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# Antimicrobial effect of ethanolic, methanolic, and ethyl acetate extracts of green tea against *Escherichia coli* and *Listeria monocytogenes*

Pantea Ramezannezhad[1], Maryam Beigomi[2], Farzin Ali-Malayeri[3], Fardin Ali-Malayeri[4], Saeide Saeidi[5*]

[1]Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran. [2]Department of Food Sciences and Technology, Zahedan University of Medical Sciences, Zahedan, Iran. [3]PhD of Feed Hygiene, Department of Animal and Poultry Health and Nutrition, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran. [4]Department of clinical biochemistry, Faculty of Medicine, Zabol University of Medical Sciences, Zabol, Iran. [5]Center of Agricultural Biotechnology, University of Zabol, Zabol, Iran

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## Abstract

**Background and aims:** One of the most important bacterial species transmitted through the use of aquatic products is *Escherichia coli*. The main aim of this study was to investigate the antimicrobial effects of ethanolic, methanolic, and ethyl acetate extracts of green tea on *Escherichia coli* and *Listeria monocytogenes*.

**Methods:** The samples of *Escherichia coli* and *Listeria monocytogenes* were purchased from the company and an antibiotic resistance pattern was determined. Finally, the minimum inhibitory concentration and minimum bactericidal concentration of ethanolic, methanolic, and ethyl acetate extracts of green tea were investigated.

**Results:** The results of this study showed that the lowest inhibitory concentration is related to ethyl acetate extract of green tea against *E. coli*, while the highest inhibitory concentration is related to methanolic extract of green tea against *Listeria monocytogenes*.

**Conclusion:** It is worth mentioning that high concentrations of ethanolic, methanolic, and ethyl acetate can be used as a natural antibacterial in fish products.

Keywords: *Escherichia coli*, *Listeria monocytogenes*, Fish, Green tea, Antimicrobial activity

Original article

## Introcuction

Fishery products are considered as healthy food because of high quality proteins, high saturated fats, vitamins, and minerals [1]. *Escherichia coli* is the

***Corresponding author**: Saeide Saeidi' Center of Agricultural Biotechnology, University of Zabol, Zabol, Iran. Email: s.saeedi12@yahoo.com Phone number: +989330040095

most common gram-negative, rod-shaped intestinal pathogen that causes fatal food poisoning in humans [2,3].

Different pathotypes of this bacterium include enterotoxigenic *E. coli*, pathogenic *Escherichia coli*, intestinal secretion of *Escherichia coli*, invasive intestinal esophagus, and hemorrhagic *Escherichia coli* [4]. Basically, *Escherichia coli* are the common cause of food poisoning. After entering the digestive tract, it moves to intestines through the mouth, connects to the mucosal cells of the intestines, and begins to replicate. When their number increases, they begin to release poison. Toxins caused by bacteria damage the intestinal mucosa and cause severe abdominal pain and diarrhea. Transmission of the disease is through food. The minimum pathogenicity level is 1010 to 108 in each gram of food [5]. The intestinal pathogenic bacteria are the major cause of diarrhea and digestive disorders in developing countries and places with poor health. This bacterium is a part of the natural flora of the intestines of warm blood animals and the presence of these microorganisms in food represents fecal contamination [6].

In the last decade, the cultivation of hydrothermal fishes has flourished in Iran. In 2010, the amount of hydrothermal fish production reached 121, 608 tons in a year [7]. Common carp breeding began in China from 1400 BC, and in Iran, this fish is cultivated in most provinces of the country. The most suitable temperature for spawning and its growth is 18-20 and 25°C, respectively [8]. Among the fishes, this one is very easy to nurture, and although it is a freshwater fish, it can live in half salty water [9]. The use of herbal additives as natural ingredients has grown exponentially to increase the speed of growth and improve the nutritional efficiency of the aquatic food industry. The group of vegetable-growth and health-promoting stimulants has several advantages over artificial growth and immune stimuli, such as availability, less damage to the environment and animals, and the possibility of generating a wide range with low price [10].

