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ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Effects of Nettle (*Urtica dioica*) Extract on Versus Pathogenic Microorganisms in Yogurt Production

Yoğurt Üretiminde Isırgan Otu (*Urtica dioica*) Ekstraktının Patojenik Mikroorganizmalara Karşı Etkileri

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Abstract

Contamination with pathogenic bacteria is the most common cause of foodborne illness and represents a public health problem worldwide. These pathogens can be controlled by adding extracts to fermented milk. In the present study, the effects of nettle extract on selected pathogenic bacteria in yogurt production were investigated. For this purpose, the antibacterial effectiveness of the extract additives in the presence of bacteria was examined and compared. Yoghurt samples, 24 different samples contaminated with 7 standard pathogen strains, were analyzed for chemical properties (pH and acidity) and antimicrobial activity after 1, 7, 14 and 21 days of storage at 4 °C. In particular, it was found that the decrease in pH and increase in acidity in the nettle extract samples after 21 days were significantly greater than in natural yogurt and yogurt samples containing nettle extract (0.5% and 1%, respectively) ($p<0.05$). Pathogens were reduced more significantly in samples treated with nettle extracts, which have antibacterial activity against pathogens. In particular, compared to natural yogurt, these samples showed significant antibacterial effects on the standard strains *Streptococcus pneumoniae* ATCC 45615, *Klebsiella pneumoniae* ATCC 70063 and *Pseudomonas aeruginosa* ATCC 27853 ($p<0.01$). Furthermore, in the samples containing nettle extract, the 1% concentration had synergistic effects on *Streptococcus pneumoniae* ATCC 45615, while the 0.5% concentration had synergistic effects on *Klebsiella pneumoniae* ATCC 70063 and *Pseudomonas aeruginosa* ATCC 27853. Overall, these results suggest that the nettle extract could be used as a natural preservative to improve the safety of yogurt and other dairy products.

Keywords: Antimicrobial activity, Physicochemical properties, Nettle extract, Yogurt

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Öz

Patojenik bakteri kontaminasyonu, küresel bir halk sağlığı sorunu olan gıda kaynaklı hastalıkların önde gelen nedenleri arasında yer almaktadır. Bu patojen mikroorganizmalar fermente süt üretiminde kullanılan ısırgan otu ekstraktları ile kontrol altına alınabilir. Bu çalışmada ısırgan otu (*Urtica dioica*) ekstraktının yoğurt üretiminde seçilmiş patojen bakteriler üzerindeki etkileri değerlendirilmiştir. Bu amaçla bakterilerin bulunduğu ortamda ekstrakt takviyelerinin antibakteriyel etkinliği araştırılmış ve karşılaştırmaları yapılmıştır. 24 farklı yoğurt örneği 7 standart patojen suşla kontamine edildikten sonra kimyasal özellikleri (pH ve asitlik) ile antimikrobiyal aktiviteleri açısından 4ºC depolama sıcaklığında ve 1., 7., 14. ve 21. depolama günlerinde analiz edilmiştir. Özellikle ısırgan otu ekstraktlı örneklerde pH değerlerinde azalma ve asitlik değerlerindeki artışın, kontrol yoğurdu ile ısırgan otu ekstraktı içeren (%0.5 ve %1) örnekler arasındaki karşılaştırmaya göre 21 gün boyunca istatistiksel olarak daha anlamlı olduğu belirlenmiştir ($p<0.05$). Patojenlere karşı antibakteriyel aktiviteye sahip olan ısırgan otu ekstraktları ile muamele edilen örneklerde patojenlerin daha belirgin şekilde azaldığı saptanmıştır. Özellikle bu örneklerden *Streptococcus pneumoniae* ATCC 45615, *Klebsiella pneumoniae* ATCC 70063 ve *Pseudomonas aeruginosa* ATCC 27853 standart suşları ile hazırlananların kontrol yoğurtla karşılaştırıldığında anlamlı antibakteriyel etki gösterdiği tespit edilmiştir ($p<0.01$). Ayrıca ısırgan otu ekstraktı içeren örnekler arasında %1'lik konsantrasyon *Streptococcus pneumoniae* ATCC 45615'e karşı daha iyi bir etki gösterirken, %0.5'lik konsantrasyon hem *Klebsiella pneumoniae* ATCC 70063 hem de *Pseudomonas aeruginosa* ATCC 27853'e karşı daha fazla etki sağladığı belirlenmiştir. Genel olarak araştırma bulguları ısırgan otu ekstraktının yoğurt ve diğer süt ürünlerinin güvenliğini arttırmak için doğal bir koruyucu madde olarak kullanılabileceğini göstermiştir.

Anahtar Kelimeler: Antimikrobiyal aktivite, Fizikokimyasal özellikler, Isırgan otu ekstraktı, Yoğurt

1. Introduction

Yogurt is a popular fermented dairy product known for its nutritional value, digestibility, and health benefits (Nguyen et al., 2017; Demirci and Gürbüz, 2023). For several years, yogurt has been enriched with certain vegetable and fruit preparations as well as some plant extracts (Hussein et al., 2011; Balthazar et al., 2015; Gahruie et al., 2015; Oliveira et al., 2015; Parsa et al., 2015; Bansal et al., 2016; Kiros et al., 2016). Moreover, according to related research, eating yogurt represents a good way to ensure the appropriate daily intake of some bioactive compounds known to prevent diseases and exert positive health benefits (Hashemi et al., 2016). Historically, humans have utilized medicinal herbs to heal various ailments in accordance with traditional practices. In fact, despite advances in modern treatment protocols, some bioactive compounds are still used for supportive therapy. In this regard, prior studies have revealed the critical role of many plant-derived substances in alleviating pain (Calixto et al., 2000; Dhouibi et al., 2020). For instance, *Urtica dioica* L. (Urticaceae), a medicinal herb commonly known as nettle or soi' in Kashmiri, which grows around the geographical coordinates of 34° 02' N-75° 20' E at 1075.5 mm annual precipitation and 2400 m above sea level, and is used for treating allergies, kidney stones, burns, anaemia, rash, internal bleeding, diabetes, etc. In addition, previous studies have shown that the substances found in nettle exhibit anticarcinogenic, anti-inflammatory, antiviral, and antioxidant effects, with flavonoid glycosides also serving to strengthen the immune system (Singh et al., 2012).

