Antimicrobial efficiency of *Allium atroviolaceum* extract on Rainbow trout in different temperature and storage time

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Abstract

Foodborne diseases in nature are usually infectious or toxic, caused by bacteria, viruses, parasites or chemicals, transmitted through food or water contaminated by the human body. To control the disease, as well as to ensure that the food is safe and healthy, its risks must be checked, detected and controlled. The goals of food industry are to produce healthy and high quality products that requires no contamination with bacteria. The use of natural preservatives such as herbal extracts and essential oils is recommended. *Allium atroviolaceum* is a family of Alliaceae and is a perennial herb which has a high nutritional value. The role of plant extracts has been evaluated as a preservative.

The total number of bacteria calculated followed by treatment of the aqueous and ethanol extractions of the *A. atroviolaceum* on rainbow trout fish fillet. 100 µl of aqueous and alcoholic extracts followed by ten times dilution used to evaluate the minimum inhibitory concentration, then 25 grams of rainbow trout fillet was submerged with the best concentrations of aqueous and alcoholic extracts. The results illustrated that the best aqueous and alcoholic extractions against *Staphylococcus aureus* was 3.125 mg ml^{-1} and 6.25 mg ml^{-1} and followed by treatment on fish both type of extraction showed inhibition on growth of bacteria compare to control group. So we believe that this plant has such a valuable effect to improve the quality and shelf life of fish.

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Keywords: *Allium atroviolaceum*, Total count of bacteria, Aqueous extraction; Ethanol extraction

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Introduction

In many countries, providing of the high quality, healthy with high shelf life of food products is one of the main needs of consumers. According to the World Health Organization, it is estimated that 600 million people die after eating contaminated food each year, with an estimated 420,000 deaths per year (WHO 2017). The fish are categorized as cold blood animals and their microbial load depends on the environment in which the fish are hunted. Fish are counted as fast food corruption because of the high percentage of unsaturated fatty acids and protein in relation to other foodstuffs, and keeping it in an inappropriate condition causes corruption, as well as a decrease in the quality of fish meat. Hence its maintenance requires a lot of speed and its maintenance is more severe than other meat (Özogul & Özogul 2006; Leisner & Gram 2014). So to protect of fish from antibacterial corruption researchers have been conducting extensive research and studies on chemicals and natural materials (Extracts and essential oils) which are imported directly or immersed in fish so they found antibacterial and antioxidant properties of these compounds (Fraser & Sumar 2014).

Rainbow trout with the scientific name *Oncorhynchus mykiss* and the English name Rainbow trout, is native to the Pacific North American, and from the Salmonidae family. The habitat of this genus is varies from oceans, small rivers to large, cool and cold lakes, sweet waters, salty waters and sea waters of temperate regions (Chen, Snow, Lawrence, Church, Narum, Devlin & Farrell 2015; Rodrigues, Silveira Alvaresb, Sampaio, Cabral, Araujo & Franco 2016).

Allium atroviolaceum plant is belong to Alliaceae family, among the herbaceous plants with onions and native to the Zagros area in Iran. This species is one of the lesser-known species of Allium. This plant has a high nutritional value and it is used as a source of vitamin and food (Khazaei, Esa, Ramachandran, Hamid, Pandurangan, Etemad & Ismail 2017).

The aim of this study was to investigate the antibacterial effect of aqueous and alcoholic extracts of *A. atroviolaceum* on rainbow trout fillets influenced by factors such as temperature, storage time and extract concentration.

Materials and Methods

Extraction

The extraction of the *Allium atroviolaceum* leaves was carried out at the Microbiology Laboratory of the Faculty of Veterinary Science, Islamic Azad University, Science and Research Branch. The leaves of *A. atroviolaceum* were collected in Borujerd and then dried at room temperature. 40 g of powdered leaves, with 120 ml of water solvent and 70% ethanol soaked and placed in a shaker for 48 hours. After styling, the solution was passed through filter paper. Then it was prepared for the preparation of two aqueous and alcoholic extracts within the Rotary Evaporator (IKA-WERKE, Italy) (Golestan Mohammadzadeh & Zarean 2016).

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Determine the best concentration of aqueous and alcoholic extract

Standard strains of *Staphylococcus aureus* (ATCC25923) and *Escherichia coli* (ATCC25922) were tested according to the Clinical and Laboratory Standards Institute (CLSI) for antibiograms assay. Initially, the standard bacteria were cultured on Brain Heart Infusion medium, After 24 hours, colonies were harvested and add to 1-cc physiologic serum to compare with half-MacFarland turbidity. Both MacFarlend's standard and suspension were read in a spectrophotometer with a wavelength of 625 nm. Antimicrobial susceptibility testing was performed based on micro-dilution broth method. 48 wells plates were used for two different extractions respectively for *S. aureus* and *E. coli*. 100 µl of the mueller hinton broth was added to all wells. Then 100 µl of aqueous and alcoholic extracts was added to the first well and then diluted to the second well and to the well number tenth. The well number 11, containing the extract and the mueller hinton broth as a negative control (-), and the last well containing the mueller hinton broth and bacteria as positive control (+). Then the plates were incubated at 35 ° C for 48 hours (Golestan *et al.*, 2016).

