(E)-cinnamaldehyde	Nootkatin
	Patchouli alcohol	Occidol acetate
	α-bisabolene oxide A	Abietadiene
	(E,E)-farnesol	*n*-heneicosane
	Chamazulene	Methyl octadecanoate

al., 2011), anti-fungal (Ayatollahi Mousavi et al., 1996), anti-inflammatory (Benedek et al., 2007), anti-hypertensive and lipid-lowering (Asgary et al., 2000), anticonvulsant and antispasmodic (Lemmens-Gruber et al., 2006), antitumor (Csupor-Löffler et al., 2009), anti-ulcer skin (Cavalcanti et al., 2006), and antiarrhythmic (Asgary et al., 2000) properties. The antibacterial properties of yarrow extract are attributed to flavonoids, saponins, and terpens (Table 1) (Mothana et al., 2009). Moreover, the administration of medicinal plants with antibiotic properties can change intestinal bacterial flora, increase growth and improve carcass quality (Tekeli et al., 2008). Therefore, the present study was carried out to determine whether yarrow (*Achillea millefolium* L.) would influence on the blood biochemical parameters, growth performance and immune parameters of rainbow trout.

Materials and Methods

Dried yarrow extract preparation: Yarrow powder was extracted with hydro-alcoholic mixture (ethanol 70% and water in 1:1 proportion) at room temperature by cold maceration method (Asgary et al., 2003). Then, this extract was filtered through Whatman filter paper. Finally, the extract was concentrated in an incubator at 50°C obtaining the dry extract.

Animal treatments: One hundred and twenty rainbow trout (with average weight and length of

Table 2. Nutrient composition of the commercial diets (Chineh Company, Iran).

Nutrient composition	Reference diet
Dry material	92
Metabolize energy (Kcal/g)	350
Crud protein	40
Ether extract (lipid)	10.5
Ash	7.9
Crude fiber	5.8
Carbohydrate	27

146.9 ± 18.1 g; 25.9 ± 2.6 cm, respectively) were randomly introduced into 4 treatments, 3 replicates with 10 fish in the 12 fibreglass tanks (200 L) with semi-closed water recirculating systems for two weeks to acclimate to the laboratory conditions (15 ± 2°C; pH: 7.4 ± 0.2; photoperiod: 16L: 8D; 20% water exchange rate/day) prior to experiments. During acclimation and experimental periods, fish were fed with prepared pellets according to commercial formulations at 2% of their body weight twice a day (Hinshaw, 1999).

The enriched diet was prepared with formulated fish feed obtained from Chineh Company, Karaj, Iran (Table 2) and yarrow extract according to the method developed by Banaee et al. (2011). Each supplemented diet was mixed with distilled water until obtaining a homogenous mixture. This mixture was passed through a meat grinder, producing extruded string shapes, which were dried in an oven at 55°C for 12 hrs and then broken to produce pellets with approximate 10 mm length. The prepared pellets were packed and stored at -20°C in a freezer until be used. The control diet was prepared using the same process, although no supplement was added.

In day of 15 and 30 of the experiment, 12 fish from each treatment were randomly sampled and anesthetized with clove powder solution (200 mg/L). Then, the blood of fish from each experimental and control groups were taken by puncturing of the caudal vessels with a syringe and the blood was stored in sterilized glass vials containing the anticoagulant 1% EDTA at 4°C. The blood was centrifuged for 15 min at 4000 rpm. The contents of digestive tract, nutritional status (gastro-intestine-somatic index) and clinical symptoms such as changes in the size of gall-bladder and liver (hepato-somatic index) as well as feeding behavior of treated fish were evaluated.

Biochemical Analysis: Biochemical parameters of plasma including glucose, total protein, albumin, globulin, cholesterol and triglyceride; aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) and creatine kinase (CK) were analyzed using the biochemical diagnostic kit (Pars-Azemon Company, Karaj, Iran). Alternative complement activity (ACH50) was evaluated based on Yano (1992) using rabbit red blood cells (RaRBC). In this method, the absorbance of the haemolysate was measured at 414 nm against distilled water to acquire the 100% lysis value. The volume of plasma producing 50% haemolysis (ACH50) was determined. Lysozyme activity was determined by the lysis of bacterial cells of *Micrococcus lysodeikticus* (Lange et al., 2001). The decrease in turbidity at 570 nm after 0.5 min and 4.5 min was recorded by spectrophotometer. Lysozyme of sample was calibrated using a standard curve determined with hen's egg white lysozyme (Sigma) in PBS. The total peroxidase activity was determined according to Cuesta et al. (2007) with modification by measuring the optical density of 3,3′,5,5′–tetramethylbenzidine hydrochloride oxidation products during particular intervals of time at 450 nm. All biochemical parameters were measured in duplicates by UV/Vis Spectrophotometer (model Unico 2100).