Over the years, man has discovered the various effects of herbal extracts. Known advantages of these extracts are the absence of harmful side effects and their wide range of effects. However, some of them, such as tea, have been infused with the daily life of humans [11]. The importance of using medicinal plants in treating diseases and preventing the growth of pathogenic bacteria is well known, but despite the many variations of these types of plants, globally or regionally, and the emergence of diseases and factors, a new pathogenic study is still ongoing [12]. Green tea is the human’s second drink after water. Green tea is used extensively in Asia, China, and Japan, which contains carotenoids, chlorophylls, polysaccharides, fat, vitamins, and elements like manganese, zinc, potassium, and polyphenols. Epithecate, Epigallocatechin, Epithecene Gallate, and Epigallocatechin 3 Galatians are four important types of polyphenols that are important in green tea. The health effects of green tea include reducing the risk of cardiovascular diseases, reducing the incidence of some cancers, controlling blood pressure, controlling body weight due to appetite loss, making probiotic effects, having antimicrobial and antiviral properties, making ultraviolet radiation protection of the sun, increasing density of bones, and making positive effects on the nervous system functions [13]. In recent years, green tea has found a good place in food and pharmaceutical industries due to its beneficial properties, including the antioxidant and antimicrobial ones [14,15]. Polyphenols are chromatic and water-soluble substances that have extensive effects on human health, including antimicrobial, anticancer, anti-inflammatory, anti-cardiovascular, and immune-enhancing ones. Among the antimicrobial effects of polyphenols, the effect of inhibitory on the growth of various bacteria and types of viruses, as well as yeast and fungi, can be mentioned [16]. Green tea is one of the natural preservatives. Polyphenylene compounds in green tea have antimicrobial activity. The resistance of bacteria to polyphenols depends on the type of bacteria and the structure of the polyphenol[17]. Antimicrobial activity of green tea is due to its polyphenolic compounds, which can be used in many ways, including protection from microbial contamination to polyphenols in industrial sizes, as well as prevention of the contamination of food products by pathogenic bacteria [18].

In 1999, a study found that catechins containing catechin, epigalocatecan, epicatecan gallate, and epigalaccatin in green tea prevent the release of a poison called verotoxin from enterohaemorrhagic *E. coli* and, consequently, inhibit the pathogenicity of this bacterium. This effect has been reported for plant polyphenols [19]. Also, in 2001, a study showed that Epigallocatecan gallate in tea reduces the transmission of the **Eolic** 600 pathogenic plasmid between R222 (donors) and Rc85 (receptor) *E.coli* K12 [20]. Green tea is one of these preservatives. Polyphenolic compounds in green tea have antimicrobial activity. The resistance of bacteria to polyphenols depends on the type of bacteria and the structure of the polyphenol. The aim of this study was to investigate the antimicrobial effects of ethanolic, methanol, and ethyl acetate extracts of green tea on *E. coli* and *Listeria monocytogenes*.

## Methods

The following bacterial strains of two antibiotic resistant pathogenic bacteria were used in this research: Escherichia coli (ATCC 35218) and *Listeria monocytogenes* (ATCC1783).

### Extraction:

20 grams of green tea was weighed and then poured for 20 minutes in antiseptic solution and germicide, benzalkonium chloride 10% in order to remove the possible microorganisms. After several rinses, in order to remove the effects of the disinfectant, the plant residues were placed in sterile Erlen and 100 ml of

alcohol 98% was added to the solution to dilute all parts of the plant. The green tea plant was then placed in a shaker for 48 hours to affect the solvent at 35°C and then the rotary machine was used to remove the solvent. Finally, the plant extracts were dissolved in DMSO (Merck-Germany) (and in order to prevent the effect of light and heat in a sterile bowl with aluminum sheet coating, it was kept in the refrigerator until doing the test [21].

To prepare the disks of extracts, the extracts were inoculated on to 25 blank discs at concentration of 25 µl. The disks were placed in an incubator at 37 °C for 24 h. Then, the effects of the disks containing the extracts against *E.coli* and *L .monocytogenes* strains were investigated.

