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ARTICLE

Antimicrobial Activity of *Hibiscus sabdariffa* L. (Roselle) Powder against Food-Borne Pathogens Present in Dairy Products: Preliminary Study

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Abstract

The antimicrobial activity of the ethanol extract of *Hibiscus sabdariffa* L. (Roselle) powder against various food-borne pathogens was tested using the lawn diffusion assay. The results showed that the ethanol extract exhibited antimicrobial activities against *Staphylococcus aureus* (total inhibition), *Salmonella enteritidis* (partial inhibition), *Listeria monocytogenes* (partial inhibition), *Escherichia coli* (partial inhibition), *Cronobacter sakazakii* (partial inhibition), and *Bacillus cereus* (partial inhibition). Therefore, it is strongly recommended that *Hibiscus sabdariffa* L. (Roselle) should be considered for use as a natural food-grade additive for the inhibition of various food-borne pathogens, including both gram-positive and gram-negative pathogens, and the improvement of the overall quality of various dairy products, including milk.

Keywords

Hibiscus sabdariffa L. (Roselle) powder, antimicrobial activity, food-borne pathogens, lawn-diffusion assay

Introduction

Hibiscus sabdariffa L. (Roselle) is an herbaceous plant of the genus *Hibiscus* of the Malvacease family, and widely cultivated in Africa, Asia, China, Egypt, Mexico, North America, Senegal, Tanzania, Thailand, and so on [1-5]. In general, *Hibiscus sabdariffa* L. (Roselle) is an annual (or perennial) herb which could grow to 2.0-2.5 m height, and the leaves of *Hibiscus sabdariffa* L. (Roselle) are arranged alternately on the cylindrical-smooth red stems with deeply 3-5 palmately lobed and 8-15 cm length [1, 3, 6, 7], and the flowers of *Hibiscus sabdariffa* L. (Roselle) are 8-10 cm in diameter. The flowers are white to pale yellow with a dark red spot at the base of each petal, and also had a stout fleshy calyx at the base (1-2 cm wide and enlarging to 3-3.5 cm), fleshy and bright red as the fruit matures [1, 6]. Table 1 shows the composition and content of calyces, leaves, and seeds of *Hibiscus sabdariffa* L. (Roselle) in detail [3-4, 6]. Until now, the red calyx of *Hibiscus sabdariffa* L. (Roselle) was used in the preparation of various beverages, because the calyx contained a variety of bioactive ingredients [1, 5, 8-10]. Based on the results of various previous studies, the main ingredients that had bioactive ingredients were polyphenolic elements which could demonstrate the antimicrobial effect (Fig. 1) [1, 4, 11-13]. Moreover, the ingredients extracted from *Hibiscus sabdariffa* L. (Roselle) showed various pharmacological activities of anticholesterol, antidiabetic,

Table 1. Composition of calyces, leaves, and seeds of *Hibiscus sabdariffa* L. (Roselle)

Hibiscus sabdariffa L. (Roselle)						
	Calyces (unit: 100 g)	Leaves (unit: 100 g)	Seeds (unit: %)			
Protein	1.9 g	3.3 g	27.78%	Crude protein		
Fat	0.1 g	0.3 g	21.85%	Crude fat		
Carbohydrate	12.3 g	12.3 g	21.25%	Carbohydrate		
Fibre	2.3 g	-	6.2%	Ash→	Potassium	1,329 mg
Vitamin C	14 mg	54 mg			Sodium	659 mg
β-Carotene	300 μg	4,135 μg			Calcium	647 mg
Calcium	1.72 μg	-			Phosphorus	510 mg
Iron	57 μg	4.8 mg			Magnesium	443 mg
Phosphorus	-	214 mg	20.84%	Palmitic acid	Major saturated fatty acids	
Thiamine	-	0.45 mg	5.88%	Stearic acid		
Riboflavin	-	0.45 mg	39.31%	Linoleic acid	Main unsaturated fatty acids	
			32.06%	Oleic acid		

antihypertensive, antitumoral, hepatoprotective, hypolipidemic, nephronprotective, renal/diuretic, and so on (Fig. 1) [1, 7, 11, 14].