Due to being rich in various minerals, vitamins, ascorbic acids, essential amino acids, and oils, nettle can play a crucial role in human nutrition (Tekin, 2018). Indeed, studies have shown that *U. dioica* powder contains three times more protein than traditional grains such as rice, wheat, or barley (Rutto et al., 2013; Adhikari et al., 2016). Moreover, due to its high calcium (169 mg/100 g) and iron (277 mg/100 g) contents, *U. dioica* represents a good source of mineral components such as potassium, phosphorus, magnesium, sodium, and zinc. It also contains fewer carbohydrates (37.4%) than wheat and barley, which indicates that it has a lower glycaemic index when compared with certain other plant foods, such as cereals and potatoes (Adhikari et al., 2016; Esposito et al., 2019). In addition, nettle has been proven to exert antibacterial, antifungal, antiviral, and antioxidative effects (Gülçin et al., 2004; Jyoti et al., 2016). Furthermore, the phenolic compounds found in nettle extract can prevent food spoilage by inhibiting various microorganisms (Grauso et al., 2020). In this study, the action of mechanisms of nettle extract, which has strong antimicrobial activity, on yogurt samples contaminated with pathogenic microorganisms were investigated. Specifically, we analysed the antibacterial activity of different concentrations of *U. dioica* extract (0.5% and 1%) against *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 45615, *Klebsiella pneumoniae* ATCC 70063, *Escherichia coli* ATCC 25292, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076 on days 1., 7., 14., and 21. of storage.

2. Materials and Methods

2.1. Materials

The nettle herbs used in this study were collected from the village of Büyük Çakırman (formerly known as Vank) in Erzincan, Turkey, in May 2021. The yogurt was produced at the Balacan™ Milk and Dairy Products Factory in Erzincan.

2.2. Preparation of the Nettle Extract

The nettle samples were collected, washed, left to dry in the shade, and then ground into powder. The extract was prepared according to the method described by Flórez et al. (2022). Briefly put, the dried leaves were extracted using 95% ethanol (Merck, Darmstadt, Germany). The extract was then stored at 4°C for 2 days before being filtered through a 45 µm membrane filter (Pall Corporation, Puerto Rico). Finally, the extract was separated from the solvent using an evaporator and transferred into dark glass bottles for storage at -20°C until required for yogurt production.

2.3. Yogurt Preparation and Contamination

Cow's milk (14% dry matter, 3.8% fat, pH of 6.65) was used to produce the yogurt. The milk was pasteurized at 85°C for 30 minutes and then cooled to 45°C. Yogurt starter culture (Mayasan®, Istanbul, Turkey) was added at a 2% (h/h) concentration (Tamime and Robinson, 1985). Six different yogurt products were produced: control,

0.5% nettle extract, 1% nettle extract, control with a pathogen, 0.5% nettle extract with a pathogen, and 1% nettle extract with a pathogen. The sensory acceptability of concentrations of the nettle extracts used in yogurt has been previously reported (Yangılar and Gülhan, 2021). Specifically, sensory evaluations have shown that 1% and 2% nettle powder can be used in yogurt production, which informed the decision to use 0.5% and 1% concentrations, respectively, in the present study. The samples were all stored in a refrigerator at 4°C. *Table 1* below shows the yogurt sample groups and codes.

Table 1. The experimental yogurt formulations and codes

Yogurt code	Yogurt contain
C	Control
$C_{0.5}$	0.5% nettle extract
C_1	1% nettle extract
P_1C	Control + *Staphylococcus aureus* ATCC 29213
P_2C	Control + *Enterococcus faecalis* ATCC 29212
P_3C	Control + *Streptococcus pneumoniae* ATCC 45615
P_4C	Control + *Klebsiella pneumoniae* ATCC 70063
P_5C	Control + *Escherichia coli* ATCC 25292
P_6C	Control + *Pseudomonas aeruginosa* ATCC 27853
P_7C	Control + *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076
$P1_{0.5}$	0.5% nettle extract + *Staphylococcus aureus* ATCC 29213
$P2_{0.5}$	0.5% nettle extract + *Enterococcus faecalis* ATCC 29212
$P3_{0.5}$	0.5% nettle extract + *Streptococcus pneumoniae* ATCC 45615
$P4_{0.5}$	0.5% nettle extract + *Klebsiella pneumoniae* ATCC 70063
$P5_{0.5}$	0.5% nettle extract + *Escherichia coli* ATCC 25292
$P6_{0.5}$	0.5% nettle extract + *Pseudomonas aeruginosa* ATCC 27853
$P7_{0.5}$	0.5% nettle extract + *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076
$P1_1$	1% nettle extract + *Staphylococcus aureus* ATCC 29213
$P2_1$	1% nettle extract + *Enterococcus faecalis* ATCC 29212
$P3_1$	1% nettle extract + *Streptococcus pneumoniae* ATCC 45615
$P4_1$	1% nettle extract + *Klebsiella pneumoniae* ATCC 70063
$P5_1$	1% nettle extract + *Escherichia coli* ATCC 25292
$P6_1$	1% nettle extract + *Pseudomonas aeruginosa* ATCC 27853
$P7_1$	1% nettle extract + *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076

2.4. Microorganisms, Culture, Antibacterial Activity

The bacterial strains were obtained from the microbiology laboratory at Mengücek Gazi Training Hospital. We analysed the antimicrobial activity of the plain samples and those with nettle extract against *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212, *Streptococcus pneumoniae* ATCC 45615, *Klebsiella pneumoniae* ATCC 70063, *Escherichia coli* ATCC 25292, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076. We passaged the standard strains in blood culture media with 5% sheep blood (bioMerieux, France). We then inoculated brain heart infusion broth (bioMerieux, France) from fresh passages into these media and finished the preparation with a DensiCHEK™ Plus densitometer (bioMerieux, France) using the 0.5 McFarland standard (1.5×10^8 microorganisms cfu/mL). The standard solutions were prepared separately for each bacterium. We added 1 mL of each solution to the three sample groups with pathogens, with a starter culture. It was passaged 10 µL of all the yogurt samples into blood culture media with 5% sheep blood (bioMerieux, France) on days 1, 7, 14, and 21 and recorded the growth of the pathogenic microorganisms. The number of pathogenic microorganisms per millilitre was evaluated and recorded.

