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RESEARCH ARTICLE

Effects of *Artemisia dracunculus* L. and *Origanum majorana* L. Extracts Added in Different Concentrations on Some Possible Foodborne Pathogenic Bacteria in Hamburger Patties

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ABSTRACT

In this study, the antibacterial effects of distinct concentrations of *Origanum majorana* L. and *Artemisia dracunculus* L. extracts added to the formulation of hamburger patties on four different foodborne pathogenic microorganism named Gram positive (*Staphylococcus aureus, Listeria monocytegenes*) and Gram negative (*Salmonella Typhimurium*, *Escherichia coli*), which are the most ones isolated from hamburger patties, were researched. Besides, the use of natural preservatives in conjunction with the additional extracts was also explored to see if the microbiological reliability of hamburger patties could be guaranteed. According to the findings of the research, *Origanum majorana* L. and *Artemisia dracunculus* L. extracts were found to have strong antibacterial effects on Gr (+) and Gr (-) pathogenic bacteria inoculated into hamburger patties, and it was discovered that they inhibited at various rates. The antibacterial impact found by the study turned out to be greater on Gr (+) bacteria as compared to Gr (-) bacteria. It was also verified that 2% *Artemisia dracunculus* L. extract provided the highest antibacterial effect on *Listeria monocytegenes* with a decrease of 3.38 log cfu/g. Furthermore, it became apparent that the antibacterial impact grew in direct proportion to the concentration applied to the extracts employed, and that the antibacterial effect generated by the Artemisia dracunculus L. extract was stronger and more potent than the *Origanum majorana* L. extract.

Keywords: Antibacterial effect, extract, *Listeria monocytogenes,* pathogen, tarragon

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Değişik Konsantrasyonlarda ilave Edilen *Artemisia dracunculus* L. ve *Origanum majorana* L. Ekstraktlarının, Hamburger Köftesinde Bulunması Muhtemel Bazı Gıda Kaynaklı Patojen Bakteriler Üzerine Etkileri

ÖZ

Bu araştırmada hamburger köftesi formülasyonuna dahil edilen farklı konsantrasyonlarda mercanköşk (*Origanum majorana* L.) ve tarhun (*Artemisia dracunculus* L.) ekstraktlarının, hamburger köftelerinden en çok izole edilen, gıda kaynaklı dört farklı Gram pozitif (*Staphylococcus aureus, Listeria monocytegenes*) ve Gram negatif (*Salmonella Typhimurium, Escherichia coli*), patojen bakteri üzerindeki antibakteriyel etkilerinin incelenmesi amaçlanmıştır. Ayrıca ilgili bitki ekstraktlarının, hamburger köftelerinde doğal koruyucular olarak kullanılabilirliği de araştırılmıştır. Araştırma sonucunda, mercanköşk ve tarhun ekstraktlarının hamburger köftelerine inoküle edilen Gr (+) ve Gr (-) patojen mikroorganizmalar üzerinde güçlü antibakteriyel etki göstererek farklı oranlarda inhibisyonuna neden olduğu tespit edilmiştir. Çalışma ile ortaya konulan antibakteriyel aktivitenin Gr (-) bakterilere kıyasla Gr (+) bakteriler üzerinde daha yüksek olduğu belirlenmiş olup, en yüksek antibakteriyel etkiyi *Listeria monocytegenes* üzerinde, 3.38 log kob/g düşüş ile %2'lik *Artemisia dracunculus* L. ekstraktının sağladığı belirlenmiştir. Kullanılan ekstraktlarda uygulanan konsantrasyonun artışına paralel olarak, antibakteriyel etkinin artış gösterdiği, ek olarak tarhun ekstraktının sağladığı antibakteriyel etkinin, *Origanum majorana* L. ekstraktına kıyasla daha yüksek ve etkili olduğu da ayrıca saptanmıştır.

Anahtar Kelimeler: Antibakteriyel etki, ekstrakt, *Listeria monocytogenes,* patojen, tarhun

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INTRODUCTION

Hamburger is a meat product that can be produced by adding beef fat, seasonings, and salt to fresh or frozen ground beef and is mostly consumed by grilling (Güner and Atasever 2010). In accordance with Turkish Standards, hamburger is defined as a mixture and is prepared in phases. In the very initial step of butchery, the body meats of veal, sheep, and lamb are removed from the bones, cartilage, tendons, lymph nodes, fascia, and nerves. Then, the meat is ground with the addition of breadcrumbs, starch, potato flour, soy proteins, edible soybean flour, flavorings, and additions, along with kidney oil, tallow, tail fat, and edible salt. After being reduced to minced beef, it is then mixed to ensure an equitable distribution (Anonymous 2002).

Ready-to-eat products that have been preserved in the cold for a long period are the ones that pose the most microbiological risks. This risk is fairly considerable because hamburger patties are a quick-consumption product. When foods are maintained at improper temperatures for an extended period of time, they become particularly suited for microbial growth, especially infections. Examples of such foods include hamburger patties. They may therefore result in microorganism-related issues, particularly food poisoning (Koçan and Halkman 2006; Şevik et al. 2021).

