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Effect of Ethanolic and Aquatic Extract of Harmal (*Peganum harmala*) on the Activity of *Staphylococcus aureus* in Minced Meat of Silver Carp (*Hypophthalmichthys molitrix*) in Different Times of Storage at Refregerated (4°C)

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Abstract In this study, the plant Peganum collected for providing its ethanolic and aquatic extract. 0.01, 0.02 and 0.35 µg/ml of it were prepared and kept at 4°C for evaluating it's effect on growth of *Staphylococcus aureus* in minced meat of Silver carp in days 0, 1, 2, 5, 7, 9, 11, 13. Results showed the ethanolic extract of *Peganum harmala* at 0.35 µg/ml has the most anti-microbial effect and also, the aquatic extract prevented the growth of *Staphylococcus aureus*. Proxide value (PV) was minimum in control during 13 days. The maximum and minimum of thiobarbiutic acid (TBA) observed at 13th day in control and day zero at 0.01 µg/ml, respectively. The minimum of fat was in control in 11th and 13th days. Maximum level of fat observed at 0.35 µg/ml at the first and 13th day. Based on results adding 0.35 µg/ml of harmal in minced meat of Sliver carp is recomended.

Keywords: *Peganum harmala, Anti-microbial effect, Ethanol and aquatic extract, Staphylococcus areus*

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1. Introduction

Aquatic animals are one of the most perishable products due to relatively high protein, and unsaturated fatty acids in muscles. Refrigerated storage method used in distribution transport of fish farms to shopping centers [22]. Iran located among the countries pioneer in the application of cold to preserve food. Keeping fish in the refrigerator lead to slow fermentation and chemical microorganisms activity, but because of the inability refrigerator temperature (4°C) to reduce the temperature of the fish to the extent necessary, changes will be undesirable and product quality reduced [27]. Therefore, the use of appropriate materials with anti-bacterial and anti-oxidant activity to improve quality, increase retention time of meat and yet avoid economic losses seems essential [31]. In recent years, due to lower sales to consumers of processed food with chemical matters, using biological preservatives are taken into consideration.

One way to achieve nutritional goals in developing countries is aquaculture. In breeding several species of carp fish, Silver carp is particularly important due to the base of the food pyramid first (phytoplankton), the rapid growth, resistance to pathogens, stress and difficult situations transportation [29]. Fish meat including perishable foods and exposure to corruption and improper temperature conditions, chemical properties and increase of microbial spoilage. In order to increase the shelf life of fish and its products physical cooling methods at low temperatures, frozen out below zero, packed under vacuum and etc. (and chemical preservatives) a variety of organic acids and their salts and biological bacteriocins, lactoperoxidase, enzymes, peptide are used.

Diseases caused by consumption of contaminated food are the most important issues related to public health. To reduce economic losses and health risks caused by these diseases, using natural materials as an effective antimicrobial compounds for the control of pathogenic bacteria and increase shelf life of processed foods, it is essential. Because consumers about the safety of foods that contain synthetic preservatives are not sure to consume natural food ingredients from natural products are used as preservatives (replace chemical preservatives) [2].

Among these, compounds derived from medicinal plant extracts have antimicrobial properties against pathogens and act as a source of antimicrobial substances. Aromatic plant extracts and essential oils obtained plants has anti-bacterial, antifungal, anticancer and antioxidant properties and are able to control the growth of pathogens and toxin production. Taking effective antimicrobial effects of essential plant oils, they can be used as an alternative antimicrobial chemicals in the food industry that have a very amounts due to the use of essential oils to inhibit the

growth of bacteria, organoleptic properties is likely to have significant adverse effects in food [21]. Considering to some negative consumer reactions to chemical and artificial preservatives, increased use of natural ingredients. This is particularly the case of products that are marketed as excellent the material is important, where the need to replace the safe and effective for treatment and chemical preservatives. Considering to potential applications of plant extracts and botanical extracts antimicrobial properties against a broad spectrum of microorganisms including bacteria, yeasts and molds have been approved.

Espand (*Peganum harmala*) due to having alkaloids has antimicrobial, anti-fungal and anti-parasitic properties. Also, it has the property of a heart beat slowing. Harmel alkaloids comprise about 4 percent of the dry weight of grain and are very important in industrial and medical. These compounds can be named harmaline, harmine, harmalol.

Another study by [13] and [25] was done to arrange antiviral and antibacterial properties of hydatid cyst. The anti-tumor effects of alkaloids harmal by [17] were introduced. Compounds extracted from this plant, have shown many health benefits. Among its properties: anti-inflammatory properties Peptulin material obtained from the harmel has been studied by EL-SAUDEL-Rifai. He proved antibacterial, antifungal, anti-parasitic dehumidifier and harmal extract [8].