Until now, among many foods, milk was generally known as a complete and nutritious food. However, if people consumed various dairy products contaminated with various food-borne poisoning bacteria, they could easily become infected and develop serious diseases [15-17]. But dairy products, including milk, can be a harbor of a number of food-borne pathogens that can adversely affect people's health, and also the outbreak of food poisoning involving dairy products have been steadily increasing each year [18]. In general, the survival ability of food-borne pathogens in dairy products including milk could be largely due to two major factors. the first is the indirect (and/or direct) touch with contaminated materials in the environment of dairy farming, and the second is the excretion from the udder of an infected mammals such as cows, goats, sheep, and other animals [15-20]. Recently, according to US FDA, unpasteurized raw milk could carry dangerous food-borne pathogens such as *Campylobacter, Escherichia coli, Listeria, Salmonella,* and others that could induce various serious diseases commonly called food poisoning [21]. For this reason, research is desperately needed to prevent various

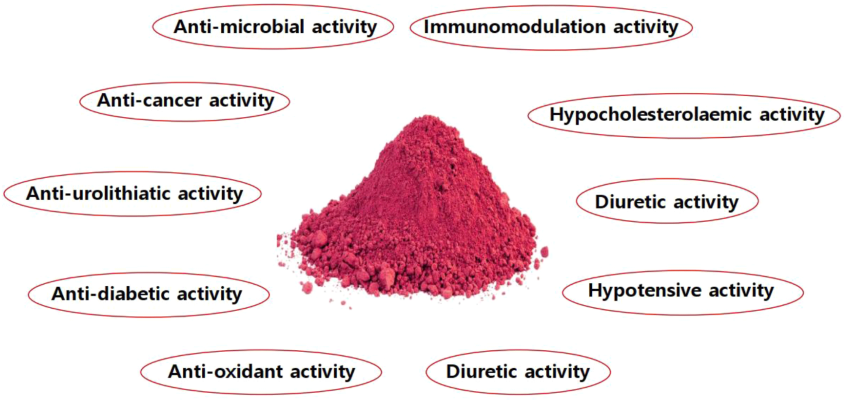

Fig. 1. Various biological activity of *Hibiscus sabdariffa* L. (Roselle) plant.

food-borne pathogens through the addition of natural substances to various dairy products including milk. Among the various types of food-borne pathogens that survived in dairy products including milk, 6 different types of food-borne pathogens that were frequently found in dairy products including milk have been selected and investigated in this study.

Hence, the major purpose of this present study was to observe the inhibition of *Hibiscus sabdariffa* L. (Roselle) against 6 different food-borne pathogens such as *Staphylococcus aureus* (Gram positive), *Salmonella* Enteritidis (Gram negative), *Listeria monocytogenes* (Gram positive), *E. coli* (Gram negative), *Cronobacter sakazakii* (Gram negative), and *Bacillus cereus* (Gram positive), and then to determine whether *Hibiscus sabdariffa* L. (Roselle) powder as a natural food-grade additives can be added to various dairy products including milk as well.

Materials and Methods

1. Ethanol extraction of *Hibiscus sabdariffa* L. (Roselle) powder

Hibiscus sabdariffa L. (Roselle) powder was produced in Poland and was purchased from Lilly Super Food (Korea). According to method of Lim et al. [22], *Hibiscus sabdariffa* L. (Roselle) powder was drenched in 95% ethanol at 25℃ for 48 hours. Then the soluble ingredients was concentrated in rotary evaporator until it was almost dry, and then filtered using a 0.22 μm filter (Millipore, Bedfor, MA, USA). Before the extracts obtained in this study was tested, it was kept in the freezer (about at −20℃).