A perennial shrub, Tarragon (*Artemisia dracunculus* L.) is one of the species in the *Asteraceae* family (Ceylan 1996). The Northern Hemisphere is home to a large population of *A. dracunculus* L., whose native country is assumed to be Siberia. Tarragon, whose leaves are 2–8 cm long and 2–10 mm wide, can reach 120-150 cm in length (Raghavan 2007). The composition of the essential oil extracted from the leaves of Tarragon has been broken down into more than thirty constituents, and anethole (81.0%), β-ocimene (9.6%), limonene (3.1%) and methyleugenol (1.8%) were listed as the primary components of these oils (Kordali et al. 2005).

When combined with mint and anise, Tarragon results in a pleasing aroma. In addition to being used dried with its stems and leaves, Tarragon L. can also be crushed or ground after drying. According to reports, *A. dracunculus* L. is appetizing and facilitates digestion by increasing digestive secretions, relieves stomach and intestinal gases, and is diuretic. Studies have shown that Tarragon L. is anthelmintic, antibacterial, effective against cramps, and helpful for stomach ailments. Plus, it is utilized in combinations for the treatment of anemia and digestive disorders as well as in antirheumatic mixtures (Azırak 2007).

A member of the *Labiatae* family, the semi-shrub plant Marjoram (*Origanum majorana* L.) belongs to the *Origanum* genus. It is a common spice that comes from the Mediterranean region and is used all around Europe. Other Greek islands, particularly Crete, as well as the south and west of Turkey are also home to a plethora of its growth (Raghavan 2007).

The chemical composition of the essential oil of Marjoram mainly consists of carvacrol (% 50-82), γ-terpinene (% 0.09- 7), linalool (% 0.04-1.9), thymol (% 0-1.9) and p-cymene (% 0.01-10.9) (Azırak 2007). Marjoram, which has been found to have potent antioxidant and antibacterial activities, additionally has been associated with sedative, cardiovascular, antiseptic, stimulant, and anthelmintic characteristics. (Wetter 2010; Azrak 2007; Raghavan 2007).

The aim of this study is to figure out the antibacterial effects of *O. majorana* L. and *A. dracunculus* L. extracts, which were included in the formulation of hamburger patties, on four different foodborne pathogenic bacteria, most of which were obtained from hamburger patties. It was also looked into whether natural preservatives could be used along with the additional extracts to guarantee the hamburger patties' microbiological dependability.

MATERIAL and METHOD

Material

The meat manufacturer in Afyonkarahisar provided the minced meat that was put to use for the production of the hamburger patties that were used in the study. The beef was the ingredient used to make the minced meat, which had a 12% fat level overall. All ingredients (breadcrumbs and onions) were purchased at a chain market, and all spices (including Tarragon and Marjoram), which were used in the preparation of hamburger patties) were purchased from a herbalist working in the province of Afyonkarahisar.

Extraction of spice extracts

A complete amount of 400 mL of 80% ethyl alcohol was then poured into the dried spices of Tarragon and Marjoram. soon after they had been weighed at 100 g each. The resulting mixes were shaken for 24 hours at 120 rpm using a shaker (Wise Shake®, SHO-2D, Thailand). The mixes were filtered using sterilized 22 mm (Whatman, UK) filter paper at the conclusion of the experiment. The alcohol and extract combination in the filtrate was then separated from one another using a rotary evaporator at 60 °C and 120 rpm (Heidolph Hei-VAP valuei Germany). After that, the extracts were maintained in dark, airtight containers at +4 °C (Akarca et al. 2019).

Bacteria strains used in the study

In the study, bacterial strains belonging to *Escherichia coli* ATCC 8739, *Listeria monocytogenes* ATCC 51774, *Staphylococcus aureus* ATCC 6538, and *Salmonella Typhimurium* ATCC 14028 species have been employed.

Production of hamburger patty

By modifying the formulation proposed by Ilhan (2010), the production of the hamburger patties utilized in the study was accomplished in accordance with the formulation listed in Table 1.

Table 1. Hamburger Patties Formulation

Component	Quantity (g)
Beef Meat	500
Onion (Grated)	49.3
Salt	14.1
Sweet Paprika Powder	1.8
Cayenne Pepper Powder	1.8
Balck Pepper	3.5
Breadcrumbs	46.9
Water	63.4

Preparation of inoculums

The pathogenic bacteria that were employed in the study were grown on non-selective media and were obtained from overnight cultures and single colonies that were growing on their own with the use of sterile loops. These were combined until uniform turbidity was generated in tubes containing Ringer's solution (Merck 1.15525, Germany). A densitometer (Biosan, 1B, Turkey) was used to adjust the density of the produced inoculum suspension so that it satisfied the 0.5 McFarland (8.17 log cfu/mL) standard (Akarca and Şevik 2022). Then, 0.5 Mc Farland standard pathogenic bacteria suspensions (6 log cfu/g) were added into the patty dough separately and the mixture was homogeneously mixed and finally, the hamburger patties were shaped.

