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Research note

매실나무(*Prunus mume*) 품종별 부산물(가지 및 잎)의 항균, 항산화 및 세포독성 잠재력 비교

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Comparison of Antibacterial, Antioxidant, and Cytotoxic Potentials of *Prunus mume* Byproducts (Branches and Leaves) in Different Cultivars

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ABSTRACT - This study investigated the antibacterial, antioxidant, and cytotoxic potentials of nonedible byproducts (i.e., branches and leaves) collected from different cultivars of *Prunus mume*. The antibacterial properties of water- or ethanol-based extracts were measured against food-borne pathogenic bacteria using an agar disc diffusion assay, and the ability of the extracts to scavenge radicals was assessed via 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)- and 2,2-diphenyl-1-picrylhydrazyl (DPPH) tests. The extracts from *P. mume* branches exhibited higher antioxidant activities than those from *P. mume* leaves, regardless of the solvents used for the extraction. Several branch-derived ethanol-based extracts showed notable antibacterial effects against *Bacillus cereus*, *Staphylococcus aureus*, and *Yersinia enterocolitica*, whereas leaf-derived extracts had no or negligible effects for suppressing food-borne pathogenic bacteria. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay revealed that no cytotoxic effects were observed at concentrations of 500 μg/mL. The findings highlight the potential of *P. mume*'s byproducts as functional and safe bioresources for value-added applications.

Keywords: Antibacterial, Antioxidant, Branch, Leaf, *Prunus mume*

Recently, as well documented by Gong et al.[1)] and Tian et al.[2)], *Prunus mume* contains a large array of bioactive compounds, such as flavonoids and phenolics, which may contribute to its valuable pharmacological activities. Such compounds have been associated with a wide range of biological activities, mainly including anticancer effects against human gastric, kidney, liver, and ovarian cancer cells and anti-inflammatory effects through the inhibition of the production of lipopolysaccharide (LPS)-induced nitric oxide (NO) and prostaglandin E_2.

To date, however, although a plethora of previous studies have been conducted, primarily focusing on the functional characterization of the edible parts of *P. mume*, such as flowers[3,4)], fruits[5-16)], and seeds[17)], there is a notable lack of studies studies regarding the antibacterial and antioxidant effects of nonedible parts, such as branches and leaves. Typically, the nonedible parts of *P. mume* are regarded as useless agricultural byproducts or wastes with low economic values. Nevertheless, the nonedible parts would contain a

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high concentration of potentially valuable bioactive constituents and thus serve as untapped sources of functional ingredients with significant biological potentials. In this context, exploring the utility of nonedible byproducts can contribute not only to reducing or eliminating environmental burdens, but also to creating value-added functional materials through the resource utilization. Additionally, the concentration and composition of bioactive compounds responsible for the biological activities, such as antibacterial and antioxidant effects, are likely to vary, depending on the different *P. mume* cultivars. As such, evaluating the antibacterial and antioxidant properties of branch or leaf extracts from different *P. mume* cultivars is essential for supporting the broader utilization of *P. mume* byproducts as sustainable resources, thereby establishing a foundation for the development of functional ingredients. This study, therefore, aimed at investigating the biological activities (i.e., the antibacterial, antioxidant, and cytotoxic properties) of branch- and leaf-derived extracts from *P. mume* for the purpose of exploring the potential of *P. mume* byproducts in making them into functional food ingredients or natural bioactive resources.

Materials and Methods

Preparation and extraction

As summarized in Table 1, branch and leaf byproducts of *P. mume* from different cultivars were provided by the Suncheon-si Horticultural Science Team (Technology Supply Project Department) (Suncheon, Korea). Branches or leaves (weighing 100 g) were mixed in 1 L of ethanol (DAEJUNG Chemicals and Metals Co., Ltd., Siheung, Korea) and heated at 60°C for 5 h. The extracts were filtered under pressure using a vacuum pump (SUNILEYELA Co., Ltd., Seongnam, Korea). The filtrates were then concentrated using a rotary evaporator (SUNILEYELA Co., Ltd.) with 80 RPM at 40°C and freeze-dried to obtain powdered samples.

Each of the powders weighing 5 g was individually combined with 50 mL of deionized water (DW) or 25% ethanol (DAEJUNG Chemicals and Metals Co., Ltd.), vortexed thoroughly, and incubated in an oven (Daewon Science Co., Bucheon, Korea) to facilitate its extraction at 25±1°C for 48 h. Following a centrifugation procedure at 1,500×g for 30 minutes at 4°C, the resultant supernatants were collected and filtered using a 0.22-μm pore size polycarbonate membrane (Millipore®, Mississauga, Ontario, Canada). Then, the filtrates originating from the water- or ethanol-based extracts were individually concentrated using a rotary evaporator (SUNILEYELA Co., Ltd.) at 2,000×g and 35°C for 3 h.