Preparing the fish

10 new rainbow trout were transferred into ice boxes to the Microbiology Laboratory of the Faculty of Veterinary Medicine, Islamic Azad University, Research Branch. After washing with water, heads and fins and abdominal staff were discharges (Golestan *et al.*, 2016).

Samples Preparation and storage time

25 grams of rainbow trout fillet was immersed with the best MIC concentrations of aqueous and alcoholic extracts and top up to 200ml and one container was set as control group. After immersion, fish fillets were packed for 24 hours (one day), 48 hours (two days), 72 hours (three days) and 168 hours (7 days), and refrigerated at + 4, and + 8 ° C. According to the set time, a test was performed and its antibacterial properties were evaluated. At first 10 g of fish fillets were weighed under sterile conditions and homogeneous with 90 cc of physiological saline solution in sterile bags to provide a uniform mixture. Then it used to prepare serial dilutions (Golestan *et al.*, 2016).

Microbial tests

For bacterial counting of the samples, 10 grams of fish fillets were weighed under sterile conditions and homogenized with 90 cc of physiological saline solution. The aforementioned solution was used to prepare the dilutions and count the bacteria at specific temperatures. For the total viable microbe evaluation Brain Hart Infusion Broth medium was used and the plates were incubated for 48 hours at 37 ° C (Golestan *et al.*, 2016).

Statistical analysis

The results of microbial analysis were descriptively analyzed using IBM SPSS Statistics 25 and repeated Measures Anova result and the variables were compared with each other.

Results

Minimum inhibitory concentration of the aqueous and alcoholic extraction

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After 48 hours' incubation, the minimum inhibitory concentration of aqueous and alcoholic extractions against *S. aureus* was 3.125 mg ml^{-1} and 6.25 mg ml^{-1} receptively. Also, the minimum inhibitory concentration results of aqueous and alcoholic extractions against *Escherichia coli* was 3.125 mg ml^{-1} and 12.50 mg ml^{-1} receptively.

Comparison of total average bacterial count between 4 and 8 degrees in different days

The Non-parametric Kruskal-Wallis test was used to examine the significant difference between extraction solvents and control group. The results showed no significant difference between extraction solvents and control group (P> 0.05). Fig 1 shows the comparison of total averages of total bacteria according to the type of extraction solvent and control group at 4 and 8 degrees Celsius. Generally, the average of the total number of bacteria counted under water extraction treatment was more than alcoholic extraction, and control group.

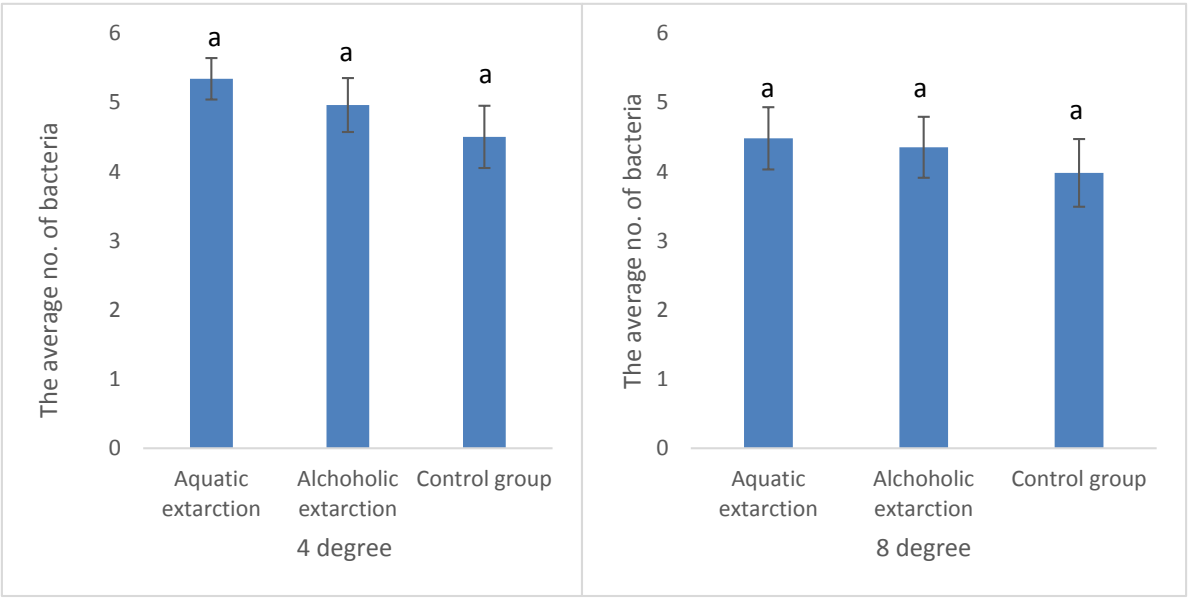

Fig 1. Comparison of the total average bacteria count based on the type of extraction solvent a) 4 degree and b) 8 degree.