Microbiological analysis

Preparation of samples for microbiological analysis

With the help of a sterilized spatula, taking 10 g of homogenized hamburger patties samples were weighed, and placed in stomacher bags. 90 mL of 0.1% buffered peptone water (Merck, 107,228, Germany) was added to these and the stomacher (Bag Mixer® 400 P-080921247) was homogenized for one minute. One milliliter (mL) of each sample was drawn out by means of automatic sterile pipettes (Eppendorf, Research Plus), and 9 mL of sterile 0.1% buffered peptone water kept in the tubes was added on top of it, and 10^{-2} dilutions were prepared. By continuing the process in this way, serial dilutions up to 10^{-5} were prepared (Halkman 2005).

***Staphylococcus aureus* species bacteria count**

Using Baird-Parker Agar (Merck 1.05406, Germany) as the medium, the spread plate method has been employed for calculating the total count of *S. aureus*. Egg yolk-tellurite emulsion (Merck 1.03755, Germany) was added on the medium that has been sterilized and cooled in an autoclave while stirring in a magnetic stirrer and was later mixed well. Afterward, the medium was poured into Petri dishes at a volume of approximately 12 mL, allowed to disperse properly and allowed to solidify. Then, 0.1 mL of prepared dilutions were inoculated into Petri dishes with the help of an automatic pipette (Eppendorf Research Plus) in double parallel. Later, with the help of a sterile drigalski spatula, the inoculated sample was spread homogeneously in the Petri dish. The petri dishes were inverted and incubated for 24-48 hours at 37°C under aerobic conditions in an incubator (Incucell, MMM, Germany) after permitting the sample to be absorbed by the medium. Black colonies that developed during incubation were identified and counted (ISO 1999).

The Petri dishes underwent a further 18 hours of incubation following the labeling of the colonies. After incubation, typical *Staphylococcus* colonies with white precipitation rings and bright black colonies that do not form zones were counted separately. A coagulase test was subsequently utilized to identify the count of *S.aureus* bacteria by counting the positive colonies from five of each type of colony (Martins et al. 2009).

***Escherichia coli* species bacteria count**

Using Violet Red Bile Agar (Merck 1.01406, Germany) as the medium, the spread plate method was employed for calculating the count of *E. coli* . After the medium has been prepared, poured into petri dishes, and those dishes have solidified, 0.1 mL was inoculated in pairs in parallel from the prepared dilutions, and it was ensured that it spread homogeneously with a drigalski spatula. After the inoculate was absorbed by the medium, the medium was added a second time but less (4-5 mL) onto the solidified medium and mixed. Then, the petri dishes were inverted and left for incubation in an incubator at 37°C for 24-48 hours. At the end of the incubation period, pink colored colonies were marked and counted (ISO 2001a; ISO 2001b).

***Listeria monocytogenes* species bacteria count**

The spread plate method with Fraser Listeria selective enrichment Broth (1.10398.0500) and Oxford Listeria

selective Agar (Merck, 1.07004, Germany) was implemented to acquire an L. monocytogenes type bacteria count. The supplements (Fraser Listeria Ammonium Iron (III) Supplement; Merck 1.00092, Germany, and Fraser Listeria Selective Supplement; Merck 1.00093, Germany) were added, and everything was thoroughly mixed, after the Fraser Listeria selective enrichment Broth had been sterilized in an autoclave and cooled to room temperature. 10 g samples were taken from each sample in sterile stomacher bags, and 90 mL of sterile Fraser Listeria selective enrichment broth was added to them and mixed in the stomacher and it was ensured that they are homogenized. Then, these pre-enrichment cultures were incubated for 24 hours in a 37℃ incubator (ISO 2017a; ISO 2017b).

At the end of the period, 1 mL each was taken from the sample and Fraser broth mixtures with the help of a sterile pipette and was transferred to tubes containing 9 mL sterile Fraser broth. This process was continued until 10^{-5} as the serial dilutions were got ready. With the help of a sterile automatic pipette, 0.1 mL of the prepared dilutions were taken and inoculated onto Oxford Listeria selective Agar surfaces. The inoculation was then spread homogeneously on the petri dish with a sterile drigalski spatula. Then, the Petri dishes were incubated at 37℃ for 24-48 hours. At the end of the period, colonies with rounded black zones on petri dishes were counted (ISO 2017a; ISO 2017b).

The Count of *Salmonella* Typhimurium

The count of *S.Typhimurium* was measured by applying spread plate method using Nutrient Broth (Merck, 105443, Germany), Rappaport Vassiliadis Salmonella (RVS) Broth (Merck 107700, Germany) and Brilliant Green Phenol Red Lactose Sucrose (BPLS) Agar (Merck, 110747, Germany). Taking 10 g of homogenized hamburger patties samples was taken and placed in sterile stomacher bags, to which 90 mL of Nutrient Broth was added and these were mixed in the stomacher and homogenized. Then, these pre-enrichment cultures were left for incubation for 24 hours in 37℃ incubator. At the end of the period, 1 mL of each inoculate and nutrient broth mixture was taken with the help of a sterile pipette and transferred into tubes containing 9 mL of sterile RVS Broth. Later, serial dilutions up to 10^{-5} were prepared. After the incubation of these dilutions at 37℃ for 24 hours, they were inoculated onto BPLS Agar surfaces with the help of an automatic pipette and spread homogeneously with the help of a drigalski spatula. Following this procedure, the petri dishes were inverted and incubated at 37℃ for 24 hours. At the end of incubation, pink colonies with red zones formed on the surface of the medium were counted (ISO, 2017c).