Growth performance: The growth parameters including weight gain percentage, specific growth rate, and feed conversion ratio and condition factor were calculated using the following formulas, on day15 and 30 day.

$$\text{Weight gain (\%)} = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100$$

Specific growth rate (SGR%) = [(Ln (final body weight)-Ln (initial body weight))/(Experimental periods)]×100

$$\text{Feed conversion ratio (FCR)} = \frac{\text{Feed intake (g)}}{\text{Wet weight gain (g)}}$$

$$\text{Condition factor (CF)} = \frac{\text{Weight (g)}}{(\text{Length (cm)})^3} \times 100$$

Statistical analysis: A significant difference in the biochemical parameters of specimens treated with the different concentrations of *A. millefolium* extracts was examined using one-way ANOVA. All the data were examined for normality (Kolmogorov-Smirnov test). The means were compared by Duncan's test and a $P<0.05$ was considered statistically significant. Statistical analyses were performed using SPSS (IBM, Release 19) software. Data are presented as mean ± SD.

Results

The hepato-somatic index (IH) was significantly decreased in fish fed with a diet containing 1% yarrow extract. No significant changes were observed during the test in spleen-somatic index (IS) and gastro-intestine-somatic index (IG) in the fish fed with diets containing yarrow extract when compared with control group. The contents of the digestive system of fish as satiety index indicated that there were no significant difference between amounts of feed was fed by experimental fish and control group (Fig.1a, b, c).

Significant changes in the WG and SGR was observed in the fish fed with a diet containing 1% yarrow extract during the experiment. The FCR was significantly decreased in fish with diets having 1% yarrow extract. The CF in the fish fed with yarrow extract was significantly higher than the control group at the end of experiment (Table 3).

There was no significant difference in glucose levels between fish fed yarrow extract and control group during experimental period. Although, oral administration of 1% yarrow extract resulted in a significant increase in total protein levels, a significant decrease in the albumin levels was observed in plasma of fish fed with 0.1% yarrow extract on day 30. Supplementing feed with 0.1 and

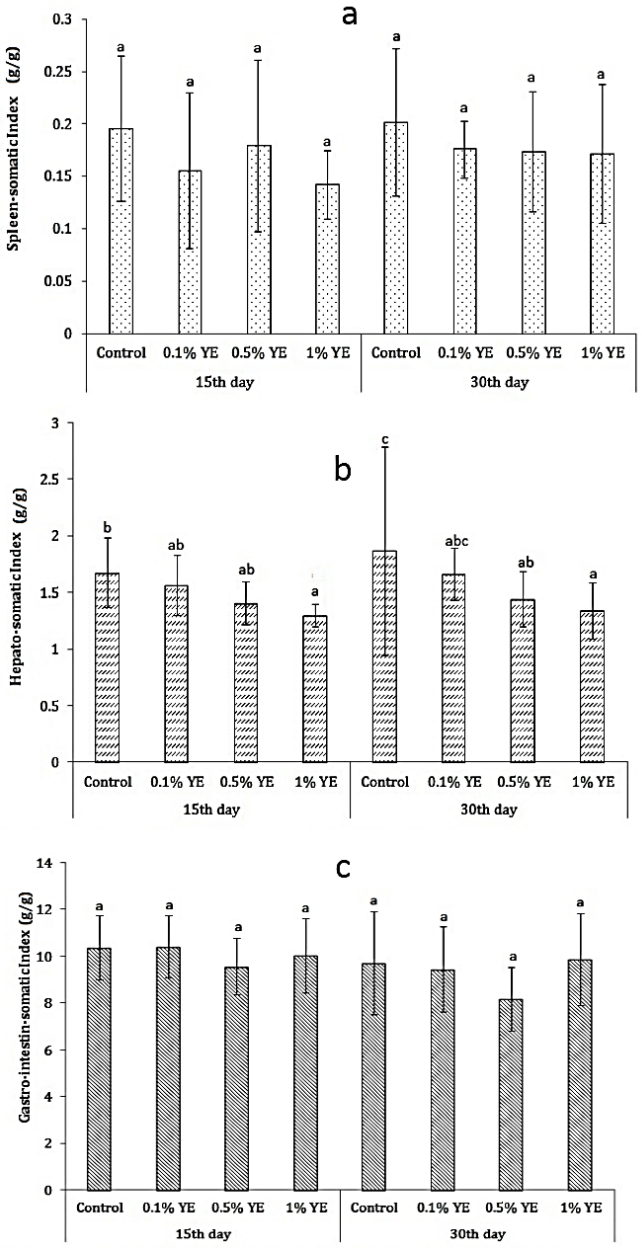

Figure 1. Effects of different concentrations of yarrow extract as feed additive on (a) percentage ratio of spleen to total weight as spleen-somatic index (IS), (b) liver weight to total weight as hepato-somatic index (IH) and (c) gastrointestinal tract weight to total weight (IG) as a satiety index or gastro-intestine-somatic index. Significant differences between treatment and control groups were represented by alphabets (one-way ANOVA, $P<0.05$). Values represent mean ± S.D.

