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Antimicrobial Activity of Pomegranate Peel and Its Applications on Food Preservation

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ABSTRACT

Multiplex PCR has shown to be a useful technique for identifying Listeria monocytogenes and Staphylococcus aureus in food items. It can also be used to trace toxins to enhance food safety. This study identifies *Listeria monocytogenes* and Staphylococcus aureus in processed beef meat products using multiplex PCR. These bacteria were found in the beef meat samples using the *16SrRNA* gene of *L. monocytogenes* and the *23S rRNA* gene of *S. aureus.* For this study, 40 processed meat samples—ten samples of each minced meat, beef sausage, beef burger, and beef kofta—were gathered from different supermarkets in the El-Menoufia governorate. One important byproduct produced during the culinary processing of pomegranates is the peels from the fruits. Due to their plentiful presence of broad-spectrum antibacterial agents and antioxidants, these peels can effectively prevent food spoilage. The primary objective of the study was to determine whether pomegranate peel extract had any antimicrobial effects on *Listeria monocytogenes* and *Staphylococcus aureus* in minced beef. Also, the antioxidant and antibacterial properties of a hydromethanolic extract from sweet orange (Citrus sinensis) peel were examined. The results indicated that an extract from the peel of the Citrus sinensis tree has antibacterial effects against *S. aureus* and *Listeria monocytogenes*.

1-INTRODUCTION

Staphylococcal enterotoxins (SEs) share structural and biological traits with members of the pyrogenic toxin super-antigen family. Food poisoning can be significantly caused by them (Larkin et al., 2009). Pomegranate has a broad spectrum of antimicrobial activity, demonstrating inhibitory qualities against molds, fungi, and both Gram-positive and Gram-negative bacteria. However, the antibacterial properties of extracts made from various pomegranate plant sections differ. PPE's antibacterial effectiveness is linked to the level of total tannins and flavonoids. PPE is well known for its antibacterial properties against infections, both bacterial and fungal (Ismail et al., 2016).

One important byproduct produced during the culinary processing of pomegranates is the peels from the fruits. These peels work well to keep food from spoiling since they are high in antioxidants and broad-spectrum antibacterial agents. Pomegranate peels' antibacterial qualities and ability to preserve food are explored in detail to provide a full manual for food processors, farmers, storage companies, and academics. Pomegranate peel extracts have been demonstrated in earlier studies to exhibit antibacterial properties against a variety of foodborne pathogens, including *Bacillus subtilis* and *Escherichia coli* (El-Sherbiny et al., 2016).

The antibacterial activity of pomegranate peel, seeds, juice, and whole fruits was investigated in a comprehensive study against seven different microorganisms. According to a study conducted by

Dahlan et al. (2010), the peel extracts exhibited the most potent inhibitory effect against the tested bacteria, including *Bacillus coagulans, Bacillus cereus, B. subtilis, Staphylococcus aureus, E. coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa.*

Citrus fruits are widely cultivated across the globe and are considered a vital commercial fruit crop. Antioxidants play a crucial role in preventing or slowing down oxidative damage in our bodies by combating free radicals. There is an increasing interest in investigating the natural antioxidants and radical scavengers that are present in plant materials that are high in polyphenolic compounds. There are several beneficial effects on human health caused by polyphenols, which are mostly attributed to their exceptional antioxidant and free radical scavenging capabilities. A popular ingredient in many beauty products, citrus fruit essential oil is renowned for its antibacterial properties (Caccioni, 1998).

Microorganisms, including bacteria, fungi, and protozoa, can be either eliminated or halted by antimicrobial compounds. They may either be microstates, which prevent the growth of pathogens, or microbicidal, which eliminates microbes. However, disinfectants are antimicrobial agents that are applied to inanimate objects. Citrus fruits and their products are famously antimicrobial due to the large levels of flavanones and polyethoxylated flavones found in them; these compounds are rare in other plant foods (Ahmed, 2006).