**Determination of the sensitivity of bacteria to plant extracts:**

Determination of bacteria susceptibility to the extracts of the plant was done by Micro broth dillution. Six wells were created in a solid culture medium and 100 µl of each well was added to the nutrient medium of Muller Hinton Broth (MHB) (Merck-Germany). Then, to the first well, 100 ml of dilute solution of the extracts of plants was added along with the addition of, 100 µl of the first well to the second one after mixing, and this was done until the last well. From the final well, 100 µl of the culture medium was removed and the 10 µl of the microbial suspension containing 107 µg / ml was added to 0.5 McFarland and incubated at 37°C for 24 hours. The first well that prevented the growth of the bacteria after insertion into the incubator was considered as the least inhibitory concentration. To ensure, from transparent wells, 10µl was transferred to the Muller Hinton Agar medium (Merck-Germany), and after 24 hours the first concentration that was able to eliminate 99.9% of the bacteria was considered as the minimum lethal concentration [22].

**Agar Well Diffusion Assay for Extracts:**

Antibacterial activity of the plant crude extracts was tested using agar well diffusion method. The test inoculums (0.5 McFarland turbidity) were spread into Muller-Hinton agar using a sterile cotton swab. The wells were made by sterile well puncture and 20 µL of the extracts was added to each well and incubated at 37°C for 24 hours. The presence of the inhibition zone was regarded as the presence of antimicrobial action. The average diameter of the inhibition zone was measured in millimeter.

**Statistical Analysis:**

Growth was compared in each experiment using repeated measures of analysis of variance (ANOVA) in SPSS version 16.0. P-value less than 0.01 were considered significant.

## Results

The results of this study showed that the lowest inhibitory concentration was related to ethyl acetate extract of green tea against *E. coli* (0.62 mg / ml) while the highest inhibitory concentration of methanolic extract of green tea was against *L. monocytogenes* (10 mg / ml).

The greatest inhibitory diameter was observed for ethyl acetate extract of green tea against *E. coli* (25±1) mm, while the highest inhibition zone against *Listeria monocytogenes* was observed at 15 ± 1 mm, and the lowest inhibition zone was equal to 6 ± 1 mm against *Listeria monocytogenes* (tables 1- 2).

**Table 1:** Minimum inhibitory concentration and minimum lethal concentration of green tea extract (mg/ml)

| | MIC/MBC methanol | MIC/MBC ethanol | MIC/MBC Ethylacetat |
|---|---|---|---|
| ***E.coli*** | 5-10 | 2.5-5 | 0.62-1.25 |
| ***L.monocytogenes*** | 10-20 | 5-10 | 2.5-5 |

**Table 2:** Zone inhibition diameter of green tea extract against *Escherichia coli* and *Listeria monocytogenes* (mm)

| | methanol | ethanol | Ethylacetat |
|---|---|---|---|
| ***E.coli*** | 14±1 | 18±1 | 25±1 |
| ***L.monocytogenes*** | 6±1 | 9±1 | 15±1 |

## Discussion

The results of this study showed that green tea extract significantly increased the growth inhibitory hole diameter in amikacin and gentamicin, and by increasing the amount of the extract, this synergistic effect increased significantly. Addition of 1.25 milligrams of green tea extract to the two antibiotic discs, norrofloxacin and sulfomethoxazole, significantly inhibited its antibacterial activity, but decreased with the increase in the dose of green tea extract to 2.5 mg [23]. In a study by Nasrollahi et al., who investigated the antifungal effect of green leaf of green tea polyphenols on Candida albicans, it was found that the antifungal activity of catechins (the most effective combination of green leaf tea) was time-dependent. The lowest inhibitory concentrations of catechins in 103×0.5 , 103×1, and 103×2 yeast after 24 hours were 12.5, 25 and 100 ml/g, respectively, and after 48 hours 6.25, -12.5, and 50 ml/g, respectively [24]. Investigating the effects of green tea on *Streptococcus mutans* and *Enterococcus ficulus*, the study of Ranjbar et al. showed that there was no significant difference in the chlorhexidine greenhouse inhibition test for chlorohexidine in the one-way analysis of variance for *Streptococcus mutans*, but less significantly of sodium hypochlorite, although, in *enterococcus ficus*, sodium hypochlorite was significantly higher than green tea and chlorhexidine [25].