2.5. Physiochemical Analysis

The pH and titratable acidity of the yogurt samples were analysed on days 1, 7, 14, and 21. We measured the pH using a pH meter (Eutech PH 150 Model) (Eutech Instruments, Singapore) according to Method No. 981.12 of AOAC (Feldsine et al., 2002). Titratable acidity (lactic acid, %) was measured as described by Kurt et al. (2012).

2.6. Statistical Analysis

For the data analysis, we conducted one-way analysis of variance (ANOVA) using IBM SPSS Statistics 22.0 software (SPSS Inc., USA).

3. Results and Discussion

Gülhan and Yangılar (2022) in their study "Determination of the antibacterial activities of stinging nettle (*Urtica dioica*) ethanol extract at different bacterial concentrations", reported MIC and MBC values were determined for different concentrations of the extract obtained from the leaves of nettles, starting from 3 mg/mL, which were prepared with serial dilutions of $1.5x10^7$, $1.5x10^6$, $1.5x10^5$ and $1.5x10^4$ cfu/mL at four different concentrations for pathogen standard bacterial strains. Considering the positive effect of nettle extract on pathogenic microorganisms, this study was designed to determine the underlying mechanisms of action on yogurt pathogens, which are common. *Table 2* show the results of the pH and acidity values of the yogurt samples during storage, respectively. We found statistically significant changes in pH between the samples ($p<0.01$). The pH values of the study samples decreased throughout the storage period. Furthermore, the plain and nettle extract control samples had lower pH values, which decreased at higher concentrations. Here, we observed fluctuations in acidity based on the extract concentration and overall increase in acidity over longer storage periods. We determined no pathogenic microorganism growth in the plain or extract yogurt samples without pathogens. Figure 1 show the statistical analysis of the growth status of the microorganisms in the plain and extract yogurt samples contaminated with ATCC strains on days 1, 7, 14, and 21.