In recent years, researchers investigated the inhibitory effects of harmal, Hashemi *et al.* [11] and in all studies antibacterial effect of harmal and the result is confirmed. *Staphylococcus aureus* is an important cause of infections acquired in hospitals and the community. The bacteria may be existing in normal flora of the skin or in the nose. It is estimated that 20 percent of people in a long time, are carriers of the bacteria. *Staphylococcus aureus* is one of the most successful pathogen bacteriaand creates a wide range of infections from simple skin infections (such as boils, carbuncle, sty or abscess) to life-threatening diseases (such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome and septicemia). Thought of the plant's antimicrobial properties, according to the proof of this plant was made on fungi, and parasites. The need to address this issue is needed antibacterial properties of various plants, because using of always extracted plants or plant material has more acceptability among patients compared to synthetic drugs. Furthermore, the bacterial infections are usually multiple medications are prescribed.

Therefore, this study was carried out to evaluate the antimicrobial effects of ethanol extract of the plant harmal (*Peganum harmala*) on the bacteria *Staphylococcus aureus* in mince meat of Silver carp (*Hypophthalmichthys molitrix*) and determining the effect of different concentrations of harmal "0.01 and 0.02 and 0.35 µg/ml" on growth of *Staphylococcus aureus* at 4°C by measuring the growth of bacteria in minced meat.

2. Materials and Methods

2.1. Preparations Extracts, Bacteria and Treatments

Dried Harmal bought from shop and ground by electric mill match. In order to extract the harmal (*Peganum harmala*) Soxhlet method was used. Thus, 30 g of powdered sample was taken within Kartush was located in the extractor device. 300 ml of solvent to be poured into the flask at 50°C was used in order to extract from for 4 to 5 hours. Solvent removal device for the removal of solvent extract were transferred to 80% ethanol at 50°C and at low pressure (337 mbar) was removed. Concentrated extract weighed and were transferred into plates of the remaining solvent at the plate and on Benmary (at 30°C) was evaporated.

2.2. Standard Bacterial Suspension Process

Standard and lyophilized bacteria strains *Staphylococcus aureus* (25923 ATCC) was provided from the collection of fungi and bacteria Iranian Research Organization for Science and Technology. Lyophilized vials of the bacteria transmitted in the liquid medium BHI (Brain heart infusion agar) at sterile conditions and were incubated for 24-48 hours at 37°C. The medium containing the bacteria for 5 minutes in the centrifuge 6000 rpm and supernatant was replaced with Ringer's solution. In order to complete separation medium of the bacteria, the solution was centrifuged again for 5 minutes. The number of bacteria in the lower liquid by turbidity at a wavelength of 620 nm. This was routine work for the equivalent of 10^4 bacteria was considered at light absorption 0.08 - 0.1. The amount was approved by surface culture *Staphylococcus aureus* bacteria to inoculate in all treatments through the action.

2.3. Preparing Standard McFarland

To prepare the solution McFarland 0.5, 99.5 ml of sulfuric acid 1% and 5% ml barium chloride 1.175% was used. The resulting solution at a wavelength of 620 nm absorption was equivalent to 0.13 -0.08. McFarland turbidity equal to half the resulting solution with a bacterial suspension equivalent to 1×10^4 was created.

2.4. Preparation of Minced Meat Treatments

2 kg of Silver carp fillets were bought from the market and with the help of a portable ice was transported to the laboratory. And after washing, using sterile gauze soaked in alcohol, surface layers of fillet with a diameter of 1 - 2 cm was removed using sterilized scalpel. Then, by using a meat grinder that was sterilized with alcohol, Silver carp meat was minced three times. In the next step, 10^4 *Staphylococcus aureus* bacteria cells were inoculated and homogenized. For the preparation control treatments, meat inoculated harmal ethanol extract at concentration 0.01, 0.02 and 0.35 µg/ml was used. Also, for each treatment 6 tubes containing 1 gr of meat were considered. And then the tubes were packaged using Parafilm and were stored in the refrigerator 4°C over 13 days of experiments.

2.5. Bacterial Tests

Staphylococcus aureus bacteria tests were performed during each 48 hrs. In order to count the bacteria *Staphylococcus aureus* from the culture medium BHI (Merck, Germany) was used. At first, 100 gr of medium weight were mixed with 1000 cc distilled water. Serial dilutions were prepared.

Samplers tip to put in place and close the lid, then medium, pipes and tip samplers put into the autoclave to be sterilized. For ethanol extract three test tubes including 0.01, 0.02, 0.35 µg/ml of harmala were assigned and fish meat with specific concentration weighted the amount of one gram. Each poured in its own tube and 4 cc distilled water throw in each tube by pipette. Afterwards, the pipes will be closed by adhesive Parafilm and each tube is homogenized for 5 min by shakers. After working, the pipes, medium and tip samplers that was previously steriled in autoclave bringing out. After cooling the tube containing fish and water 0.01, 1 cc of liquid picked up by the sampler. Each treatment was repeated for two and after culture standard plates were selected and counted. The bacteria count in minced meat treatments were done once every two days.