All microbiological analyzes were repeated at 0^{th}, 1^{st}, 2^{nd}, and 3^{rd} hours in the same way, and the counting results were calculated as a standard weighted average at consecutive dilutions, and the results are given as "log cfu/g".

Experimental design and statistical analysis

The factorial structure of the research design, which was totally random, is 4 x 4. Factors are time (zeroth, 1st, 2nd, and 3rd hours) and patty examples (control, M1, M2, T1 and T2). Duncan's multiple range tests (SPSS, version 23) were used to analyze factors to find differences (P0.05) between samples over time. Replications were used to totally randomize the design.

RESULTS and DISCUSSION

The count of *Staphylococcus aureus*

The initial *S. aureus* measurements were reported to range from 6.07 to 6.17 log cfu/g in hamburger patties created through adding extracts of Tarragon and Marjoram at various ratios (Table 2; $p>0.05$). This amount increased by 0.32 log cfu/g in the control sample after three hours ($p>0.05$). On the other hand, it was shown that there was a decline in the samples throughout this time to which spice extract was added at various rates ($p<0.05$). It was figured out that the highest decrease was in the samples to which 2% Tarragon extract was added with a rate of 2.27 log cfu/g. This was followed by the samples produced by adding 1% extracts of Tarragon at 1.91 log cfu/g, Marjoram at 1.41 log cfu/g, 2%, and by the samples produced with the addition of 1% extracts of Marjoram at a ratio of 1.31 log cfu/g (Table 2).

Other samples were confirmed to be statistically significant ($p<0,05$) aside from the control sample, which displayed a time-dependent decline in the quantity of *S. aureus* bacteria inoculated into the hamburger samples. The same time period also revealed statistical significance for *S. aureus*-inoculated hamburger samples, and all time periods but the zeroth hour ($p<0.05$) displayed statistical significance for the decline in bacterial counts.

Corresponding to the findings of this research, Sağdıç (2003) looked into the effects of *Origanum majorana* L. species used in food production on four different food-borne pathogenic bacteria and assessed the inhibition effect. The study found that *S. aureus* is the bacteria that is most vulnerable to the Marjoram. Shan et al (2007) investigated the antibacterial effect of a total of 46 plant and spice extracts on 5 foodborne pathogenic bacteria (*B. cereus, L. monocytogenes, S. aureus, E. coli* and *S. Anatum*). The study results suggested that compared to Gr (-) bacteria, Gr (+) bacteria are more sensitive and stated that the most resistant bacteria are *E. coli* and the most sensitive bacteria is *S. aureus*. In addition to that, in a study by Lacroix et al (2006), it was detected that *E. coli* O157:H7, food pathogens of the spice Marjoram., showed high antimicrobial activity against

food pathogens such as *L. monocytegenes, S. Typhimurium* and *S. aureus*. In another study, in which *S. aureus* was inoculated into feta cheese with Marjoram. and Cinnamon extracts added, it has been reported that Cinnamon and Marjoram extracts have an inhibitory effect on *S.aureus* (Kahraman 2017).

Table 2. Time-dependent variation in inoculated *Staphylococcus aureus* counts (0/25g Log cfu/g)

Samples	Initial microorganism load	0.Hour	1.Hour	2. Hour	3.Hour
Control	<1	6.07±0.01^{Aa}	6.92±0.03^{Aa}	6.70±0.02^{Aa}	6.39±0.01^{Aa}
Tarragon 1%	<1	6.14±0.03^{Aa}	5.91±0.02^{ABab}	4.80±0.02^{Bb}	4.23±0.03^{Bbc}
Tarragon 2%	<1	6.17±0.01^{Aa}	5.76±0.03^{ABb}	4.54±0.01^{Bb}	3.90±0.05^{Cc}
Marjoram 1%	<1	6.14±0.02^{Aa}	6.08±0.02^{Aa}	5.49±0.03^{ABab}	4.83±0.03^{Bb}
Marjoram 2%	<1	6.17±0.01^{Aa}	5.99±0.01^{ABab}	5.36±0.03^{ABab}	4.76±0.04^{Bb}

A-C (→): Values shown with different letters on the same line differ from each other at the p<0.05 level
a-c (↓) : Values shown with different letters on the same column differ from each other at the p<0.05 level

The count of Escherichia coli

Introductory *E. coli* counts were observed to vary between 6.47 and 6.50 log cfu/g in hamburger patties that had Tarragon and Marjoram extracts added at various rates (Table 3; p>0.05). Three hours later, it was discovered that the quantity of *E. coli* had decreased in all samples. With the exception of the control sample, it was found that this drop was statistically significant in the other samples (p<0.05). In a similar way, it was decided that the decrease in bacterial counts in the hamburger patties inoculated with *E. coli* in the same period of time was statistically significant in all other time periods except the zeroth time (p<0.05). It was obtained that the highest decrease was observed in hamburger patties produced by adding 2% Tarragon extract at a rate of 2.85 log cfu/g. and this was followed by the samples produced by adding 1% Tarragon extract at a rate of 2.09 log cfu/g, and the samples produced by adding 2% of Marjoram extract at a rate of 1.09 log cfu/g (Table 3), respectively.