2. Six different food-borne pathogens

In this study, 6 different food-borne pathogens were tested. *Staphylococcus aureus* ATCC 6538, *Salmonella* Enteritidis 110, *Listeria monocytogenes* ATCC 51776, *E. coli* 23716, *Cronobacter sakazakii* KCTC 2949, and *Bacillus cereus* ATCC 10876 were provided by KU Center for Food Safety and Department of Public Health, College of Veterinary, Konkuk University (Seoul, Korea). Six different food-borne pathogens were grown on nutrient agar (Oxoid, UK) for 24 hours. Colonies were transferred into tubes containing cryopreservation fluid, and they were stored in the deep freezer (about at −70℃) until use.

3. Antimicrobial activity measured using lawn-diffusion assay

According to method of Lim et al. [22], the inhibition of Hibiscus sabdariffa L. (Roselle) were tested on Staphylococcus aureus ATCC 6538, Salmonella Enteritidis 110, Listeria monocytogenes ATCC 51776, E. coli 23716, Cronobacter sakazakii KCTC 2949, and Bacillus cereus ATCC 10876 using by the lawn-diffusion assay. Six different food-borne pathogens were cultured for 24 hours at 37℃ in Mueller-Hinton broth (Difco Laboratory, USA). Immediately cultivated strains were adjusted to 0.5 McFarland using Mueller-Hinton broth (Difco Laboratory). It was then spread to Mueller-Hinton agar (Difco Laboratory) using sterile cotton swabs, and the negative control (0 μL), 1×

(10 μL), 2× (20 μL), and 3× (30 μL) of Hibiscus sabdariffa L. (Roselle) extract were quickly dropped directly onto the surface of the Mueller-Hinton agar (Difco Laboratory), respectively. And then, it was incubated at 37℃ for 24 hours, and the zone of inhibition was visually verified.

Results and Discussion

Fig. 2 showed the inhibition at various concentrations (control, 1×, 2×, and 3×) extracted from *Hibiscus sabdariffa* L. (Roselle) powder with ethanol using the lawn-diffusion assay. These results obtained in this study showed that extracts from *Hibiscus sabdariffa* L. (Roselle) powder using ethanol showed the inhibition against 6 different food-borne pathogens such as *Staphylococcus aureus* ATCC 6538 (total inhibition), *Salmonella* Enteritidis 110 (partial inhibition), *Listeria monocytogenes* ATCC 51776 (partial inhibition), *E. coli* 23716 (partial inhibition), *Cronobacter sakazakii* KCTC 2949 (partial inhibition), and *Bacillus cereus* ATCC 10876 (partial inhibition) (Fig. 2). Namely, the inhibition was demonstrate regardless of the increase in the concentration of extracts from *Hibiscus sabdariffa* L. (Roselle) powder using ethanol (Fig. 2). As a result, *Hibiscus sabdariffa* L. (Roselle) powder showed the ability to inhibit both gram-positive and gram-negative food-borne pathogens. Therefore, this was assessed to be a sufficient possibility as an additive to dairy products including milk.

According to Riaz and Chopra [1], the antimicrobial activity of *Hibiscus sabdariffa* L. (Roselle) extract was generally known to be caused by the flavonoids. Namely, it took the capability for making the complex with the bacterial cell walls and the permeability of bacterial cell surface to the extract [1].

Also, Jung et al. [23] reported that the antimicrobial activity of *Hibiscus sabdariffa* L. (Roselle) was influenced by different extraction solvent such as water and ethanol. The antimicrobial activity of *Hibiscus sabdariffa* L. (Roselle) water and ethanol extracts was tested with *Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538 and *E. coli* ATCC 8739 and the inhibition of *Hibiscus sabdariffa* L. (Roselle) ethanol extract against *B. subtilis* and *S. aureus* was slightly higher than *Hibiscus sabdariffa* L. (Roselle) water extract [23]. But this difference was not significant [23]. Therefore, in this study, *Hibiscus sabdariffa* L. (Roselle) was extracted using ethanol.

