

Antibacterial activity of Some Medicinal Plants against Fish Pathogenic *Aeromonas* spp. Isolated from Farmed Common Carp (*Cyprinus carpio*)

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□ ABSTRACT □

A total of 18 aqueous, methanolic and ethanolic extracts of 6 plant species that are collected from local environments of Lattakia- Syria were screened for antibacterial activity against three species of bacterium *Aeromonas* pathogenic to common carp (*Cyprinus carpio*). The three species *Aeromonas hydrophila*, *A. caviae* and *A. sobria* were isolated from infected carp in freshwater fish farm located in Dam 16 Tishreen in Lattakia. It was showed that all plant extracts exhibit different degrees of activity against tested bacteria. All bacterial strains in the present study were highly sensitive to all extracts of garlic (*Allium sativum* L.), thyme (*Thymus vulgaris* L), and onion (*Allium cepa* L). Moderate activity of aqueous but not alcoholic extracts of water mint (*Mentha aquatica* L) were observed against tested bacteria. In contrary, alcoholic extracts of eucalyptus (*Eucalyptus globules*) and sage (*Salvia officinalis*) exhibited moderate activity while the aqueous ones were weak. In general, isolates of both species *A. caviae* and *A. sobria* were more sensitive to the studied extracts when compared with *A. hydrophila*. Minimum inhibitory concentration (MIC) was determined for three of the studied plants. The MIC values of plant extracts against tested bacteria ranged from 1.0 to 10 mg/ml for garlic, 2.0 to 15 mg/ml for onion and 13 to 25mg/ml for thyme extracts.

Key words: *Aeromonas*, carp, antibacterial, extract, garlic, onion, thyme, sage, watermint, eucalyptus.

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الفعالية ضد الجرثومية لبعض النباتات الطبية تجاه الأيرومونات العامل المرض للأسماك المعزول من الكارب الشائع المستزرع

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□ ملخص □

درست الخواص ضد جرثومية ل 18 خلاصة مائية و ميثانولية و إيثانولية تعود إلى 6 أنواع نباتية جمعت من البيئات المحلية في اللاذقية- سوريا تجاه ثلاثة أنواع لجرثوم الأيرومونات العامل الممرض لسمك الكارب الشائع. عزلت الأنواع الثلاثة للأيرومونات *Aeromonas hydrophila* و *A. caviae* و *A. sobria* من أسماك كارب مصابة في مزرعة سمكية مائية عذبة (سد 16 تشرين- اللاذقية). أظهرت كل الخلاصات درجات مختلفة من الفعالية تجاه الجراثيم المختبرة. كانت كل السلالات المدروسة حساسة جداً لخلاصات الثوم و الزعتر الشائع والبصل. على حين أظهرت فعالية متوسطة للخلاصات المائية للنعناع المائي تجاه الجراثيم المختبرة و لكن ليس للخلاصات الكحولية له. على العكس فإن الخلاصات الكحولية للكينا (أوكاليتوس) و المرمية أظهرت فعالية متوسطة على حين الخلاصات المائية كانت ضعيفة الفعالية. بشكل عام، كانت سلالات النوعين *A. sobria* و *A. caviae* كليهما أكثر حساسية للخلاصات المدروسة إذا ما قيست بالنوع *A. hydrophila*. حدد التركيز المثبط الأصغري لثلاثة من النباتات المدروسة. راوحت قيم التركيز المثبط الأصغري للخلاصات النباتية تجاه الجراثيم المدروسة بين 1.0 و 10 ملغ/مل لخلاصات الثوم، وبين 2.0 و 15 ملغ/مل لخلاصات البصل، وبين 13 و 25 ملغ/مل لخلاصات الزعتر.

الكلمات المفتاحية: الأيرومونات، كارب، ضد جرثومي، خلاصة، ثوم، بصل، زعتر، ميرمية، نعناع مائي، كينا.

Introduction.

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At the present time, the increasing demand for fish, and other aquatic food organisms is the main factor behind growing aquatic animal husbandry or aquaculture (**Naylor and Burke, 2005**). By 2015, and according to the Food and Agriculture Organization (FAO), aquaculture is predicted to account for 39% of total global seafood production by weight (**FAO, 2005**).

Common carp (*Cyprinus carpio*) or what so called ornamental fish together with other carp species in family of Cyprinids are considered as the most freshwater cultured fish worldwide. It was estimated, in 1989, that the world aquaculture production of common carp alone reached $9.87.10^5$ metric tons, representing 13.5% of Cyprinids production. Moreover, the production of Cyprinids reached more than 5 million metric tons, representing more than 74.6% of the freshwater global finfish production in the same above year (**Kestemont, 1995**).

However, the intensive and wide culturing of fish has paralleled with the emergence of microbial diseases. There is growing concern about increased fish disease as critical factor influencing aquaculture and leading cause of economic losses.

Although fish can be affected by a number of other diseases like erythrodermatitis, enteric redmouth disease and epizootic ulcerative syndrome, hemorrhagic septicemias caused by *Aeromonas hydrophila* is still the most important because of its wide distribution and outcomes (**Jeney and Jeney, 1995; Thune et al., 1993; Grizzle and Kirya, 1993**).