Table 2 pH and titratable acidity values of yogurt samples during storage

	pH				Titratable acidity (%)			
Samples	**1st day**	**7th day**	14**th day**	21**th day**	**1st day**	**7th day**	14**th day**	21**th day**
C	4.31±0.01^{bc,A}	4.20±0.00^{a,B}	4.16±0.00^{a,C}	4.08±0.00^{a,D}	1.17±0.00$^{a-e,B}$	1.28±0.00$^{a-c,A}$	1.31±0.00$^{c-l,A}$	1.35±0.00$^{b-g,A}$
$C_{0.5}$	4.32±0.00^{ab,A}	4.15±0.01^{b,B}	4.12±0.01^{b,C}	4.02±0.00^{b,D}	1.15±0.00$^{a-f,B}$	1.33±0.00^{a,A}	1.34±0.02$^{a-g,A}$	1.36±0.01$^{a-f,A}$
C_1	4.30±0.00^{bc,A}	4.13±0.01^{bc,B}	4.09±0.01^{e,C}	3.97±0.00^{e,D}	1.17±0.00$^{a-e,D}$	1.33±0.00^{a,C}	1.39±0.00$^{a-c,B}$	1.40±0.01^{ab,A}
P_1C	4.34±0.01^{ab,A}	4.13±0.01^{cd,B}	4.11±0.00^{c,C}	4.05±0.00^{c,D}	1.06±0.02$^{g-h,C}$	1.34±0.00$^{e-f,B}$	1.19±0.01^{m,B}	1.27±0.02^{l,A}
P_2C	4.29±0.01$^{b-d,A}$	4.11±0.01^{cd,B}	4.11±0.00^{c,B}	4.05±0.00^{c,C}	1.10±0.01$^{d-h,C}$	1.17±0.00$^{e-f,B}$	1.20±0.01$^{l-m,B}$	1.28±0.02$^{h-i,A}$
P_3C	4.36±0.01^{a,A}	4.12±0.01^{cd,B}	4.10±0.00^{d,C}	4.00±0.00^{d,D}	1.11±0.00$^{d-g,B}$	1.17±0.00$^{b-e,A}$	1.27±0.02$^{h-l[illegible]A}$	1.30±0.02$^{g-i,A}$
P_4C	4.26±0.01$^{c-e,A}$	4.09±0.01^{de,B}	4.04±0.00^{j,C}	3.97±0.00^{j,D}	1.02±0.02^{h,B}	1.24±0.01$^{b-e,A}$	1.24±0.06^{lm,A}	1.30±0.00$^{g-i,A}$
P_5C	4.22±0.01$^{e-g,A}$	4.10±0.00^{de,B}	4.06±0.00^{i,C}	3.99±0.00^{i,D}	1.14±0.01$^{b-g,B}$	1.28±0.02$^{a-c,A}$	1.31±0.04$^{d-h,A}$	1.33±0.02$^{d-h,A}$
P_6C	4.24±0.01$^{d-f,A}$	4.08±0.01$^{d-f,B}$	4.07±0.00^{h,C}	4.00±0.00^{h,D}	1.08±0.09$^{f-h,B}$	1.24±0.09$^{b-e,AB}$	1.30±0.00$^{f-i,A}$	1.32±0.02$^{f-i,A}$
P_7C	4.25±0.01$^{b-d,A}$	4.15±0.01^{b,B}	4.10±0.00^{d,C}	3.98±0.00^{d,D}	1.07±0.01$^{f-h,D}$	1.15±0.02^{f,C}	1.22±0.02$^{k-m,B}$	1.33±0.02$^{d-h,A}$
$P1_{0,5}$	4,16±0.00$^{h-i,A}$	4.06±0.00$^{f-h,B}$	4.16±0.00^{a,A}	3.95±0.00^{a,C}	1.23±0.02^{a,A}	1.29±0.00^{ab,B}	1.33±0.01$^{b-h,B}$	1.41±0.01^{a,A}
$P2_{0,5}$	4.14±0.01$^{h-j,A}$	4.04±0.01$^{g-j,C}$	4.07±0.00^{h,B}	3.93±0.00^{h,D}	1.19±0.01$^{a-c,D}$	1.24±0.00$^{b-d,C}$	1.29±0.01$^{f-j,B}$	1.34±0.01$^{c-g,A}$
$P3_{0,5}$	4.05±0.07^{l,A}	4.01±0.01^{kl,AB}	4.00±0.00^{m,AB}	3.91±0.00^{m,B}	1.15±0.02$^{a-f,B}$	1.29±0.00^{ab,A}	1.35±0.05$^{a-g,A}$	1.36±0.02$^{a-f,A}$
$P4_{0,5}$	4.07±0.07$^{k-l,A}$	4.00±0.01^{kl,B}	4.00±0.00^{m,B}	3.89±0.00^{m,C}	1.21±0.04^{ab,B}	1.30±0.00^{ab,A}	1.32±0.01$^{c-h,A}$	1.36±0.02$^{a-f,A}$
$P5_{0,5}$	4.06±0.01^{l,A}	3.98±0.01^{l,B}	3.98±0.00^{n,B}	3.86±0.00^{n,C}	1.19±0.02$^{a-c,C}$	1.32±0.01^{a,B}	1.38±0.01$^{a-d,A}$	1.40±0.01^{ab,A}
$P6_{0,5}$	4.16±0.01$^{h-i,A}$	4.01±0.01^{jk,B}	4.01±0.00^{l,A}	3.86±0.00^{l,A}	1.17±0.05$^{a-e,B}$	1.24±0.01$^{b-e,B}$	1.40±0.00^{ab,A}	1.41±0.02^{a,A}
$P7_{0,5}$	4.12±0.04$^{i-k,A}$	4.05±0.01^{fi,C}	4.09±0.00^{f,B}	3.91±0.00^{f,D}	1.11±0.02$^{c-g,B}$	1.33±0.02^{a,A}	1.40±0.04^{a,A}	1.39±0.01$^{a-c,A}$
$P1_1$	4.21±0.01$^{e-g,A}$	4.07±0.01$^{e-g,B}$	4.08±0.00^{g,B}	3.97±0.00^{g,C}	1.10±0.01$^{d-h,C}$	1.24±0.01$^{b-e,B}$	1.23±0.03^{lm,B}	1.32±0.02$^{c-i,A}$
$P1_2$	4.19±0.07$^{f-h,B}$	4.05±0.04$^{f-i,B}$	4.07±0.00^{h,B}	4.01±0.00^{h,A}	1.09±0.08$^{e-h,B}$	1.19±0.04$^{d-f,AB}$	1.24±0.01$^{i-m,A}$	1.32±0.02$^{f-i,A}$
$P1_3$	4.19±0.01$^{g-h,A}$	4.03±0.00$^{h-k,B}$	4.01±0.00^{m,BC}	3.92±0.00^{m,C}	1.14±0.01$^{b-g,C}$	1.23±0.02$^{b-e,B}$	1.28±0.01$^{g-k,AB}$	1.33±0.02$^{d-h,A}$
$P1_4$	4.09±0.01$^{j-l,A}$	4.02±0.00$^{j-k,B}$	3.97±0.00^{o,C}	3.90±0.00^{o,D}	1.15±0.02$^{a-f,C}$	1.22±0.00$^{c-e,B}$	1.20±0.01$^{l-m,B}$	1.31±0.02$^{f-i,A}$
$P1_5$	4.12±.0.01$^{i-k,A}$	4.03±0.00$^{i-k,B}$	4.03±0.00^{k,B}	3.88±0.00^{k,C}	1.18±00.1$^{a-d,B}$	1.31±0.00^{a,A}	1.34±0.01$^{a-g,A}$	1.38±0.01$^{a-d,A}$
$P1_6$	4.15±0.01$^{h-i,A}$	4.03±0.00$^{i-k,B}$	3.98±0.00^{n,C}	3.86±0.00^{n,D}	1.11±0.02$^{c-g,C}$	1.20±0.00$^{d-f,B}$	1.36±0.01$^{a-f,A}$	1.38±0.05$^{a-e,A}$
$P1_7$	4.26±0.01$^{b-d,A}$	4.00±0.00^{kl,C}	4.03±0.00^{k,B}	3.89±0.00^{k,D}	1.08±0.02$^{f-h,C}$	1.23±0.02$^{b-e,B}$	1.37±0.07$^{a-e,A}$	1.37±0.02$^{a-f,A}$