2.6. Analysis of the Extract (GC/MS)

The methanol extract obtained from harmal was analysis by GC connected to (GC/MS). Inhibition coefficients were calculated using each of the separate elements and mass spectra and compare them with standard.

Chromatography set used was type 6890 Agilent column to the 30 m, the inner diameter of 0.25 nm and layer thickness 0.25µm of the 5HP-MS. Column temperature program was formulated in way: The initial temperature of the oven 50°C stop at this temperature for an increase of 5 minutes, the temperature gradient is 3°C per minute, temperature up to 240°C at 15°C per minute, increasing DeMatha 300°C and 3 min Stop temperature. Injection chamber temperature was 290°C and helium as the carrier gas flow rate (flow) 0.8 ml per min was used (Lawrence 1988). Agilent mass spectrometer voltage 5973 model 70eV, ionization method (EL) and ion source temperature was 220°C.

2.7. Statistical Analysis

Statistical analysis was performed using the software Spss 16. One-way ANOVA was used to determine significant differences between treatments and Duncan test at a significance level was used for statistical comparison $p<0.05$.

2.8. Measuring the Peroxide Value (PV)

Samples of fish oil extracted carefully in 250 ml Erlenmeyer flasks weight of the sand and about 25 ml of acetic acid: chloroform (chloroform to acetic acid ratio 3:2) was added to the flask contents. Then 0.5 ml of saturated solution of potassium iodide, 30 ml and 0.5 ml added to one percent starch and iodine value released was titrated with sodium thiosulfate solution normal 0.01. The peroxide was calculated from the following equation.

$$\text{Weight of the oil} / 1000 \times \text{ normality} \times \text{consumption of thiosulfate} = \text{Peroxide value}.$$

2.9. Measuring Thiobarbituric Acid (TBA)

TBA was measured by a colorimetric method. 200 mg of minced fish sample was transferred to a 25 ml flask and then brought to the volume of 1-butanol. 5 ml of the above mixture into the dry tube inserted lid (TBA reagent by dissolving 200 mg of TPA in 100 ml of solvent 1-butanol after filtering is obtained). Capped tube in a water bath with a temperature of 95°C for 2 hrs and then cooled at room temperature. Then the absorbance (As) at 530 nm against distilled water (Ab) was read. The amount of TBA (MDA mg in fish tissue kg) was based on the relationship below:

$$\text{TBA} = (\text{As} - \text{Ab}) \times 200 / 50.$$

2.10. Measuring Fat

A certain amount of homogenized sample is then mixed with sand for 6 hours at 100°C (5.1 h at 125°C) was put up quite a moisture evaporates. Then work on it and poured the contents in a distillation device interface Laboratory was placed in the tube. It was exactly weighed (A) and inside it spilled 250 cubic centimeters chloroform and the distillers) interface and refrigerant pipes (connected and cold water inlet valve is opened and continued distillation to extract the fat completely. Then chloroform Rotary machine or oven completely evaporated in balloons and balloons weighed again (B) and the total amount of fat sample was calculated from the following equation:

$$\text{Fat}(\%) = \frac{(\text{B} - \text{A}) \times 100}{\text{Sample weigth}}.$$

3. Results

The number of bacteria in different treatments zero-day refrigerated storage at 4°C using ethanol extract harmal reduce the density of *Staphylococcus aureus* in minced meat Silver carp. So that all affected treatments extract significantly lower bacterial density compared to control, but there was no significant difference between treatments by the extract. The use of ethanol harmal extract reduced the density of *Staphylococcus aureus* in minced meat of Silver carp. So that all affected treatments extract significantly lower bacterial density compared to control treatment ($P<0.05$), but treatment dose of 0.35 µg/ml effect is better. The use of ethanol harmal extract reduced the density of *Staphylococcus aureus* in minced meat of Silver carp. So that all affected treatment extract significantly lower than the control treatment, but bacterial density at dose 0.35 µg/ml treatment effect was better. The use of ethanol extract harmal reduced the density of *Staphylococcus aureus* in minced meat of Silver carp. So that all affected treatments extract significantly lower bacterial density compared to control treatment, but treatment dose of 0.35 µg/ml effect was better (Table 1).

Peroxide value (PV), fat (%) and tubarbiotic acid (TBA) levels showed different changes in Silver carp minced meat during 13 days storage at refregerated temperature (4°C) and there was significant difference in some treatments (different cncentrations of ethanolic harmal) and days ($P < 0.05$) (Table 2).

Staphylococos aureus count in Silver carp minced meat decreased significantly in harmal extract treatments than control ($P < 0.05$). Based on obtained results, concentration

0.35 µg/ml of harmal extract showed the best effects compared to other treatments ($P < 0.05$) (Table 3).

Peroxide value (PV), fat (%) and tubarbiotic acid (TBA) levels showed different changes in Silver carp minced meat storage at refregerated temperature (4°C) during 13 days and there was significant difference in some treatments (different cncentrations of aquatic harmal) and days ($P < 0.05$) (Table 4).