In a study, Ehrich et al. (1995) investigated the effects of 38 kinds of spices against pathogenic bacteria such as *S. epidermidis, E. coli* and some mold types. The study results indicated that Tarragon and Marjoram. spices were among the spices that displayed the most antimicrobial effect among the extracts. Moreover, in the study carried out by Pişkin (2007), it was maintained that the essential oil obtained from the Marjoram plant showed the highest antimicrobial effect against *E. coli* with a concentration range of 0.4-0.0007 µl/ml. Turhan (2015) executed a study investigating the antimicrobial effect of some spice essential oils on E. coli and found that Marjoram plant affirmed the highest antimicrobial effect on *E.coli* and expressed that Marjoram plant formed the largest inhibition zones. Turhan (2015) also revealed in the same study that 5-10-15 µL of Marjoram essential oil completely inhibits bacterial growth.

Another study focused on the sausage-infused essential oil of Marjoram to find out how it affected inhibition. In the investigation, bacteria from the species *B. subtilis, S. choleraensis, S. flexneri, S. aureus*, and *E. coli* were employed. According to the results of the research, gram negative bacteria can be inhibited much more easily by using Marjoram essential oil (Busatta et al., 2008). The results of similar studies on the subject are similar to the results obtained in this study.

Table 3. Time-dependent variation in inoculated *Escherichia coli* counts (0/25g Log cfu/g)

Samples	Initial microorganism load	0.Hour	1.Hour	2. Hour	3.Hour
Control	<1	6.47±0.03^{Aa}	6.40±0.03^{Aa}	6.20±0.04^{Aa}	6.00±0.03^{Aa}
Tarragon 1%	<1	6.47±0.02^{Aa}	5.52±0.02^{ABb}	4.68±0.03^{Bb}	4.38±0.02^{Bb}
Tarragon 2%	<1	6.51±0.01^{Aa}	5.25±0.04^{Bb}	4.27±0.04^{BCb}	3.66±0.02^{Cc}
Marjoram 1%	<1	6.51±0.04^{Aa}	6.30±0.03^{Aa}	6.08±0.02^{Aa}	5.63±0.02^{Bab}
Marjoram 2%	<1	6.50±0.04^{Aa}	6.17±0.05^{ABab}	5.68±0.03^{Bab}	5.41±0.03^{Bab}

A-C (→): Values shown with different letters on the same line differ from each other at the p<0.05 level
a-c (↓) : Values shown with different letters on the same column differ from each other at the p<0.05 level

The count of *Salmonella Typhimurium*

Through the application of two different ratios of Marjoram and Tarragon. extract, it was conceivable to acquire initial count of *S. Typhimurium* bacteria type inoculated into hamburger patties that differed between 6.55 and 6.52 log cfu/g (Table 4; p>0,05). All samples established a downward trend in *S. Typhimurium* bacteria throughout the course of the three-hour storage period, and all samples other than the control sample exhibited this decline to be statistically significant (p<0.05). Over and above, it was revealed that the decrease in the count of bacteria in the same time period of the samples inoculated with *S. Typhimurium* type bacteria was also statistically significant in all other time periods except the first hour (p<0,05). It was resolved that the highest decrease among the samples was in the samples produced by adding 2% Tarragon extract with a value of 2.66 log cfu/g (p<0.05). It was specified that this

sample was followed by the sample adding 1% Tarragon extract with a value of 2.38 log cfu/g and the other was produced by adding 2% of Marjoram extract with a value of 1.74 log cfu/g, respectively ($p<0.05$).

Consistent with the results of this research, Chaudhry et al. (2007) investigated the antibacterial effect of Origanum majorana L. (Marjoram) plant for Gram (-) bacteria in research they performed in Pakistan. It has been uttered that the chemical compounds such as thymol and carvacrol in the content of this plant, have antimicrobial effects on other Gr (-) bacteria species, especially *Salmonella* supsp. *and E. coli.* In a study undertaken by Özcan and Erkmen (2001), the effects of nine distinct plants on 11 various microorganisms, including *S. Typhimurium*, were analyzed. The researchers concluded that the antimicrobial activity of the Marjoram plant is extremely strong. Four different foodborne pathogenic bacteria, including *S. Typhimurium, L. monocytogenes, Y. enterocolitica* and *B. Cereus*, were addressed by Bonyadian and Moshtaghi (2008) in order to assess the antibacterial effect of Tarragon The most sensitive bacteria, according to the findings of this study, is *S. Typhimurium.*

Govaris et al. (2010) identified the antibacterial effect of Marjoram essential oil by inoculating *Salmonella* subsp. into minced meat produced from lamb and after 12 days of storage, they found that 0.6% Marjoram essential oil exhibited a strong antimicrobial effect. According to Babacan et al. (2012), Marjoram extract possesses strong antibacterial effect on *Salmonella* serotypes.

