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Dissertation for Degree of Doctor of Philosophy

New Substances with Preservative Activity from Food Materials and Medicinal Herbs

by

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This is to certify that the dissertation of

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Contents

List of Tables

List of Figures

ABSTRACT

New Substances with Preservative Activity from Food Materials and Medicinal Herbs

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The medicinal herbs and food sources used in the present study were collected from all around the nation. According to the Food composition table published by RDA(Rural Development Administration) and MOHW(Ministry of Health and Welfare), 170 kinds of food materials such as cereals, potatoes and starches, pulses, seeds, vegetables, spice, seasonings, oils and fats, fishes and shellfishes, seaweeds, mushrooms, and fruits etc. and 190 kinds of medicinal herbs were purchased from domestic market of medicinal herbs. The deterioration and the decay of food material and food by putrefactive and food borne pathogenic microorganisms cause profound damage to public health and enormous economic loss. So it is very important to develop preservatives to prevent food contamination. However, current sterilization such as physical treatment and preservation by artificial additives brings changes of food properties and problems of food

safety and residual toxicity, etc. This study focused on the search of antimicrobial agent originated from natural products for food preservation. The purpose of this study was to develop new food additives for the prevention from preservative active substances of food materials and medicinal herbs. For these purpose, total 360 extracts from food sources and medicinal herbs extracted were tested for preservative activity using paper disk method and macrobroth dilution method. The chemical structures of these compunds were identified with the data obtained from spectrophotometry such as ^{1}H-NMR, ^{13}C-NMR, and the potentiality as effective preservatives were examined by comparing with common synthetic preservatives. Among total 360 natural extracts from food sources and medicinal herbs, *S. miltiorrhizae, S. flavescens, C. cassia*, and *E. japonicum* were selected as the candidates for the natural preservative. The four antimicribial substances showed a stronger antimicrobial activity than synthetic preservatives such as benzoic acid, propionic acid, sorbic acid and dehydroacetic acid. For the purpose of increasing the antimicrobial activity, the synergistic effect of synthetic and natural preservatives was examined. The synergistic effect of combining benzoic acid, sorbic acid and natural preservatives against foodborne pathogens was especially high. Application of purified preservatives to the target foods resulted in the prolonged shelf life with a significant decrease in number of viable cells. These natural preservatives in this study could be useful for natural and artificial food preservation.

INTRODUCTION

Food preservatives are natural or synthetic chemicals that are added to foods to retard and suppress undesirable chemical and physical changes in food. However, the food preservatives in the narrower meaning are called antimicrobialpreservatives which function by the inactivation of microorganisms or the delay or prevention of microbial growth. Processes of preservation may be generally classified as drying, heating, refrigeration, and the use of chemicals or other particular agents. The traditional methods including the use of salt (one of the oldest preservatives), sugar, and smoking, vinegar for pickling meats and vegetables have been used for food preservation.

People since the earliest ages have experimented with methods for successful food preservation, because most foods remain edible for only a brief period of time. To date, this endless desire and efforts have been succeeded. As food production has dramatically increased over recent decades, the usage of food preservatives to keep the products fresh and flavorsome has increased in parallel. Food preservation is the process of treating and handling food in such a way as to stop or greatly slow down spoilage toprevent foodborne illnesseswhile maintaining nutritional value, density, texture and flavor. Common preservation processes including 1) physical methods such as heating, drying, freezing, 2) fermentation by beneficial microorganisms, 3) addition of chemical preservatives have been used (1-3).

The purpose of using preservatives which inactivate microorganisms or delay or prevent microbial growth is similar to the use of disinfectant; however, the mode of action is not sterilization of microbes but bacteriostatic action or inactivation of enzymes inducing fermentation. Therefore, preservatives affect specific microbes by the action based on chemical composition and microbial metabolism with restoring the appearance and flavor of food (1-6). There are 18 food preservatives such as dehydroacetic, sorbic, benzoic, esters of p-hydroxybenzoic, propionic acids etc. approved by current Korea Food Sanitation Act (7) and Korea Food Additives Code (8). Among these, most preservatives, except esters of p-hydroxybenzoic, are organic acids or their salts which are kind of acidic preservatives.

