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ARTICLE

Antibacterial Activity of Crude *Aronia melanocarpa* (Black Chokeberry) Extracts against *Bacillus cereus*, *Staphylococcus aureus*, *Cronobacter sakazakii*, and *Salmonella* Enteritidis in Various Dairy Foods: Preliminary Study

Dong-Hyeon Kim[1†], Hyun-Woo Lim[1†], Se-Hyung Kim[1], Jun-Man Lee[1], Jung-Whan Chon[1,2†], Kwang-Young Song[1,3*], Dongryeoul Bae[1], Jinhyun Kim[1], Hyunsook Kim[4], and Kun-Ho Seo[1†]

[1]Center for One Health, College of Veterinary Medicine, Konkuk University, Seoul, Korea
[2]College of Veterinary Medicine, Oklahoma State University, Stillwater, OK, USA
[3]Dept. of Biological Engineering, Yanbian University of Science & Technology, Yanji, China
[4]Dept. of Food & Nutrition, College of Human Ecology, Hanyang University, Seoul, Korea

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†These authors contributed equally to this study.

*Corresponding author :
Kwang-Young Song
Center for One Health, College of Veterinary Medicine, Konkuk University, Seoul, Korea, and Dept. of Biological Engineering, Yanbian University of Science & Technology, Yanji, China.
Tel : +82-2-450-4121
Fax : +82-2-3436-4128
E-mail : drkysong@gmail.com

ORCID
Dong-Hyeon Kim
0000-0003-0585-2432
Hyun-Woo Lim
0000-0001-6013-8106
Se-Hyung Kim
0000-0003-4747-7394
Jun-Man Lee
0000-0002-6639-6648
Jung-Whan Chon
0000-0003-0758-6115
Kwang-Young Song
0000-0002-5619-8381
Dongryeoul Bae
0000-0002-4754-5580
Jinhyun Kim
0000-0002-5268-2723
Hyunsook Kim
0000-0001-7345-4167
Kun-Ho Seo
0000-0001-5720-0538

Abstract

In this study, the antibacterial activity of *Aronia melanocarpa* (black chokeberry) ethanol extract against *Bacillus cereus, Staphylococcus aureus, Cronobacter sakazakii*, and *Salmonella* Enteritidis was investigated using the spot-on-lawn assay. The results showed that this extract exhibited antibacterial activities against *Bacillus cereus* (complete inhibition) and *Staphylococcus aureus* (partial inhibition), but did not inhibit the growth of *Cronobacter sakazakii* and *Salmonella* Enteritidis. This study shows that the *Aronia melanocarpa* (black chokeberry) ethanol extract was more effective against Gram-positive bacteria than Gram-negative bacteria. Hence, it is suggested that *Aronia melanocarpa* could be a useful food supplement, and could be utilized as a naturally derived additive for maintaining the safety of various dairy products. Furthermore, future research should be conducted to examine the possibility of using such products as functional ingredients for improving the anti-oxidative and anti-inflammatory activities of food products.

Keywords

Aronia melanocarpa (black chokeberry), antibacterial activity, *Bacillus cereus*, *Staphylococcus aureus*

Introduction

Recently, the usability of natural extracts has dramatically increased for improving human's health (Jakobek *et al.,* 2007; Jurikova *et al.,* 2017). Especially, among various natural compounds, Aronia has received important attention in recent years due to their various positive effects for human's health (Kokotkiewicz *et al.,* 2010; Park and Hong, 2014; Banjari *et al.,* 2017). And Aronia could be classified into three types of by color; (1) Red chokeberry as *Aronia arbutifolia.* (2) Purple chokeberry as *Aronia prunifolia*, and (3) Black chokeberry as *Aronia melanocarpa* (Chrubasik *et al.,* 2010; Borowska and Brzoska, 2016). Especially, among three types, many researchers had a focus on *Aronia melanocarpa* (black chokeberry) (Borowska and Brzoska, 2016; Sic Zlabur *et al.,* 2017). In general, *Aronia melanocarpa* (black chokeberry) was originated from the eastern parts of North America and Canada, and then was introduced into Europe about a century ago (Chrubasik *et al.*, 2010; Jurikova *et al.*, 2017). Since the middle of the 20th century, *Aronia melanocarpa* (black chokeberry) have been cultivated and medicinally used in the former Soviet Union (Chrubasik *et al.*, 2010; Borowska *et al.*, 2017) (Fig. 1). According to heretofore known, *Aronia melanocarpa* (black chokeberry) contained many bioactive compounds such as anthocyanins, carotenoids, fatty acids, flavonoids, phenolic compounds, vitamins and so on (Bräunlich *et al.*, 2013; Nguyean and Hwang, 2016; Sic Zlabur *et al.*, 2017). Furthermore, *Aronia melanocarpa* (black chokeberry) have a greater content of phenolic constituents than most of the other berries, because of reflecting antioxidative potency (Chrubasik *et al.*, 2010; Banjari *et al.*, 2017) (Fig. 2). Until now, the most common *Aronia melanocarpa* (black chokeberry) products were powder, syrup, juice, fruit jelly, fruit tea, liquor, wind, and so on (Chrubasik *et al.*, 2010). Therefore, it would be the best way for the application of *Aronia melanocarpa* (black chokeberry) to add to various juices of other fruit species for improving both the organoleptic and nutritional characteristics of the final product (Kokotkiewicz *et al.*, 2010; Banjari *et al.*, 2017).