The field of infectious disease diagnosis has been revolutionized by PCR. To address the cost and diagnostic capacity limitations of the test, multiplex PCR has been developed. This variant allows for the amplification of more than one target sequence using multiple pairs of primers in a single reaction tube, enabling the detection of viral, bacterial, and other infectious agents simultaneously. Although the production of sensitive and specific multiplex assays was found to be difficult in earlier studies, more recent work has offered systematic methodologies and technical advancements for easier test design. Key advancements include the selection of oligonucleotide primers and the use of PCR. These developments, along with others aimed at enhancing sensitivity, specificity, and automation, have led to a surge in publications on the application of multiplex PCR for diagnosing infectious agents, particularly those targeting viral nucleic acids (Hwang et al., 2007).

The purpose of this study was to determine whether *Listeria monocytogenes* and *Staphylococcus aureus* were present in beef products using multiplex PCR. Additionally, the effects of extracts from orange and pomegranate peels on these microorganisms were to be evaluated in minced beef.

2-MATERIAL AND METHODS:

A multiplex PCR investigation was performed on 40 beef meat products (10 pieces of minced beef meat, Kofta, Burger, and Sausage) to ascertain the prevalence of *Listeria spp*. and *Staphylococcus aureus*.

Samples were randomly selected for participation in the study. The samples included (minced beef meat, Kofta, Burger, and Sausage). All samples were collected from 2023-2024 from El-menoufia governorates under complete aseptic conditions.

2.1.-Bacteriological examination:

2.1.1. Preparation of samples (ISO 4833-1, 2013):

Precisely, 225 milliliters of 0.1% sterile peptone water were used to homogenize 25 grams of each sample aseptically in a stomacher (Colworth, 400) for 1.5 minutes. Following this, tenfold serial dilutions were generated.

2.1.2. *Staphylococcus aureus* count (ISO/TS 11133-1,2006).

Using Baird Parker agar enriched with potassium tellurite and egg yolk emulsion, Staphylococcus aureus colonies were counted on average after plates were incubated for one to three days at 37 °C.

2.1.3. Quantification of *L. monocytogenes*:

After homogenizing each sample with 90 ml of buffered Listeria enrichment broth, a 10 ml aliquot was set aside. Every sample was presumed to be positive for L. monocytogenes (based on the appearance of typical colonies on LMPM).

2.2. The Extract Process of Pomegranate Peel and Orange Peel:

According to Dahham et al. (2010), one of the most used techniques for pomegranate peel extraction is the methanol extraction method. Using an electric blender, the fine peel powders are initially produced in this technique, and they are then oven-dried for 24 hours at 40°C. The powders are then sieved through a 24-mesh screen, and 250 ml of 80% methanol is used to extract 10 g of the powder sample over 24 hours at room temperature (around 25°C). After filtering, the finished extract is put to use. The powdered sample (10 g) is extracted with 100 ml of distilled water and employed if the aqueous extract

is required for usage. The biochemical makeup of PPEs is said to be significantly influenced by the extraction process. Typically, the traditional methods require

2.3. Molecular methods of detection for detection of *Staphylococcus aureus* and *Listeria monocytogenes.*

2.3.1. Extraction of DNA: (Sambrook et *al.*, 1989) according to **Emerald Amp GT PCR mastermix (Takara)** Code No. **RR310A**kit .

2.3.2. oligonucleotide primers used in qPCR Metabion (Germany) provided three sets of primers. As seen in Table (1), they have a specific sequence and amplify a certain product.

Table 1. Oligonucleotide primers sequences.

Target gene	Primer sequence (5'-3')	Length of amplified product (bp)	Reference
S. aureus 23S rRNA	AC GGAGTTACAAAGGACGAC	1250	**Bhati et al., 2016**
	AGCTCAGCCTTAACGAGTAC		
L. monocytogenes 16S rRNA	ggA CCg ggg CTA ATA CCg AAT gAT AA	1200	**Kumar et al., 2015**
	TTC ATg TAg gCg AgT TgC AgC CTA		

3.4.2. Cycling conditions of the primers during PCR

Temperature and time conditions of the two primers during PCR are shown in Table (3).

