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Water extracts of pomegranate peel and hojicha tea leaf inhibit the growth of methicillin-resistant staphylococcus aureus

Natsumi Nakamura[1]
Yusuke Komiya[2+]
Hisaya K Ono[3]
Keizo Arihara[4]

[1,2,4]*Department of Animal Science, School of Veterinary Medicine, Kitasato University, Towada, Aomori 034-8628, Japan.*
[1]*Email: ag107093@gmail.com*
[2]*Email: komiya@vmas.kitasato-u.ac.jp*
[4]*Email: arihara@vmas.kitasato-u.ac.jp*
[3]*Department of Zoonoses, School of Veterinary Medicine, Kitasato University, Towada, Aomori 034-8628, Japan.*
[3]*Email: hisaono@vmas.kitasato-u.ac.jp*

(+ Corresponding author)

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) strains capable of causing infections and food poisoning have emerged worldwide in response to the use of various antimicrobial agents. To date, several drugs have been used to combat these bacteria. We focused herein on functional foods (particularly food waste products), which are safer and exhibit fewer side effects than drugs. The objective of present study is to elucidate the growth inhibitory effects of several food waste products on *Staphylococcus aureus* using three different *Staphylococcus aureus* strains, JCM2413 as the standard strain, and MW2 and N315 as MRSA strains. One percent water extracts of ten food waste products (grapefruit peel, pineapple leaf, dragon fruit peel, persimmon peel, turnip leaf, pomegranate peel, apple peel, potato peel, hojicha tea leaf, and banana peel) were added to the medium, and colonies were counted after a 24-hour incubation. Significant decreases were observed in MW2 and N315 colonies with pomegranate peel and hojicha tea leaf extracts. By contrast, no significant difference was observed in JCM2413 in any of the extracts. We demonstrated that water extracts of pomegranate peel and hojicha tea leaves possess bacteriostatic activity specific to MRSA. Our findings help to improve food safety with reducing food loss. However, further studies are needed to elucidate the detailed mechanism of the effects of pomegranate peel and hojicha tea on MRSA.

Contribution/Originality: The originality of this study is to demonstrate the specific antibacterial activity of aqueous extracts from pomegranate peel and hojicha tea leaves against methicillin-resistant Staphylococcus aureus (MRSA). Our findings contribute to the improvement of food safety and the promotion of sustainable energy production through the utilization of food waste products.

1. INTRODUCTION

Staphylococcus aureus is a ubiquitous gram-positive bacterium, known worldwide to cause food-borne diseases, such as staphylococcal food poisoning (SFP), and a variety of infectious diseases ranging from skin infections to fatal systemic infections [1]. They secrete a variety of toxins and enzymes, including heat- and proteolysis-stable protein toxins (staphylococcal enterotoxins (SEs)) that underlie their pathogenicity [2]. New SEs continue to be identified, and SFP outbreaks are being reported worldwide, rendering SFP a continuing important public health and food safety issue [3].

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged worldwide in response to the use of various antimicrobial agents and is associated with poorer clinical outcomes than methicillin-susceptible *Staphylococcus aureus* (MSSA) [4, 5].

The emergence of MRSA as a significant concern is evident as it has infiltrated the community and is no longer limited to being primarily a nosocomial pathogen [6, 7]. The increasing cases of vancomycin-resistant MRSA, antibiotic-resistant tuberculosis, and other bacterial infections highlight the extremely challenging scenario that humanity faces in combating bacterial infections. Consequently, collaborative efforts have been initiated to discover antimicrobial compounds from natural sources and traditional medicines.