Table 1. Category of branch or leaf extracts originated from *P. mume*

Category	Source (origin)
ABW	Water-based extracts from the branch of *Napwol Hongmae*
BBW	Water-based extracts from the branch of *Neungsu Cheongmae*
CBW	Water-based extracts from the branch of *Neungsu Hongmae*
DBW	Water-based extracts from the branch of *Mi-inmae*
EBW	Water-based extracts from the branch of *Seonammae*
FBW	Water-based extracts from the branch of *Unyongmae*
GBW	Water-based extracts from the branch of *Okyeom*
HBW	Water-based extracts from the branch of *Josaeng Namgo*
IBW	Water-based extracts from the branch of *Cheonmae*
JBW	Water-based extracts from the branch of *Chunil Yahong*
ABE	Ethanol-based extracts from the branch of *Napwol Hongmae*
BBE	Ethanol -based extracts from the branch of *Neungsu Cheongmae*
CBE	Ethanol -based extracts from the branch of *Neungsu Hongmae*
DBE	Ethanol -based extracts from the branch of *Mi-inmae*
EBE	Ethanol -based extracts from the branch of *Seonammae*
FBE	Ethanol -based extracts from the branch of *Unyongmae*
GBE	Ethanol -based extracts from the branch of *Okyeom*
HBE	Ethanol -based extracts from the branch of *Josaeng Namgo*
IBE	Ethanol -based extracts from the branch of *Cheonmae*
JBE	Ethanol -based extracts from the branch of *Chunil Yahong*
ALW	Water-based extracts from the leaf of *Napwol Hongmae*
BLW	Water-based extracts from the leaf of *Neungsu Cheongmae*
CLW	Water-based extracts from the leaf of *Neungsu Hongmae*
DLW	Water-based extracts from the leaf of *Mi-inmae*
ELW	Water-based extracts from the leaf of *Seonammae*
FLW	Water-based extracts from the leaf of *Unyongmae*
GLW	Water-based extracts from the leaf of *Okyeom*
HLW	Water-based extracts from the leaf of *Josaeng Namgo*
ILW	Water-based extracts from the leaf of *Cheonmae*
JLW	Water-based extracts from the leaf of *Chunil Yahong*
ALE	Ethanol-based extracts from the leaf of *Napwol Hongmae*
BLE	Ethanol -based extracts from the leaf of *Neungsu Cheongmae*
CLE	Ethanol -based extracts from the leaf of *Neungsu Hongmae*
DLE	Ethanol -based extracts from the leaf of *Mi-inmae*
ELE	Ethanol -based extracts from the leaf of *Seonammae*
FLE	Ethanol -based extracts from the leaf of *Unyongmae*
GLE	Ethanol -based extracts from the leaf of *Okyeom*
HLE	Ethanol -based extracts from the leaf of *Josaeng Namgo*
ILE	Ethanol -based extracts from the leaf of *Cheonmae*
JLE	Ethanol -based extracts from the leaf of *Chunil Yahong*

Evaluation of antioxidant properties

The antioxidant activities of each of the water- or ethanol-based extracts from *P. mume* branches or leaves were evaluated according to a protocol described in a study conducted by Park et al.[18] and Yang et al.[19] for measuring 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; Sigma-Aldrich® Co., St Louis, MO, USA) and 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich® Co.) radical-scavenging capabilities, respectively.

Determination of antibacterial activities

Following the agar disc diffusion method described in a study conducted by Hyun et al.[20], the antibacterial effects of the extracts were assessed in order to determine the diameter (mm) of clear zones imposed by water- or ethanol-based extracts from *P. mume* branches and leaves with different cultivars against a total of 23 food-borne pathogenic bacteria, including *Bacillus cereus* (ATCC 10876, ATCC 13061, and ATCC 14579), *Cronobacter sakazakii* (ATCC 12868, ATCC 29004, and ATCC 29544), *Escherichia coli* (ATCC 35150, ATCC 43889, and ATCC 43890), *Listeria monocytogenes* (ATCC 19114 and ATCC 19117), *Salmonella enterica* serovar Enteritidis (ATCC 13076), *Salmonella enterica* serovar Typhimurium (ATCC 19585, ATCC 43971, and DT104), *Shigella flexneri* (KCTC 2517, KCTC 22192, and NCCP 10852), *Staphylococcus aureus* (ATCC 23235, ATCC 25923, and ATCC 40050), and *Yersinia enterocolitica* (ATCC 23715 and ATCC 55075). All the bacterial stocks used in this study were obtained from the bacterial culture collections of the Sunchon National University (Sunchon National University, Suncheon, Korea). Individually, all the stock cultures were stored in a mixture of 1 mL of tryptic soy broth (TSB; Difco® Laboratories Inc., Detroit, MA, USA) and 0.5 mL of 50% glycerol (Junsei Chmical Co., Ltd., Kyoto, Japan) at -80°C and were activated in TSB, followed by 24 h of incubation at 37°C. Following the centrifugation at 3,000×g for 3 min at 4°C, each of the bacterial pellets was washed with 0.1 M phosphate-buffered saline (PBS; BIONEER® Corp., Daejeon, Korea) and resuspended in 1 mL of 0.1 M PBS. Separately, the bacterial aliquots (i.e.,100 μL) were diluted in 9.9 mL of 0.1 M PBS, which corresponded to the viable populations of 10^7-10^8 CFU/mL.

After being autoclaved at 121°C for 20 min, TSB supplemented with 0.75% agar (Difco® Laboratories Inc.) was cooled in a water bath (JEIO TECH® Co. Ltd., Daejeon, Korea) at 45°C for 30 min and inoculated, corresponding to bacterial populations of 10^5-10^6 CFU/mL, with 100 μL of each of the bacterial solutions held in 0.1 M PBS. The bacterial fluids were poured into a sterile petri dish and solidified, followed by being left at 25±1°C. Then, paper discs (diameter: 8 mm; SPL life sciences Co. Ltd., Pocheon, Korea) were placed on the surface of the agar plates, and 20 μL of each of the water or ethanol-based extracts from *P. mume* branches or leaves at a dose range of 100, 200, 300, and 400 mg/mL was loaded onto each disc, followed by 24 h of incubation at 37°C.

Measurement of cytotoxic levels

Caco-2 cells were seeded and cultured in each well of a 96-well plate according to a method described in a study conducted by Han et al.[21]. Briefly, the water- or ethanol-based extracts obtained from *P. mume* branches or leaves were diluted in the Dulbecco's Modified Eagle's Medium (DMEM; Lot 26418007; Corning® Inc., Manassas, VA, USA) and added to each of the wells containing Caco-2 cells, which were adjusted to approximately 10^5-10^6 cells per well, at a range of concentrations (i.e., 31.25, 62.50, 125.00, 250.00, and 500.00 μg/mL), followed by 24 h of incubation at 37°C under 5% CO_2. The fluids contained in each of the wells were replaced with 100 μL of DMEM containing a 5-mg/mL 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; MTT; Sigma-Aldrich® Co.) solution, followed by 1 h of incubation at 37°C. The MTT solution was discarded, and 100 μL of dimethyl sulfoxide (DMSO; Sigma-Aldrich® Co.) was added to each well to solubilize the formazan crystals. The plate was then incubated at 37°C under 5% CO_2 for an additional 15 min. Absorbance was measured at 570 nm and 620 nm using a microplate reader (Spectronic 20® Genesys™ spectrometer, Spectronic Instruments, Inc., Rochester, NY, USA) to quantify the cell's viability.

