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Antimicrobial Effect of Roselle (*Hibiscus sabdariffa* L.) Petal Extracts on Food-Borne Microorganisms

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Abstract In this study, we investigated the antimicrobial effect of 14 different herbal petal extracts on various foodborne and food spoilage bacteria. Herbal petal extracts were prepared with 70% ethanol followed by sequential hexane, chloroform, ethyl acetate, n-butanol, and water fractionation. Antimicrobial activity was highest in the ethanol fraction from roselle (*Hibiscus sabdariffa* L.) petals as determined by the paper disc method. The roselle ethanol extract retarded the growth of food spoilage bacteria in *kimbap* (rice rolled in dried laver). Foodborne microorganisms (e.g. *Bacillus cereus* and *Clostridium perfringens*), on the other hand, were most efficiently inhibited by the ethyl acetate fraction of the roselle petal extract as determined by growth inhibition curves. Our study shows that roselle petals harbor antimicrobial activity against foodborne and food spoilage microorganisms. The critical ingredient is highly enriched in the ethyl acetate fraction of the extract.

Keywords: roselle (*Hibiscus sabdariffa* L.), antimicrobial activity, food-borne microorganism

Introduction

Historically, the word 'herb' (Latin: '*herba*', grass) refers to common grass. In modern English, however, 'herbs' generally incorporate any plant, whose stem, leaves, flower, roots, or seeds have medicinal value. Herbs hence comprise annual plants, biennial plants, perennial plants (i.e. evergreen trees and deciduous trees), and bulbous plants (1).

While herbs have a long history as taste and aroma additives in Western meals (1), their benefits for the human health have only recently been established, which resulted in a trend targeting them as medicine rather than spices. Herbs harbor proven activity against conditions that can only be cured with difficulty, such as allergies, chronic pains, hyperlipidemia, cancer, arthritis, and cardiac diseases (2-4) and they have the added benefit of minimal side effects. One out of three Americans uses herbs in form of alternative medicine, which is reflected in the sales of herbs now exceeding 2 billion dollars per year in the US (5). It is hence of great interest to further examine and establish the medicinal properties of herbs and the specific characteristics of their active ingredients to fully harness the great diversity of herbal applications ranging from their use as drugs to food and drink preservatives.

In this study, we investigated antimicrobial effects of herbal extracts obtained from 14 different herb petals marketed for human consumption. Using fractionation, we found that the ethyl acetate extract from roselle (*Hibiscus sabdariffa* L.) petals has strong antimicrobial activity against foodborne and food spoilage bacteria.

Materials and Methods

Herbs and chemicals We tested selected herbs, including chamomile (*Anthemis nobilis* L.), thyme (*Thymus* spp.), tennel (*Foeniculum vulgare* M.), lavender (*Lavandula* spp.), lemon balm (*Melissa officinalis* L.), rose hip (*Rosa canina* L.), rosemary (*Rosmarinus officinalis* L.), lemongrass (*Cymbopogon citratus.*), jasmine (*Jasminium officinale*), pepermint (*Mentha piperita* L.), blue mallow (*Malva sylvestris* L.), orange passion flower, rose flower, and roselle (*Hibiscus sabdariffa* L.). Ampicillin was purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents originated from commercial suppliers and were of the highest grade available.

Bacterial strains *Escherichia coli* O-157:H7 ATCC 25922, *Bacillus cereus* ATCC 11778, *Clostridium perfringens* ATCC 13124, and *Vibrio parahaemolyticus* ATCC 17802 were purchased from the Korean Culture Center of Microorganisms (Seoul, Korea) and the Biological Resource Center (Daejeon, Korea) (Table 1).

Preparation of herbal extracts Dried herbal petals were mixed 1:10 w/v with 70 % ethanol and shaken with 150 rpm at 50°C for 48 hr. The plant material was then removed with gauze and the liquid extract was concentrated in a rotary vacuum evaporator ((N-1000; Eyela, Tokyo, Japan) (6). Following freeze-drying (Ilshin, Korea), we added distilled water to the powder (500 mg/mL) and serially extracted the ingredients with (in that order) hexane, chloroform ($CHCl_3$), ethyl acetate (EtOAc), n-butyl alcohol (n-BuOH), and water (H_2O) (Fig. 2). Each fraction was solidified by concentration and freeze-drying. Double-distilled water was added (100 mg/mL) and all the samples were filtered through 0.45 mm PVDF filter (Millipore, Billerica, MA, USA).