Table (2): Cycling conditions of the primers during cPCR.

Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
S. aureus 23S rRNA	94˚C 5 min.	94˚C 30 sec.	55˚C 1 min	72˚C 1.2 min.	35	72˚C 12 min.
L. monocytogenes 16S rRNA	94˚C 5 min.	94˚C 30 sec.	60˚C 1 min.	72˚C 1.2 min.	35	72˚C 12 min.

3-RESULTS AND DISCUSSION

Symptoms of staphylococcal food poisoning usually appear 1 to 6 hours (usually 2 to 4 hours) after consuming infected food and include nausea, vomiting, diarrhea, weakness, and general malaise. Although the sickness is usually not lethal, shock and dehydration are possible side effects. Recovery usually happens in a day or two, but occasionally it can take longer (Aydin et al., 2011).

Meat products are often linked to S. aureus poisoning outbreaks, with contamination potentially occurring during handling, preparation, post-processing, or if left unrefrigerated for an extended period, allowing for the multiplication of *S. aureus* and the production of enterotoxins. The handling of ready-to-eat meat products can introduce a high number of microorganisms, including coagulase-positive *S. aureus*, which are known food poisoning agents (Le Loir et al., 2003). While heat treatment can eliminate bacteria, the heat-resistant enterotoxins may still cause food poisoning. In a recent study (Table 4), it was found that pomegranate peel extract at 2.5% and orange peel extract at 2.5% inhibited the growth of S. aureus by 1.36±.02 and 2.04±.03, respectively, aligning with the findings of Ali et al. (2019) who demonstrated the inhibitory effects of pomegranate peel extract on *S. aureus* and *Salmonella*. The antimicrobial properties of pomegranate peels were also evaluated under high-pressure conditions (300 and 600 MPa) by Alexandre et al. (2019).

At 300 MPa, the extraction procedure produced the maximum levels of phenolic content and antioxidant activity, which enhanced the antibacterial activity against pathogenic microorganisms. It was discovered that the pomegranate peel extract has a significant tannin content, which makes it efficient against strains of *Staphylococcus aureus*. Pomegranate peel extract at 2.5% and orange peel extract at 2.5% both suppressed the development of *Listeria monocytogenes* by 1.30±.04 and 2.04±.03 in the current experiment (Table 5).

Table 4. Effect of pomegranate peel and orange peel on Staphylococcus isolates

Groups/storage period	Control	pomegranate peel 2.5%	orange peel 2.5%	P value
1st day	5.61± .046 [a]	5.41±.04 [b]	5.54± .029[ab]	.031
3rd day	6.67±.03[a]	4.33± .03[b]	4.49±.01[b]	.001
6th day	7.74±.02 a	3.14± .03[b]	3.32± .07[b]	.000
9th day	S	2.05±.04	2.04±.03	.43
12th day	S	1.36±.02	S	-
13th day	S	S	S	-

Means in the same row with different superscripts (a,b, and c) are statistically significant (p≤ 0.05)., Means ±SE=Standard error

Table 5. Effect of pomegranate peel and orange peel on *Listeria monocytogenes* isolates:

Groups/storage period	Control	pomegranate peel 2.5%	orange peel 2.5%	P value
1st day	5.67± .06	5.51±.05	5.66± .03	0.433
3rd day	6.74±.88[a]	4.33± .038[b]	4.49±.01[b]	.006
6th day	7.94±.10 [a]	3.45± .28[b]	3.52± .33[b]	.004
9th day	S	2.05±.04	2.04±.03	.45
12th day	S	1.30±.04	S	-
13th day	S	S	S	-

The means that have distinct superscripts (a, b, and c) in the same row indicate statistical significance (p≤ 0.05)., Standard error = Means ± SE

Table (6): Sensory evaluation for pomegranate peel and orange peel on *Staphylococcus aureus* isolates:

Group	Days	color	odor	Appearance	Overall acceptability	consistency	Grade
Control	Zero-day	V	V	V	V	V	Acceptable
	3rd day	G	G	G	G	G	Unacceptable
	6th day	S	S	S	S	S	Spoiled
	9th day	S	S	S	S	S	spoiled
	12th day	S	S	S	S	S	Spoiled
	15th day	s	S	S	S	s	Spoiled
pomegranate peel	Zero-day	v	V	V	V	V	Very good
	3rd day	g	G	G	G	g	Good
	6th day	g	G	G	G	g	Good
	9th day	g	G	G	G	g	Acceptable
	12th day	g	G	G	G	g	Acceptable
	15th day	u	U	U	U	U	Unacceptable
orange peel	Zero-day	v	V	V	V	v	Very good
	3rd day	g	G	G	G	g	Good
	6th day	g	G	G	G	g	Good
	9th day	g	G	G	G	g	Acceptable
	12th day	u	U	U	U	u	Unacceptable
	15th day	s	S	S	S	s	Spoiled

V: very good , g: good, u: unacceptable, s: spoiled

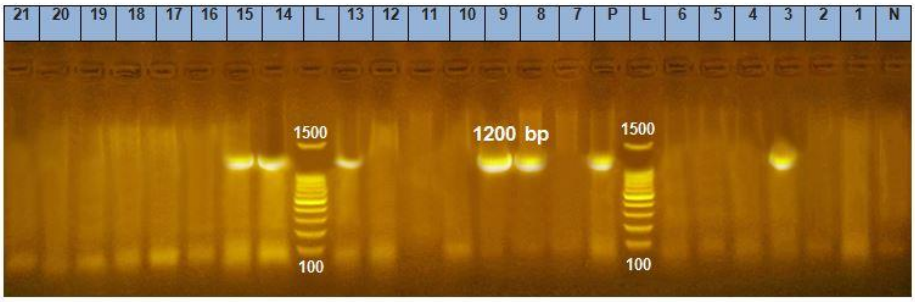

Fig 1. Agarose gel electrophoresis demonstrating the multiplex PCR test for isolates of *Staphylococcus aureus* and *Listeria monocytogenes* from beef meat products. *L. monocytogenes* was amplified at 1200 bp in lanes 1 through 21 (sample no. 3,8,9,13,14, and 15).

Table 7. Sensory evaluation for pomegranate peel and orange peel on *Listeria monocytogenes* isolates:

Group	Days	color	odor	Appearance	Overall acceptability	consistency	Grade
Control	Zero-day	v	V	V	V	V	Very good
	3rd day	g	G	G	G	G	Acceptable
	6th day	u	U	U	U	U	Un acceptable
	9th day	s	S	S	S	S	Spoiled
	12th day	s	S	S	S	S	Spoiled
	15th day	s	S	S	S	S	Spoiled
pomegranate peel	Zero-day	v	V	V	V	V	Very good
	3rd day	G	G	G	G	G	Good
	6th day	g	G	G	G	G	Good
	9th day	g	G	G	G	G	Acceptable
	12th day	g	G	G	G	G	Acceptable
	15th day	u	U	U	U	U	Acceptable
orange peel	Zero-day	v	V	V	V	V	Very good
	3rd day	G	G	G	G	G	Good
	6th day	g	G	G	G	G	Good
	9th day	u	U	U	U	u	Unacceptable
	12th day	S	S	S	S	s	Spoiled
	15th day	S	S	S	S	S	Spoiled

V: very good, g: good, u: unacceptable, s: spoiled

Table 8. PCR amplification results of examined beef meat products.