Statistical approaches

All the experiments were repeated three times with duplicate plates. The obtained results were expressed as the mean±standard deviation. Significant (P<0.05) differences among the treated and nontreated groups were determined using the analysis of variance (ANOVA) with the Duncan's multiple range test (SAS Institute Inc., Cary, NC, USA).

Results and discussion

The ABTS and DPPH radical-scavenging activities of *P. mume* branch and leaf extracts prepared using either water or ethanol as solvents at a concentration of 0.2 mg/mL are illustrated in Fig. 1. ABTS activities of extracts from *P. mume* branches at the level of 87.218-99.743% were significantly (P<0.05) higher than those from *P. mume* leaves, ranging from 19.985% to 62.783%, regardless of the extraction solvents. Similarly, with the exception of EBW (19.478±5.942%), BBW (24.168±6.557%), and FBW (24.170±6.765%), all *P. mume* branch extracts exhibited their strong DPPH-scavenging capability at a range of 31.417

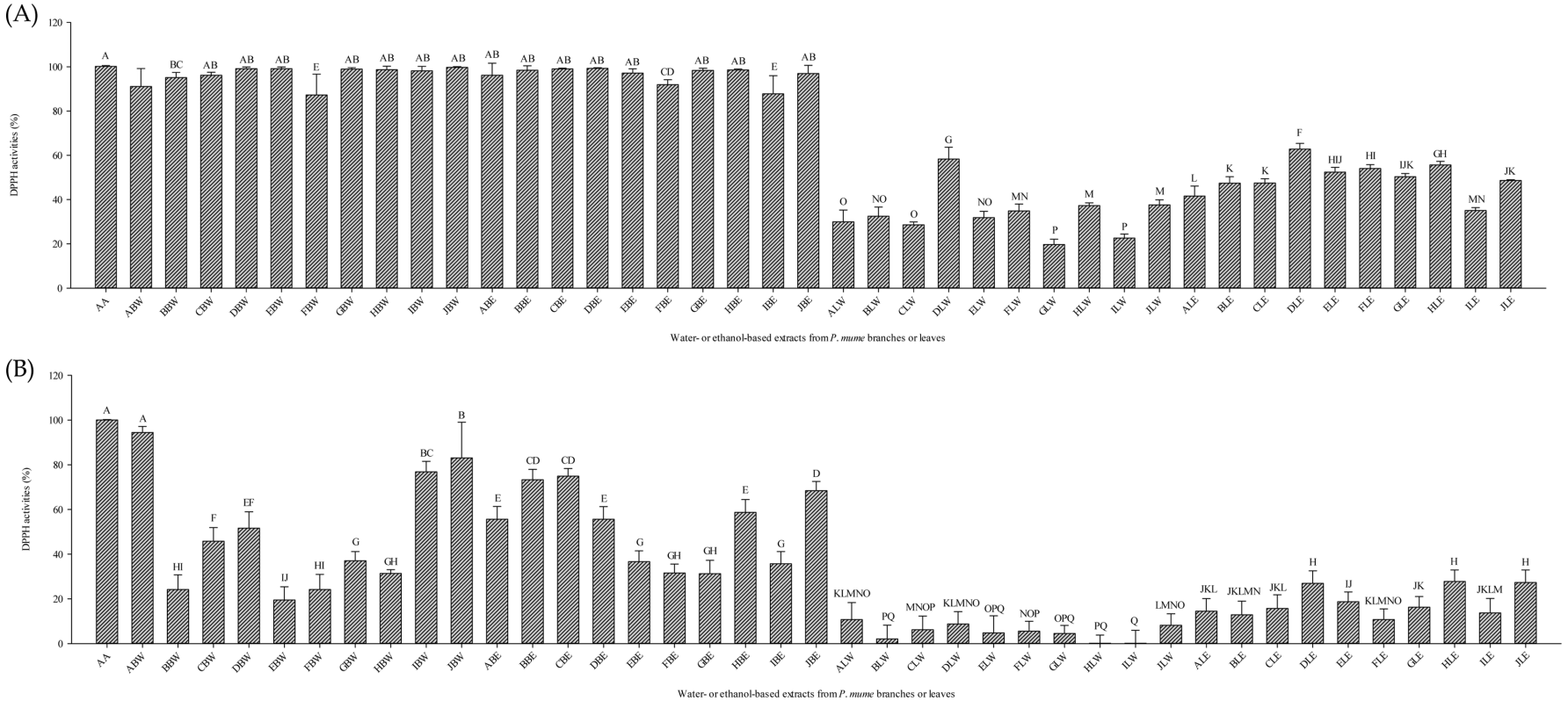

Fig. 1. (A) ABTS and (B) DPPH activities of water- or ethanol-based extracts (concentration: 0.2 mg/mL) originated from the branches or leaves of *P. mume*.