Table 1 illustrates the Duncan's test of comparing the average of bacteria counted on different days at 4 ° C for different extraction solvents and the control group. As the results show, there was a significant difference between the days based on the aqueous extraction and the lowest average number of bacteria counted on the third day. There was also a significant difference in alcohol extraction solvent between different days, and there was no significant difference between the first, second and fourth days, and there was a significant difference between the third day with other days and the lowest average number of bacteria counted was on the third day. Also There was a significant difference between different days in the control group and there was no significant difference between the second and fourth days and between the third and the first days, and on the second and fourth days the highest average number of bacteria was counted and on the third the lowest mean bacteria counting was measured.

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Table 1. Comparison of the Mean ± SD of bacteria counted on different days at 4 ° C in different extraction solvents and control group

Day of experiments	Aqueous extraction	Alcoholic extraction	Control group
Day 1	5.49 [ab]	5.54 [a]	3.46 [b]
Day 2	5.51 [ab]	5.81 [a]	5.85 [a]
Day 3	3.89 [b]	2.10 [b]	2.15 [b]
Day 7	6.49 [a]	6.39 [a]	6.55 [a]

Duncan's test results are shown in Table 2 for comparison between the average numbers of bacteria counted on different days at 8 °. There was a significant difference between different days for aqueous extraction, the highest average number of bacteria counted on the seventh and second day. And the lowest number of bacteria on the first and third day was measured.

Table 2. Comparison of the Mean ± SD of bacteria counted on different days at 8 °C in different extraction solvents and control group

Day of experiments	Aqueous extraction	Alcoholic extraction	Control group
Day 1	3.71 [b]	5.43 [b]	3.37 [b]
Day 2	5.46 [a]	5.58 [b]	5.99 [a]
Day 3	2.02 [b]	0.001 [c]	0.002 [c]
Day 7	6.55 [a]	6.39 [a]	6.56 [a]

Significant differences were observed in alcohol extraction, and the highest average of bacteria counted on day 7 was measured. There was no significant difference between the first and second days and there was a significant difference between the third day and the other days and the lowest average number of bacteria counted on the third day. There was a significant difference between different days in the control group and there was no significant difference between the second and fourth days, and there was a significant difference between days 3 and 1 with other days, and the lowest average was measured on the third day.

Discussion

Microbial decay causes toxins and so infections or food poisoning in human body through the entry of bacteria into the host body. Hence, the goals of each food industry are to produce a healthy, high quality product with no contamination of the products by microorganisms, bacteria and fungi (Fraser & Sumar 2014). Among food products, meat and its products are suitable for microbial activity. Factors such as pH, O_2 and high humidity support the growth of a wide variety of microorganisms (Casaburi, Piombino, Nychas, Villani & Ercolini 2015; Remenant, Jaffres, Dousset, Pilet, & Zagorec 2015). Food additives are deliberately added to food products and increase the shelf-life and food safety, and also prevent growth, corrosion and diseases caused by organisms (Tomaska & Brooke-Taylor 2014). By conducting numerous studies with plants, valuable compounds such as phenolic compounds, anti-microbial polyphenols were obtained (Balasundram, Sundram & Samman 2006). The use of ethanolic extract of grapefruit juice inhibits the growth of *Enterobacteriacea*, *S. aureus*, *Salmonella*, yeast and molds in beef at 4 ° C (Sagdic, Ozturk, Yilmaz & Yetim 2011). The genus Allium is the largest and most important genus of the Alliaceae family. This genus is found in seasonal regions and in Iran, 52 species of 800 species have been distributed in the plains and mountainous areas of Azerbaijan. Valuable compounds such as onions, garlic, leeks and onions in these species have led to the use of these compounds as an additive and