Antibiotics remain the method of choice for treating bacterial diseases; however, some food products, including garlic and ginger, display antibacterial activities. Bhatwalkar, et al. [8] reported that various compounds found in garlic exert antibacterial activity against a variety of bacteria, including MRSA [8]. Garlic organosulfur compounds, including diallyl sulfides and allicin, exert their antibacterial activity mainly by inactivating bacterial enzymes and altering the cell membrane. Ginger and its constituents also play a vital role in preventing microbial growth and act as antimicrobial agents [9]. Gingerol and shagelol have been identified as effective therapeutic agents. Although food functionality has attracted attention, food loss and waste have become growing problems in recent years.

Sustainable Development Goals (SDGs) are a set of global goals for achieving sustainable health at every level, from the biosphere level to the local community. Addressing food loss and waste contributes to human health and ecosystems by reducing its negative impact on the environment. Approximately 1.3 billion and of food is wasted annually worldwide, and 6.12 million tons in Japan. In Japan, the Ministry of the Environment recommends innovations to enhance recycling and minimize food loss. These reports led us to wonder whether food waste products harbored effective anti-growth activity against *Staphylococcus aureus*.

In this study, we aimed to investigate the functionality of food waste products against *Staphylococcus aureus* by examining the growth-inhibitory activity of water extracts obtained from ten food waste products on standard and methicillin-resistant strains of *Staphylococcus aureus*.

2. MATERIALS AND METHODS

2.1. Bacterial Strains and Growth Conditions

We used JCM2413 as the standard *Staphylococcus aureus* strain, and MW2 and N315 as MRSA strains. Bacteria were cultured in Luria-Bertani (LB-Miller) Miller broth medium (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) at 37 °C for 24 h with shaking. Bacteria were diluted (10^{-1} to 10^{-8}) in trypto-soya broth (TSB) medium (Nissui Pharmaceutical Co., Tokyo, Japan).

2.2. Preparation of Water Extracts from Food Waste Products

Grapefruit, pineapple, dragon fruit, persimmon, turnip, pomegranate, apple, potato, hojicha, and banana peels were obtained for extraction. Each waste product was cut into small pieces, weighed, and homogenized in sterile water (4X each sample volume). Homogenized samples were centrifuged (4 °C, 4000 rpm, 30 min), supernatants were sterilized by filtration, and sterile extracts were stored until used at -20 °C.

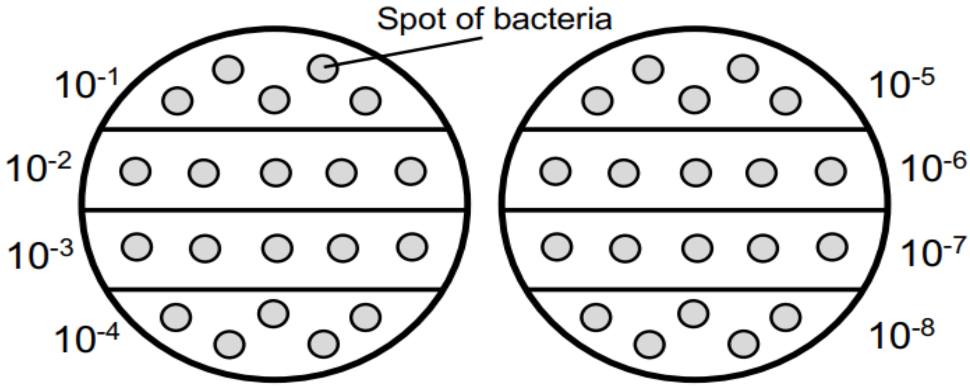

Figure 1. Schematic representation of the experimental design. Culture plates supplemented with each extract were separated into four sections, and each diluted bacteria (10^{-1} to 10^{-8}) was seeded onto five arbitrary points per section (Gray dot).

2.3. Experimental Procedures

The study design is provided in Figure 1. Briefly, extract was added (to 1%) to nutrient agar medium (Nissui Pharmaceutical Co., Ltd.). Sterile water was used as control. Each plate was subdivided into four sections, and each diluted bacteria (10^{-1} to 10^{-8}) was seeded at five arbitrary points per section. Colonies were counted after 24-hour incubation at 37 °C.