Thiripurasundari et al. [24] showed the antimicrobial activity against *Staphylococcus* spp. by using aqueous extracts of seed of *Ricinus communis* and aqueous extracts of leaves of *Tridax procumbens, Hibiscus sabdariffa, Majorana hortensis* and *Origanum majorana*. Of the five different plants tested, the aqueous seed extracts showed zone of inhibition against *Staphylococcus* spp [24].

The extracts of *Hibiscus sabdariffa* L. (Roselle) showed a wide range of antimicrobial activity against various food-borne pathogens [25]. Higginbotham et al. [25] examine the antimicrobial activity of *Hibiscus sabdariffa* L. (Roselle) aqueous extracts against *E. coli* O157:H7 and *Staphylococcus aureus* in a microbiological medium and milk of various fat concentrations. It was shown that the potential use of *Hibiscus sabdariffa* L. (Roselle) extracts to inhibit the growth of food-borne pathogens in various beverages

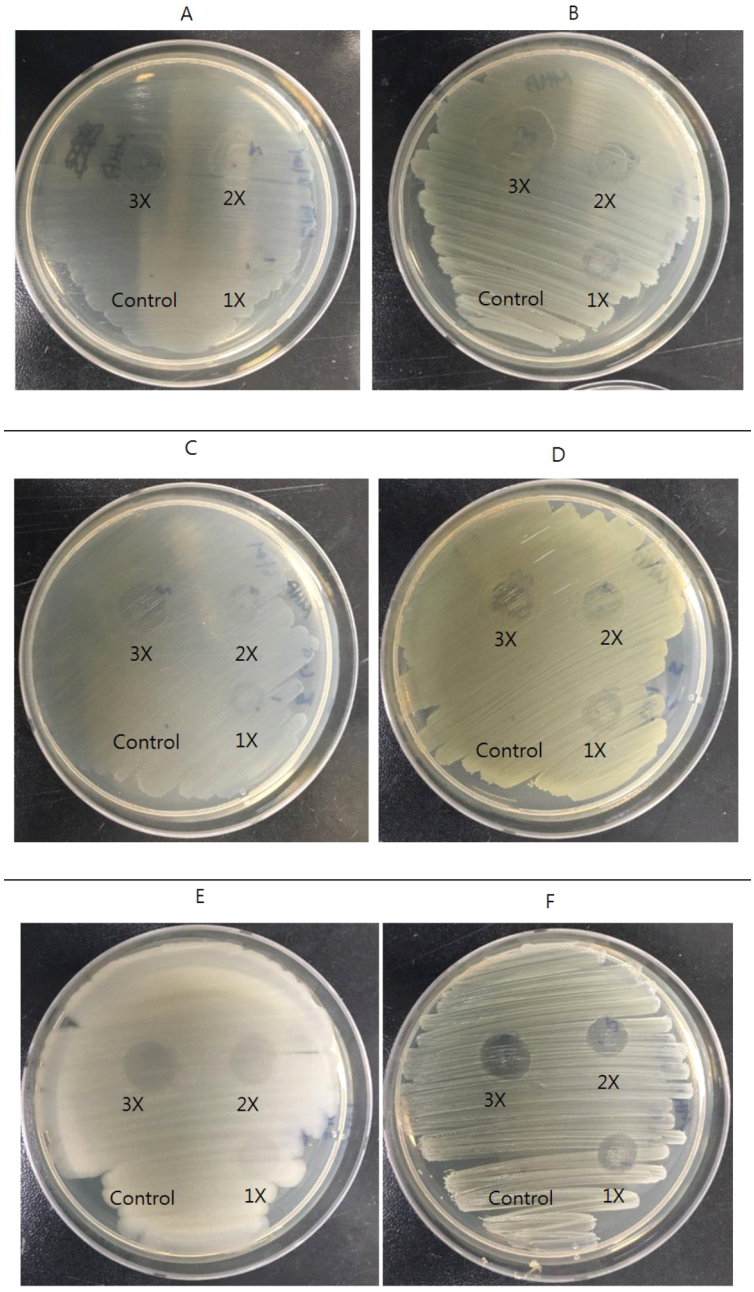

Fig. 2. The antimicrobial activity on various concentration (control, 1X, 2X, and 3X) of extracts from *Hibiscus sabdariffa* L. (Roselle) powder using ethanol against *Listeria monocytogenes* ATCC 51776 (A), *Salmonela* Enteritidis 110 (B), *Escherichia coli* 23716 (C), *Cronobacter sakazakii* KCTC 2949 (D), *Bacillus cereus* ATCC 10876 (E), and *Staphylococcus* aureus ATCC 6538 (F) investigated by the lawn-diffusion assay.

and foods. According to Chao and Yin [26], ethanol and aqueous extracts of *Hibiscus sabdariffa* L. (Roselle) (5 or 10 mg added to 100 g of ground beef or 100 mL of apple juice) showed dose-dependent antimicrobial activity against *B. cereus, E. coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* serovar Typhimurium, and *S. aureus* after 3 days of storage conditions, with ethanol extracts demonstrating greater antimicrobial

activity [26]. Navarro Garcia et al. [27] reported that that the minimum inhibitory concentrations for aqueous extracts of calyx of *Hibiscus sabdariffa* L. (Roselle) was 0.5 and 1.0 mg/mL for *S. aureus* ATCC 6358 and *E. coli* ATCC 8937, respectively. Also, the use of aqueous extracts of *Hibiscus sabdariffa* L. (Roselle) (100%, vol/vol) as a wash on lettuce *against