Foodborne pathogenic bacteria could be transmissible to humans through milk and dairy products, and also could cause various disease (Vasavada, 1988; Zeinhom and Abdel-Latef, 2014). Hence, attention would be focused on milk and dairy food contaminated with foodborne pathogenic bacteria, because milk was considered as a high quality and nutrition-rich food for human beings (Theresa and Nicklas, 2003). Especially, the high quality of milk is mainly referred to its composition and hygiene that are provided during the production and storage of milk (El-Baz *et al.*, 2017). However, if the hygienic conditions are not applied properly, the milk could be contaminated by microorganisms leading to its early spoilage (Oliver *et al.*, 2005; Nanu *et al.*, 2007). Furthermore, milk and dairy products could harbor a variety of microorganisms and also could be important sources of foodborne pathogens (El-Baz *et al.*, 2017). The presence of foodborne pathogens in milk and dairy products would be due to excretion from the udder of an infected animal and to direct/indirect contact with contaminated sources in the dairy farm environment (Vasavada, 1988; Oliver *et al.*,

Fig. 1. *Aronia melanocarpa* known as the black chokeberries was originated from eastern North America and was commonly found in wet woods and swamps (Banjari *et al.*, 2017).

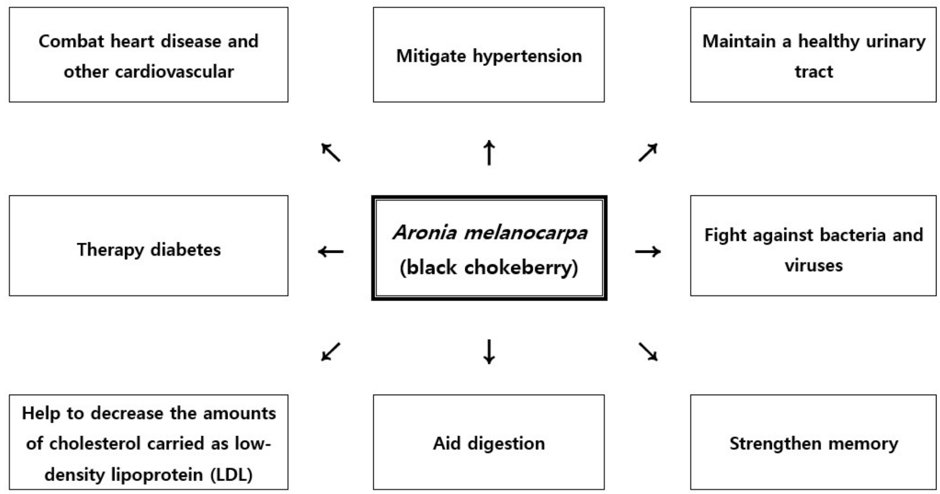

Fig. 2. Various bioactive effects of *Aronia melanocarpa* (black chokeberry) for improving human's health (Banjari *et al*., 2017; Jurikova *et al*., 2017; Sic Zlabur *et al*., 2017).

2005; Nanu *et al.,* 2007; Zeinhom and Abdel-Latef, 2014; El-Baz *et al.,* 2017). In this study, among various foodborne poisoning bacteria, 4 kinds of foodborne poisoning bacteria - *Staphylococcus aureus, Cronobacter sakazakii, Salmonella* Enteritidis., and *Bacillus cereus* - which occur most frequently in milk and dairy products were selected and investigated.

Until now, there was very few studies about the inhibition against various foodborne pathogenic bacteria using the crude extracts from *Aronia melanocarpa* (black chokeberry). Therefore, this study was aimed at investigating the antibacterial activity of *Aronia melanocarpa* (black chokeberry) on *Staphylococcus aureus, Cronobacter sakazakii, Salmonella* Enteritidis., and *Bacillus cereus* so as to improve the quality of various dairy products by adding them as natural food additives.