For decades, antimicrobial agents have become an urgent need to control such fish diseases. However, overuse of antimicrobials in aquaculture systems either chemotherapeutically to treat fish diseases or as medicated feeds have led to the development of drug resistant bacteria and occurrence of multiple antibiotic resistance among different types of bacteria (**McPherson et al., 1991; Alderman and Hastings, 1998**). Depending on the mechanism the antimicrobial resistance is developed by, plasmid resistance can be transferred horizontally to the same or different species. Resistance acquired by chromosome mutations, however, can only be passed vertically within the same species. Irrespective of mechanism, resistance can be transferred to pathogenic bacteria leading to reduced efficacy of antibiotic in treatment of diseases caused by resistant pathogens either clinical for human or subclinical for animal. In the same context, it has been found that most of drug-resistant strains of *A. hydrophila* isolated from farmed carp have transferable R-plasmids (**Akashi and Aoki, 1986**). Having *A. hydrophila* possesses two to nine plasmids (**Ansary et al., 1992**), the spread of drug resistance among *Aeromonas* spp. is of concern because of the role played by these bacteria in human beings as emergent pathogens (**Chang and Bolton, 1987; Ansary et al., 1992**). On the other hand, there are environmental effects as consequence of the intensive usage of antimicrobials in aquaculture mainly as medicated feed. These effects can be summarized by the fact of accumulation of drugs residues and their metabolites in aquatic systems and their influence on aquatic indigenous bacteria by extended exposure for those chemicals (**Petersen et al., 2002**). Moreover, antimicrobial residues may also accumulate in the flesh of fish and subsequently in human tissues as final consumers (**Witte et al, 1999; Alderman and Hastings, 1998**).

Due to the reasons above, several attempts have been made to develop alternative medicines against pathogens that infect fish and other aquaculture animals. It has been proven earlier that the phytomedicines may serve as effectively therapeutic agents against antimicrobial resistant bacteria (**Cowan, 1999**). Hence, herbal products are of great interest as a promising source to control disease problems in fish culture. Many studies have

documented the potency of different herbs or plants to inhibit or kill fish pathogenic bacteria including *Aeromonas* spp. (Sudagar and Abasali, 2010; Aboud, 2010; Turker *et al.*, 2009; Direkbusarakom, 2004; Muniruzzaman and Chowdhury, 2004; Bansemir *et al.*, 2006; Dubber and Harder, 2008). In comparison, medicinal plants to be used as alternative therapeutic agents have many advantages over synthetic chemotherapeutics. Besides their advantages such as minimized side effects and cheaper source, the plant medicinal agents are natural substances that do not threat fish health, human health or the environment (Gabor *et al* 2010; Punitha *et al.*, 2008).

Aim of the study.

This research was designed according to extensive search and survey of the most medical plants that are expected to be bioactive and have antibacterial properties worldwide. Therefore, native representatives of some such plants were included in the study whereas ineffective ones were excluded. The aim of the present study was to investigate antibacterial effects of aqueous and alcoholic extracts of some medical plants against fish bacterial pathogens in order to find alternative drugs for treating of bacterial infection of common carp. Consequently, the following study investigated the possibility of use some of medicinal plants as phytoadditive in carp farm exploiting the availability and low coast of all the plants used in the study in local environments and markets all times throughout a year.

Material and methods.

Plant collection and extraction: Fresh samples of five different medicinal plants were collected from various regions of Lattakia- Syria during years 2008-2009. Nomenclature of plants collected, common names and parts used are shown in Table 1. Sufficient quantities of collected plants were oven dried at 40°C and fine grounded. Aqueous, ethanolic and methanolic extractions were made. For aqueous extraction, 25 grams from powdered plant sample were macerated in 250 ml of distilled water at room temperature for ten days, filtered using Watman No. 2 filter paper under vacuum. For alcoholic extractions, 25 grams of plant sample were extracted with 50 ml of 80% methanol and ethanol at 60°C for 24 hours and evaporated under vacuum. All extracts were allowed to dry in vacuum and weighed. Final concentration of 100 mg/ml of an extract in distilled water was obtained for tests of antibacterial assay. All tests were carried in laboratories of department of botany in the faculty of science- Tishreen University.

Table 1: The studied medicinal plants, their classification and nomenclature, common names, and parts used for extraction.

Common name	Scientific name	Part used
Eucalyptus	<i>Eucalyptus globules</i>	Leaves
Garlic	<i>Allium sativum.</i>	Rhizome
Onion	<i>Allium cepa</i>	Rhizome
Sage	<i>Salvia officinalis</i>	Leaves
Thyme	<i>Thymus vulgaris</i>	Leaves
Water Mint	<i>Mentha aquatica</i>	Leaves

Bacterial strains and antibacterial screening:

The bacterial species used were: *Aeromonas hydrophila*, *Aeromonas caviae* and *Aeromonas sobria*. All bacterial isolates were isolated from infected carp fish (common carp- *Cyprinus carpio*) farmed in Dam of 16 Tishreen- Lattakia during the period 2007-2008. These three pathogenic species belonged to genus *Aeromonas* are causative agents of septicemia and other diseases in carp fish (Cipriano, 2001). By definition, they are short, gram-negative, motile bacilli with a single flagellum that fermented glucose with or without the production of gas (Brenner *et al.*, 2005).