1% yarrow extract resulted in significantly increased in globulin levels on day 30 ($P<0.05$). Triglyceride levels in the plasma of the fish fed with diets having 0.5% and 1% yarrow extract showed a significant decrease during the test. Cholesterol levels

Table 3. Growth performance of rainbow trout fed with yarrow extract as a supplement.

Growth Performance	YE% of g/kg	Experimental periods	
		15th day	30th day
Weight gain (%)	0.0	27.45±7.45a	56.63±8.09[a]
	0.1%	35.80±11.85ab	70.81±18.77[b]
	0.5%	28.33±6.53a	67.23±11.43[ab]
	1%	39.57±8.74b	69.79±9.36[b]
Specific growth rate (%/day)	0.0	1.61±0.38a	1.49±0.17[a]
	0.1%	2.02±0.59ab	1.77±0.37[b]
	0.5%	1.66±0.33a	1.71±0.23[ab]
	1%	2.21±0.42b	1.76±0.18[b]
Food conversion rate	0.0	1.73±0.54b	1.59±0.24[b]
	0.1%	1.40±0.62ab	1.33±0.37[ab]
	0.5%	1.63±0.34ab	1.35±0.27[ab]
	1%	1.18±0.33a	1.28±0.17[a]
Condition factor	0.0	0.85±0.06[a]	0.89±0.06[a]
	0.1%	0.92±0.07[a]	1.00±0.09[b]
	0.5%	0.93±0.13[a]	0.98±0.06[b]
	1%	0.93±0.0.9[a]	0.98±0.08[b]

Effects of different concentrations of yarrow extract on growth index were analyzed using a one-way ANOVA. Significant differences between treatment and control groups were represented by alphabets (P<0.05). Values represent mean ± SD.

significantly decreased in plasma of fish fed with diets having 0.5% and 1% yarrow extract on day 30 of the test (P<0.05), (Table 4).

There was a significant increase in the total complement activity in plasma of fish fed with food enriched with 1% of yarrow extract on day 30 of trial (P<0.05). No significant changes were observed in the ACH50 activity on day 15 (Table 4).

The activity of AST significantly decreased in plasma of fish fed with 0.1% and 0.5% yarrow extract on day 30 of experiment (P<0.05). However, AST activity in plasma of the fish fed with diets having 1% yarrow extract significantly increased on day 15 of test (P<0.05). The activity of ALT significantly increased in plasma of fish fed with 0.5 and 0.1% yarrow extract on 15 and 30 days when compared with control group, respectively. LDH activity in plasma of the fish fed with diets enriched with 0.1% and 1% yarrow extract was significantly lower than control group on day 30 of the experiment. The activity of ALP significantly increased in plasma of fish fed with 1% yarrow extract on day 15 of experiment. The activity of CPK significantly decreased in plasma of fish fed with 1% yarrow extract during experimental period (P< 0.05), (Table 5).

A significant decrease in the peroxidase activity was observed in plasma of fish fed with 0.5% and 1% yarrow extract on day 15 (P< 0.05). Also, there was no significant change in peroxidase activity on day 30 of test. No significant changes in lysozyme activity in plasma of fish fed with yarrow extract supplement were observed when compared with control group during experimental periods (P> 0.05), (Table 5).

Discussion

In this study, rainbow trout were fed with diets enriched with 0.1%, 0.5%, and 1% yarrow extract for 30 days. During the experimental periods, no mortality or changes in the appetite of the fish were observed. Platel et al. (2002) evidenced the favorable effect of medicinal plants on digestion and a stimulating effect on bile secretion and the activity of pancreatic enzymes. Moreover, adding plants extracts to the diet can affect the fish ability to find

Table 4. Plasma biochemical parameters in rainbow trout fed with yarrow extract as a supplement.