Table 1. Bacteria count of *Staphylococcus aureus* (cfu/g) at different concentrations of ethanolic harmal extract in minced meat Silver carp storaged at 4°C during 13 days

Treattments / Duration	Control	DMSO	Ethanol 0.01	Ethanol 0.02	Ethanol 0.35
Zero	4.06 ± 0.07^{a}	4.05 ± 0.13 ^{a}	3.98 ± 0.18^{a}	3.96 ± 0.19^{a}	3.97 ± 0.18^{a}
1th day	4.54 ± 0.11^{a}	4.24 ± 0.09^{a}	2.96 ± 0.205^{a}	2.7 ± 0.02^{a}	2.45 ± 0.04^{a}
2th day	5.82 ± 0.11^{c}	5.45 ± o.30^{c}	4.79 ± 0.08 ^{b}	4.53 ± 0.81^{ab}	4.2 ± 0.08^{a}
5th day	6.42 ± 0.27^{b}	6.41 ± 0.05 ^{b}	5.88 ± 0.01^{a}	5.58 ± 0.06^{a}	5.51 ± 0.09^{a}
7th day	6.42 ± 0.28^{c}	7.49 ± 0.04^{c}	7.32 ± 0.10^{c}	6.70 ± 0.03^{b}	6.22 ± 0.10^{a}
9th day	9.02 ± 0.17^{c}	8.42 ± 0.04^{c}	7.63 ± 0.14^{b}	7.45 ± 0.01^{ab}	7.17 ± 0.7^{a}
11th day	10.82 ± 0.17^{c}	10.62 ± 0.08^{bc}	10.31 ± 0.11^{ab}	10.28 ± 0.11^{ab}	10.08 ± 0.28^{a}
13th day	12.06 ± 0.07^{c}	11.94 ± 0.35^{c}	10.79 ± 0.12^{b}	10.73 ± 0.95^{b}	10.46 ± 0.11^{a}

Table 2. Biochemical indices changes in Silver carp minced meat storaged in refregerated (4°C) at different concentrations of ethanolic harmal extract during 13 days.

Biochemical indices / Treatments	Fat (%)	PV (meg/kfat)	TBA (mg MDA/K)
Control zero day ethanol	2.35±0.05^{C}	0.91±0.01^{B}	0.57±0.005^{A}
Control 7th day ethanol	2.12±0.04^{B}	5.11±0.04^{H}	3.67±0.02^{F}
Control 13th day ethanol	1.42±0.03^{A}	7.22±0.07^{I}	5.24±0.04^{G}
Control zero day ethanol 0.01	2.84±0.04^{D}	0.87±0.01^{AB}	0.64±0.007^{A}
Control 7th day ethanol 0.01	2.83±0.03 ^{D}	3.9±0.08^{D}	2.69±0.8^{C}
Control 13th day ethanol 0.01	2.8±0.05 ^{D}	4.95±0.02^{G}	3.37±0.02^{E}
Control zero day ethanol 0.02	2.83±0.08 ^{D}	0.84±0.07^{A}	0.63±0.003^{A}
Control 7th day ethanol 0.02	2.78±0.03 ^{D}	4.06±0.02^{E}	2.53±0.05B^{C}
Control 13th day ethanol 0.02	2.78±0.04 ^{D}	4.92±0.03^{G}	3.33±0.08 ^{E}
Control zero day ethanol 0.35	2.8±0.05 ^{D}	0.89±0.01^{AB}	0.63±0.004^{A}
Control 7th day ethanol 0.35	2.78±0.07 ^{D}	3.53±0.06^{C}	2.38±0.45^{B}
Control 13th day ethanol 0.35	2.75±0.04 ^{E}	4.35±0.02^{F}	2.95±0.03 ^{D}

Table 3. *Staphylococos aureus* density changes in Silver carp minced meat at different concentrations of aquatic harmal extract storaged in refregeator (at 4°C) during 13 days

Treatments / Duration	Control	DMSO	Aquatic 0.01 (µg/ml)	Aquatic 0.02 (µg/ml)	Aquatic 0.35 (µg/ml)
Zero	4.06 ± 0.07^{a}	4.05 ± 0.13^{a}	3.98 ± 0.18^{a}	3.9 ± .19^{a}	3.9 ± 0.18^{a}
1th day	4.54 ± 0.11^{d}	4.2 ± 0.09^{c}	3.66 ± 0.07^{b}	3.5 ± 0.65^{ab}	3.38 ± 0.8^{a}
2th day	5.86 ± 0.11^{c}	4.45 ± 0.3^{b}	4.68 ± 0.26^{a}	4.8 ± 0.35^{a}	4.7 ± 0.26^{a}
5th day	6.42 ± 0.28 ^{b}	6.41 ± 0.51^{b}	5.6 ± 0.11^{a}	5.45 ± 0.50^{a}	5.48 ± 0.15^{a}
7th day	7.50 ± 0.28^{b}	7.49 ± 0.04^{b}	7.74 ± 0.03^{b}	7.54 ± 0.72^{b}	6.98 ± 0.17^{a}
9th day	9.02 ± 0.17^{d}	8.94 ± 0.04^{d}	8.73 ± 0.11b^{c}	8.48 ± 0.052^{ab}	8.36 ± 0.23^{a}
11th day	10.82 ± 0.17^{c}	10.63 ± 0.8^{bc}	10.46 ± 0.08^{ab}	10.45 ± 0.18^{ab}	10.32 ± 0.13^{a}
13th day	12.06 ± ^{06a}	11.94 ± 0.03^{a}	12.02 ± 0.21^{a}	11.88 ± 0.05^{a}	12.09 ± 0.02^{a}