Product	Sample	*Listeria monocytogenes*	*S.aureus*
Minced beef meat	1	-	-
	2	-	+
	3	+	+
	4	-	+
	5	-	+
	6	-	-
	7	-	+
	8	+	+
	9	+	+
	10	-	-
Burger	11	-	-
	12	-	+
	13	+	+
	14	+	+
	15	+	-
	16	-	+
	17	-	-
	18	-	-
	19	-	+
	20	-	-
sausage	21	-	+
	22	-	-
	23	-	-
	24	+	-
	25	-	+

	26	+	+
	27	+	+
	28	-	-
	29	+	-
	30	-	+
Kofta	31	-	-
	32	-	+
	33	+	-
	34	-	+
	35	-	+
	36	-	+
	37	+	+
	38	+	+
	39	+	-
	40	+	+

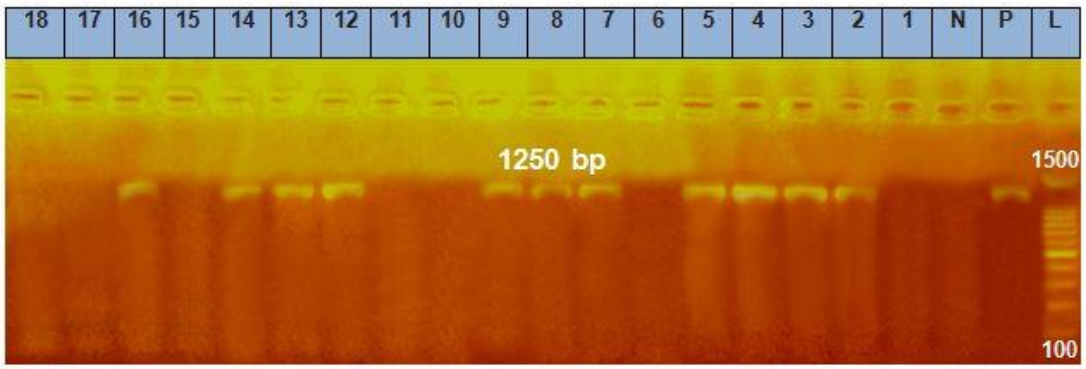

Fig (2): Agarose gel electrophoresis demonstrating the multiplex PCR test for *Staphylococcus aureus* and *Listeria monocytogenes* that were isolated from beef meat products. *Staphylococcus aureus* lanes 1 through 18 were amplified at 1250 bp (sample nos. 2,3,4,5,7,8,9,12,13,14, and 16).

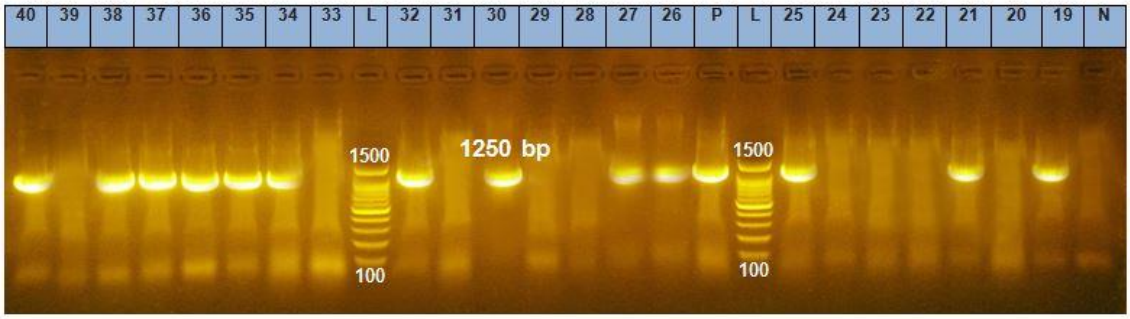

Fig (3): Multiplex PCR test for *Listeria monocytogenes* and *Staphylococcus aureus* isolated from beef meat product using agarose gel electrophoresis. *Staphylococcus aureus* was amplified at 1250 bp in lanes 19 through 40 (sample no. 19-21-25-26-27-30-32-34-35-36-37-38-40).