2.4. Statistical Analysis

Data are expressed as means ± standard error of the mean (SEM) and were analyzed using one-way analysis of variance followed by Tukey–Kramer multiple-comparison testing. Statistical significance was set at $p < 0.05$. Statistics were calculated using Excel-Toukei ver.7.0 (Social Survey Research Information Co., Ltd., Tokyo, Japan).

3. RESULTS

In the MW2 strain, a significant decrease in the number of colonies was observed compared to the control group with exposure to pomegranate peel and hojicha tea leaf at 10^{-3}, 10^{-4}, and 10^{-5} bacterial dilutions Figure 2A and Table 1). At bacterial concentrations of 10^{-1} and 10^{-2}, colony numbers were uncountably large. At bacterial concentrations of 10^{-3} and 10^{-4}, supplementation with grapefruit peel, pineapple leaf, dragon fruit peel, persimmon peel, and apple peel tended to increase colony numbers compared to controls.

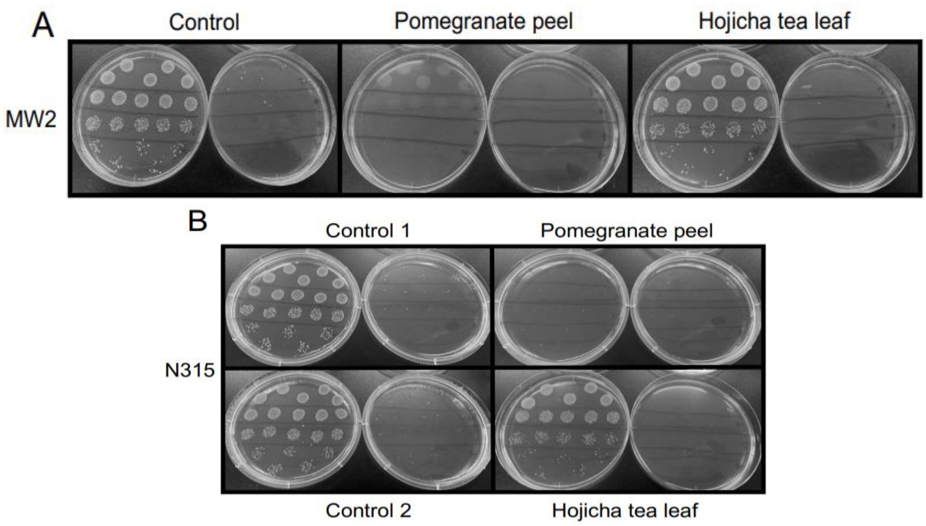

Figure 2. Images of MW2 (A) and N315 (B) colonies after 24-hour incubation at 37 °C in control, pomegranate peel, and hojicha tea leaf groups. Controls for hojicha tea leaf and pomegramate peel assessments were conducted independently in the N315 strain.

Table 1. The number of colonies of MW2.

Food samples	Dilution rate of MW2							
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Control	-	-	124.8±11.0	19.6±3.5	2.4±0.51	0.40±0.40	0.0	0.0
Grapefruit peel	-	-	148.0±10.7	23.0±1.8	2.0±0.70	0.40±0.40	0.0	0.0
Pineapple peel	-	-	152.4±10.1	25.6±1.6	1.4±0.40	0.60±0.60	0.0	0.0
Dragon fruit peel	-	-	148.0±14.2	22.6±0.75	2.4±0.81	0.20±0.20	0.0	0.0
Persimmon peel	-	-	132.6±7.9	27.2±1.7	1.0±0.63	0.0	0.0	0.0
Turnip leaf	-	-	-	32.6±4.3	1.8±0.37	0.40±0.40	0.0	0.0
Pomegranate peel	-	-	0.0**	0.0**	0.0*	0.0	0.0	0.0
Apple peel	-	-	119.8±4.3	21.6±3.7	1.6±0.60	0.20±0.20	0.0	0.0
Potato peel	-	-	100.0±6.3	20.8±1.8	1.2±0.20	1.0±0.31	0.20±0.20	0.0
Hojicha tea leaf	-	-	79.2±6.1*	6.2±1.0*	0.20±0.20	0.20±0.20	0.0	0.0
Banana peel	-	-	109.4±3.9	11.2±0.86	0.60±0.40	0.20±0.20	0.20±0.20	0.0

Note: Results are presented as mean ± SEM (**$P < 0.01$, *$P < 0.05$ vs control).