Table 4. Changes of biochemical indices in Silver carp minced meat at different concentrations of aquatic harmal extract storaged at refregerator temperature (4°C) during 13 days

Biochemical indices / Treattments	Fat (%)	PV (meg/kfat)	TBA (mg MDA / K)
Control Zero day aquatic	2.35 ± 0.05^{C}	0.991 ± 0.002^{A}	0.57 ± 0.005^{A}
Control 7th day aquatic	2.12 ± 0.04^{B}	5.11 ± 0.04^{F}	3.67 ± 0.02^{G}
Control 13th day aquatic	1.42 ± 0.03^{A}	7.22 ± 0.07^{H}	5.24 ± 0.04^{I}
Control zero day aquatic 0.01	2.75 ± 0.05^{E}	0.849± 0.06^{A}	0.62 ± 0.003^{A}
Control 7th day aquatic 0.01	2.75 ± 0.02^{E}	4.22 ± 0.02^{D}	2.74 ± 0.04^{C}
Control 13th day aquatic 0.01	2.72 ± 0.04^{DE}	3.35 ± 0.06^{B}	3.55 ± 0.03^{F}
Control zero day aquatic 0.02	2.65 ± 0.035^{D}	0.854 ± 0.03^{A}	0.63 ± 0.005^{A}
Control 7th day aquatic 0.02	2.69 ± 0.04D^{E}	24.16 ± 0.04^{D}	2.831 ± 0.02^{D}
Control 13th day aquatic 0.02	2.68 ± 0.04D^{E}	5.33 ± 0.03^{G}	3.77 ± 0.07^{H}
Control zero day aquatic 0.35	2.85 ± 0.05^{F}	0.912 ± 0.003^{A}	0.6 ± 0.004^{A}
Control 7th day aquatic 0.35	2.67 ± 0.02^{D}	3.65 ± 0.05^{C}	2.25 ± 0.05^{B}
Control 13th day aquatic 0.35	^{F}2.83 ± 0.03	4.77 ± 0.03^{E}	3.13 ± 0.08^{E}

4. Discussion

A modern social and economic change as well as international food trade at the global level has raised risk of food-borne diseases more than the past. Therefore, access to healthy food with high durability, recalls the use of chemical and natural preservatives in food [20].

Nowadays, due to increased consumer interest in natural products usage and also spreed of digestive and respiratory diseases and different kinds of cancer, extensive research has been done on the use of extracts and essential oils [15]. Drugs used in traditional folk medicine have long been prevalent in communities and in recent years the use of herbal medicines is growing on food storage [4]. The compounds derived from medicinal plant extracts have anti-bacterial and acts anti-microbial substances against pathogens. For example, the effect of alcoholic extract of *Thymus vulgaris* to prevent the growth of *Staphylococcus aureus* represents replacement of essential oils and extracts of medicinal plants [28].

Food poisoning caused by *Staphylococcus aureus* in most countries, the incidence of poisoning is primarily placed in listing 3 primarily poisoning [12]. Hence, this study was carried out with the aim at detecting the effect of ethanolic harmal extract on *Staphylococcus aureus* activity.

The results of this study indicated that ethanolic harmal extract has antibacterial activity against *Staphylococcus aureus*. The results of the analysis of ethanolic harmal extract indicated chemical compounds alkaloides such as Harmalyn, harmalol and harmine have antibacterial properties. Alkaloids are nitrogen complex combinations and have strong physiological effects. The most of alkaloids because of benefits their mental health benefits known as known very strong painkillers and sedatives such as morphine, cocaine, nicotine as well as a number of alkaloids are highly toxic. The therapeutic effects of medicinal plants are due to the presence of secondary metabolites that has pharmacological effects. Alkaloids with diverse biological effects, ie, the secondary metabolites that are important in medicine as pharmacologic agents. Zargari [32] studied the anti- viral and anti-microbial effects of harmel beta-carbonyl alkaloids and found that these alkaloids have basic combinations that stopped the reaction basic reactions in the virus and microbes.

Darabpoor *et al.* [7], Studied on different parts of harmal on bacteria that were resistant to antibiotics and concluded that the effect of exctact than the bacteria *Escherichia coli* and *Staphylococcus typhi* impact to an amount equal to (0.625). Hashemi *et al.* [11] studied antibacterial activity of methanol and ethanol harmal effect on *Pseudomonas aeroginas* strains and concluded that ethanol extract of harmal had beneficial effects in comparison with methanol.