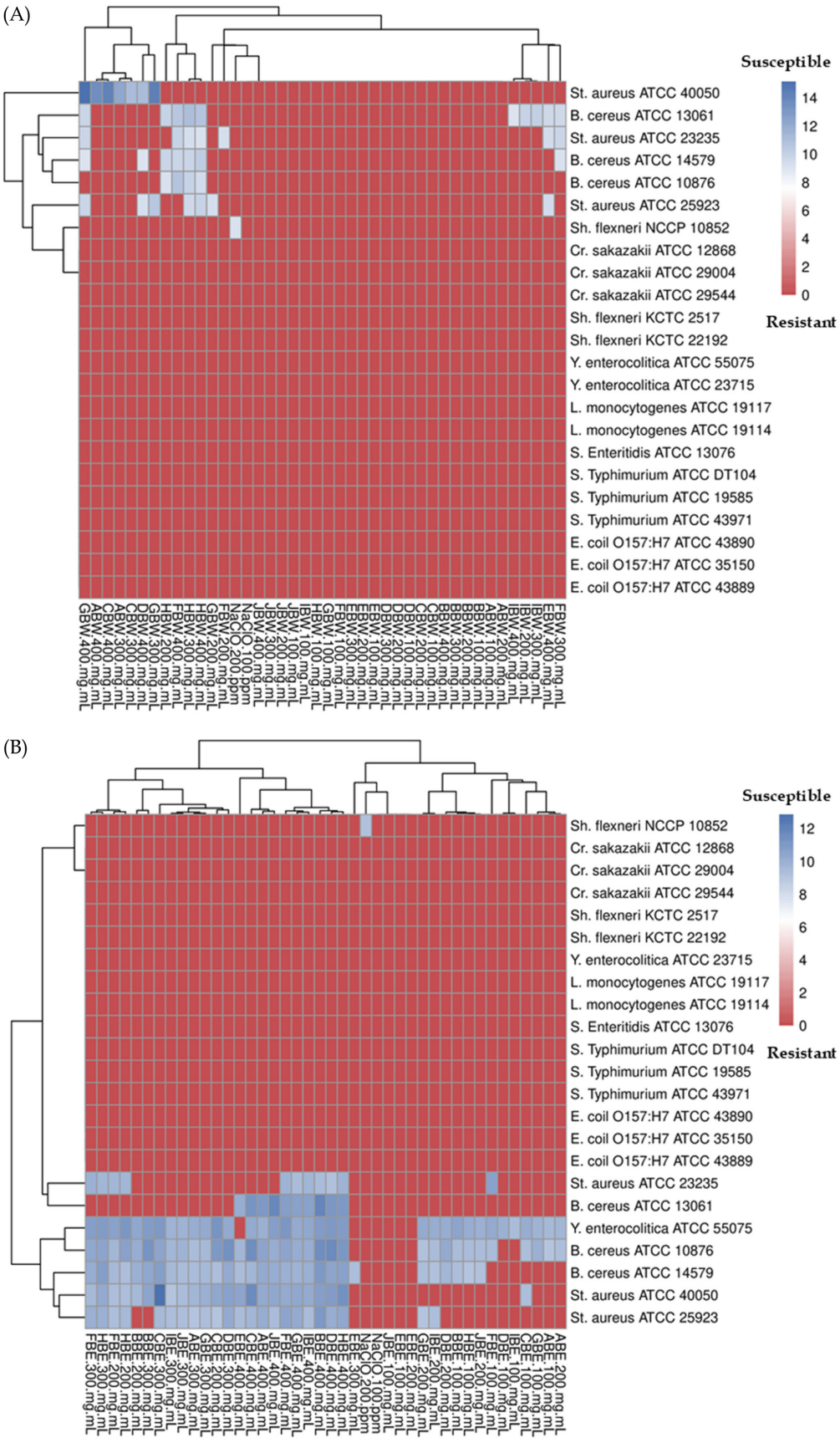

Fig. 2. Antibacterial activities of (A) Water- and (B) Ethanol-based extracts originated from the branches of *P. mume*.

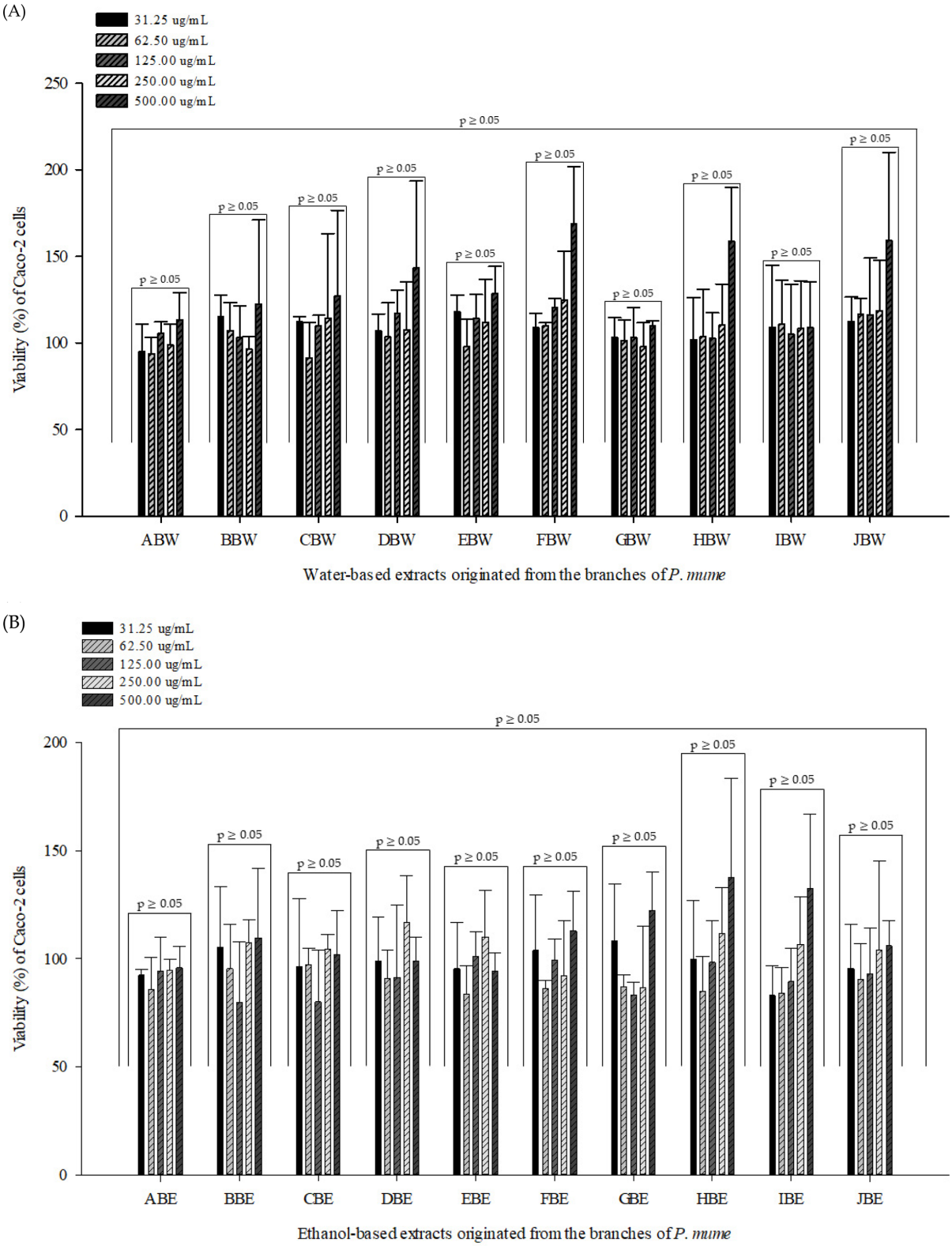

Fig. 3. Viability (%) of Caco-2 cells exposed to 31.25-500.00 μg/mL (A) water- or (B) ethanol-based extracts originated from the branches of *P. mume*.