$^{a-o}$Means followed by different letters in the same column are significantly different (*$p<0.05$); $^{A-D}$Means followed by different capital letters in the same row are significantly different (*$p<0.05$). C: control; $C_{0.5}$: 0.5% nettle extract; C_1: 1% nettle extract; P_1C: *Control+Staphylococcus aureus ATCC 29213;* P_2C: Control+*Enterococcus faecalis* ATCC 29212; P_3C: Control+*Streptococcus pneumoniae* ATCC 45615; P_4C: Control+*Klebsiella pneumoniae* ATCC 70063; P_5C: Control+*Escherichia coli* ATCC 25292; P_6C: Control+*Pseudomonas aeruginosa* ATCC 27853; P_7C: Control+*Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076; $P1_{0.5}$: 0.5% nettle extract+*Staphylococcus aureus ATCC 29213;* $P2_{0.5}$: 0.5% nettle extract+*Enterococcus faecalis* ATCC 29212; $P3_{0.5}$: 0.5% nettle extract+*Streptococcus pneumoniae* ATCC 45615; $P4_{0.5}$: 0.5% nettle extract + *Klebsiella pneumoniae* ATCC 70063; $P5_{0.5}$: 0.5% nettle extract+*Escherichia coli* ATCC 25292; $P6_{0.5}$: 0.5% nettle extract+*Pseudomonas aeruginosa* ATCC 27853; $P7_{0.5}$: 0.5% nettle extract+*Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076; $P1_1$: 1% nettle extract+*Staphylococcus aureus ATCC 29213;* $P2_1$: 1% nettle extract+*Enterococcus faecalis* ATCC 29212; $P3_1$: 1% nettle extract+*Streptococcus pneumoniae* ATCC 45615; $P4_1$: 1% nettle extract+*Klebsiella pneumoniae* ATCC 70063; $P5_1$: 1% nettle extract+*Escherichia coli* ATCC 25292; $P6_1$: 1% nettle extract+*Pseudomonas aeruginosa* ATCC 27853; $P7_1$: 1% nettle extract+*Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076

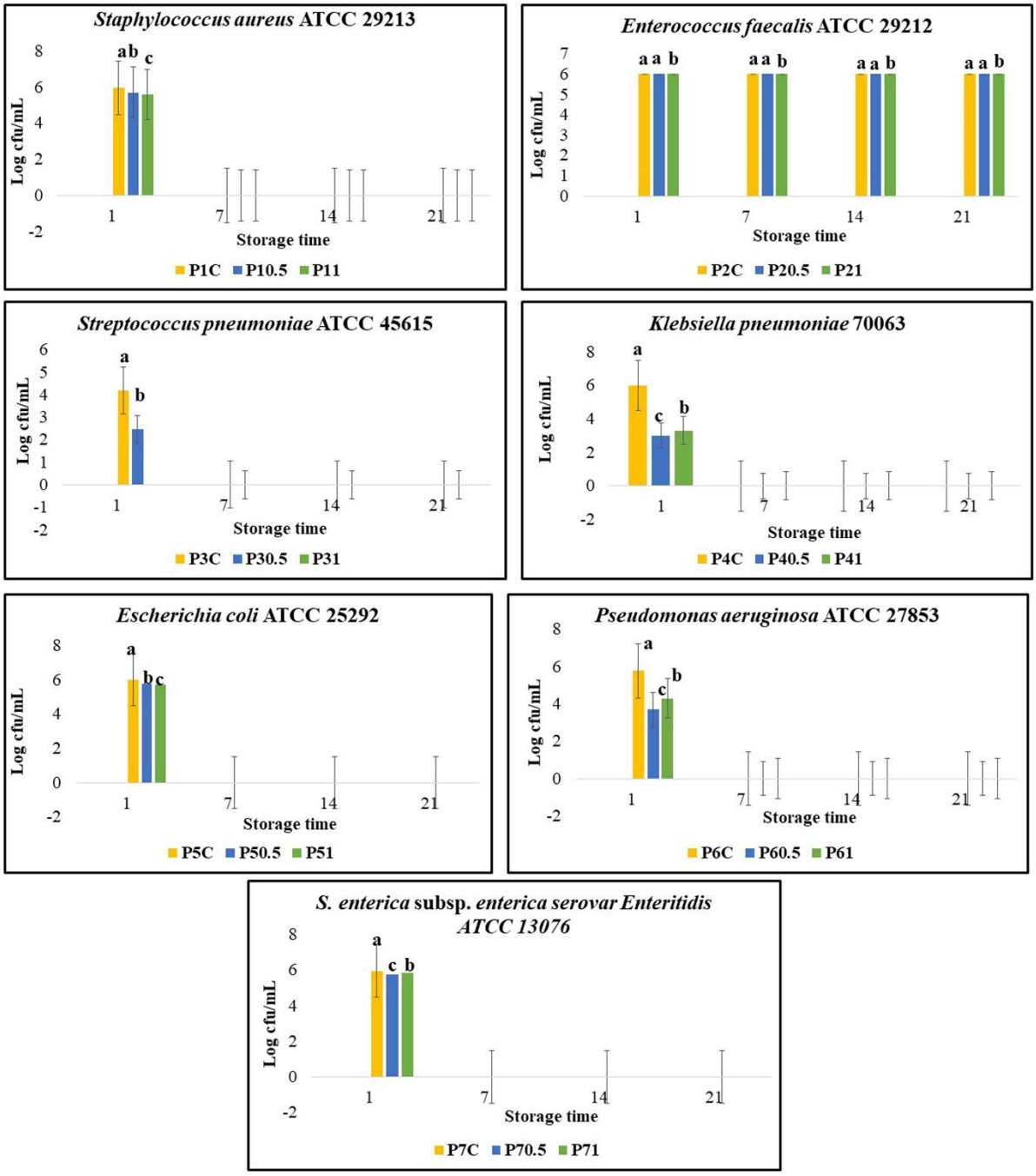

Figure 1. Antibacterial effects of the pathogen in control yogurt and supplemented with 0.5% or 1% nettle extract during storage.