Zhangyi *et al.* [33] examined pharmacological effects of harmala alkaloids and the results showed the inhibitory properties of these compounds on the bacteria. Liujian [18] studied antibacterial effects of hydroalchoholic extract obtained from harmala on several species of Gram-positive bacteria and concluded that the harmal extract has been inhibited effect at 0.05 mg/ml concentration.

For this reason, this study was designed to investigate the effect of ethanolic extract harmal on *Staphylococos aureus* and was tested on bacteria concentrations at 0.01, 0.02 and 0.35 µg/ml on bacteria. The results showed that ethanol at concentration 0.35 µg/ml has the greatest impact on bacteria and reduce bacterial activity during 13 days. Results showed that the bacteria is highly sensitive at the beginning than the ethanol extract and reduces considerably the activity. The mechanism of inhibitory effect on bacteria is by reducing bacterial activity, reduce the rate of growth and reproduction of bacteria that this effect can be due to the presence of alkaloids and phenolic compounds present in the harmal extract. Mainly phenolic compounds are responsible for the antimicrobial properties of extracts and essential oils [26]. The results of the antibacterial effects of the high content of phenolic compounds and flavonoids harmal in this study are consistent with results of previous reporting [33]. High antimicrobial activity due to the presence of alkanes and phenolic extracts and essential oils in harmala members and the genus extract. Phenols and flavonoids abundant is major reason for bioactivity and physiological function of harmal and medicinal plants [23]. In general, it is expected that the plant against Gram-positive bacteria compared to gram-negative bacteria are more active. This finding was according to Asgarpanah & Ramezanpour [3], which examined anti-bacterial features in the harmal and concluded that alkalonoids present in harmal such as Harmalyn had inhibitory effect on Gram-positive bacteria growth such as *Bacillus pumilus*. In the present studies in 0.35 µg/ml level on every day of the ethanol extract of minced meat stored at refrigerator temperature has inhibitory effects on the bacteria *Staphylococcus aureus.* For the ethanol extract significantly increased by increasing the concentration of the bacterial population. In this study, the bacterial activity significantly decreased over time. In general, there are limiting factors in food matrix that can affect their antibacterial extract performance over time. The most important limiting factors can be mentioned the following: 1- Food processing conditions 2- Food storage temperatures 3- pH combination food 4- The solubility 5- Limited stability during storage plant extracts 6- Factors related to food such as microbial activity and microbial interactions in food. In general it can be expected that the active compounds in ethanol harmal extract can be used as an inhibitor of bacterial growth. But for maximum inhibitory concentration can vary depending on conditions. So that any amount of active effective components in herbal extracts exceed the impact is greater.

4.1. Thiobarbituric Acid Values (TBA)

The amount of TBA in ethanolic harmal extract treatments for hindering extract fat oxidation was significantly lower compared to control. The results of this study showed that TBA had an increasing trend in all treatments over time.

The difference is that the observed changes in control than other treatments, and this difference was significant ($P<0.05$). Also in treatments containing ethanolic harmal extract ascending TBA changes compared to the control treatment has been slower process. Study results showed that TBA minimum values TBA 0.5 mg MDA/K) is related to the time zero control and maximum values of it in control in 13th day were 5.24. Results indicate that TBA

from day zero to day 13 was in the allowed range and are standard treatments containing ethanol harmal extract and only amount of TBA exceeded in control than the standard (5mg MDAK) on day 13. Although the TBA in comparison with the control sample containing ethanol extract harmal changes has been less but the rate of increase has been observed in both the treatment and control samples. Minimal change in treatments containing 0.35 ethanol extract. This suggests that the effect of harmal with defined dose was effective on the chemical changes spoilage of fat and to appear with increasing dose of harmal extract can more effectively prevent spoilage of corruption and chemical changes. The high content of thiobarbituric acid in control samples indicates the high level of corruption in fat. So based on the results of this study ethanol extract of harmal decreased the rate of formation of peroxide. But due to the increased amount of peroxide in control, fat oxidation occurred more quickly.

4.2. Peroxide Value (PV)

In this study, the amount of peroxide at different times and in samples containing ethanol extra harmal has been increased in control. Peroxide measured in Silver carp maintained in all 4 treatment groups increased significantly at 13^{th} day ($P<0.05$). Similar experiments in this field reported by Rezai *et al.* (2003). That the peroxide value in golden gray mullet in ice increased during the storage period of 13 days significantly ($p < 0.05$). Also Alghazeer *et al*. (2008) examined the impact of green tea over the course of 26 weekly maintenance in Atlantic mackerel (*Scomber scombrus*) at -10 and -80°C that showed increased number in peroxide value. The minimum amount of peroxide in this study was observed in control baseline. It can be concluded peroxide in each case is the minimum amount of PV in the treatment and control on day zero of maintanin and a maximum related to 13 days of maintenance with the control treatment equal to 7.22.