-94.478% significantly (P<0.05) higher than the *P. mume* leaf extracts (0.177-27.783%). As shown in Fig. 1B, ABW (94.478±2.669%) was the most effective for scavenging DPPH-mediated radicals, followed by JBW (83.055±16.000%), IBW

(76.807±4.714%), CBE (74.895±3.445%), and BBE (73.320 ±4.554).

Fig. 2 shows the antibacterial activities of water- (the supplementary Table S1) or ethanol-based (the supplementary Table S2) extracts originating from *P. mume* branches and leaves against a total of 23 food-borne pathogenic bacteria. Previously, we found that the usage of 100-200 ppm sodium hypochlorite (NaOCl), as the positive control group, represented no or little effects for inhibiting the growth of food-borne pathogenic bacteria. Overall, although all extracts from *P. mume* leaves were found to have negligible or no antibacterial activities (*data not shown*), *B. cereus*, *St. aureus*, and *Y. enterocolitica* were susceptible to *P. mume* leaf extracts. With the exception of BBW and JBW, the water-based extracts from *P. mume* branches exhibited a degree of antibacterial activities against a limited group of gram-positive bacteria, such as *B. cereus* and *St. aureus*, with the formation of clear zones of 9.04-10.94 mm and 9.20-14.33 mm, respectively. Particularly, BBE produced clear zones of 11.87±0.43 mm against *B. cereus* at its concentration of 400.00 mg/mL, followed by JBE (11.80±1.42 mm), DBE (11.63±0.38 mm), and HBE (11.09±0.29 mm). Similarly, profound growth-inhibitory activities were observed when *St. aureus* was treated with several ethanol-based extracts, such as CBE, HBE, and BBE, with the concomitant clear zones exceeding 12.87±1.58, 11.36±0.67, and 11.06±0.57 mm, respectively. Additionally, CBE has been demonstrated for its strong ability to inhibit the growth of *Y. enterocolitica*, as evidenced by measurable clear zones of 11.25±0.34 mm at its dose of 200.00 mg/mL.

Fig. 3 depicts the viability of Caco-2 cells exposed to water- or ethanol-based extracts obtained from the branches of *P. mume*. Commonly, there were no significant ($P \geq 0.05$) differences in the viable population of Caco-2 cells treated with water- and ethanol-based extracts from different *P. mume* cultivars, irrespective of all the concentrations used. Although the viability of Caco-2 cells was inversely proportional to decreasing concentrations of *P. mume* extracts, the cell line still remained viable, ranging from 95.749% to 159.041%, at the onset of water and ethanol-based extracts at concentrations of up to 500.00 μg/mL. Generally, the cell viability exceeding 100% reflects a potential metabolism- or proliferation-stimulatory effect of the tested samples, although an experimental variability inherent to the assay used may have contributed to the results (i.e., the accumulation of cell metabolites and/or waste products in the negative control cell groups)[22].

In this study, the branch-derived extracts showed marked antioxidant activities, which was superior to the leaf-derived extracts, and were able to scavenge the radicals in a concentration-dependent manner. A similar trend has been reported in previous studies that investigated the antioxidant properties of woody plant materials, where lignified tissues were found to be rich in phenolic compounds associated with the enhanced radical-scavenging capacity[23,24]. The *P. mume* fruit was found to exhibit profound antibacterial activities against *B. cereus* and *St. aureus*, which may be primarily attributed to its abundant contents of bioactive compounds, such as chlorogenic acid, citric acid, and epicatechin, as reflected by the formation of clear zones being 12.7±0.3 and 17.7±0.3 mm, respectively[15]. Mitani et al.[11] also determined that the principal phenolic constituents, such as caffeic acid, chlorogenic acid, and ferulic acid, identified in *P. mume* fruits were effective for inactivating a wide diversity of pathogenic and/or spoilage bacteria, contributing to a reduction in viable cell counts to less than 20% of the initial loads. Although flavonoids and phenolics may contribute to the strong antioxidant activity of the *P. mume* branch-derived extracts, this remains speculative because their contents were not quantitatively analyzed in the present study.

Branch-derived extracts exhibited selective inhibitory effects against *B. cereus* and *St. aureus*. In contrast, gram-negative bacteria were less susceptible, which may be attributed to the presence of an outer membrane that can restrict the penetration of hydrophobic compounds[25]. Such a structural feature has been recognized as a key factor for reducing the effectiveness of plant-derived antimicrobial agents, such as carvacrol[26], eugenol[27], and thymol[28], against gram-negative bacteria. According to Burt[29], gram-negative bacteria, such as *E. coli* and *Salmonella* sp., tend to be more resistant to plant-derived antimicrobial agents (i.e., essential oils) than gram-positive bacteria primarily due to the presence of an additional permeability barrier imposed by the outer membrane. Additionally, in a study of Suručić et al.[30] who investigated the antibacterial effects of plant-derived polyphenolic extracts against both gram-positive and gram-negative bacteria, the lower susceptibility of *E. coli* than *St. aureus* may be attributed to the limited diffusion of some polyphenolic compounds, such as punicalagin, through gram-negative outer membrane porins.

In conclusion, this study determined that the nonedible byproducts of *P. mume*, particularly branches, possessed notable antibacterial and antioxidant properties, with no detectable cytotoxic effects. Regardless of the extraction solvents, the ABTS- and DPPH-mediated radical-scavenging capacity of the branch-derived extracts was consistently superior to that of the leaf-derived extracts. Additionally, several water- or ethanol-based extracts from *P. mume* branches (i.e., GBW, CBW, BBE, and JBE) showed measurable antibacterial activities against certain gram-positive bacteria, with the concomitant formation of clear zones of up to 15.17,

14.20, 11.87, and 11.87 mm, respectively. Importantly, exposing Caco-2 cells to both water- and ethanol-based extracts did not compromise the viability of the human cell line. Taken together, all the findings highlight *P. mume* branches as an underutilized, but promising bioresource that may be valorized as a functional ingredient with antibacterial and antioxidant properties. Further studies focusing on the identification of active compounds present in the extracts from *P. mume* branches and leaves are warranted to support broader food-related and industrial applications.