Biochemical Parameters	YE% of g/kg	Experimental periods	
		30th day	15th day
Glucose (mg/dL)	0.0 %	74.64±26.41[a]	89.58±4.42[a]
	0.1%	64.53±11.46[a]	83.46±7.41[a]
	0.5%	73.32±22.68[a]	87.20±8.30[a]
	1%	62.59±13.17[a]	83.08±5.56[a]
Total protein (g/dL)	0.0 %	4.54±0.55[a]	4.77±0.87[a]
	0.1%	4.31±0.42[a]	4.52±0.59[a]
	0.5%	4.32±0.48[a]	4.45±0.41[a]
	1%	4.84±0.47[b]	4.37±0.91[a]
Albumin (g/dL)	0.0 %	3.00±0.40[b]	2.84±0.18[a]
	0.1%	2.36±0.56[a]	2.89±0.09[a]
	0.5%	3.00±0.51[b]	2.88±0.48[a]
	1%	2.77±0.62[ab]	2.88±0.11[a]
Globulin (g/dL)	0.0 %	1.53±0.36[a]	1.93±0.82[a]
	0.1%	1.95±0.67[b]	1.63±0.61[a]
	0.5%	1.32±0.35[a]	1.56±0.39[a]
	1%	2.07±0.43[b]	1.49±0.90[a]
Cholesterol (mg/dL)	0.0 %	222.11±41.41[b]	220.77±29.07[ab]
	0.1%	226.75±34.86[b]	258.18±52.80[b]
	0.5%	182.22±36.49[a]	178.16±42.49[a]
	1%	168.43±38.39[a]	191.25±49.81[a]
Triglyceride (mg/dL)	0.0 %	167.38±39.68[b]	166.97±33.09[b]
	0.1%	125.01±38.83[a]	169.77±12.43[b]
	0.5%	104.04±40.61[a]	129.60±17.07[a]
	1%	114.29±28.02[a]	126.02±26.34[a]
ACH50 (U/mL)	0.0 %	311.89±9.05[a]	321.44±7.86[a]
	0.1%	321.89±43.96[a]	315.67±11.05[a]
	0.5%	344.45±19.18[a]	314.34±10.06[a]
	1%	344.45±46.22[a]	366.00±30.56[b]

Effects of different concentrations of yarrow extract on biochemical parameters. Significant differences between treatment and control groups were represented by alphabets (one-way ANOVA, $P<0.05$). Values represent mean ± SD.

food by stimulating their sense of smell and encourage them to eat more (Adams, 2005).

Our results indicate that feeding of fish with 1% yarrow extract significantly improved growth performance. Some compounds in medicinal plants extracts including bioflavonoids can positively induce effects on growth performance and general health of fish (Yilmaz et al., 2006). Similar results are found in cichlid, *Cryptoheros nigrofasciatus*, (Cek et al. 2007); red seabream, *Pagrus major* (Ji et al. 2007); and rainbow trout (Bohlouli Oskoii et al., 2012) which were fed with diets supplemented with medicinal plants extracts.

Although, the results indicate that administration period of yarrow extract had a significant effect on glucose levels in plasma, hypoglycemic effect of yarrow on rats with diabetes mellitus indicates this plant's effects on against in regulating blood sugar in these animals (Sadeghi et al., 2009). These results may be attributed to the hypoglycemic activity of

Table 5. Plasma enzymatic activities in plasma of rainbow trout fed with yarrow extract as supplement.

Biochemical Parameters	YE% of g/kg	Experimental periods	
		15th day	30th day
AST (U/L)	0.0 %	87.27±12.65[a]	87.49±32.49[b]
	0.1%	97.49±13.94[a]	63.23±16.61[a]
	0.5%	96.75±16.18[a]	63.15±10.65[a]
	1%	121.89±36.22[b]	69.91±13.59[ab]
ALT (U/L)	0.0 %	12.86±3.67[a]	17.27±2.65[a]
	0.1%	12.57±4.39[a]	23.60±8.39[b]
	0.5%	19.11±5.52[b]	17.64±4.44[a]
	1%	12.79±3.87[a]	18.08±4.45[a]
LDH (U/L)	0.0 %	808.89±103.17[a]	735.60±237.85[b]
	0.1%	884.44±169.41[a]	523.05±203.21[a]
	0.5%	882.22±116.76[a]	700.57±251.85[ab]
	1%	890.37±205.86[a]	493.96±94.42[a]
CPK (U/L)	0.0 %	177.31±23.81[b]	207.27±27.47[b]
	0.1%	167.11±12.29[ab]	199.15±27.53[b]
	0.5%	160.34±31.61[ab]	199.99±37.76[b]
	1%	150.85±28.84[a]	151.19±31.27[a]
ALP (U/L)	0.0 %	18.89±5.59[a]	25.93±4.45[a]
	0.1%	25.12±9.63[a]	23.69±12.64[a]
	0.5%	34.41±20.40[ab]	21.95±4.12[a]
	1%	45.44±25.92[b]	20.22±11.65[a]
Peroxidase (U/mL)	0.0 %	138.11±10.78[b]	139.44±10.49[a]
	0.1%	129.67±11.68[ab]	126.11±11.21[a]
	0.5%	120.00±10.10[a]	119.56±9.41[a]
	1%	120.44±9.38[a]	163.44±86.12[a]
Lysozyme (U/mL)	0.0 %	123.68±8.38[a]	120.67±10.83[a]
	0.1%	124.78±9.13[a]	125.11±11.95[a]
	0.5%	128.22±11.10[a]	126.00±14.01[a]
	1%	129.44±10.10[a]	124.22±18.46[a]