A significant decrease in N315 colony numbers relative to the control was observed with exposure to pomegranate peel and hojicha tea leaf extracts at 10^{-4} bacteria concentrations (Figure 2B and Table 2). Although statistical analyses were not conducted due to the large number of control group colonies, few colonies were obtained in the pomegranate peel group at 10^{-1} to 10^{-3} bacteria concentrations. Grapefruit peel, pineapple leaf, dragon fruit peel, persimmon peel, and stock leaf extracts tended to increase colony numbers compared with the control.

Table 2. The number of colonies of N315.

Food samples	Dilution rate of N315							
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Control 1	-	-	-	29.8±2.2	3.6±0.60	0.0	0.0	0.0
Grapefruit peel	-	-	-	40.6±3.3	3.8±1.5	0.40±0.24	0.0	0.0
Pineapple peel	-	-	-	32.2±1.5	4.0±0.90	0.60±0.40	0.0	0.0
Dragon fruit peel	-	-	-	36.2±2.7	3.4±0.87	0.40±0.24	0.20±0.20	0.0
Persimmon peel	-	-	-	38.0±2.1	4.2±0.58	0.60±0.24	0.20±0.20	0.0
Turnip leaf	-	-	-	45.4±2.9	7.6±0.87	0.80±0.49	0.0	0.0
Pomegranate peel	0.0	0.0	0.0	0.0**	0.0	0.0	0.0	0.0
Control 2	-	-	-	40.4±2.2	3.2±0.73	0.40±0.24	0.0	0.0
Apple peel	-	-	-	43.6±2.2	4.4±0.40	0.60±0.40	0.0	0.0
Potato peel	-	-	-	56.2±5.2	5.8±1.0	0.60±0.24	0.20±0.20	0.0
Hojicha tea leaf	-	-	75.0±5.9	9.4±1.0**	0.40±0.24	0.0	0.0	0.0
Banana peel	-	-	-	41.8±2.3	4.6±1.0	0.0	0.0	0.0

Note: There were two controls (Control 1 and 2) because of independent experiment. Results are presented as mean ± SEM (**$P < 0.01$ vs control).

No significant differences were observed between extract and control treated JCM2413 strains (Table 3).

Table 3. The number of colonies of JCM2413.

Food samples	Dilution rate of JCM2413							
	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}
Control	-	-	121.3±32.4	8.6±1.2	0.6±0.24	0.0	0.0	0.0
Grapefruit peel	-	-	-	3.4±0.68	0.20±0.20	0.0	0.0	0.0
Pineapple peel	-	-	-	12.2±1.5	1.0±0.45	0.0	0.0	0.0
Dragon fruit peel	-	-	-	17.0±4.3	1.0±0.32	0.40±0.24	0.0	0.0
Persimmon peel	-	-	-	10.0±1.5	0.40±0.24	0.0	0.0	0.0
Turnip leaf	-	-	-	11.4±1.5	5.2±4.0	0.0	0.0	0.0
Pomegranate peel	-	-	95.0±33.3	5.6±5.6	0.0	0.0	0.0	0.0
Apple peel	-	-	-	38.4±23.9	0.60±0.24	0.0	0.0	0.0
Potato peel	-	-	106.0±12.7	12.6±1.5	1.4±0.51	0.80±0.37	0.0	0.0
Hojicha tea leaf	-	-	73.2±3.7	10.8±0.73	0.60±0.24	0.0	0.0	0.0
Banana peel	-	-	-	10.0±1.3	1.4±0.68	0.0	0.20±0.20	0.0

Note: Results are presented as mean ± SEM.