The antibacterial activity of the plant extracts was determined by modified agar well diffusion method (Owais *et al.*, 2005). Pure culture of each bacterial isolates was grown in sterile Tryptic Soy Agar (TSA) and incubated for 18-24 hours at 37°C. Several colonies grown on TSA were suspended in 5 ml of Mueller–Hinton broth and the density of this suspension was adjusted to be 1.5×10^8 cells/ ml (0.5 McFarland standards). Mueller Hinton agar plates were inoculated with a bacterial suspension by using sterile cotton swabs. Wells of 5mm diameter were punched in the agar and filled with 40µl of 100 mg/ml of a plant extract for each. Sterilized distilled water was used as negative control. Five different antibiotics were also used for positive control. The antibiotics and their concentrations were ciprofloxacin (5µg), cefixime (5µg), lincomycin (15µg), neomycin (30µg) and trimethoprim- sulphamethoxazole (25µg). Results were recorded by determining the diameter of inhibition zones after incubation for 24 h at 37°C.

Result of Antibacterial activity was categorized as follows: (-), no inhibition; (+), inhibition zone (IZ): 10 – 15 mm; (++) , IZ:16 – 20 mm; (+++) , IZ:21 – 25 mm; (++++), IZ: > 25 mm.

Minimum Inhibitory Concentration (MIC) value determination. Minimum inhibitory concentrations (MIC) of extracts that showed high antibacterial activity in aqueous phase (i.e.: garlic, onion, and thyme) were determined by agar dilution method (NCCLS, 1997). Stock concentration of the plant extract was prepared by using sterilized distilled water in the ratio of 1:1 which was then diluted with equal volume of sterilized saline (0.9% sodium chloride, pH 7.0). Serial dilutions of each extract were prepared; one ml of each dilution was well mixed with 9 ml of molten sterilized Muller Hinton agar (around 45° C) and poured into Petri dishes. After solidification, each plate was overlaid with 0.5 ml of a test bacterial suspension that was pre cultured on Muller Hinton broth and adjusted to 10^8 cfu/ml (McFarland standards). After incubation at 37°C for 24 hr, results were recorded on the basis of bacterial growth and the MIC was defined as the lowest antibacterial concentration of test plant extract that can inhibit the growth of the tested bacteria.

Results.

In the present study, eighteen crude extracts obtained by alcoholic and aqueous solvents of six different plants were screened for their antibacterial activity against three fish pathogenic species belonging to bacterial genus *Aeromonas*. Results of antibacterial effect of the tested extracts on *A. hydrophila*, *A. caviae* and *A. sobria* are presented in Table 2. The garlic extracts (both aqueous and alcoholic) were the most active followed by onion and thyme although the tow latter were so effective. The ranges of inhibition zone diameter for garlic, onion and thyme were 36- 25, 20- 13, and 19- 12 mm respectively. The extracts of the rest three plants sage, water mint and eucalyptus exhibit variable activity against tested bacteria. For water mint, only aqueous extract appeared to be somewhat effective against *A. caviae* and *A.sobria*. In contrary, alcoholic extracts of eucalyptus (*Eucalyptus globules*) and sage (*Salvia officinalis*) exhibited moderate activity while the

aqueous one was weak. In comparison, strains of both species *A. caviae* and *A. sobria* were more sensitive to extracts studied rather than *A. hydrophila*. On the other hand, all tested bacterial isolates were sensitive to the five antibiotic used as control. For *A. hydrophila*, minimum inhibitory concentration (MIC) value were ranged from 1.0 to 10 mg/ml for garlic, 3.0 to 10 mg/ml for onion and 18.5 to 25mg/ml for thyme extracts. (table 3). However, MIC values for other two species *A. caviae* and *A. sobria* were slightly lower than that recorded for *A. hydrophila*.

Table 2: Antibacterial activities of aqueous, ethanolic and methanolic extracts of 6 plants against *Aeromonas* spp. isolated from infected carp.

Plant	Extract	<i>Aeromonas</i> species		
		<i>A. hydrophila</i>	<i>A. caviae</i>	<i>A. sobria</i>
Eucalyptus	Aqueous	-	-	-
	Ethanolic	-	++	+
	Methanolic	+	+	++
Garlic	Aqueous	++++	++++	++++
	Ethanolic	++++	++++	++++
	Methanolic	++++	++++	++++
Onion	Aqueous	+++	+++	+++
	Ethanolic	+++	+++	++
	Methanolic	+++	+++	++
Sage	Aqueous	-	+	+
	Ethanolic	++	+++	+++
	Methanolic	++	+++	+++
Thyme	Aqueous	+++	+++	+++
	Ethanolic	++	++	++
	Methanolic	++	++	++
Water Mint	Aqueous	-	++	++
	Ethanolic	-	+	+
	Methanolic	-	+	+

(-), no inhibition; (+), zone of inhibition 10 – 15 mm; (++) ,zone of inhibition 16 – 20 mm; (+++) ,zone of inhibition 21 – 25 mm; (++++), > 25 mm.