5. Conclusion

In the present study ethanol extract at level 0.35 µg/ml on every day of the of minced meat *Hypophthalmichthys nobilis* stored at refrigerator temperature has inhibitory effects on the bacteria Staphylococcus aureus. As well as with increase ethanol extract the concentration of the bacterial population in silver carp minced meat increased significantly. This indicates that by increasing the concentration of the extract, the bacterial activity reduced significantly and ethanol extract showed the inhibitory effect. But after a few days it had not very noticeable effect of extract and the bacterial population will increase significantly. Using of matters having anti-bacterial and antioxidant activity to increase quality and maintance life in fish meat. Due to the adverse effects of synthetic antioxidants used today as natural antioxidants to replace synthetic antioxidants, is highly recommended. Plant extracts are an important source of natural antioxidant. Since using different methods in order to minimize bacterial spoilage and oxidative seafood and health is important in economic terms and further studies on evaluating the anti-bacterial of natural plant extracts in fish and fishery products is suiable and nesseary. The concentration required the use of essential oils should be pay more attentioned. The results showed that the samples containing ethanol extract of the plant's antibacterial and antioxidant effects on chemical and biological agents have been effective. So that the shelf life of fish increased from zero to thirteen days compared to control samples that corresponded to our hypothesis about the effects of chemical. The ethanol extracts of harmal impact on Staphylococcus aureus a similar on the microbial load, peroxide index PV (meq/Kfat) and Thiobarbitoric acid (TBA mg MDA/K). In general, considering to the above results, we can use the 0.35 µg/ml of ethanolic harmal extract to increase the shelf life of minced meat Silver carp considered appropriate and recommended.

References

[1] Abbas, K.A. and Saleh Olasekan, A.M. (2009). The relationship between water and water activity and fish spoilage during cold storge: A review.J.Food, Agric, environ, 7: 86-90.

[2] Akhondzadeh Basti, A., Razavilar, V., Misaghi, A., Abasifar, R., Radmehr, B. and Sigarodi, F. (2004). Evaluation of *Zataria Multiflora* on *Slamonella typhimurium* in heart broth. *J. Medical Plants*, March, 9: 85-93.

[3] Asgharpanah, Zh. and Ramzani, F. (2012). An overview on the characteristics of the chemical and pharmaceutical harmal on several species of bacteria. Pharmaceutical Journal. Sixth year, 22: 1573-1580.

[4] Ayoughi, F., Barzegar, M., Sahari, M.A. and Naghdibdi, H. (2011). Chemical compositions of essential oils of *Artemisia dracunculus* l. andendemic *Matricaria chamomilla* l. and an evaluation of their antioxidative effects. J Agr Sci Tech 13: 79-88.

[5] Charls, V. (2006). Weekly medicine today. Translated by Afsharpoor, S. Isfahan University of Medical Sciences, and PP: 5-10.

[6] Cenci - Goga, B.T., Karama, M., Rossitto, P.V., Morgonet, R.A. and Cullor, J.S. (2003). Enteroxidant production by *Staphylococcus aureus* isolated journal of food protection, 66(9): 1693-1696.

[7] Darabpoor, E., Poshyouhian, N., Bavi, A., Motamed, I.H. and Seyyed Nejad, S.M. (2011). Antibacterial activity of different Parts of *Peganum harmala* L, growing in Iran against multi-drug resistant bacteria Excl.J, 10: 252-263.

[8] Diba, K., Gerami, M. and Hassanpour, L. (2009). The amount of inhibition of alcoholic extract of the plant's seeds *Candida* and *Aspergillus* species in vivo. Medical Journal. University of Medical Sciences, 20 (4): 271-277.

[9] Diba, K., Germani Shoar, M., Shabakhori, M. and Khorshidvand, Z. (2011). Anti fungal activity of alcoholic extract of *Peganum harmala* seeds.J.Med.plants pes, 5(23): 5550-5554.

[10] Fatorusso, E. and Taglaialatea-Scafati, O. (2008). Modern alkaloids: structure, isolation, synthesis and biology, Wiley- VCH Verlag GmbH & Co. KGaA, 4-25: 341-345.

[11] Hashemi, A., Shams, M., Barati, M. and Hamedani, A. (2010). Antibacterial effects of methanol and harmal on the standard mice and *Pseudomonas areoginosia*. Arak University of Medical Sciences. Fourteenth year, 4: 18-22.

[12] Jagadeesh Babu, A., Rupasundari, A., Sankar Reddy, B. and Sravnthi, M. (2012). Studies on the antimicrobial effectiveness of essential oils of garlic, clove and cinnamon on *Staphylococcus aureus* in chicken meat patties. Tamil Nadu J vet Anim Sci, 8 (1): 45-49.

[13] Kang, F. (1994). In vitrocidal effect of Chinese traditional herbs against *Echinoccus granulosus* proto scoliosis. Endemicdis - Bul, 9: 22-34.