Effects of different concentrations of yarrow extract on biochemical parameters (Enzyme activity). Significant differences between treatment and control groups were represented by alphabets (one-way ANOVA, $P<0.05$). Values represent mean ± SD.

herbal extracts to increase the level of plasma insulin and improvement of peripheral metabolism of glucose (Awad, 2010) or may be attributed to the activation of glycogen synthesis and healthy hepatic function (Ji et al., 2007). Decreased glucose levels in the blood of rainbow trout fed with silymarin extract (Banaee et al., 2011) and African catfish fed with onion and garlic extracts are reported (Al-Salahy, 2002).

Albumin and globulin make up most of the protein within the body and are measured in the total protein of the plasma. Total protein, albumin and globulin tests are used to monitor the course of diseases in immune disorders, liver dysfunction and impaired kidney activity. At the end of the test, the levels of albumin in plasma of the fish fed with diets containing 0.1% yarrow significantly decreased. The 0.1% of yarrow extract was able to significantly increase the total protein levels on day 30. Oral administration of 0.1 and 1 % yarrow extract significantly increased the globulin levels in plasma of fish on day 30. Although an increased level of total protein, albumin and globulin in plasma of the fish fed with diets enriched with *Echinacea purpurea* and *Silybum marianum* was reported by Bohlouli Oskoii et al. (2012) and Banaee et al. (2011), no significant changes are reported in the levels of albumin and total protein in plasma of fish fed with diets enriched with onion and garlic extract (Al-Salahy, 2002; Naeiji et al., 2013)..

Quercetin is the most important flavonoid in yarrow (Saednia et al., 2009) that prevents the biosynthesis of cholesterol by inhibiting the activity of fatty acid synthesis (Yamamoto and Oue, 2006). Therefore, the

decreased cholesterol levels in the blood of fish fed with 0.5 and 1% yarrow extract at the end of the test, and decreased levels of triglycerides in plasma of the fish fed with diets having 0.5% and 1% yarrow extract during the test may be attributed to the influence of quercetine on the synthesis of cholesterol. The influence of long term use of yarrow in decreasing triglyceride, total cholesterol and low-density lipoprotein (LDL) and increasing the high density lipoprotein (HDL) in the blood of human volunteers verifies this issue (Asgary et al., 2000). The cholesterol synthesized in liver transfers to other tissues of the body through LDL, but HDL transports the cholesterol of peripheral tissues to liver. So, the increased excretion of cholesterol through bile (Asgary et al., 2000) decreases the cholesterol level in blood of the fish fed with yarrow. Decreases in cholesterol and triglyceride levels are also reported in blood of rainbow trout and catfish respectively fed with silymarin extract (Banaee et al., 2011) and onion and garlic extracts (Al-Salahy, 2002).

The complement system is an essential and effective part of the innate immune system. It can rapidly distinguish and opsonize bacteria for phagocytosis by specialized phagocytes or destroy them directly by membrane disorder (Ahmadi et al., 2014). The increase in the complement activity (ACH50) observed in plasma of fish fed with diets enriched with 1% of yarrow extract on day 15 may help to identify and eliminate bacterial agents by phagocytosis. Increases in the total complement activity were reported in fish fed with a diet enriched with *Punica granatum*, *Chrysanthemum cinerariaefolium* and *Zanthoxylum schinifolium* extracts (Harikrishnan et al., 2010), *S. marianum* (Ahmadi et al., 2012) and *Nasturtium nasturtium* extracts (Asadi et al., 2012).