Table 4. Time-dependent variation in inoculated *Salmonella Typhimurium* counts (0/25g Log cfu/g)

Samples	Initial microorganism load	0.Hour	1.Hour	2. Hour	3.Hour
Control	<1	6.55 ± 0.02^{Aa}	6.45 ± 0.03^{Aa}	6.44 ± 0.03^{Aa}	6.40 ± 0.02^{Aa}
Tarragon 1%	<1	6.52 ± 0.01^{Aa}	6.07 ± 0.04^{Aab}	4.92 ± 0.02^{Bb}	$4.14B\pm0.03^{bc}$
Tarragon 2%	<1	6.51 ± 0.02^{Aa}	5.49 ± 0.02^{Bb}	4.80 ± 0.05^{Cb}	3.85 ± 0.05^{Dc}
Marjoram 1%	<1	6.54 ± 0.03^{Aa}	6.44 ± 0.01^{Aa}	5.59 ± 0.01^{Bab}	5.11 ± 0.06^{Bb}
Marjoram 2%	<1	6.52 ± 0.01^{Aa}	6.27 ± 0.03^{ABab}	5.27 ± 0.04^{Bab}	4.78 ± 0.02^{Cbc}

A-D (→): Values shown with different letters on the same line differ from each other at the $p<0.05$ level
a-c (↓) : Values shown with different letters on the same column differ from each other at the $p<0.05$ level

The count of *Listeria monocytogenes*

It was judged that the initial *L. monocytogenes* count in hamburger patties produced by adding Tarrgon and Marjoram extracts at differing ratios rank between 6.55 and 6.52 log cfu/g (Table 5; $P>0.05$). During the three-hour study period, the count of *L. monocytogenes* bacteria decreased in all samples. Whereas, this decrease was found to be statistically significant in the other samples except for the control sample ($P<0.05$). In addition, it was decided that the decrease in the count of bacteria occurring in the same time period in hamburger patties inoculated with *L. monocytogenes* bacteria was also statistically significant in all other time periods except the first hour ($P<0.05$). It was evaluated that the greatest decrease occurred in the samples was in the samples produced by adding 2% Tarragon extract at a rate of 3.38 log cfu/g. ($P<0.05$). It was considered that this sample was followed by the samples produced by adding 1% *A. dracunculus* L. (Tarragon) extract at a rate of 2.59 log cfu/g, the samples produced by adding 2% *O. majorana* L. (Marjoram) extract at a rate of 1.44 log cfu/g, and by the samples produced by adding 1% Marjoram extract, respectively (Table 5).

In a study conducted by Ting and Deibel (1992), which examined the impact of various spices on the reproduction of *L. monocytegenes*, it came to light that the *O. majorana* L. plant is successful. Dadalıoğlu and Evrendilek (2004) investigated the antimicrobial effect of Marjoram on food-borne pathogenic bacteria such as *Salmonella* subsp., *E. coli, S. aureus* and *L. monocytogenes*. According to the study results, Marjoram is an effective antibacterial. According to Al-Joboury (2015), *S. aureus, S. Typhimurium, E. coli, B. abortus*, and *L. monocytogenes* are just a few of the foodborne pathogens that Marjoram extract is effective against. What is more, Ökmen et al. (2017) reported that *O. majorana* L. extract has a strong antibacterial effect on *L. monocytogenes*. The results obtained from similar studies on the subject show parallelism with the data obtained in this study.

The antibacterial effect exerted by the extracts of Tarragon and Marjoram is likely due to the reaction of these components with sulfhydryl groups or as a result of enzyme inhibition of oxidized compounds through more nonspecific interactions with proteins. Consequently, it damages the integrity of the cell wall by affecting pH homeostasis and the balance of inorganic ions (Lambert et al. 2001).

There are a couple of exceptions, yet studies have demonstrated that Gr (+) bacteria are more susceptible to plant extracts than Gr (-) bacteria (Burt 2004; Kalemba and Kunicka 2003). In addition to the cell wall elements present in gram negative bacteria, the inclusion of an extra layer of lipopolysaccharide may result in the formation of an intact plasma membrane that has a higher capacity for buffering and hydrophobicity around the cell wall. As a result, it can prevent simple phenolic compounds from acting in a way that makes bacteria more resistant to flavonoids and polyphenols (Yashaswini and Arvind 2018; Du et al. 2011). These assertions are supported by the findings of this investigation.