E. coli* O157:H7 and sprouts against *S. enterica* was performed, and bacterial populations of approximately 4 Log CFU of *E. coli* O157:H7 and *S. enterica* per g were eliminated after 24 h [28]. Methanol extracts of the calyces of *Hibiscus sabdariffa* L. (Roselle) demonstrated the antimicrobial activity against *Bacillus cereus, Bacillus stearothermophilus, Clostridium sporogenes, E. coli, Klebsiella pneumoniae, Micrococcus luteus, Pseudomonas* spp. *Serratia marcescens,* and *S. aureus* at concentrations of 0.30 to 1.30 mg/mL [29]. Bokaeian et al. [30] examine the antimicrobial activity of flower extract of *Hibiscus sabdariffa* L. (Roselle) using ethanol against antibiotic-resistant *E. coli* and *Staphylococcus aureus* isolated from the urinary tract infection. *E. coli* showed the resistance to tetracyclin, erythromycin and cefixime, whereas *Staphylococcus aureus* showed the resistance to vancomycin and cefixime [30]. Also the highest value of the minimum inhibitory concentrations was exhibited to be 20 mg/mL against two *E. coli*, whereas the least value of the minimum inhibitory concentrations and to be 1.25 mg/mL against three *Staphylococcus aureus* [30]. Calyces of *Hibiscus sabdariffa* L. (Roselle) showed strong antibacterial properties for *Acinetobacter baumannii*, a multi-drug resistant bacteria [31]. The potential for *Hibiscus sabdariffa* L. (Roselle) to be used as an antimicrobial agent has been identified [31, 32].

In conclusion, this study demonstrated the potentiality of *Hibiscus sabdariffa* L. (Roselle) powder to inhibit the growth of *Listeria monocytogenes* ATCC 51776, *Salmonella* Enteritidis 110, *E. coli* 23716, *Cronobacter sakazakii* KCTC 2949, *Bacillus cereus* ATCC 10876, and *Staphylococcus aureus* ATCC 6538. Hence, *Hibiscus sabdariffa* L. (Roselle) could be directly used in the preparation of functional dairy products as well as naturel food additives with antimicrobial activity. To meet these needs, it is strongly required that further research should be intensively conducted in the future.

Conflict of Interest

The authors declare no potential conflict of interest.

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