AST, ALT, LDH, ALP and CK are found in various tissues. When diseases or injuries affect these tissues, the cells are destroyed and these enzymes are released into plasma (Banaee et al., 2011). Although, oral administration of 0.1 and 0.5% yarrow extract resulted in a significant decrease in the activity of AST on day 30, a significant increase in AST activity in plasma of fish fed with enriched with 1% yarrow extract on day 15. Treatment with 0.5 % yarrow extract resulted in a significant increase in AST activity on day 15. Our results show that ALP activity significantly increased in the plasma of fish fed with enrich diet with 1% yarrow extract when compared with the control group at day 15, no significant changes were observed in ALP activity at day 30. LDH measurement is used to detect tissue disorders and as an aid in the diagnosis of tissue damage. The LDH activity also significantly decreased after the oral administration of 0.1 and 1% yarrow extract on day 30. A significant decrease were observed in the CK activity in plasma of common carp fed with enrich diet with 1% marshmallow flower extract when compared with the control group during experimental period. The most important of the biological mechanisms of flavonoids have been attributed to their antioxidant properties (Oteiza et al., 2005). Due to anti-radical and anti-oxidant properties of the yarrow extract, its administration might prevent lipid peroxidation of cell membranes and inhibit the release of foresaid enzymes into the plasma. Therefore, yarrow may be useful in treatment and prevention of diseases caused by oxidative stress derived from its rich content in flavonoids. A low concentration of silymarin (Banaee et al., 2011) and garlic (Al-Salahy, 2002; Naeiji et al. 2013) in diet of fish has been evidenced to regulate the plasma activities of AST, ALT, ALP, CK and LDH. More hydrophilic flavonoids can interact at the membrane surface through hydrogen bonding; can act to reduce the access of oxidants and per-oxidants and protect the structure and function of cellular membranes (Oteiza et al., 2005).

Lysozymes are a family of enzymes with antibacterial activity characterized by the ability to damage the cell wall of bacteria (Ahmadi et al., 2014). Our results showed that fish fed with diets containing yarrow extract had no effects on lysozyme activity. Studies of Sivaram et al. (2004) showed that no significant changes were observed in serum lysozyme activity of cultured grouper, *Epinephelus tauvina*, fed with diets enriched with

Ocimum sanctum, *Withania somnifera* and *Myristica fragrans* extracts.

Peroxidases play an important role in defense system against extracellular bacterial and parasitic pathogens. Myeloperoxidase and eosinophil peroxidase are important active peroxidases in immune system of fish and found in the cytoplasmic granules of neutrophils and eosinophil, respectively. If the leukocytes are stimulated, peroxidase activity will increase in plasma (Ahmadi et al., 2014). The results revealed that the administration of yarrow extract to fish significantly decrease the peroxidase activity. Antibacterial compounds that exist in the yarrow extract may be prevented stimulation of leukocytes. Ahmadi et al. (2012) and Asadi et al. (2012) found that the administration of *S. marianum* and *N. nasturtium* extract had no effects on peroxidase activity of fish.

Since the change in blood biochemical parameters is a useful clinical tool to predict and monitor the health in organisms, these factors can also be used for determining drug safety. Preclinical research on the biochemical factors and growth performance as pharmacology indices of yarrow revealed that this herbal medicine may be useful by providing benefit in the treatment of some disease of aquatic animals. Therefore, in clinical studies should be focused on the medical effects of yarrow.

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Reference

Adams C. (2005). Nutrition-based health. Feed internat. 2: 25-28.

Ahmadi K., Banaee M., Vosoghei A.R., Mirvaghefei A.R., Ataeimehr B. (2012). Evaluation of the immunomodulatory effects of silymarin extract (*Silybum marianum*) on some immune parameters of rainbow trout, *Oncorhynchus mykiss* (Actinopterygii: Salmoniformes: Salmonidae). Acta Ichthyologica Et Piscatoria, 42 (2): 113-120.

Ahmadi K., Mirvagefei A.R., Banaee M., Vosoghei A.R. (2014) Effects of Long-term Diazinon Exposure on Some Immunological and Haematological Parameters in Rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792). Toxicology and Environmental Health Sciences, 6(1): 1-7.

Al-Salahy M.B. (2002). Some physiological studies on the effect of onion and garlic juices on the fish, *Clarias lazera*. Fish Physiology and Biochemistry; 27: 129–142.

Asadi M.S., Mirvaghefei A.R., Nematollahi M.A., Banaee M., Ahmadi, K. (2012). Effects of Watercress (*Nasturtium nasturtium*) extract on some immunological parameters of rainbow trout (*Oncorhynchus mykiss*). Open Veterinary Journal; 2(1):32-39.

Asgary S., Naderi G.A., Gjanadi A.R., Gharipour M., Golban S. (2003). The effect of chamomile, yarrow, hawthorn on the increased resistance of red blood cells and protection from thiols groups (-SH) againstto oxidizing materials. Journal of Medicinal and Aromatic Plants, 6: 41-48.