Table 5. Time-dependent variation in inoculated *Listeria monocytogenes* counts (0/25g Log cfu/g)

Samples	Initial microorganism load	0.Hour	1.Hour	2. Hour	3.Hour
Control	<1	6.55 ± 0.02^{Aa}	6.50 ± 0.02^{Aa}	6.50 ± 0.03^{Aa}	6.15 ± 0.01^{Aa}
Tarragon 1%	<1	6.52 ± 0.03^{Aa}	5.61 ± 0.04^{ABb}	4.74 ± 0.06^{Bb}	3.93 ± 0.02^{Cbc}
Tarragon 2%	<1	6.55 ± 0.03^{Aa}	5.30 ± 0.06^{ABb}	3.99 ± 0.08^{Bc}	3.17 ± 0.05^{Bc}
Marjoram 1%	<1	6.55 ± 0.04^{Aa}	6.41 ± 0.07^{Aa}	6.17 ± 0.04^{ABab}	5.79 ± 0.03^{Bab}
Marjoram 2%	<1	6.52 ± 0.02^{Aa}	6.11 ± 0.05^{ABab}	5.79 ± 0.03^{Bab}	5.08 ± 0.02^{Bb}

A-C (→): Values shown with different letters on the same line differ from each other at the $p<0.05$ level
a-c (↓) : Values shown with different letters on the same column differ from each other at the $p<0.05$ level

CONCLUSION

The antimicrobial effects of *O. majorana* L. (Marjoram) and *A. dracunculus* L. (Tarragon) extracts added to hamburger patties on four different foodborne pathogenic bacteria, Gr (+) (*S. aureus, L. monocytegenes*) ve Gr (-) (*S. Typhimurium, E. coli*), were investigated in this research. As a result, it was concluded that Marjoram and Tarragon extracts exhibited strong antibacterial effects on inoculated Gr (+) and Gr (-) bacteria and caused inhibition at different rates. It was ended that the antibacterial effect revealed by the study was higher on Gr (+) bacteria compared to Gr (-) bacteria, and it was identified that 2% Tarragon extract supplied the highest antibacterial effect on *L. monocytogenes* with a decrease of 3.38 log cfu/g. It was ascertained that the antibacterial effect enhanced concurrently with the application of more extracts at a particular concentration level. In addition, it has been uncovered that compared to the Marjoram extract, the antibacterial effect of Tarragon extract is higher and more effective.

Consumer preferences have shifted as a consequence of studies revealing that synthetic preservatives used in food manufacturing around the world cause serious medical conditions, particularly cancer. Manufacturers were forced to concentrate on products generated with more natural ingredients as a result. Furthermore, there have been more studies on spices as a result of the identification of the protective implications of spices in foods by demonstrating antimicrobial activity.

The necessity of employing spices in foods becomes evident when taking into account the adverse effects of chemical and synthetic preservatives employed in the food business on the human body. Spices are capable of being used in place of non-organic preservatives in foodstuffs. In this way, it has been demonstrated by the studies that the consumption of organic products can be increased and the public health can be increased.

Consequently, the results of this study demonstrated that the application of Marjoram and particularly Tarragon plants can enhance the microbiological quality of hamburger patties, which are popular both internationally and in Turkey. It is believed that by using this technique, food poisoning that can arise from consumption of these patties can be decreased while the safety of hamburger patties, which present a risk to the public's health, can be organically increased.

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REFERENCES

Akarca, G., Sevik, R. (2022). Biological Activities of *Citrus limon* L. and *Citrus sinensis* L. Peel Essential Oils. J Essent Oil Bear Plant., 24(6): 1415-1427.

Akarca, G., Tomar, O., Güney, İ., Erdur, S., & Gök, V. (2019). Determination of sensivity of some food pathogens to spice extract. J Food Sci. Technol., 56(12): 5253-5261.

Al-Joboury, & Th. MA. (2015). Effect of crude extract of Origanum vulgare on the inhibition of some pathogenic bacteria and causing spoilage of food. Al-Anbar J. Vet. Sci., 8(2): 6527-28.

Anonymous. (2002). Turkish Standards Institute meatball and hamburger patty standard, TS 1058.0 Ankara, Türkiye.

Bonyadian M., & Moshtaghi H. (2008). Bactericidal activity of essential oils of some plants against *Bacillus cereus, Salmonella typhimurium, Listeria monocytogenes* and *Yersinia enterocolitica*. Res J Microbiol., 3: 648-653.

Burt S. (2004). Essential oils: Their antibacterial properties and potential applications in foods A review. Int. J. Food Microbiol, 94: 223-253.

Busatta C., Vidal RS., Popiolski AS., Mossi AJ., Dariva C., Rodrigues MR., Corazza FC., Corazza ML., Vladimir Oliverira J. & Cansian RL. (2008). Application of 81 *Origanum majorana* L. essential oil as an antimicrobial agent in sausage. Food Microbiol., 25(1): 201–211.

Chaudhry, NMA., Saeed, S., & Tarıq, P. (2007). Antibacterial effects of oregano (Origanum Vulgare) against gram negative bacili. Pak. J. Bot., 39: 609-613.

Cowan, MM. (1999). Plant products as antimicrobial agents. Clinical Microbiol. Rev., 12: 564−568.

Dadalıoğlu I., & Evrendilek G. (2004). Chemical compositions and antibacterial effects of essential oils of Turkish Oregano (*Origanum minutiflorum*), Bay Laurel (*Laurus nobilis*), Spanish Lavender (*Lavandula Stoechas* L.) and Fennel (*Foneniculum vulgare*) on common foodborne pathogens. J. Agric. Food Chem., 52: 8255-8260

Du, WX., Olsen, CW., Avena-Bustillos, RJ., Friedman, M., & Mc. Hugh, TH. (2011). Physical and antibacterial properties of edible films formulated with apple skin polyphenols. J Food Sci., 76(2): 149-155.