P_1C: *Control+Staphylococcus aureus ATCC 29213;* P_2C: Control+*Enterococcus faecalis* ATCC 29212; P_3C: **Control+*Streptococcus pneumoniae*** ATCC 45615; P_4C: Control+*Klebsiella pneumoniae* ATCC 70063; P_5C: Control+*Escherichia coli* ATCC 25292; P_6C: Control+*Pseudomonas aeruginosa* ATCC 27853; P_7C: Control+*Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076; $P1_{0.5}$: 0.5% nettle extract+*Staphylococcus aureus ATCC 29213;* $P2_{0.5}$: 0.5% nettle extract+*Enterococcus faecalis* ATCC 29212; $P3_{0.5}$: 0.5% nettle extract+*Streptococcus pneumoniae* ATCC 45615; $P4_{0.5}$: 0.5% nettle extract+*Klebsiella pneumoniae* ATCC 70063; $P5_{0.5}$: 0.5% nettle extract+*Escherichia coli* ATCC 25292; $P6_{0.5}$: 0.5% nettle extract+*Pseudomonas aeruginosa* ATCC 27853; $P7_{0.5}$: 0.5% nettle extract+*Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076; $P1_1$: 1% nettle extract+*Staphylococcus aureus ATCC 29213;* $P2_1$: 1% nettle extract+*Enterococcus faecalis* ATCC 29212; $P3_1$: 1% nettle extract + *Streptococcus pneumoniae* ATCC 45615; $P4_1$: 1% nettle extract+*Klebsiella pneumoniae* ATCC 70063; $P5_1$: 1% nettle extract+*Escherichia coli* ATCC 25292; $P6_1$: 1% nettle extract+*Pseudomonas aeruginosa* ATCC 27853; $P7_1$: 1% nettle extract+*Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076

On day 1, for the control samples containing pathogenic microorganisms, we detected growths of 5.95, 6, 4.20, 6, 6, 5.77 and 5.95 log cfu/mL for *Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212,

Streptococcus pneumoniae ATCC 45615, *Klebsiella pneumoniae* ATCC 70063, *Escherichia coli* ATCC 25292, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC13076, respectively. On day 1 for the extract samples with pathogenic microorganisms, all the pathogenic microorganisms showed intense growth, except for *S. pneumoniae* in the 1% nettle extract sample. Additionally, *S. pneumoniae* showed less growth in the control samples than in the other microorganisms at 4.20 log cfu/mL.

For the 0.5% nettle extract samples with pathogens, decreased growth was observed for all the strains, except for *E. faecalis* ATCC 29212, compared to that of the control sample with pathogens. The lack of change in the growth rate of *E. faecalis* is attributed to the fact that this bacterium is particularly resistant to external environmental conditions. These samples showed 2.47, 3 and 3.69 log cfu/mL growths for *S. pneumoniae*, *K. pneumoniae*, and *P. aeruginosa*, respectively, with a statistically significant decrease in these strains ($p < 0.05$). The *S. aureus*, *E. coli*, and *S. enterica* strains also exhibited lower growth than the control sample, at 5.69, 5.77 and 5.77 log cfu/mL, respectively ($p<0.05$). For the 0.5% nettle extract samples, *Streptococcus pneumoniae* ATCC 45615 had the lowest growth at 2.47 log cfu/mL on day 1. The decrease in the number of bacteria will likely be reflected in the clinical results. We can state that yogurt ingredients with and without nettle will also cause a decrease in the number of pathogenic microorganisms that mix with our flora. Decreasing the amount of pathogenic bacteria will prevent or hinder the colonization of pathogenic bacteria. This may lead to control of the number of pathogenic microorganisms in yogurt samples and thus extend the shelf life of the product.

The antimicrobial effects of the various concentrations of the nettle extracts increased against some of the microorganisms, with the highest effect occurring at 1%. For this group, all the microorganisms, except for *E. faecalis* ATCC 29212, exhibited lower growth than the control group ($p<0.05$). Again, these samples demonstrated complete inhibition of growth for *S. pneumoniae*. A lower decrease in the growth of *K. pneumoniae* and *P. aeruginosa* compared to that in the 0.5% group was observed, at 3.30 and 4.30 log cfu/mL, respectively. For *S. aureus*, *E. coli*, and *S. enterica*, the growths were 5.60, 5.69, and 5.84 log cfu/mL, respectively, which were lower than those of the control group. However, compared to those in the 0.5% nettle extract group, the growth of *S. aureus* and *E. coli* was low, and the growth of *S. enterica* ATCC 13076, *P. aeruginosa* ATCC 27853, and *K. pneumoniae* ATCC 70063 was greater. On days 7, 14, and 21, the growth of all ATCC strains was inhibited, except for that of *E. faecalis*. Unlike the other strains, *E. faecalis* survived in all the yogurt samples. Significant results were found in terms of antibacterial effect on the 1st day of storage for each of the pathogenic microorganisms we used to contaminate the samples, but no growth was detected except *Enterococcus faecalis* ATCC 29212 on the 7th, 14th and 21st days of storage. The difference in statistical results may be due to this.

The results of the statistical analysis of the growth status of microorganisms in the passages of plain yogurt contaminated with ATCC strains and yogurts contaminated with extract on 1, 7, 14, and 21 are shown in Figure 1. According to the results obtained here, the yogurt samples with 0.5% and 1% nettle extract had fewer pathogen cells than the contaminated control samples during fermentation and storage. In our samples, the number of pathogenic bacteria in the study groups significantly decreased compared to that in the controls throughout storage, with or without the extract. The 1% extract sample had a better effect on the pathogens. Among the samples contaminated with *S. pneumoniae*, 4.15 log cfu/mL for the controls and 2.47 log cfu/mL for the 0.5% extract sample on day 1 were obtained. The antibacterial effects of the yogurt samples during storage are given in *Figure 2*.

The pH of the yogurt samples contaminated with pathogens fluctuated, while the pH of the contaminated samples treated with the nettle extract decreased with increasing concentration. All the contaminated groups, including the plain and extract samples (0.5 and 1%), had lower pH values than did the control samples. The researchers produced yogurts with various moringa extract concentrations (0.2%, 0.4%, and 0.8%) and examined their antimicrobial effects against *Staphylococcus aureus* ATCC 43300, *Bacillus subtilis* NCTC 3610, *Escherichia coli* ATCC 35150, *Saccharomyces cerevisiae*, and *Aspergillus fumigatus*. The authors reported a decreased pH across all samples, with a higher pH for controls than for samples treated with moringa extract (Saad and Elkhtab, 2019).