Table 3: Minimum Inhibitory Concentration (MIC) (mg/ml) values of aqueous extracts of three plants against *Aeromonas* spp. isolated from infected carp.

Plant	Range of MIC values (mg/ml)		
	<i>A. hydrophila</i>	<i>A. caviae</i>	<i>A. sobria</i>
Garlic	1.0- 10	0.5 - 10	0.5 - 10
Onion	3.0- 10	2.5- 15	2- 15
Thyme	18.5 - 25	13- 22	13- 19

Discussion.

Actually, very few references can be found on antibacterial activity of medical plants against pathogens of cultured fish especially carp. Therefore, results of the somehow specific present study is, in some situations, compared with results of other studies carried on other fish species in order to consider any published finding that might be helpful.

Antibacterial activity: Antimicrobial activity of plant extracts is mostly due to their essential oil fraction or sulphur containing compounds in aqueous phase (Gould, 1996). In contrast to eucalyptus and water mint, sage, thyme, onion and garlic are common in Syria as medicinal plants used in folk medicine for treating different health problems.

Bioactivity of garlic is far related to its components allicin, ajoene, thiosulfinates and a wide range of other organosulphata compounds (Ledezma and Apitz-Castro, 2006; Hunter *et al.*, 2005). Garlic is one of the most studied medical plants and has showed bactericidal effects against human pathogenic bacteria such as *Staphylococcus epidermis* and *Salmonella typhi* (Arora and Kaur, 1999), enterotoxigenic *Escherichia coli* (E.T.E.C) (Kumar and Sharma, 1982), *Clostridium botulinum* (Dewitt *et al.*, 1979) and others. On the other hand, (Musa *et al.* 2008) observed that garlic was active against *A. hydrophyla* as well as other many bacteria isolated from fish and shrimp. More recently, garlic extract showed to be active against three *Aeromonas* species *A. hydrophila*, *A. caviae* and *A. sobria* isolated from seafood (Durairaj *et al.*, 2009). Actually, our result of high activity of garlic extracts (aqueous and alcoholic) against those three pathogenic species of *Aeromonas* isolated from infected carp is in full agreement with many other studies carried on other fish or aquarium animals.

The antimicrobial effect of onion is mainly due to sulfoxide allicin (Vohora *et al.*, 1973). High activity of onion, in the present study, is agreed with report of (Muniruzzaman and Chowdhury 2004) who found that onion (*Allium cepa*) was highly effective against *A. hydrophila* and other tow fish pathogenic bacteria *Pseudomonas fluorescence* and *Edwardsiella tarda*. However, (Musa *et al.* 2008) found little effect of onion (zone of inhibition: 10 – 19 mm) on *Aeromonas* spp. isolated from cultured fish.

The following compounds are thought to be behind the antimicrobial (bacteria, viruses and fungi) activity of thyme thymol, carvacrol, γ -terpinene, *p*-cymene and caffeic acid (Cowan, 1999; Juliano *et al.*, 2000). Thyme extracts and essential oil have showed antibacterial activity against pathogenic *E. coli* O157:H7 (Yasar *et al.*, 2005; Burt and Reinders, 2003; Cosentino *et al.*, 1999), *Salmonella typhimurium* (Yasar *et al.*, 2005; Cosentino *et al.*, 1999; Hammer *et al.*, 1999), *Staphylococcus aureus* (Yasar *et al.*, 2005; Cosentino *et al.*, 1999; Farag *et al.*, 1989), and *Listeria monocytogenes* (Yasar *et al.*, 2005; Smith-Palmer *et al.*, 1998), *Yersinia enterocolitica*, *Klebsiella pneumonia* and *Enterococcus faecalis* (Yasar *et al.*, 2005), and *Bacillus subtilis* and *Shigella sonnei* (Fan and Chen, 2001). In addition to that, only aqueous extracts of thyme significantly inhibited the growth of *Helicobacter pylori*, (Tabak *et al.*, 1996), *E. coli*, *Klebsiella pneumonia*, *Enterobacter cloacae*, and *Staphylococcus aureus* a (El Astal *et al.*, 2005). As related to fish pathogens, recent study revealed the efficacy of *Thymus vulgaris* essential oil in inhibiting and reducing the numbers of pathogenic bacteria in cultured fish in Chile (Navarrete *et al.*, 2010). However, moderate activity of *Thymus vulgaris* extract on *Aeromonas hydrophyla* isolated from common carp was recently found by Mohamad and Abasal (2010) in Iran. As it is obvious above, *Aeromonas* spp. are not included in most such studies. However, the clear activity of thyme appeared in the present study is harmonized with most of studies carried on other bacterial species.