4. DISCUSSION

This study revealed that aqueous extracts of pomegranate peel and hojicha tea leaves possess bacteriostatic activity specific to methicillin resistant *Staphylococcus aureus* strains.

Hojicha is a traditional Japanese tea made by roasting green tea leaves. Zhao et al. reported that green tea extracts possess antibacterial effects against *Staphylococcus aureus* [10]. Epigallocatechin gallate (EGCg), the major constituent of tea catechins, shows potent bactericidal activity against both MRSA and MSSA. EGCg not only suppressed the growth of both MRSA and MSSA, but also significantly reduced their resistance to increased ionic strength and decreased osmotic pressure in the surrounding environment. It's important to note that this effect is not exclusive to MRSA.

Our results showed that the effect of hojicha tea leaf extract was specific to MRSA strains, and specific components of hojicha tea leaves not included in green tea may contribute to this effect. The antibacterial activity of green tea are altered by heat treatment, and it has been suggested that the antibacterial elements generated in green tea infusion during heating consist of low monomer polymers of catechin [11]. Therefore, the functional components of hojicha tea leaves may be specific components produced by further heat treatment.

Pomegranate (Punica granatum), one of the oldest known fruits, belongs to the Lythraceae family. It is believed to have originated in Persia, and several varieties are cultivated. At present, pomegranates are grown in many Middle Eastern, Asian, European, and Western countries, including the United States of America. Pomegranate peel extract contains abundant polyphenols, flavonoids, and tannins.

Feng et al. [12] reported that polyphenols and tannins correlate positively with antibacterial strength against *Staphylococcus aureus*, while tannins alone contribute to MRSA inhibition, implying a crucial role for tannins in suppressing bacteria [12]. Unfortunately, pomegranate peels contain toxic substances, such as pelletierine, an alkaloid that is harmful to the body. Therefore, its use as an antimicrobial substance in food products would be limited without purification of the antimicrobial components. Interestingly, a green synthesis of selenium nanoparticles using pomegranate peel extract showed promising antimicrobial activities against several bacteria, including *Staphylococcus aureus*, with low cytotoxicity in normal human cell lines [13].

Continued research is expected to lead to enhanced mechanistic understanding and development of technologies allowing production of food-derived antimicrobial products with fewer side effects.

Bacteria evolve various mechanisms to survive exposure to antimicrobial agents. For example, they prevent the influx of drugs by changing the composition of their outer membranes, pump out toxins that have entered the bacteria (efflux pump), change the antimicrobial's point of action (DNA or RNA mutation), enzymatically degrade the antimicrobial (e.g. use of β-lactamase), form biofilms, etc. The reason significant reduction in colony numbers was only observed in MRSA is that hojicha tea leaf and pomegranate peel extracts inhibited the function of MRSA-specific proteins and genes such as penicillin-binding protein (PBP) and β-lactamase, which are absent in standard *Staphylococcus aureus* [14, 15].

A limitation of this study was that the mechanism of antibacterial activity of hojicha tea leaf and pomegranate peel extracts was not investigated. We revealed that the effects of these extracts were specific to MRSA strains. However, their functional components and pathways remain undetermined. Further studies are required to clarify the detailed mechanisms underlying the antibacterial activity of hojicha tea leaf and pomegranate peel extracts against MRSA.

5. CONCLUSION

We revealed that water extracts from pomegranate peel and hojicha tea leaves exhibit specific antigrowth activity against MRSA.

Our findings contribute to improving food safety with reducing food loss; however, further studies are required to elucidate the detailed mechanism underlying the effects of pomegranate peel and hojicha tea leaves on MRSA.

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