A CRediT authorship contribution statement

Sang-Woo Lee: Formal analysis, Investigation, Writingoriginal draft.

Se-Gyeong Yoon: Formal analysis, Investigation.

Yu-Bin Hwang: Formal analysis, Investigation.

Inyong Kim:Writing-review & editing.

Suncheon-si Horticultural Science Team (Technology Supply Project Department): Writingreview & editing.

Jae-Hyun Yoon: Conceptualization, Project administration, Supervision.

Conflict of interests

The authors declare no potential conflict of interest.

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국문요약

본 연구는 매실나무(*Prunus mume*) 품종별 부산물(즉, 가지 및 잎)의 활용 가능성을 평가하기 위하여 물 또는 주정으로 추출한 시료의 항균·항산화 및 세포독성 특성을 비교하였다. 항산화 활성은 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS)- 및 2,2-diphenyl-1-picrylhydrazyl (DPPH) 라디칼 소거능으로 측정하였고, 항균 활성은 23종 식품매개 병원성 세균을 대상으로 한 agar disc diffusion 방법으로 확인하였으며, 또한 Caco-2 세포를 이용한 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) 분석으로 세포독성 여부를 평가하였다. 그 결과로서 가지 추출물은 용매의 종류와 관계없이 잎 추출물보다 유의적으로(P<0.05) 더 높은 항산화 활성을 보였다. ABTS 소거능은 가지 87.2-99.7% 및 잎 19.7-62.8% 수준으로 확인되었으며, DPPH 소거능 역시 가지 추출물이 전반적으로 우수한 반면(19.5-94.5%), 잎 추출물은 그보다 낮은 범위를 나타내었다(0.2-27.8%). 항균 활성의 경우 잎 추출물은 항균 효과가 없거나 미미한 반면, 일부 가지 유래 주정 추출물은 바실러스 세레우스(*Bacillus cereus*), 황색포도상구균(*Staphylococcus aureus*) 및 여시니아 엔테로콜리티카(*Yersinia enterocolitica*)에 대하여 비교적 뚜렷한 클리어존을 형성하였다. 한편 세포독성은 500 μg/mL까지 독성이 관찰되지 않아 그 안전성이 확인되었다. 종합하면 매실나무 부산물 중 "가지"는 항산화 활성이 우수하고 선택적 항균 효과를 보이며 세포독성이 낮아 기능성 소재 또는 천연 생리 활성 자원으로의 부가가치화 가능성이 크다고 사료된다.

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Supplementary Table S1. Antibacterial activities of water-based extracts originated from the branches of *P. mume*

Bacterium	NaOCl (ppm)		ABW (mg/mL)				BBW (mg/mL)			
	100	200	100	200	300	400	100	200	300	400
E. coli O157:H7 ATCC 35150	-	-	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43889	-	-	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43890	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 43971	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 19585	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC DT104	-	-	-	-	-	-	-	-	-	-
S. Enteritidis ATCC 13076	-	-	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19114	-	-	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19117	-	-	-	-	-	-	-	-	-	-
St. aureus ATCC 40050	-	-	-	-	12.18±0.24	13.51±1.93	-	-	-	-
St. aureus ATCC 23235	-	-	-	-	-	-	-	-	-	-
St. aureus ATCC 25923	-	-	-	-	-	-	-	-	-	-
Y. enterocolitica ATCC 23715	-	-	-	-	-	-	-	-	-	-
Y. enterocolitica ATCC 55075	-	-	-	-	-	-	-	-	-	-
Sh. flexneri NCCP 10852	-	-	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 22192	-	-	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 2517	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29544	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29004	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 12868	-	-	-	-	-	-	-	-	-	-
B. cereus ATCC 14579	-	-	-	-	-	-	-	-	-	-
B. cereus ATCC 13061	-	-	-	-	-	-	-	-	-	-
B. cereus ATCC 10876	-	-	-	-	-	-	-	-	-	-

Supplementary Table S1. (continuous from the previous page) Antibacterial activities of water-based extracts originated from the branches of *P. mume*

Bacterium	CBW (mg/mL)				DBW (mg/mL)				EBW (mg/mL)			
	100	200	300	400	100	200	300	400	100	200	300	400
E. coli O157:H7 ATCC 35150	-	-	-	-	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43889	-	-	-	-	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43890	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 43971	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 19585	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC DT104	-	-	-	-	-	-	-	-	-	-	-	-
S. Enteritidis ATCC 13076	-	-	-	-	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19114	-	-	-	-	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19117	-	-	-	-	-	-	-	-	-	-	-	-
St. aureus ATCC 40050	-	-	11.11±0.55	14.20±3.31	-	-	-	11.08±0.48	-	-	-	-
St. aureus ATCC 23235	-	-	-	-	-	-	-	-	-	-	-	9.44±0.54
St. aureus ATCC 25923	-	-	-	-	-	-	-	9.39±0.53	-	-	-	9.52±0.06
Y. enterocolitica ATCC 23715	-	-	-	-	-	-	-	-	-	-	-	-
Y. enterocolitica ATCC 55075	-	-	-	-	-	-	-	-	-	-	-	-
Sh. flexneri NCCP 10852	-	-	-	-	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 22192	-	-	-	-	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 2517	-	-	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29544	-	-	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29004	-	-	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 12868	-	-	-	-	-	-	-	-	-	-	-	-
B. cereus ATCC 14579	-	-	-	-	-	-	-	9.04±0.17	-	-	-	-
B. cereus ATCC 13061	-	-	-	-	-	-	-	-	-	-	-	9.62±0.14
B. cereus ATCC 10876	-	-	-	-	-	-	-	-	-	-	-	-

Supplementary Table S1. (continuous from the previous page) Antibacterial activities of water-based extracts originated from the branches of *P. mume*