The major components of sage are camphor α -pinene, β -pinene, 1,8-cineole and α -tujone (Marino *et al.*, 2001). The following bacterial pathogens have been found to be

inhibited by extracts of sage in different studies, *E. coli* (Farag *et al.*, 1989; Smith-Palmer *et al.*, 1998), *Salmonella typhimurium* (Hammer *et al.*, 1999; Shelef *et al.*, 1984), *Staphylococcus aureus* (Shelef *et al.*, 1984; Smith-Palmer *et al.*, 1998), and *Listeria monocytogenes* (Smith-Palmer *et al.*, 1998). Yasar *et al.* (2005) found moderate effect of sage against eighteen bacterial species with being the *Enterobacter aerogenes* and *Staphylococcus aureus* the most affected. However, El Astal *et al.* (2005) found that aqueous extracts of sage and thyme at the different concentrations had an obvious activity against many Gram negative and Gram positive bacteria except *Aeromonas* spp. (not included in that study). Weak activity of sage extract against *A. hydrophila* obtained in the present study is similar to that recently reported by Mohamad and Abasal (2010) who found weak activity of sage extract against *A. hydrophila* isolated from carp. However this was not the state of *A. caviae* and *A. sobria* both that were found to be more sensitive to alcoholic extracts of sage in our study.

It was showed that eucalyptus contains tannin compound that is active against a variety of bacteria and viruses (Cowan, 1999) and eucalyptus oil has then been recommended and used as topical treatment of genital herpes (Schnitzler *et al.*, 2001). Many studies have revealed eucalyptus to be with antibacterial activity towards different species of bacteria other than *Aeromonas* spp.. For instance, human pathogenic bacteria such as *Staphylococcus aureus* and to less extent *E. coli* (Ghalem and Mohamed, 2008), *S. aureus* and *Bacillus subtilis* (Babayi *et al.*, 2004), *S. aureus*, *Proteus* spp., *Pseudomonas* spp., and *Klebsiella* spp. has been inhibited by leaf extracts of eucalyptus (Trivedi and Hotchandani, 2004). Giving studies carried on sensitivity of *Aeromonas* spp. to bioactive components of eucalyptus are so rare, the present study revealed activity of eucalyptus extract against tested species of *Aeromonas* although it was weak and restricted to alcoholic one.

As related to the result of water mint, it is worth mentioning first that water mint or the species *Mentha aquatica* is a wild type of mint and different from the other edible types of peppermint or spearmint (There are 25 species of the genus *Mentha* e.g.: *Mentha pulegium*, *Mentha arvensis*) that is generally regarded as the world oldest medicine, and used as culinary herb. The worldwide distributed *Mentha aquatica* together with *Mentha pulegium* are well known as aromatic plants (Mahboubi and Haghi, 2008). Menthol is the primary component of the essential oil of peppermint (mint) and has antibacterial properties (Sivropoulou *et al.*, 1995). Essential oil obtained from *Mentha aquatica* and *Mentha piperita*. exhibited broad-spectrum antibacterial activity against disease pathogens (Mimica-Dukic *et al.*, 2003). Musa *et al.* (2008) observed no activity of edible mint *Mentha arvensis*. Against a variety of microorganisms including *A. hydrophila* isolated from fishes and shrimps.

MIC values: The values of MIC for garlic and onion obtained by the current study are still within ranges reported in other related studies for *Aeromonas* spp.; these values ranged from 1.0 to 10 mg/ml for garlic, 2.0 to 10 mg/ml for onion and 13 to 25mg/ml for thyme extracts. For instance, (Muniruzzaman and Chowdhury 2004), reported related ranges of MIC values of garlic extracts and onion against *A. hydrophila* and were 0.6- 10 and 2.5 - 10 mg/ml respectively. Similarly, MIC values of garlic extracts against *A. hydrophila* were 13.24 mg/ml in study of (Chatchawanchontera *et al.* 2008) who found garlic extract to be inhibitor to *A. hydrophila* isolated from infected fish at concentration 2.64, 13.24 mg/ml. More recently, Aboud (2010) found that garlic preparations at concentration of 800 mg/l can be used effectively to eliminate pathogenic *A. hydrophila* in tilapia fish.

In summary, the current study revealed the potency of garlic, thyme and onion as natural agents to inhibit the carp pathogenic *Aeromonas* spp. The finding of the present study is in full agreement with results of other studies (Chatchawanchonteera *et al.*, 2008; Muniruzzaman and Chowdhury, 2004; Navarrete *et al.*, 2010). Furthermore, many studies carried on fish diets supplemented with phytoadditives of bioactive plants have showed the efficacy of the three above plants in improvement of survival and immunity status of different types of cultured fish and animals (Mohamad and Abasal, 2010; Gabor *et al.*, 2010). Other study revealed the possibility of raw and squeezed garlic at 200 mg/l to treat Trichodiniasis in eel (Madsen *et al.*, 2000). In an experimental study performed by Aly *et al.* (2008), Nile tilapia fish diet was supplemented with garlic in doses of 10 and 20 g/kg feed; significant improvements were observed in the immunological state, the meat quality indices and in shelf life as well as better survival rates in that fishes which artificially were infected with *A. hydrophila*. Similar result was before obtained by (Shalaby *et al.* 2006) who compared the effects of garlic and chloramphenicol Nile tilapia and found significantly increases of final weight and specific growth rate directly linked with the inclusion levels of garlic and chloramphenicol.