Materials and Methods

1. Materials and extraction

The powder of *Aronia melanocarpa* (black chokeberry) manufactured in Finland was purchased from Kiantama OY (Finland). The dried powder of *Aronia melanocarpa* (black chokeberry) was macerated in 95% ethanol for 48 hours with occasionally stirring at ambient temperature. And then, the soluble ingredients were concentrated by rotary evaporator at 50℃ until dryness, and the yield was obtained by ethanol extraction type. These stock solutions were filtrated through 0.2 mm Millipore and stored at −20℃ before use.

2. Bacterial strains and culture condition

Staphylococcus aureus ATCC6538, *Cronobacter sakazakii* KCTC2949, *Salmonella* Enteritidis 110, and *Bacillus cereus* ATCC10876 were obtained from Center for One Health, College of Veterinary, Konkuk University in Seoul, Korea. These bacteria were grown on nutrient agar (NA) (Oxoid, UK) overnight. Colonies were transferred into tubes containing cryopreservation fluid according to the instruction of the manufacturer (Original Microbiology Bead Storage System, STS, Technical Service Consultants Limited, UK). The beads were stored at −70℃ until use.

3. Antibacterial susceptibility testing

The antibacterial activity of *Aronia melanocarpa* (black chokeberry) were tested on *Staphylococcus aureus* ATCC6538, *Cronobacter sakazakii* KCTC2949, *Salmonella* Enteritidis 110, and *Bacillus cereus* ATCC10876 using by the spot-on-lawn assay with some modifications (Cadirci and Citak, 2005). All test bacteria were cultured on Mueller-Hinton broth (MHB; Difco) and incubated at 37℃±0.5 for one day. The culture broth was diluted using MHB to 0.5 McF and spread onto Mueller-Hinton agar (MHA; Difco) using sterilized cotton swabs. A total of negative control (0 μL), 1X (10 μL), 2X (20 μL), and 3X (30 μL) of *Aronia melanocarpa* (black chokeberry) extract was directly dropped onto the surface of the MHA, respectively. The plates were incubated for one day at 37±0.5℃, and the inhibition zone was observed.

4. Statistical analysis

All experiments were carried out independently in triplicate experiments. The inhibition of various concentration of *Aronia melanocarpa* (black chokeberry) ethanol extracts against *Staphylococcus aureus* ATCC6538, *Cronobacter sakazakii* KCTC2949, *Salmonella* Enteritidis 110, and *Bacillus cereus* ATCC10876 were evaluated by one-way analysis of variance (ANOVA). Statistical significance was accepted at the p=0.05 level.

Results and Discussion

The ethanol extract of *Aronia melanocarpa* (black chokeberry) showed various levels of antibacterial activity when tested by the spot-on-lawn assay (Fig. 3). These results obtained showed that ethanol extract of *Aronia melanocarpa* (black chokeberry) exhibited antibacterial activities against *Bacillus cereus*, and the inhibitory activity of *Aronia melanocarpa*'s (black chokeberry) ethanol extracts was shown as a whole regardless of the increase in the concentratin (Fig. 3). Although the final result of antimicrobial activity of *Aronia melanocarpa*'s (black chokeberry) extract by ethanol against *Staphylococcus aureus* was negative, it did not seem to be effective at all (Fig. 3). Hence, it is likely that the higher concentration, the more effective it will be. Whereas the other two bacteria - *Cronobacter sakazakii,* and *Salmonella* Enteritidis - did not show any inhibition by the ethanol extract of *Aronia melanocarpa* (black chokeberry) (Fig. 3). Also, Park and Hong (2014) reported that the physiological activities of *Aronia melanocarpa*'s (black chokeberry) extracts was affected by various extraction solvent such as hot water, methanol, and ethanol. The yield of *Aronia melanocarpa*'s (black chokeberry) extracts by ethanol extract was 84.50%, by hot water extract was 84.05%, and by methanol extract was 76.20%, respectively. But, regardless of the extraction solvents,