Bacterium	FBW (mg/mL)				GBW (mg/mL)				HBW (mg/mL)			
	100	200	300	400	100	200	300	400	100	200	300	400
E. coli O157:H7 ATCC 35150	-	-	-	-	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43889	-	-	-	-	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43890	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 43971	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 19585	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC DT104	-	-	-	-	-	-	-	-	-	-	-	-
S. Enteritidis ATCC 13076	-	-	-	-	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19114	-	-	-	-	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19117	-	-	-	-	-	-	-	-	-	-	-	-
St. aureus ATCC 40050	-	-	-	-	-	-	14.33±1.21	15.17±0.47	-	-	-	-
St. aureus ATCC 23235	-	9.38±0.29	9.75±0.64	10.00±0.63	-	-	-	9.77±0.34	-	-	9.30±0.40	9.20±0.14
St. aureus ATCC 25923	-	-	-	-	-	9.40±0.49	10.52±0.48	9.78±0.18	-	-	9.56±0.17	9.95±0.37
Y. enterocolitica ATCC 23715	-	-	-	-	-	-	-	-	-	-	-	-
Y. enterocolitica ATCC 55075	-	-	-	-	-	-	-	-	-	-	-	-
Sh. flexneri NCCP 10852	-	-	-	-	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 22192	-	-	-	-	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 2517	-	-	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29544	-	-	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29004	-	-	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 12868	-	-	-	-	-	-	-	-	-	-	-	-
B. cereus ATCC 14579	-	-	9.12±0.19	9.75±0.93	-	-	-	9.08±0.16	-	9.85±1.37	9.72±0.27	10.26±0.74
B. cereus ATCC 13061	-	-	9.61±0.32	10.53±0.22	-	-	-	9.83±0.18	-	10.01±0.06	10.94±0.95	10.69±0.67
B. cereus ATCC 10876	-	-	-	10.68±1.70	-	-	-	-	-	9.20±0.60	9.87±0.13	9.44±0.93

Supplementary Table S1. (continuous from the previous page) Antibacterial activities of water-based extracts originated from the branches of *P. mume*

Bacterium	IBW (mg/mL)				JBW (mg/mL)			
	100	200	300	400	100	200	300	400
E. coli O157:H7 ATCC 35150	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43889	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43890	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 43971	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 19585	-	-	-	-	-	-	-	-
S. Typhimurium ATCC DT104	-	-	-	-	-	-	-	-
S. Enteritidis ATCC 13076	-	-	-	-	-	-	-	-
L. *monocytogenes* ATCC 19114	-	-	-	-	-	-	-	-
L. *monocytogenes* ATCC 19117	-	-	-	-	-	-	-	-
St. *aureus* ATCC 40050	-	-	-	-	-	-	-	-
St. *aureus* ATCC 23235	-	-	-	-	-	-	-	-
St. *aureus* ATCC 25923	-	-	-	-	-	-	-	-
Y. enterocolitica ATCC 23715	-	-	-	-	-	-	-	-
Y. enterocolitica ATCC 55075	-	-	-	-	-	-	-	-
Sh. *flexneri* NCCP 10852	-	-	-	-	-	-	-	-
Sh. *flexneri* KCTC 22192	-	-	-	-	-	-	-	-
Sh. *flexneri* KCTC 2517	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29544	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29004	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 12868	-	-	-	-	-	-	-	-
B. *cereus* ATCC 14579	-	-	-	-	-	-	-	-
B. *cereus* ATCC 13061	-	9.88±0.89	10.06±0.29	9.09±0.09	-	-	-	-
B. *cereus* ATCC 10876	-	-	-	-	-	-	-	-

Supplementary Table S2. Antibacterial activities of ethanol-based extracts originated from the branches of *P. mume*

Bacterium	NaOCl (ppm)		ABE (mg/mL)				BBE (mg/mL)			
	100	200	100	200	300	400	100	200	300	400
E. coli O157:H7 ATCC 35150	-	-	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43889	-	-	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43890	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 43971	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 19585	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC DT104	-	-	-	-	-	-	-	-	-	-
S. Enteritidis ATCC 13076	-	-	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19114	-	-	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19117	-	-	-	-	-	-	-	-	-	-
St. aureus ATCC 40050	-	-	-	-	9.69±0.28	10.26±0.77	-	9.53±0.41	10.55±0.43	10.70±0.26
St. aureus ATCC 23235	-	-	-	-	-	-	-	-	-	9.41±0.12
St. aureus ATCC 25923	-	-	-	-	9.04±0.010	10.1±0.63	-	-	-	11.06±0.57
Y. enterocolitica ATCC 23715	-	-	-	-	-	-	-	-	-	-
Y. enterocolitica ATCC 55075	-	-	9.61±0.21	9.73±0.23	10.15±0.09	10.00±0.22	10.55±0.09	10.40±0.26	10.90±0.66	10.90±0.37
Sh. flexneri NCCP 10852	-	-	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 22192	-	-	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 2517	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29544	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29004	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 12868	-	-	-	-	-	-	-	-	-	-
B. cereus ATCC 14579	-	-	-	-	9.43±0.32	10.18±0.46	9.33±0.07	10.00±0.82	10.32±0.16	11.05±0.30
B. cereus ATCC 13061	-	-	-	-	-	11.06±0.46	-	-	-	11.87±0.43
B. cereus ATCC 10876	-	-	9.12±0.14	9.31±0.17	9.42±0.14	10.50±0.83	9.52±0.12	10.10±0.16	11.23±0.03	11.56±0.61

Supplementary Table S2. (continuous from the previous page) Antibacterial activities of ethanol-based extracts originated from the branches of *P. mume*