VI- Conclusion. The present study provides, to our knowledge for the first time, information about antibacterial activities of Syrian edible and inedible plants against pathogen *Aeromonas* spp. of cultured carp in order to verify the efficacy of active herbs as alternative and natural source of medicines for treatment of bacterial fish diseases. These can be used as phytoadditives in fish farms. Furthermore, phytoadditives appear to be reasonable alternative solutions to substitute synthetic antimicrobials used in aquaculture without any undesired effects upon recent related studies. Recently, studies have showed the potential of such herbs as growth promoters, immunostimulants, immunomodulators, as well as antioxidants. Additional reason evokes using bioactive herbs in control of fish diseases is the emergence of resistance in pathogenic bacteria due to heavy uses of antibiotics in aquaculture as well as other risks resulted from antibiotics residues in produced meat.

References.

- Aboud, O. A. E. (2010) Application of some Egyptian medicinal plants to eliminate *Trichodina* sp. and *Aeromonas hydrophila* in tilapia (*Oreochromis niloticus*). *Researcher*:2(10): 12-16.
- Akashi, A. and Aoki, T. (1986) Characterization of transferable R plasmids from *Aeromonas hydrophila*. *Bull. Jpn. Soc. Sci. Fish.* 52(4): 649-655.
- Alderman, D.J. and Hastings, T. S. (1998) Antibiotic use in aquaculture: development of antibiotic resistance potential for consumer health risks. *Int. J. Food. Sci. Technol.*, 33: 139-155.
- Aly, S. M., Abdel Atti, N. M., and Mohamed, M. F. (2008) Effect of garlic on the survival, growth, resistance and quality of *Oreochromis niloticus*; 8th International Symposium on Tilapia in Aquaculture, 2008, 276-296.
- Ansary, A. R., Haneef, M., Torres, J. L. and Yadev, M. (1992) Plasmids and antibiotic resistance in *Aeromonas hydrophila* isolated in Malaysia from healthy and diseased fish. *J. Fish Dis.* 15(2): 191-196.
- Arora, D. S. and Kaur, J. (1999) Antimicrobial activity of spices. *Int. J. Antimicro Agents*, 12: 257-262.

- Babayi, H., Kolo, I., Okogun, J. I. and Ijah, U. J. J. (2004) The antimicrobial activities of methanolic extracts of *Eucalyptus camaldulensis* and *Terminalia catappa* against some pathogenic microorganisms. *BIOKEMISTRI* 16(2):106-111.
- Bansemir, A., Blume, M., Schröder, S. and Lindequist, U. (2006) Screening of cultivated seaweeds for antibacterial activity against fish pathogenic bacteria. *Aquaculture*, 252: 79-84.
- Borchardt, J. R., Wyse, D. L., Sheaffer, C. C., Kauppi, K. L., Fulcher, R. G., Ehlke, N. J., Biesboer, D. D. and Bey, R. F. (2008) Antimicrobial activity of native and naturalized plants of Minnesota and Wisconsin. *Journal of Medicinal Plants Research*. 2(5), 98-110.
- Brenner, D.J., Krieg, N.R. and Staley, J.R. (2005) Bergey's manual of systematic bacteriology, Springer, USA, vol. 2, part B, pp.557-578.
- Burt, S. A. and Reinders, R. D. (2003) Antibacterial activity of selected plant essential oils against *Escherichia coli* O157:H7. *Letters in Applied Microbiology* 36:162-167.
- Chang, B. J. and Bolton, S. M. (1987) Plasmids and resistance to antimicrobial agents in *Aeromonas sobria* and *Aeromonas hydrophila* clinical isolates. *Antimicrob. Agents Chemother.* 31(8): 1281-1282.
- Chatchawanchontea, A; Wangboonskul, J; Trongwanishnam, K; and Buttasri, A. 2008 antimicrobial activity of guava leaf and garlic extracts against *Aeromonas hydrophila* and *Streptococcus* spp. isolated from infected fish. *KKU Vet J.*18(1):46-53.
- Cipriano, R.C. (2001) *Aeromonas hydrophila* and motile Aeromonad septicemias of fish. Fish Dis Leaflet 68, United States Department of the Interior fish and wildlife service, Division of Fishery research. Washington, DC, pp.1-25.
- Cosentino, S., Tuberoso, C. I. G., Pisano, B., Satta, M., Mascia, V., Arzedi, E. and Palmas, F. (1999) *In vitro* antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. *Letters in Applied Microbiology* 29:130-135.
- Cowan, M. M. (1999) Plant products as antimicrobial agents. *Clin. Microbiol.* 12: 564-582.
- Dewitt J.C., Notermans S., Gorin N., *et al.* (1979) Effect of garlic oil or onion oil on toxin production by *Clostridium botulinum* in meat slurry. *J Food Prot.* 42: 222-224.
- Direkbusarakom, S. (2004) Application of medicinal herbs to aquaculture in Asia. *Walailak Journal of Science and Technology*, 1(1): 7-14.
- Dubber, D. and Harder, T. (2008) Extracts of *Ceramium rubrum*, *Mastocarpus stellatus* and *Laminaria digitata* inhibit growth of marine and fish pathogenic bacteria at ecologically realistic concentrations. *Aquaculture*, 274: 196-200.
- Durairaj, S., Srinivasan, S. and Lakshmanaperumalsamy, P. (2009) *In vitro* Antibacterial Activity and Stability of Garlic Extract at Different pH and Temperature. *Electronic Journal of Biology*, 5(1): 5-10.
- El Astal, Z. Y., Ashour, A. E. R. A. and Kerrit, A. A. M. (2005) Antimicrobial activity of some medicinal plant extracts in Palestine. *Pak J Med Sci.* 21 (2): 187-193.
- Fan, M. and Chen, J. (2001) Studies on antimicrobial activity of extracts from thyme. *Wei Sheng Wu Xue Bao.* 41:499-504.
- FAO Fishery Information Data and Statistics Unit. FISHSTAT+Databases and Statistics. Rome, Italy: Food and Agriculture Organization of the United Nation; 2005.
- Farag, R. S., Daw, Z. Y., Hewedi, F. M. and El-Baroty, G. S. A. (1989) Antimicrobial activity of some Egyptian spice essential oils. *Journal of Food Protection* 52:665-667.