Antimicrobial tests and inhibitory effects After overnight culturing, each tested microorganism strain was inoculated in 100 μL of the appropriate agar media. Three milligram of herbal extract were loaded on 10-mm sterilized paper

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Table 1. Microbial strains, media, and incubation temperatures used for the antimicrobial tests

Microorganisms		Media used	Incubation Temp. (°C)
Gram positive			
Bacillus cereus	ATCC 11778	NB & NA[1)]	30
Clostridium perfringens	ATCC 13124	RCMB & RCMA[2)]	37
Gram negative			
Escherichia coli O157:H7	ATCC 25922	LBB & LBA[3)]	37
Vibrio parahaemolyticus	ATCC 17802	LBB & LBA	37

[1)]NB & NA; Nutrient broth & nutrient agar.
[2)]RCMB & RCMA; reinforced clostridial medium broth & reinforced clostridial media agar.
[3)]LBB &LBA; LB broth & LB agar.

discs (Advantech, Osaka, Japan) in close contact with growth media and incubated for 18 hr at a cultivation temperature adjusted to meet each test strain needs (Table 1). *C. perfringens* (ATCC 13124) was cultivated anaerobically (MARK-II; ANOXOMAT™, Netherlands) (7, 8). To quantify the degree of growth inhibition, each strain was grown in appropriate media (optical density at 660 nm = 0.04) and subjected to the contents of each fraction at 1 mg/mL for 24 hr with shaking (200 rpm), after which the OD_{660} was measured again (Mini photo 518 spectrophotometer, TAITEC, Japan). *C. perfringens* (ATCC 13124) was incubated in a jar without shaking.

Application of hibiscus extract to food To test for properties protecting against putrefaction, roselle petal extract (500 mg/mL) was sequentially diluted with double-distilled water to 10,000, 1,000, 100, 10, and 1 ppm. A 1-g slice of *kimbap* (rice rolled in dried laver) was soaked in the diluted herb extract or double distilled water for 5 sec and subsequently placed into a petri dish, then incubated at 37°C for 48 hr. The slice was then soaked in 10 mL of 0.85% (w/v) NaCl and the number of food spoilage microorganisms counted in Colony Forming Unit (CFU) using the Standard Agar Plate Count Method (9, 10) in nutrient agar (Difco Laboratories, Detroit, MI, USA).

Table 2. Antimicrobial activity of herbal petal extracts (70% ethanol[1)])

	Escherichia coli O157:H7
Roselle (*Hibiscus sabdariffa* L.)	++++[2)]
Thyme (*Thymus* spp.)	-
Tennel (*Foeniculum vulgare* M.)	
Lavander (*Lavandula* spp.)	-
Lemon balm (*Melissa officinalis* L.)	-
Rose hip (*Rosa canina* L.)	-
Rosemary (*Rosmarinus officinalis* L.)	++
Lemongrass (*Cymbopogon citratus*.)	-
Jasmine (*Jasminium officinale*.)	-
Peppermint (*Mentha piperita* L.)	-
Blue mallow (*Malva sylvestris* L.)	-
Orange Passion flower	-
Rose flower	-
Chamomile (*Anthemis nobilis* L.)	-

[1)]Each strain was inoculated on an agar plate and 3 mg of herb extract was loaded on a 10-mm sterilized paper disc.
[2)]+, degree of antimicrobial activity; -, no activity.

Results and Discussion

Identification of herb with antimicrobial activity Antimicrobial activity of ethanol extracts from 14 different herbs was tested with *E. coli* O157:H7 ATCC 25922. As shown in Table 2, roselle extract had the highest antimicrobial activity, while extracts from rosemary and jasmine were less effective. Clear zone formation reached 70% of the ampicillin controls (3 mg/disc) with the roselle extract (Fig. 1). This is consistent with previous studies, reporting the ethanol extract from dried roselle leaves reducing aflatoxin formation (11). Another study found the same extract to be ineffective against night crawber (*Lumbricus terrestris*) (12). Further analyses of the ethanol fraction of roselle petals (including analysis of its inhibitory effect against fungi and worm) need to be done to isolate the active ingredient of roselle.