Asgary S., Naderi G.H., Sarrafzadegan N., Mohammadifard N., Mostafavi S., Vakili R. (2000). Antihypertensive and antihyperlipidemic effects of *Achillea wilhelmsii*. Drugs, Experimental and Clinical Research, 26:89–93.

Awad E.S. (2010). Studies on plant based dietary supplements for control of *Aeromonas hydrophila* infections in rainbow trout (*Oncorhynchus mykiss* Walbaum). A thesis of PhD. Scholl of Life Sciences, Heriot Watt University, Edinburgh, UK. 204 p.

Ayatollahi Mousavi S.A., Abdollahi H., Kazemipour N. (1996). Investigation of antifungal activity of 10 methanol extracts of medicinal herbs. Journal of Kerman University, Medic Science, 3(3): 115-122.

Banaee M., Sureda A., Mirvaghefi A.R., Ahmadi K. (2011). Effects of diazinon on biochemical parameters of blood in rainbow trout (*Oncorhynchus mykiss*). Pesticide Biochemistry and Physiology, 99: 1-6.

Banaee M. (2010). Influence of silymarin in decline of sublethal diazinon-induced oxidative stress in rainbow trout (*Oncorhynchus mykiss*). Ph.D. Thesis, Aquaculture and Environmental Department, Natural Resource Faculty, Natural Resource and Agriculture

Collage, Tehran University, Iran, 149 p.

Banaee M., Sureda A., Mirvaghefi A.R., Rafei G.R. (2011). Effects of long-term silymarin oral supplementation on the blood biochemical profile of rainbow trout (*Oncorhynchus mykiss*). Fish Physiology and Biochemistry, 37: 887-896.

Benedek B., Kopp B., Melzig M.F. (2007). *Achillea millefolium* L. is the anti-inflammatory activity mediated by protease inhibition? Journal of Ethnopharmacology, 113(2):312–317.

Bohlouli Oskoii S., Tahmasebi Kohyani A., Salati A.P., Parseh A., Sadeghi E. (2012). Effects of dietary administration of *Echinacea purpurea* on growth indices and biochemical and hematological indices in rainbow trout (*Oncorhynchus mykiss*) fingerlings. Fish Physiology and Biochemistry, 38: 1029-1034.

Cavalcanti A.M., Baggio C.H., Freitas C.S., Rieck L., de Sousa R.S., Da Silva-Santos J.E. (2006). Safety and antiulcer efficacy studies of *Achillea millefolium* L. after chronic treatment in Wistar rats. Journal of Ethnopharmacology, 107(2): 277-84.

Cek S., Turan F., Atik E. (2007). Masculinization of convict cichlid (*Chichlasoma nigrofasciatum*) by immersion in *Tribulus terrestris* extract. Aquaculture International, 15(2): 109–119.

Csupor-Löffler B., Hajdú Z., Zupkó I., Réthy B., Falkay G., Forgo P. (2009). Antiproliferative effect of flavonoids and sesquiterpenoids from *Achillea millefolium* L. on cultured human tumour cell lines. Phytotherapy Research, 23(5): 672-676.

Cuesta A., Vargas-Chacoff L., García-López A., Arjona F.J., Martínez-Rodríquez G., Mesequer J., Macera J.M., Esteban M.A. (2007). Effect of sex-steroid hormones, testosterone and estradiol, on humoral immune parameters of gilthead seabream. Fish and Shellfish Immunology, 23 (3): 693-700.

Divyagnaneswari M., Christybapita D., Dinakaran M.R. (2007). Enhancement of nonspecific immunity and disease resistance in *Oreochromis mossambicus* by *Solanum trilobatum* leaf fractions. Fish & Shellfish Immunology; 23(2): 249-259.

Ebrahimi M., Farajpour M., Hadavand H., Bahmani K., Khodaiyan F. (2012). Essential oil variation among five *Achillea millefolium* ssp. *elbursensis* collected from different ecological regions of Iran. Annals of Biological Research, 3 (7): 3248-3253.

Harikrishnan R., Kim J.S., Han Y.J., Heo J., Balasundaram C., Heo M.S., Kim M.C. (2010). Effect of traditional Korean medicinal (TKM) triherbal extract on the innate immune system and disease resistance in *Paralichthys olivaceus* against *Uronema marinum*. Veterinary Parasitology, 170: 1-7.

Hinshaw J.M. (1999). Trout Production (Feeds and Feeding Methods). SRAC Publication No. 223. 4 p.