- Gabor, Erol-Florian., Şara, A. and Barbu, A. (2010) The effects of some phytoadditives on growth, health and meat quality on different species of fish. *Animal Science and Biotechnologies*, 43 (1): 61-65.
- Ghalem, B. R. and Mohamed, B. (2008) Antibacterial activity of leaf essential oils of *Eucalyptus globulus* and *Eucalyptus camaldulensis*. *African Journal of Pharmacy and Pharmacology*. 2(10): 211-215.
- Gould, G. W. (1996) Industry preservatives on use of natural antimicrobials and inhibitors for food applications. *J. Food Prot.* 59 (suppl); 82-86.
- Grizzle, J. M. and Kirya, Y. (1993) Histopathology of gill, liver and pancreas and serum enzyme levels of channel catfish infected with *Aeromonas hydrophila* complex. *J. Aquat. Anim. Health* 5: 36-50.
- Hammer, K. A., Carson, C. F. and Riley, T. V. (1999) Antimicrobial activity of essential oils and other plant extracts. *Journal of Applied Microbiology* 86:985-990.
- Hunter, R., Cairra, M. and Stellenboom, N. (2005) Thiolsulfinate allicin from garlic: Inspiration for a new antimicrobial agent. *Ann of the New York Acad of Sc.* 1056: 234-241.
- Jeney, Z. Jeney, G. (1995) Recent achievements in studies on diseases of common carp (*Cyprinus carpio* L). *J. Aquac.* 129: 397-420.
- Juliano, C., Mattana, A. and Usai, M. (2000). Composition and *in vitro* antimicrobial activity of the essential oil of *Thymus herba-barona* Loisel growing wild in Sardinia. *Journal of Essential Oil Research* 12:516-522.
- Kestemont, P. (1995) Different systems of carp production and their impacts on the environment. *Aquaculture* 129 : 347-372.
- Kumar, A. and Sharma, V.D. (1982) Inhibitory effect of garlic (*Allium sativum* Linn.) on enterotoxigenic *Escherichia coli*. *Indian J Med Res.* 76: 66-70.
- Ledezma, E. and Apitz-Castro, R. (2006) Ajoene the main active compound of garlic (*Allium sativum*): a new antifungal agent. *Rev. ibe de micol: órg de la Asoc. Esp de Espe en Micol.*, 23 (2): 75-80.
- Madsen, H.C.K., Buchmann, K. and Møllergaard, S. (2000) Treatment of trichodiniasis in eel (*Anguilla anguilla*) reared in recirculation systems in Denmark: alternatives to formaldehyde. *Aquacult.* 186:221-231.
- Mahboubi, M. and Haghi, G. (2008) Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. *J. Ethnopharmacol.*, 119, 325-327.
- Marino, M., Bersani, C. and Comi, G. (2001) Impedance measurements to study the antimicrobial activity of essential oils from *Lamiaceae* and *Compositae*. *International Journal of Food Microbiology* 67:187-195.
- McPhearson, R. M., DePaola, A., Zywno, S. R., Motes, M. L. and Guarino, A. M. (1991) Antibiotic resistance in Gram-negative bacteria from cultured catfish and aquaculture ponds. *Aquaculture* 99(3/4): 203-211.
- Mimica-Dukic, N., B. Bozin, M. Sokovic, B. Mihajlovic and Matavuli, M. (2003) Antimicrobial and antioxidant activities of three *Mentha* species essential oils. *Planta Med*, 69: 413-419.
- Mohamad, S. and Abasal, H. (2010) Effect of plant extracts supplemented diets on immunity and resistance to *Aeromonas hydrophyla* in common carp (*Cyprinus carpio*). *Research Journal of Animal Science*, 4 (1): 26- 34.
- Muniruzzaman, M. and Chowdhury, M. B. R. (2004) Sensitivity of fish pathogenic bacteria to various medicinal herbs. *Bangladesh J Vet Med* 2: 75- 82.