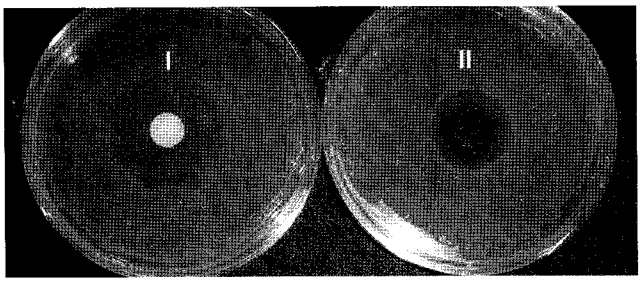

Fig. 1. Comparison of the antimicrobial activity of roselle petal extract and ampicillin. *E. coli* O157:H7 ATCC 25922 was inoculated in agar media. I, ampicillin; II, roselle extract.

Antimicrobial activity of herb fractions To further examine the antimicrobial activity, we fractionated the ethanol roselle extract with several solvents (Fig. 2). While the hexane and the chloroform ($CHCl_3$) fraction did not show any antimicrobial activity against gram-negative or gram-positive bacteria, the ethyl acetate (EtOAc), n-butyl alcohol (n-BuOH), and water (H_2O) fractions were clearly effective

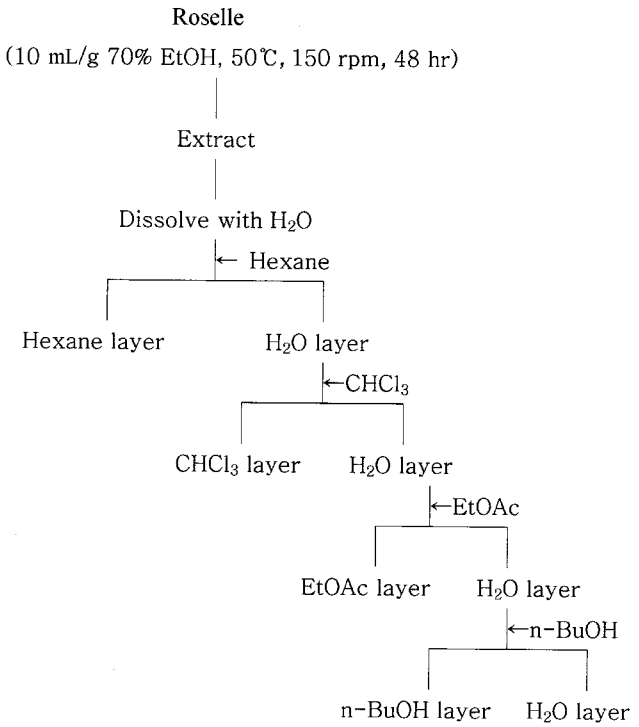

Fig. 2. Fractionation of the roselle petal extract.

against both gram-negative and gram-positive food-poisoning bacteria (Table 3). Ethyl acetate, n-BuOH, and H_2O fractions with confirmed antimicrobial activity were added to growth media (1 mg/mL) and the inhibitory effects were measured (Fig. 3). Water and n-BuOH fractions showed the highest *E. coli* (optical) density after 4 hr of incubation, which remained unchanged until 24 hr. The EtOAc fraction inhibited growth between 0-8 hr of the incubation, but the bacteria gradually increased between 8 to 24 hr to ultimately reach similar levels compared to treatment with n-BuOH and H_2O fractions (Fig. 3a). The H_2O fraction showed no growth inhibition of *B. cereus* for incubations longer than 2 hr, but n-BuOH showed growth inhibitory activity between 0 and 10 hr. The EtOAc fraction showed dramatic growth inhibition throughout the entire 24 hr of the incubation (Fig. 3b). Growth of *C. perfringens* was markedly suppressed by the EtOAc fraction, while addtion of the n-BuOH and H_2O fractions had no effect between 0 and 4 hr (Fig. 3c). *V. parahaemolyticus* incubated with H_2O fraction accumulated rapidly up to 4 to 6 hr of incubation and growth continued gradually thereafter. Addition of the EtOAc and n-BuOH fractions, however, resulted in visible growth inhibition (Fig. 3d). Neither the EtOAc fraction, nor the n-BuOH or the H_2O fraction showed any growth inhibitory effect on *P. aeruginosa* (data not shown). Previously, it has been reported that oil extracted from seeds of roselle inhibited growth of *B. anthracis* but not *Proteus vulgaris* and *P. aeruginosa* (13), suggesting that roselle extracts from petals and seeds have similar antibacterial action.