[14] Kluytmans, J., Vanbelkum, A. and Verborugh, H. (1996). Nasal carriage of *staphylococcus aureus*: epidemilogy, underlying mechanism, and associated risks. Clin. Microbial. Rev, 10(3): 505-520.

[15] Lahooji, A., Mirabolfathi, M. and Karami Osboo, R. (2010). Effect of *Zataria multiflora* and *Satureja hortensis* essential oils,

Thymol and carvacrol on growth of *Fusarium graminearum* isolates and deoxynivalenol production. Iran J Pl Path, 46 (1): 37-50 (In Persian).

[16] Lamchauri, F., Settaf, A., Cherah, Y., Zemzami, M., Lyoussi, B., Zaid, A., Atif, N. and Hassar, M. (1999). Anti bacterial principels from *Peganum harmala* seeds. Therapy, 54 (6): 753-758.

[17] Lamchouri, F. (1999). Anti tumor principles from *Peganum harmala* seeds. J Therapie, 56 (6): 753-758.

[18] Liujian, X. and Hu, H. (2007). Bin 2, Zhaoguo- lin 1 (Department of life science, 2 college of chemistry and chemical Engineering, longdong university, ingyng, Gansu 745000, China; effect of Alkaloid of *Peganum multistum* bobr on seed ger mination of cucumber (*Cucumis sativusl*); plant physiology communication, 2007-02.

[19] Mahmoud, B.S.M., Yamazaki, K., Miyashita, K., Shin, I.I. and Suzuki, T. (2006). A new Technology of fish pereservation by combined treatment with electrolysed NaCl solution and essential oil compound food chem., 99: 656-662.

[20] Moradi, B., Mashak, Z., Akhondzadeh Basti, A., Moradi, B. and Bearing, A. (2012). The survey of the effect of *Cuminum cyminum* l. Essential oil on the growth of *Bacillus cereus* in a food model system. J Med Plants, 11(8): 93-102 (In Persian).

[21] Mousavi, T. (2005). Study of behavior of *Staphylococcus aureus* during the manufacture and ripening of Iranian White cheese. Thesis of food hygiene (Ph.D) Azad University of Tehran, jan; 237: 60-70. 529-9.(4 thed).

[22] Perez-Alonso, F., Arias, C. and Aubourg, S.P.(2003). Lipid deterioration during chilled storage of Atlantic pomfret (*Brama brama*). Eur J Lipid Sci Techno; 105: 661-7.

[23] Quavee, C. L., Planob, L.R.W., Pantuso, T. and Bennett, C.B. (2008). Effects of extracts from Italian medicinal plants on plants on planktonic growth, biofilm formation and adherence of methicillin-resistant *Staphylococcus aureus*, Journal of Ethnopharmacology, 118: 418-428.

[24] Radmehr, B. (2004). Growth modeling of *Staphylococcus aureus* in combination *Zataria multiflora* and growth factors. Thesis of food hygiene (Ph.D) University of Tehran, Aug, 237: 54-57.

[25] Rashan, L.J. (1989). In vitro antiviral activity of the aqueous extracts from the seeds of *P. harmala*. Fitoterapia; 64: 365-367.

[26] Rezei, M. and Hamzeh, A. (2010). Antioxidant and antibacterial effects of Sodium alginate coating enriched with thyme essential oil on rainbow trout (*Onchorhynchus mykiss*) fillets during refrigerated storage, J. Iranian Nutrition Sciences and food industry, 3: 11-20.

[27] Rezai, M., Sahari, M.A., Moeini, S., Safari, M. and Ghaffaari, F. (2003). Comparison quality oil of anchovy Kilka in two methods of transport and cold storage. Iranian J of Fisheries Sci; 3: 97-108 [In Persian].

[28] Sherafati, R. and Rafeeian, M. (2012). Evaluation of antioxidant properties of *Thymus vulgaris* Shirazi and it's antimicrobial effect on *Staphylococcus aureus.* Journal of Mazandaran University of Medical Sciences, 22: 88-94.

[29] Siddaiah, D., Vidya, S.R.G., Raju, C.V. and Chandrasekhar, T.C. (2001). Changes in lipids, proteins and kamaboko forming ability of Silver carp (*Hypophthalmichthys molitrix*) mince during frozen storage. Food Res Int, 34 (1): 47-53.

[30] SharbaTkori, M. (2007). Study of cidal effect of alcoholic extract of *Peganum harmala* seeds on *Echinococcus granulosus* protoscolex. (Dissertation) Tehran University of Medical Sciences.

[31] Yin, M.C. and Cheng, W.S. (2003). Antioxidant and antimicrobial effects of four garlic drived organosulfur compounds in ground beef. Meat Sci, 63: 23-8.

[32] Zargari, A. (1988). Medicinal plants.Tehran university press, Iran (2): 619.

[33] Zhangyi, W. (2007). Department of Pharmacy, Chang Chunmedical College; study on Antibacterial efffect extract from *Peganum harmala*; Changchung Medial Journal, 04.