- Musa, Wei. L. S., Seng, C. T., Wee, W and Leong, L. K. (2008) Potential of Edible Plants as Remedies of Systemic Bacterial Disease Infection in Cultured Fish. *Global Journal of Pharmacology* 2 (2): 31-36.
- National Committee for Clinical Laboratory Standards (NCCLS). (1997) Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. Tentative standard M31-T. National Committee for Clinical Laboratory Standards, Wayne, Pa.
- Navarrete, P., Toledo, I., Mardones, P., Opazo, R., Espejo, R., and Romero, J. (2010) Effect of *Thymus vulgaris* essential oil on intestinal bacterial microbiota of rainbow trout, *Oncorhynchus mykiss* (Walbaum) and bacterial isolates. *Aquaculture Research*, 41, e667-e678.
- Naylor, R. and Burke, M. (2005) Aquaculture and ocean resources: raising tigers of the sea. *Annual Review of Environmental Resources*, 30: 185-218.
- Owais, M., Sharad, K.S., Shehbaz, A. and Saleemuddin, M. (2005) Antibacterial efficacy of *Withania somnifera* (ashwagandha) an indigenous medicinal plant against experimental murine salmonellosis. *Phytomedicine* 12: 229-235.
- Petersen, A., Andersen, J. S., Kaewmak, T., Somsiri, T. and Dalsgaard, A. (2002) Impact of integrated fish farming on antimicrobial resistance in a pond environment. *Appl. Environ. Microbiol.*, 68: 6036–6042.
- Punitha, S.M.J., Babu, M.M., Sivaram, V., Shankar, V.S., Dhas, S.A., Mahesh, T.C., Immanuel, G. and Citarasu, T. (2008) Immunostimulating influence of herbal biomedicines on nonspecific immunity in Grouper *Epinephelus tauvina* juvenile against *Vibrio harveyi* infection. *Aquacult. Int.*, 16: 511-523.
- Schnitzler, P., Schon, K. and Reichling, J. (2001) Antiviral activity of Australian tea tree oil and eucalyptus oil against herpes simplex virus in cell culture. *Pharmazie*, 56:343-337.
- Shalaby, A. M., Khattab, Y. A. and Abdel R, A. M. (2006) Effects of garlic (*Allium sativum*) and chloramphenicol on growth performance, physiological parameters and survival of Nile tilapia (*Oreochromis niloticus*); *J. Venom. Anim. Toxins incl. Trop. Dis.* 12(2):172-201.
- Shelef, L. A., Jyothi, E. K. and Bulgarelli, M. A. (1984) Growth of enteropathogenic and spoilage bacteria in sage-containing broth and foods. *Journal of Food Science* 49:737-740.
- Sivropoulou, A., Kokkini, S., Lanaras, T. and Arsenakis, M. (1995) Antimicrobial activity of mint essential oils. *Journal of Agricultural and Food Chemistry* 43:2384-2388.
- Smith-Palmer, A., Stewart, J. and Fyfe, L. (1998) Antimicrobial properties of plant essential oils and essences against five important food-borne pathogens. *Letters in Food Microbiology* 26:118-122.
- Sudagar, M. and Abasali, H. (2010) Effect of plant extract supplemented diets on immunity and resistance to *Aeromonas hydrophila* in common carp (*Cyprinus carpio*). *Research Journal of Animal Science* 4 (1): 26-34.
- Tabak, M., Armon, R. Potasman, I. and Neeman, I. (1996) *In vitro* inhibition of *Helicobacter pylori* by extracts of thyme. *J. Appl. Bacteriol.* 80:667-672
- Thune, R. L., Stanley, L. A. and Cooper, K. (1993) Pathogenesis of Gram – negative bacterial infection in warm water fishes. *Annu. Rev. Fish Dis.* 3: 37- 68.
- Trivedi, N. A. and Hotchandani, S. C. (2004) A study of the antimicrobial activity of oil of Eucalyptus. *Indian. J. Pharmacol.* 36 (2): 93-95.

- Turker, H., Yildirim, A. B. and Karakaş, F. B. (2009) Sensitivity of Bacteria Isolated from Fish to Some Medicinal Plants. *Turkish Journal of Fisheries and Aquatic Sciences* 9: 181-186.
- Vohora, S. B., Rizwan, M. and Khan, J. A. (1973) Medicinal uses of common Indian vegetables. *Planta Med.* 23:381–393.
- Witte, W., Klare, I. and G. Werner. (1999) Selective pressure by antibiotics as feed additives. *Infection*, 27 (Suppl. 2):35–38.
- Yasar, S., Sagdic, O. and Kisioglu, A. N. (2005) *In vitro* antibacterial effects of single or combined plant extracts. *Journal of Food, Agriculture & Environmen.*3 (1): 3 9 - 4 3.