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prophylactic medicine from ancient times (Fritsch Blattner & Gurushidze 2010; Choi & Cota-Sanchez 2010). In 2017, Khazaei *et al*., under laboratory conditions, investigated the effect of methanol extract of *A. aroviolaceum* on breast and liver cancer cells. After examining of laboratory samples, it was observed that the herb has medicinal and therapeutic properties (Khazaei *et al.,* 2017). Also in 2014, Lorigooini *et al*., analyzed the antiplatelet effect of *A. atroviolaceum* oil by using arachidonic acid and adenosine triphosphate as a marker of platelet aggregation (Lorigooini, Kobarfard & Ayatollahi 2014). According to the high level of rainbow trout production as a popular food, these fish are now found in lakes and rivers of all continents in the world except the Antarctic. After catching and the fish death, complex changes occur within the fish due to enzymatic, chemical and microbial activity. With its death, the immune system is weakened and bacteria easily multiply and rapidly spread to the tissues (Gram & Dalgaard 2002). In the current investigations on the results of total bacteria showed that the immersion of rainbow trout fillets in two aqueous and alcoholic extracts under certain temperature conditions reduced the growth of the bacterial cells and even no growth occur at some time. This is an indication of the great effect of the herbaceous medicinal plant, which inhibits the growth of bacteria. Marjoram oil also showed antimicrobial and antioxidant effects by adding this plant on rainbow trout fillet (Yasin & Abou-Taleb 2007). In 2008, Etemadi *et al*. examined the anti-bacterial and antioxidant effects of rosemary extract (0.1%) on rainbow trout packed in vacuum. After performing microbiological, chemical and sensory analyzes, they found that rosemary extract significantly ($p < 0.05$) postponed lipid oxidation in treated fish. Also, by adding 0.1% Rosemary extract, the total amount of bacteria and cold-blooded bacteria dropped below the established limit for the level of corruption (7 log cfu g^{-1}), so that the microbial degradation in this sample were significantly lower than control ($P < 0.05$), which means that the antioxidant and anti-bacterial properties of rosemary extract are effective (Etemadi, Rezaei & Abedian 2008). Oraei *et al*. (2011) examined the effect of antibacterial coatings (by adding cinnamon essential oil in chitosan coating) on the shelf life of rainbow trout in refrigerated conditions. The results of microbial evaluation showed that the essential oil of cinnamon and chitosan coating had a significant synergistic effect ($P < 0.05$) on the number of bacteria. Also, these two compounds significantly ($P < 0.05$) decreased the amount of volatile nitrogen beds in coated samples (Oraei Motalebi, Hoseini & Javan 2011).

Conclusion

The final result of this study showed that *Allium atroviolaceum* has an antimicrobial effect in addition to high nutritional value. This medicinal herb controls and prevents the growth of total bacteria and in certain temperature conditions. Therefore, it can be said that *A. atroviolaceum* plant, in addition to high nutritional value, medicinal and anti-cancer properties, can be used as an antimicrobial agent, and can use in food

factories and marine products (fish), especially salmon Rainbow.

Conflict of interests

The authors declare that there is no conflict of interest.

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ارزیابی اثرات ضد میکروبی عصاره گیاه *Allium atroviolaceum* در ماهی قزل آلای رنگین‌کمان در دما و زمان نگهداری متفاوت

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چکیده

بیماری‌های ناشی از مواد غذایی معمولاً عفونی یا سمی هستند، که ناشی از باکتری‌ها، ویروس‌ها، انگل‌ها و یا مواد شیمیایی است که از طریق غذا یا آب آلوده شده به انسان منتقل می‌شود. برای کنترل بیماری، همچنین برای اطمینان از اینکه غذا سالم است، خطر آن باید بررسی، شناسایی و کنترل شود. اهداف صنایع غذایی تولید محصولات سالم و با کیفیت بالا است که آلوده به باکتری ها نباشد. استفاده از نگهدارنده‌های طبیعی مانند عصاره‌های گیاهی و روغن های ضروری توصیه می‌شود. *Allium atroviolaceum* از خانواده Alliaceae است و گیاه چند ساله است که دارای ارزش تغذیه ای بالایی است. در این تحقیق تعداد کل باکتری‌ها پس از استخراج عصاره های آبی و اتانولی از *Allium atroviolaceum* در ماهی قزل آلای رنگین کمان محاسبه شد. ۱۰۰ میکرولیتر عصاره های آبی و الکلی با ۱۰ بار رقت برای ارزیابی حداقل غلظت مهاری استفاده شد، سپس ۲۵ گرم ماهی قزل آلا رنگین کمان با بهترین غلظت عصاره‌های آب و الکلی آمیخته شد. نتایج نشان داد که بهترین غلظت استخراج آب و الکل در برابر استافیلوکوکوس اورئوس ۳/۱۲۵ میلی‌گرم در میلی‌لیتر و ۶/۲۵ میلی‌گرم در میلی لیتر در لیتر بود و پس از آن بررسی روی ماهی هر دو نوع استخراج نشان دهنده مهار رشد باکتری در مقایسه با گروه شاهد بود. بنابراین ما معتقدیم که این گیاه دارای اثر با ارزش است تا کیفیت و عمر ماهی را بهبود بخشد.

کلمات کلیدی: *Allium atroviolaceum*، شمارش کل باکتری، استخراج آبی، استخراج اتانولی

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