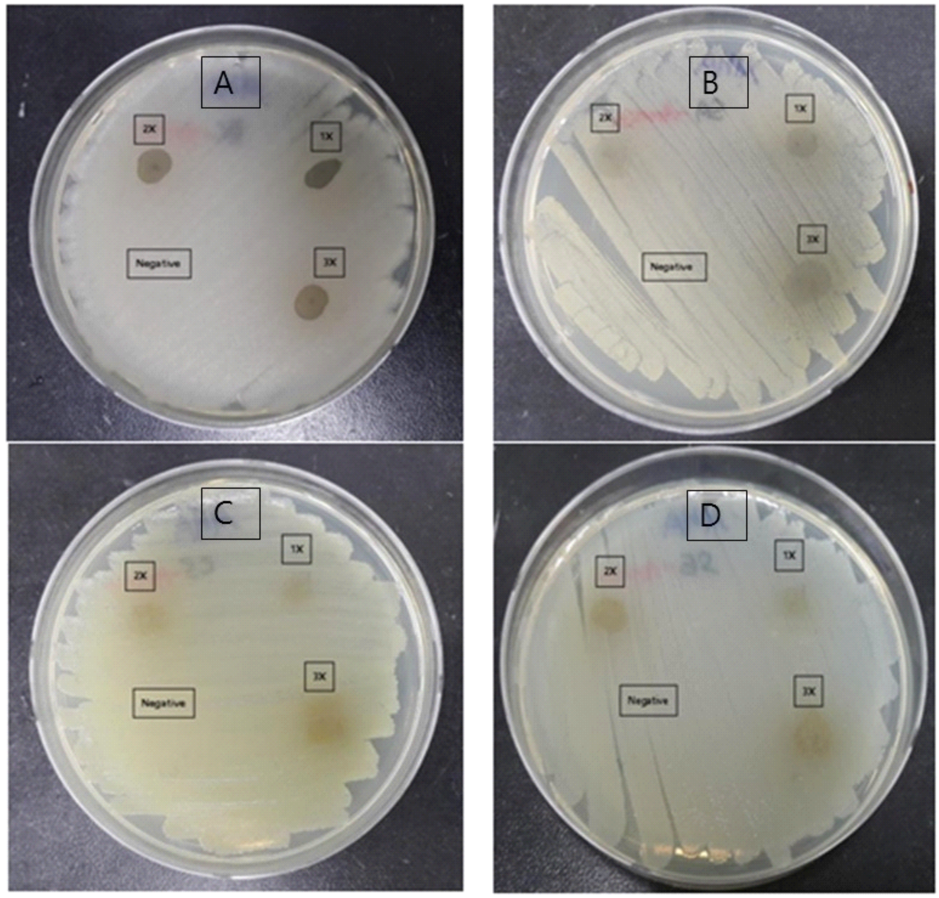

Fig. 3. The antibacterial activity on various concentration of *Aronia melanocarpa*'s (black chokeberry) ethanol extracts against *Bacillus cereus* ATCC10876 (A), *Staphylococcus aureus* ATCC6538 (B), *Cronobacter sakazakii* KCTC2949 (C), and *Salmonella* Enteritidis 110 (D) tested by the spot-on-lawn assay.

the total sugar content was 35.56~37.68 g/100 g (Park and Hong, 2014). Therefore, *Aronia melanocarpa* (black chokeberry) was extracted with ethanol in this study.

Banjari *et al.* (2017) reported that *Aronia melanocarpa*'s (black chokeberry) extracts could have various bioactive compound for improving human's health (Fig. 4). In general, the efficacy comparison of natural materials was carried out by solvent extraction (methanol, ethanol, water, and so on) (Jakobek *et al.,* 2007; Borowska *et al.*, 2017). According to Park and Hong (2014), the total anthocyanin content by methanol extract was 395.10 mg/100 g, by ethanol extract was 318.61 mg/100 g, and by hot water extract was 252.82 mg/100 g, respectively. Also, the among various anthocyanin composition, the cyanidin-3-galactoside was 364.65 mg/100 g, the cyanidin-3-arabinoside was 163.06 mg/100 g, and the cyanidin-3-glucoside was 35.69 mg/100 g by the methanol extract (Park and Hong, 2014).

Also, the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activities by methanol and ethanol extracts at 100~1,000 μg/mL was 8.90~69.21%, 7.96~70.01%, respectively, and the superoxide radical scavenging activities of all the extracts would be improved