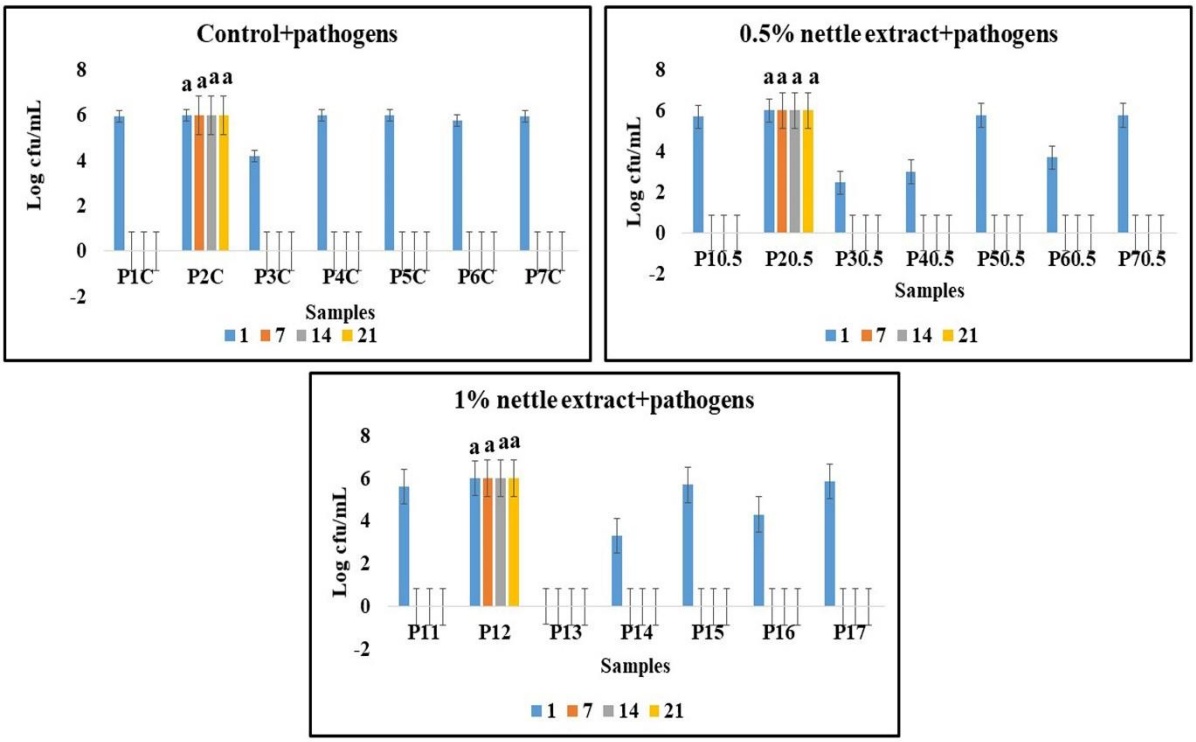

Figure 2. The antibacterial effects of yogurt samples among themselves during storage.

P_1C: *Control+Staphylococcus aureus ATCC 29213;* P_2C: Control+*Enterococcus faecalis* ATCC 29212; P_3C: Control+*Streptococcus pneumoniae* ATCC 45615; P_4C: Control+*Klebsiella pneumoniae* ATCC 70063; P_5C: Control+*Escherichia coli* ATCC 25292; P_6C: Control+*Pseudomonas aeruginosa* ATCC 27853; P_7C: Control + *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076; $P1_{0.5}$: 0.5% nettle extract+*Staphylococcus aureus ATCC 29213;* $P2_{0.5}$: 0.5% nettle extract+*Enterococcus faecalis* ATCC 29212; $P3_{0.5}$: 0.5% nettle extract+*Streptococcus pneumoniae* ATCC 45615; $P4_{0.5}$: 0.5% nettle extract+*Klebsiella pneumoniae* ATCC 70063; $P5_{0.5}$: 0.5% nettle extract+*Escherichia coli* ATCC 25292; $P6_{0.5}$: 0.5% nettle extract+*Pseudomonas aeruginosa* ATCC 27853; $P7_{0.5}$: 0.5% nettle extract+*Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076; $P1_1$: 1% nettle extract+*Staphylococcus aureus ATCC 29213;* $P2_1$: 1% nettle extract+*Enterococcus faecalis* ATCC 29212; $P3_1$: 1% nettle extract + *Streptococcus pneumoniae* ATCC 45615; $P4_1$: 1% nettle extract+*Klebsiella pneumoniae* ATCC 70063; $P5_1$: 1% nettle extract+*Escherichia coli* ATCC 25292; $P6_1$: 1% nettle extract+*Pseudomonas aeruginosa* ATCC 27853; $P7_1$: 1% nettle extract+*Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076

Acidity is a key factor for yogurt, as it affects shelf life and acceptability (Otaibi and Demerdash, 2008). During storage, the activity of lactic acid bacteria results in various metabolic activities and biochemical changes, which lead to residual lactic acid production from lactose and significantly increase acidity (Akgün et al., 2020). The researchers explored acidity in samples of dough, and a traditional Iranian yogurt beverage, and found the following trend for the acidity of increasing order: control group (from 1.80 to 2.60), doogh+5% nettle extract (from 1.77 to 2.45), doogh+10% nettle extract (from 1.72 to 2.35), doogh+5% nanoencapsulated nettle extract (from 1.70 to 2.30), and doogh+10% nanoencapsulated nettle extract (from 1.70 to 2.10). Like in our study, the authors also found higher acidity and lower pH in the control samples than in the extract samples (Amiri et al., 2021).