Ji S., Takaoka O., Jeong G., Lee S., Ishumaru K., Seoka M., Takii K. (2007). Dietary medicinal herbs improve growth and some non-specific immunity of red sea bream Pagrus major. Fishery Science, 73(1): 63-69.

Khalili B., Rafieian M., Hejazi S.H., Yusefi H.A., Yektaian N., Shirani-Bidabadi L. (2011). Effect of *Achillea millefolium*, *Artemisia absinthium* and *Juglansregia* leaves extracts on *Trichomonas vaginalis* in vitra. Journal of Shahrekord University of Medical Sciences, 12(4(Suppl 1)): 62-69.

Lange S., Gumundsdottir B.K., Mangadottir B. (2001). Humoral immune parameters of cultured Atlantic halibut (*Hippoglossus hippoglossus* L.). Fish and Shellfish Immunology, 11 (6): 523-535.

Lemmens-Gruber R., Marchart E., Rawnduzi P., Engel N., Benedek B., Kopp B. (2006). Investigation of the spasmolytic activity of the flavonoid fraction of *Achillea millefolium* L. on isolated guinea-pig ilea. Arzneimittelforschung, 56(8): 582-588.

Mazandarani M., Behmanesh B., Rezaei M.B. (2007). Ecological factors, chemical composition and antibacterial activity of essential oil from *Achillea millefolium* L. in the north of Iran. 55th International Congress and Annual Meeting of the Society for Medicinal Plant Research, Graz, Austria, September 2-6, 2007. Planta Medica, 73:170.

Mothana R., Lindequist U., Geraenert R., Bednarski P. (2009). Studies of the in vitro anticancer, antimicrobial and antioxidant potentials of selected Yemeni medicinal plants from Island Soqotra. BMC Complementary and Alternative Medicine, 9: 1-7.

Naeiji N., Shahsavani D., Baghshani H. (2013). Effect of dietary garlic supplementation on lipid peroxidation and protein oxidation biomarkers of tissues as well as some serum biochemical parameters in common carp *Cyprinus carpio*. Fisheries Science, 79: 699-705.

Platel K., Rao A., Saraswahi G., Srinivasan K. (2002). Digestive stimulant action of three Indian spice mixes in experimental rats. Nahrung, 46(6): 394-398.

Rao Y.V., Das B.K., Jyotyrmayee P., Chakrabarti R. (2006). Effect of *Achyranthes aspera* on the immunity and survival of *Labeo rohita* infected with *Aeromonas*

hydrophila. Fish and Shellfish Immunology, 20(3): 263-273.

Saednia S., Yassa N., Rezaeipoor R., Shafiee A., Gohari A.R., Kamalinejad M., Goodarzy S. (2009). Immunosuppressive principles from *Achillea talagonica*, an endemic species of Iran. Daru, 17(1): 37-41.

Saeidnia S., Gohari A.R., Mokhber-Dezfuli N., Kiuchi F. (2011). A review on phytochemistry and medical properties of the genus *Achillea*. Daru, 19(3): 173-186.

Sivaram V., Babu M.M., Citarasu T., Immanuel G., Murugadass S., Marian M.P. (2004). Growth and immune response of juvenile greasy groupers (*Epinephelus tauvina*) fed with herbal antibacterial active principle supplemented diets against Vibrio harveyi infections. Aquaculture, 237(1-4):9-20.

Tekeli A., Kutlu H.R., Celik L., Var I., Yurdakul E., Avcy A. (2008). The use of propolis as an alternative to antibiotic growth promoters in Broiler diets. Proceedings of 23th Worlds Poultry Congress, June 30 - July 4, Brisbane, Australia, pp: 482-482.

Yakhkeshi S., Rahimi S., Hemati M. (2012). Effects of yarrow (*Achillea millefolium* L.), antibiotic and probiotic on performance, immune response, serum lipids and microbial population of broilers. Journal of Agricultural Science and Technology, 14(4): 799-810.

Yamamoto Y., Oue E. (2006). Antihypertensive effect of quercetin in rats fed with a high-fat high-sucrose diet. Bioscience, Biotechnology, and Biochemistry, 70(4): 933-939.

Yano T. (1992). Assay of hemolytic complement activity. In: J.S. Stolen, T.C. Fletcher, D.P. Anderson, S.C. Hattari, A.F. Rowley (Eds.). Techniques in fish immunology. SOS Publications, Fair Haven, New Jersey, USA. 2: 131-141

Yilmaz E., Genc M.A., Cek S., Mazlum Y., Genc E. (2006). Effects of orally administered Ferula coskunii (Apiaceae) on growth, body composition and histology of common carp, *Cyprinus carpio*. Journal of Animal and Veterinary Advances, 5(12): 1236-1238.