Bacterium	CBE (mg/mL)				DBE (mg/mL)				EBE (mg/mL)			
	100	200	300	400	100	200	300	400	100	200	300	400
E. coli O157:H7 ATCC 35150	-	-	-	-	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43889	-	-	-	-	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43890	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 43971	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 19585	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC DT104	-	-	-	-	-	-	-	-	-	-	-	-
S. Enteritidis ATCC 13076	-	-	-	-	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19114	-	-	-	-	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19117	-	-	-	-	-	-	-	-	-	-	-	-
St. aureus ATCC 40050	9.37±0.05	10.30±0.12	12.87±1.58	11.69±0.54	-	-	10.60±0.50	10.87±0.56	-	-	-	10.50±1.25
St. aureus ATCC 23235	-	-	-	-	-	-	-	9.25±0.28	-	-	-	-
St. aureus ATCC 25923	-	9.02±0.01	9.60±0.33	9.70±0.42	-	-	10.20±1.49	10.04±0.33	-	-	-	9.42±0.18
Y. enterocolitica ATCC 23715	-	-	-	-	-	-	-	-	-	-	-	-
Y. enterocolitica ATCC 55075	10.10±0.28	11.25±0.34	10.95±0.55	10.22±0.10	10.01±0.27	10.16±0.21	10.28±0.33	11.04±0.28	-	-	-	-
Sh. flexneri NCCP 10852	-	-	-	-	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 22192	-	-	-	-	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 2517	-	-	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29544	-	-	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29004	-	-	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 12868	-	-	-	-	-	-	-	-	-	-	-	-
B. cereus ATCC 14579	-	9.29±0.29	10.70±0.26	9.78±0.10	-	9.66±0.13	10.24±0.15	10.44±0.64	-	-	9.31±0.26	9.41±0.29
B. cereus ATCC 13061	-	-	-	11.22±0.10	-	-	-	11.14±0.54	-	-	-	10.07±0.58
B. cereus ATCC 10876	9.33±0.09	11.01±0.34	10.43±0.12	11.49±1.56	-	10.17±0.28	11.35±0.70	11.63±0.38	-	-	-	9.76±0.06

Supplementary Table S2. (continuous from the previous page) Antibacterial activities of ethanol-based extracts originated from the branches of *P. mume*

Bacterium	FBE (mg/mL)				GBE (mg/mL)				HBE (mg/mL)			
	100	200	300	400	100	200	300	400	100	200	300	400
E. coli O157:H7 ATCC 35150	-	-	-	-	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43889	-	-	-	-	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43890	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 43971	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 19585	-	-	-	-	-	-	-	-	-	-	-	-
S. Typhimurium ATCC DT104	-	-	-	-	-	-	-	-	-	-	-	-
S. Enteritidis ATCC 13076	-	-	-	-	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19114	-	-	-	-	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19117	-	-	-	-	-	-	-	-	-	-	-	-
St. aureus ATCC 40050	-	9.29±0.20	9.63±0.25	10.24±0.11	-	-	9.91±0.03	10.31±0.21	-	9.32±0.17	10.22±0.08	11.36±0.67
St. aureus ATCC 23235	10.57±0.95	9.70±0.10	10.00±0.16	9.89±0.20	-	-	-	9.43±0.28	-	9.72±0.33	9.71±0.18	9.90±0.49
St. aureus ATCC 25923	-	9.39±0.22	9.99±0.54	10.50±0.30	-	9.13±0.06	9.34±0.24	10.20±0.65	-	9.57±0.10	9.97±0.61	10.36±0.18
Y. enterocolitica ATCC 23715	-	-	-	-	-	-	-	-	-	-	-	-
Y. enterocolitica ATCC 55075	10.19±0.40	10.57±0.26	10.75±0.18	11.09±0.38	9.79±0.44	10.25±0.13	10.20±0.15	10.20±0.26	10.09±0.11	11.19±0.11	10.88±0.36	10.93±0.31
Sh. flexneri NCCP 10852	-	-	-	-	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 22192	-	-	-	-	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 2517	-	-	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29544	-	-	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29004	-	-	-	-	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 12868	-	-	-	-	-	-	-	-	-	-	-	-
B. cereus ATCC 14579	-	9.59±0.31	10.16±0.46	10.03±0.61	-	9.32±0.36	9.58±0.20	9.82±0.46	9.13±0.05	9.38±0.31	10.71±0.60	10.56±0.78
B. cereus ATCC 13061	-	-	-	10.81±0.31	-	-	-	10.62±0.93	-	-	-	11.06±0.57
B. cereus ATCC 10876	9.40±0.22	9.77±0.32	10.38±0.13	10.50±0.17	10.00±0.74	9.25±0.14	9.61±0.40	10.07±0.40	9.47±0.16	10.58±0.33	10.45±0.18	11.09±0.29

Supplementary Table S2. (continuous from the previous page) Antibacterial activities of ethanol-based extracts originated from the branches of *P. mume*

Bacterium	IBE (mg/mL)				JBE (mg/mL)			
	100	200	300	400	100	200	300	400
E. coli O157:H7 ATCC 35150	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43889	-	-	-	-	-	-	-	-
E. coli O157:H7 ATCC 43890	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 43971	-	-	-	-	-	-	-	-
S. Typhimurium ATCC 19585	-	-	-	-	-	-	-	-
S. Typhimurium ATCC DT104	-	-	-	-	-	-	-	-
S. Enteritidis ATCC 13076	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19114	-	-	-	-	-	-	-	-
L. monocytogenes ATCC 19117	-	-	-	-	-	-	-	-
St. aureus ATCC 40050	-	-	9.10±0.08	10.27±0.15	-	-	9.45±0.02	10.39±0.20
St. aureus ATCC 23235	-	-	-	9.73±0.72	-	-	-	-
St. aureus ATCC 25923	-	9.37±0.30	9.37±0.09	9.40±0.30	-	-	9.29±0.17	9.47±0.37
Y. enterocolitica ATCC 23715	-	-	-	-	-	-	-	-
Y. enterocolitica ATCC 55075	9.52±0.05	10.07±0.10	9.89±0.36	10.35±0.07	-	10.03±0.11	9.86±0.02	10.70±0.58
Sh. flexneri NCCP 10852	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 22192	-	-	-	-	-	-	-	-
Sh. flexneri KCTC 2517	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29544	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 29004	-	-	-	-	-	-	-	-
Cr. sakazakii ATCC 12868	-	-	-	-	-	-	-	-
B. cereus ATCC 14579	-	9.21±0.01	9.65±0.43	10.03±0.89	-	9.24±0.11	9.43±0.12	10.06±0.59
B. cereus ATCC 13061	-	-	-	10.61±0.73	-	-	-	11.80±1.42
B. cereus ATCC 10876	-	9.46±0.34	9.74±0.04	10.12±0.31	-	9.40±0.34	9.83±0.35	10.10±0.59