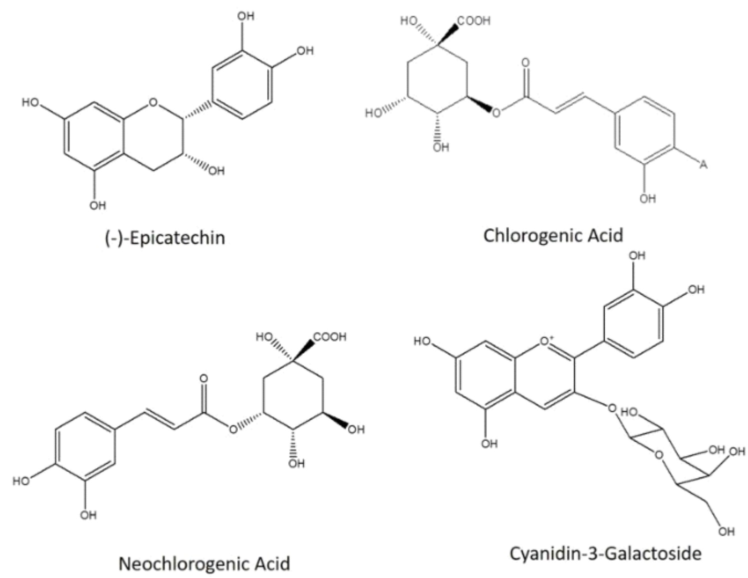

Fig. 4. Various bioactive compounds isolated from *Aronia melanocarpa* (black chokeberry) (Banjari *et al*., 2017).

with an increase in the treatment concentration (Park and Hong, 2014). The ferric reducing antioxidant power (FRAP) by methanol and ethanol extract at 100~1,000 μg/mL was 67.32~812.78 μM, 57.14~817.87 μM, respectively (Park and Hong, 2014). The tyrosinase inhibitory activity by ethanol extract was 23.03~33.82% (100~1,000 μg/mL) and it was higher than that of the other extracts (Park and Hong, 2014). Especially, the cancer cell growth inhibition activity by ethanol extract was 76.86% at 1,000 μg/mL on HeLa cell line and it was significantly higher than hot water and methanol extracts (Park and Hong, 2014).

Especially, through increasing outbreak of various drug-resistant pathogenic bacteria, the potential antibacterial activity by the unknown plant-derived substance became increasingly interested (Savoia, 2012; Liepina *et al.,* 2013). Liepina *et al.* (2013) reported that the antimicrobial activity of *Aronia melanocarpa* (black chokeberry) and *Sorbus aucuparia* extracted by aqueous and ethanolic method was exhibited antibacterial activity against Gram-positive bacteria *Bacillus cereus* and *Staphylococcus aureus*, but they did not have antifungal influence. In this study, although a similar result was obtained in *Bacillus cereus*, *Staphylococcus aureus* was not inhibited.

Also the extracts inhibited the growth of Gram-negative bacterium *Pseudomonas aeruginosa* but did not have influence on *Escherichia coli* (Liepina *et al.,* 2013). In this study, similar trends in previous studies were found, and *Cronobacter sakazakii* and *Salmonella* Enteritidis were not inhibited.

In general, ethanolic extracts demonstrated equal or larger antibacterial effects than aqueous extracts (Liepina *et al.,* 2013). Also, according to several previous literatures, better results usually have been obtained with alcoholic than with aqueous extracts (Krisch *et al.*, 2008; Chitra *et al.,* 2012; Liepina *et al.,* 2013). Since ethanol was the most commonly used organic solvent, the finished products could be relatively safely used

(Low Dog, 2009).

Furthermore, according to the report of Banjari *et al.* (2017), *Aronia melanocarpa* (black chokeberry) had been reported to possess (1) anti-achlorhydia, (2) anti-hypertension, (3) anti-hemorrhoids, (4) anti-convalescence, (5) anti-avitaminoses, (6) anti-atherosclerosis, (7) anti-diabetes, and so on (Fig. 2).

Over all, *Aronia melanocarpa* (black chokeberry) berry juice and plant extract has displayed evidence as a potent modulator of hyperglycemia-related oxidative stress which is directly correlated with its complications, in particular, cardiovascular disease (Jurikova *et al.,* 2017). Also, people with increased cardiovascular risk (i.e., with abdominal obesity, mild hypercholesterolemia, grade I hypertension) seem to benefit more from the consumption of *Aronia melanocarpa* (black chokeberry) berry juice and extract (Jakobek *et al.,* 2007; Sic Zlabur *et al.,* 2017).

In conclusion, this study demonstrated the potential of *Aronia melanocarpa* (black chokeberry) to inhibit the growth of *Bacillus cereus* and *Staphylococcus aureus* as antimicrobial activity, except for *Cronobacter sakazakii* and *Salmonella* Enteritidis. Hence, this study indicated that *Aronia melanocarpa* (black chokeberry) might be applicable in natural medicine and food as a source of antibacterial products, and also could be a useful for functional food material in the food industry. Additionally, the *Aronia melanocarpa* (black chokeberry) fruit and its extract appear to have a multitude of beneficial effects against other disease conditions, which could potentially be explored and scientifically substantiated through systematic studies and investigations.

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