Application of herbal extracts in food preservation

Anti-food putrefaction effect of roselle extract was tested in the herb extract concentration of 10,000, 1,000, 100, 10, or 1 ppm. A *kimbap* slice treated with double distilled water contained food spoilage bacteria of 2.3×10^9 CFU/mL. However, the roselle extracts showed anti-food putrefaction effect and viable microorganim counts in the *kimbap* slice treated with the herb extract concentration of 10,000 ppm were 0.3×10^9 CFU/ mL (Table 4). The calyces of roselle are commonly consumed in cold beverages and hot drinks (12). Red anthocyanin pigments in the calyces are also used as food coloring agents (14). Because roselle extracts were effective against food putrefaction, their use as a spice may prove benefitial in food and drink preservation.

Many reports have been published on the contents of the different parts of roselle (12). The petals, for example, have been found to contain gossytrin, a glycoside (15). It is also known that the petals' dry weight is made up of 65% mucilage comprising galactose, galacturonic acid and rhamnose (12). Further research is required needed to study the constituents of roselle petals extracts and to

Table 3. Antimicrobial activity of fractions obtained from roselle petal extracts[1)]

	Gram positive		Gram negative	
	ATCC 11778	ATCC 13124	ATCC 25922	ATCC 17802
Hexane	-	-	-	-
$CHCl_3$	-	-	-	-
EtOAc	++++[2)]	++++	++++	++++
n-BuOH	+++	++	+++	++++
H_2O	++	++	++	++++

[1)]Each strain was inoculated on an agar plate and 3 mg of herb extract was loaded on a 10-mm sterilized paper disc.
[2)]+, degree of antimicrobial activity; -, no activity.

Table 4. Effect of the roselle petal extracts on food spoilage bacteria

	Control[1)]	1 ppm[2)]	10 ppm	100 ppm	1,000 ppm	10,000 ppm
CFU/mL	2.3×10^9	1.8×10^9	1.9×10^9	1.1×10^9	0.8×10^9	0.3×10^9

[1)]Control: treated with double distilled water.
[2)]Treated with the roselle extract.