In parallel with our study, other researchers have investigated the antimicrobial effects of different extracts on yogurt samples. In another study, *Citrus aurantium* L. flower (*Bahar narang* extracts: 500, 1000, and 2000 ppm) was used to produce yogurt, after which its antimicrobial properties were analysed in vitro. The authors reported strong antimicrobial effects against *Escherichia coli* O157:H7, *P. aeruginosa*, *S. aureus*, and *B. cereus*, with the highest effect occurring at a concentration of 2.000 ppm (Hashemi et al., 2016). Ertürk and Demirkol (2014) reported that yogurt samples with *Urtica dioica* and *Camellia sinensis* extracts had the greatest antibacterial effects on *P. aeruginosa* and *E. coli* (Ertürk and Demirkol, 2014).

El-Gammal et al. (2017) investigated yogurt samples contaminated with *Moringa oleifera* extract (0.1, 0.2, 0.3, and 0.4%) for the treatment of gram-positive bacteria, such as *S. aureus*, *E. faecalis*, and *B. cereus*. The authors highlighted the insufficient antimicrobial effect of aqueous moringa extract against gram-negative bacteria (*E. coli* and *S. typhimurium*) (El-Gammal et al., 2017). Ahmed et al. (2014) explored the vitality of *E. coli* and *L.*

monocytogenes during storage in yogurt samples. They reported titratable acidity values of 0.9% and 1.36%, and these pathogens were completely unobserved on days 9 and 12. The authors associated this inhibition with the high acidity of yogurt (Ahmed et al., 2014). Other researchers studied the genus *Escherichia* and found that pathogenic *E. coli* organisms were significantly more acid-tolerant than non-pathogenic strains (Gorden and Small, 1993; Arnold and Kaspar, 1995; Massa et al., 1997). Here, we observed that the vitality of the microorganisms decreased with increasing acidity. However, the *Enterococcus faecalis* ATCC 29212 strain maintained its vitality. Determination of coliform bacteria is a standard test that is required by the International Dairy Federation (Mossel *et al.*, 1995). Therefore, the ability of *E. coli* O157:H7 to survive in highly acidic foods is a crucial issue for public health (Massa et al., 1997).

Eom et al. (2017) investigated the antimicrobial effects of Panax ginseng marc extract (0.5% and 1.0%) against three gram-positive and on three gram-negative strains of yogurt. The 1% extract sample was found to be more effective than the controls against *S. aureus* 1573, *B. cereus* KCCM 11341, *L. monocytogenes* H7962, *E. coli* O157:H4 FRIK 125, and *E. sakazakii* ATCC 51329, but not against *S. typhimurium* 15. They found that *B. cereus* KCCM 11341 and *E. sakazakii* ATCC 51329 were most susceptible to the 1% ginseng marc extract on day 1. The authors reported increased antibacterial activity at higher concentrations, similar to our findings (Eom et al., 2017).

For the plain, 0.5% extract, and 1% extract samples on day 1, *S. aureus* had growth values of 5.95, 5.69, and 5.60 log cfu/mL, respectively. The researchers analysed the antimicrobial effects of yogurt samples containing *Moringa oleifera* leaf extracts (0.2, 0.4, and 0.8%) on against *Staphylococcus aureus* ATCC 43300, *Bacillus subtilis* NCTC 3610, *Escherichia coli* ATCC 35150, *Saccharomyces cerevisiae*, and *Aspergillus fumigatus*. The authors found the maximum activity against all tested microorganisms for the 0.8% extract sample (Saad and Elkhtab, 2019). Kalpana et al. reported a stronger antimicrobial effect against *S. aureus* for *Moringa oleifera* leaf extracts (Kalpana et al., 2013). Yogurt samples containing different extracts (*Urtica diocia*, *Laurus nobilis*, *Nigella sativa*, and *Camellia sinensis*) were produced and increased pH values were found. The authors also highlighted that the *C. sinensis* extract had a weak antibacterial effect against *S. aureus*, while the other extracts had no antibacterial effect (Ertürk and Demirkol, 2014).

For the 1% extract sample, *Streptococcus pneumoniae* showed no growth on day 1. Our findings demonstrated a higher antibacterial effect at greater extract concentrations. The researchers studied the effects of aqueous aloe vera gel extract (5% and 10%) or *L. casei* on *E. coli* in yogurt samples and reported a significant decrease in the number of pathogenic bacteria compared with that in the controls ($p<0.05$). The number of *E. coli* was significantly lower in the extract samples and probiotic yogurt than in the control group. The author also noted that, for the extract samples, the presence of probiotic bacteria had no significant effect on *E. coli* ($p>0.05$) (Niko et al., 2016).

4. Conclusions

Our findings revealed that the 1% nettle extract was more effective against *Staphylococcus aureus* ATCC 29213, *Streptococcus pneumoniae* ATCC 45615, and *Escherichia coli* ATCC 25292, but not against *Enterococcus faecalis* ATCC 29212. On the other hand, the 0.5% nettle extract had synergistic effects on *Klebsiella pneumoniae* ATCC 70063, *Pseudomonas aeruginosa* ATCC 27853, and *Salmonella enterica* subsp. *enterica* serovar Enteritidis ATCC 13076. Nettle is an easily available, low-cost herb that contains bioactive substances with significant antimicrobial properties. Considering this, nettle can have a critical impact on preserving valuable fermented products, such as yogurt. This strain shows great promise for industrial use, although further research into *Enterococcus faecalis* ATCC 29212 is still needed, particularly for acidic foods. The antibacterial effects of nettle extracts have been well-studied, but data on yogurt production generated from these extracts are scarce. Thus, our research reserves originality in this regard and should shed light on future research on this subject.

Limitations of The Study

The study was conducted on many yogurt samples due to the high number of pathogenic bacteria studied: yogurt without nettle additives and yogurt with 0.5% and 1% nettle, the results were analysed on days 1, 7, 14, and 21. Therefore, injections at different concentrations could not be performed. This situation constitutes the limiting aspect of our study.

Ethical Statement

There is no need to obtain permission from the ethics committee for this study

Conflicts of Interest

The authors declares that they have no conflict of interest

Authorship Contribution Statement

Concept; Design; Data Collection or Processing; Statistical Analyses; Literature Search; Writing, Review and Editing: Yangılar, F. and Gülhan B.

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