Ehrich, J., Baurmann, U. & Thomann,R. (1995). Antimicrobial effect of CO spice expacts from summer savory to cinnamon .Lebensmitteltechnik, 27(11): 51-53.

Govaris, A., Solomakos, N., Pexara, A., & Chatzopoulou PS. (2010). The Antimicrobial effect of oregano essential oil, nisin and their combination against *Salmonella Enteritidis* in minced sheep meat during refrigerated storage. Int. J. of Food Microbiol.,137: 175-180.

Halkman, K. (2005). Food Microbiology Applications. Başak Printing and Promotional Services Ankara-Turkiye.

İlhan, E. (2010). Effect of Rosemary extract utilization on storage stability of hamburger patties formulated with different beef trimming ratios. Msc. Thesis. Ankara University , Ankara, Turkiye.

ISO. (1999). International Standard Organization, 6888-1 Horizontal Method for the Enumeration of Coagulase-positive Staphylococci Technique using Baird Parker Agar Medium. Geneva, Switzerland.

ISO. (2001a). International Standard Organization. 16649-1:2001 Mirobiology of food and animal feeding stuffs–Hortizonal method for the enumeration of beta-glucuronidasepositive *Escherichia coli*- Part 1: Colony count technique at 44 degrees C using membranes and 5-bromo- 4-chloro-3-indolyl beta–D-glucuronide. Geneva, Switzerland.

ISO. (2001b). International Standard Organization. 16649-2:2001 Microbiology of food and animal feeding stuffs – Hortizonal method for the enumeration of beta-glucuronidasepositive Escherichia coli- Part 2: Colony count technique at 44 degrees C using 5- bromo-4-chloro-3-indolyl beta – Dglucuronide. Geneva, Switzerland

ISO. (2017a). International Standard Organization, 11290-1:2017 Microbiology of the food chain, Horizontal method for the detection and enumeration of *Listeria monocytogenes* and of *Listeria* spp. - Part 1: Detection method. Geneva, Switzerland.

ISO. (2017b). International Standard Organization. 11290-2:2017 Microbiology of the food chain, Horizontal method for the detection and enumeration of *Listeria monocytogenes*. Geneva, Switzerland

ISO. (2017c). International Standard Organization, 6579-1:2017 Horizontal method for the detection, enumeration and serotyping of *Salmonella*. Geneva, Switzerland

Kahraman A. (2017) Examination of some food pathogens' reproductive and survival abilities at feta cheese which had been added Marjoram and Cinnamon. Msc. Thesis. Afyon Kocatepe University. Afyonkarahisar, Turkiye.

Kalemba, DAAK., & Kunicka, A. (2003). Antibacterial and antifungal properties of essential oils. Curr Med Chem., 10(10): 813-829.

Lacroix, M., Saucier, L., Caillet, S. & Qussalah. (2006). Inhibitory effects of selected plant essential oils on the growth of four pathogenic bacteria: *E.coli* O157:H7, *Salmonella typhimurium, Staphylococcus aureus* and *Listeria monocytogenes*. Food Cont., 18(5): 414-420

Lambert, RJW., Skandamis, PN., Coote, PJ., & Nychas, GJE. (2001). A study of theminimum inhibitory concentration and mode of action of oregano essential oil,thymol and carvacrol. J Appl. Microbiol., 91: 453−462.

Martins, IM., Kabuki, DY., & Kuaye, Y. (2009). Determination and characteriztion of pathogens found in dairy products. Revista do Instituto de Medicina Tropical de Adolfo Lutz, 68(3): 359-365.

Ökmen G., Arslan A., Vurkun M., & Ceylan O. (2017). Antimicrobial and antioxidant activities of different spices. Kötekli, Electronic Microbiology Journal, 15(1): 16-28.

Özcan, M., & Erkmen, O. (2001). Antimicrobial activity of the essential oils of Turkish plant spices. Eur. Food Res. Technol., 212: 658-660.

Pişkin Ç. (2007). The determination of antimicrobial effects of some spices plants of *Lamiaceae*. MSc. Thesis. Secuk University, Konya, Turkiye.

Sağdıç, O. (2003). Sensitivity of four patogenic bacteria to Turkish Thyme and Oregano hydrosols. Lebensm.-Wiss.u.-Technol., 36:467-473.

Shan, B., Cai, Y., Brooks, JD. & Corke, H. (2007). The ın vitro antibacterial activity of dietary spice and medicinal herb extracts. Int. J Food Microbiol., 117: 112-119.

Ting, WTE., & Deibel, KE. (1992). Sensitivity of *Listeria monocytogenes* to spices at two temperatures. J. Food Safety., 12(2): 129-137.

Turhan D. (2015). Investigation of the antimicrobial effect of some essential oils against Staphylococcus aureus and Escherichia coli. Msc. Thesis. Istanbul Technical University. Istanbul, Turkiye.

Yashaswini, P., & Arvind. (2018). Antimicrobial properties of orange (*Citrus reticulata* var. *kinnow*) peel extracts against pathogenic bacteria. Int J Curr Microbiol App Sci., 7(3): 737-746.