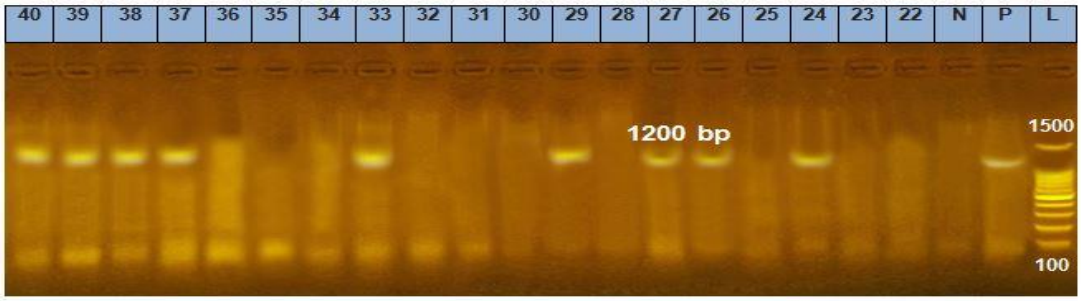

Fig (4): Multiplex PCR test for *Listeria monocytogenes* and *Staphylococcus aureus* isolated from beef meat product using agarose gel electrophoresis. *L. monocytogenes* was amplified at 1200 bp in lanes 19 to 40 (sample no. 24-26-27-29-33-37-38-39-40).

This result is in line with the research by Yehia et al. (2011), which showed that pomegranate peel water extract efficiently inhibited the growth of both Gram-positive and Gram-negative bacteria, such as *Brucella spp.*, *Rhodotorula glutinis*, *E. coli*, *B. subtilis*, *Enterobacter aerogenes*, and *Serratia marcescens.*

The present study (Table 6, 7) found that adding 2.5% pomegranate peel extract and 2.5% orange peel extract to minced meat enhanced its quality and prolonged its shelf life. Nair et al .(2018) support this conclusion. Pomegranate peel extract, which contains bioactive compounds including tannins with ellagic and gallic acids, is a useful by-product for the food preservation sector. Pomegranate peel extract is rich in several bioactive components, which have been tried and tested as natural food preservation additives in the past. Tests on pomegranate peel extract alone or in conjunction with edible coatings and films for food preservation have been conducted. Food packaging has made extensive use of environmentally friendly active packaging systems made of biopolymers such as proteins, lipids, and polysaccharides since the early Tehranifar et al. (2011).

According to the current study (Table 6, 7), adding 2.5% orange and 2.5% pomegranate peel extract to minced beef improved its quality and extended its shelf life. This conclusion is supported by Nair et al. (2018). Pomegranate peel extract is a valuable by-product for the food preservation industry since it includes bioactive ingredients including tannins with ellagic and gallic acids. Numerous bioactive components found in pomegranate peel extract have been investigated and tested as natural food preservation additives in the past. Pomegranate peel extract has been tested on its own or in combination with edible coatings and films for food preservation. Since the advent of environmentally friendly active packaging systems composed of biopolymers including proteins, lipids, and polysaccharides, food packaging has made widespread use of these systems (Nair et al.,2018).

Given that listeriosis is a foodborne bacterial zoonosis with a global impact, the new work has important clinical implications. The importance of the discoveries is highlighted by the greatest outbreak of human listeriosis, which was connected to the ingestion of tainted meat products. Furthermore, a new multiplex PCR technique for the identification of *S. aureus* and *L. monocytogenes* in beef meat products yields fast and precise results, consistent with earlier findings by Hwang et al. (2007) and Normanno et al. (2005).

Because of inadequate sanitary procedures during processing and storage, *S. aureus* and *listeria monocytogenes* are frequently detected in meat products. In this study, there were seven (17.5%), five (12.5%), and five (12.5%) instances of *S. aureus* overall in 40 samples of meat products (minced meat, burger, sausage, and kofta).

CONCLUSIONS

It is advised to use natural substances with antibacterial qualities in the production process to improve safety and quality. It may be concluded from the study's results that the examined plant extracts don't provide any extra advantages in terms of preventing foodborne pathogens while the sausage is mature.

Data Accessibility

This article contains the data that this study used to support its conclusions.

Conflicts of Interest

The writers say they have no competing interests.

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