Reports showed that green tea polyphenols inhibited the growth of *Streptococcus mutans* - *Staphylococcus aureus* and *Escherichia coli* [26,27].

There are conflicting reports about the antimicrobial activity of green tea extract against pathogenic bacteria. Hara and Ishigami have reported that *Salmonella typhimurium* and Campylobacter jejuni are resistant to green tea extract, while others expressed the susceptibility of *Salmonella typhimurium* to the aqueous extract of green tea [28].

In the study of Nodoost and colleagues, the inhibitory diameter of green tea extract against *Listeria monocytogenes* - *Bacillus cereus* - Salmonella typhimurium and *Escherichia coli* was 0.05±10.29, 0.02±10.61, 12.3 ± 0.03 and 12.1 mm ±0.03, respectively [29].

The antimicrobial activity of green tea extract has been expressed in several studies [30,31].

One of the researches that have been used with regard to the use of green tea for the preservation of fishery products is the one entitled "The inhibitory effects of green tea polyphenols on microbial growth and the amount of volatile nitrogen vapors in the muscle of yellow tuna during ice storage by Norwick et al." (2001) [32].

In another research, Mohammadzadeh and Rezaei investigated the effect of green tea polyphenols on microbial and chemical changes of rainbow trout while keeping in ice, along with using green tea extract at a concentration of 600ppm to prevent and delay the microbial corruption of trout rainbow recommended during ice storage [33].

In a study by Boran et al., who investigated the antimicrobial activity of green tea against fish pathogens, the results showed that tea seed extract and saponins are good inhibitors of *Listonella anguillarum* [34].

In the study of Anita et al., the antimicrobial effect of aqueous-acetonic and ethanolic extracts of green tea on *Streptococcus mutans* and *Lactobacillus acidophilus* bacteria was investigated. The results showed that MIC of green tea extract against *Streptococcus mutans* and Lactobacillus was 0.2% and 0.3 %, while the MBC extract of green tea was 0.8% and 0.9% against the same bacteria. The inhibitory hole diameter for 30 µl containing 300 mg of green tea extract and control against *Streptococcus mutans* was 18.33 and 14.67 mm, while the inhibition zone with the same concentration of green tea and control against Lactobacillus acidophilus was 12.67 and 7.33 mm [35].

In the study of Vasudeo and Sonika, which investigated the antimicrobial activity of green tea, the results showed that chloroform and petroleum ether extracts had a strong inhibitory effect on Pseudomonas aeruginosa and Bacillus subtilis bacteria, and the minimum inhibitory concentration of chloroform extract was 25 µg / ml [36].

In the study of Farooqui et al., the minimum inhibitory concentration of methanolic extract of green tea against *S. auresus*-MRSA-*S.pyogenes*-*E.coli*-*S.enterica* (MDR)-*S.enterica* (S) -*P.Aeruginosa*-*A.buamanni*- *K.pneumoniae*- *C.freuendii*- *E.cloacae*- *B.subtilis*- *S.pneumoniae*- *Micrococcus*- *S.paratyphi A*- *Shigella*- *H.pylori* (RAC) - *H.pylori* (S) and *C.jejuni* was 0.39-0.39-ND- 5. 1.25- 2.5- 5 <-5 <-5-5- 5- 0.78- 0.78- 0.39- 1.25- ND- 2.5- 2.5-5 <-mg / ml (37).

By examining the antimicrobial activity of ethanolic green tea extract against *E. coli*, the study of Sepehri et al. indicated that the highest inhibitory concentration of green tea against *E. coli* was 10 mg / ml [38].

## Conclusion

According to the results of this research, it can be said that green tea extract with different solvents inhibits the growth of *E. coli* and *Listeria monocytogenes*, which are two pathogenic bacteria in fish. In addition, controlling these bacteria, food corruption and harmful economic effects can be reduced.

## Conflict of Interest

The authors declare that they have no conflict of interest.

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