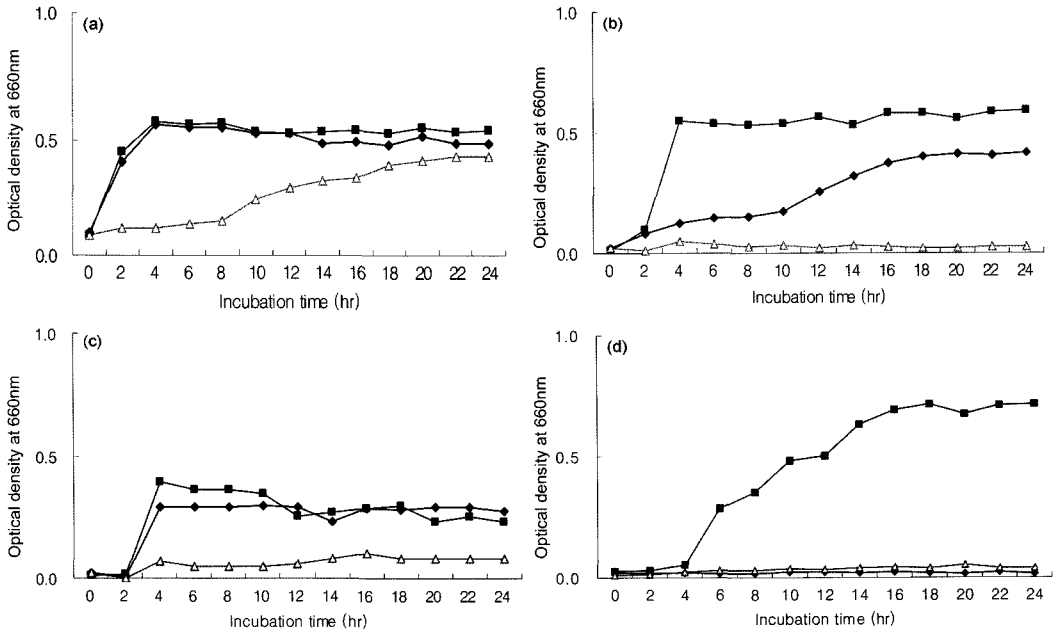

Fig. 3. Inhibitory effects of roselle extract fractions against foodborne microorganisms during a 24-hr culturing period. a, *E. coli* O 157:H7; b, *B. cereus*; c, *C. perfringens*; d, *V. parahaemolyticus*. The triangles show the inhibitory effect of the EtOAc fraction. The squares show the inhibitory effect of the H_2O fraction. The diamonds show the inhibitory effect of the BuOH fraction.

determine the compounds active against foodborne and food spoilage bacteria.

Acknowledgments

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References

1. Lue KH, Lee SS. The Illustrated Book of New Vegetable. Herbworld Press, Seoul (1998)
2. Tyler VE. The Honest Herbal, 3rd ed. Haworth Press Inc., Binghampton, New York, USA. pp. 57-73 (1993)
3. Tyler VE. Herbs of Choice: The Therapeutic Use of Phyto-medicinals. Haworth Press, New York, NY, USA. pp. 126-145 (1994)
4. Balchin ML. Essential oils and "aromatherapy": their modern role in healing. J. Rus. Soc. Health 117: 324-329 (1997)
5. Eisenberg DM, Kessler RC, Foster C, Norlock FE, Calkins DR, Delbanco TL. Unconventional medicine in the United States, preference, costs and patterns of use. New Engl. J. Med. 328: 246-252 (1993)
6. Lee KS, Kim MG, Lee KY. Antimicrobial effect of the extracts of cactus *Chounnyouncho* (*Opuntia humifusa*) against food borne pathogens. J. Korean Soc. Food Sci. Nutr. 33: 1268-1272 (2004)
7. Davidson PM, Parish ME. Methods for testing the efficacy of food antimicrobials. Food Technol. 43: 148-155 (1989)
8. Bae JH. Antimicrobial effect of *Sophora angustifolia* extracts on food-borne pathogens. Food Sci. Biotechnol. 14: 311-316 (2005).
9. Montanari G, Borsari A, Chiavari C, Ferri G, Zambonelli C, Grazia L. Morphological and phenotypical characterization of *Bacillus sporothermodurans*. J. Appl. Microbiol. 97: 802-809 (2004)
10. Lee JY, Kim YS, Shin DH. Antimicrobial synergistic effects of linolenic acid in combination with preservatives against food-borne bacteria. Food Sci. Biotechnol. 13: 323-327 (2004)
11. El-Shayeb NM, Mabrook SS. Utilization of some edible and medicinal plants to inhibit aflatoxin formation. Nutr. Rep. Int. 29: 273-282 (1984)
12. Ali BH, Wabel NA, Blunden G. Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: A Review. Phytother. Res. 19: 369-375 (2005)
13. Gangrade H, Mishra SH, Kaushal R. Antimicrobial activity of the oil and unsaponifiable matter of red roselle. Indian Drug. 16: 147-148 (1979)
14. Esselen WB, Sammy GM. Roselle: a natural red colorant for foods? Food Prod. Dev. 7: 80-82 (1975)
15. Seshadri TR, Thakur RS. Constitution of gossytrin, a new glycoside from the flower petals of *Hibiscus sabdariffa*. J. Indian Chem. Soc. 38: 649-651 (1961)