Preservatives should satisfy following requisites: 1) having strong, evident, and lasting antimicrobial activity; 2) being effective at small amount; 3) not to have adverse affects; 4) easy to use and obtain; 5) inexpensive. However, more important requisites are as follows; 1) non-toxic when accumulated in the body. However, there have been few preservatives to fit all the requirements listed above. The preservatives with antimicrobial activity affect cell wall, protoplast membrane, enzyme or protein etc. to kill the microorganisms or inhibit the growth of microorganisms. The approved preservatives used may be harmful to humans. Therefore, even the preservatives are approved to use, using dosage, characteristics, toxicity of the preservatives are now defined by Korea Food Sanitation Act and Korea Food Additives Code. Many preservatives are regulated under the food additives amendment, added to Korea Food Additives Code. The amendment strengthened the law to ensure the safety of all new ingredients that manufacturers add to foods. Under these rules, a food manufacturer must get KFDA approval before using a new preservative, or before using a previously approved preservative in a new way or in a different amount. In its petition for approval, the manufacturer must demonstrate to KFDA that the preservative is safe for consumers, considering: the cumulative effect of the preservative in the diet; the probable amount of the preservative that will be consumed with the food product, or the amount of any substance formed in or on the food resulting from use of the preservative; the cumulative effect of the preservative in the diet; the potential toxicity (including cancer-causing) of the preservative when ingested by humans or animals. Also, a preservative may not be used to deceive a consumer by changing the food to make it appear other than it is. The food additive regulations require the preservative to be of food grade and be prepared and handled as a food ingredient. Also, the quantity added to food must not exceed the amount needed to achieve the manufacturer's intended effect.

Product spoilage caused by microorganisms is serious problem not only in food industry but also in agriculture, medical science, and logistics. There continues to be an increased public concern about the use of food additives, including preservatives, resulting from a perception that some of them may have deleterious effects on health. However, as eating habits have changed with an emphasis on what has been popularly termed a `healthy diet', there is at the same time a concern that reduction in preservative usage could lead to loss of safety and protection from food poisoning. While some preservatives are coming under increasing regulatory pressure others, particularly more natural ones, are receiving increased attention and gaining in importance and acceptability. Therefore, there is a need for developing safe preservatives (6,9).

The natural preservatives from natural foods and herbal medicines are plant extracts with phytochemicals, special proteins or enzymes, organic acids, bacteriocins which are part of components or metabolites. Since plants are known to contain various effective ingredients and produce antimicrobial substances as a means of self-defense, attempts have been made to discover the active ingredients of antimicrobial activity in plant sources. Although studies to investigate the substances with antimicrobial activity in plants found in Korea and studies on the use of these substances were conducted, studies on the identification of the active ingredients with the antimicrobial activity are still lacking. Therefore, there is a need for screening of antimicrobial substances from the traditional food without any safety concern for food (10-14).

The public concern about the use of natural substances with various activities widely distributed in the world is getting increased. However, the studies about activities, screening, isolation and purification techniques are still in progress. And also, there are lacks of the technologies about identification of structure, and in vivo test (15). Therefore, there is a problem in developing the nutraceutical food and applying to medicinal substances using natural substances. Especially, the examination of relationship between structure and function of biologically active substances is now important issue for developing new medicines from natural substances. The concern about developing new medicines from natural substances and nutraceutical food will be continuously increased. It will continue to study for the development of nutraceutical food. The safety of new food additives is still in public concerns and theres is a strong need for substituting natural additives without any toxicity for chemical additives with adverse effects (16-17).

According to starting with WTO (World Trade Organization) system and joining OECD (Organization of Economic Cooperation and Development), there are open market for agricultural products. Therefore, the best thing to raise the food industry is to develop new value added materials from traditional and natural foods. Since there are a lot of edible plants and herbs, and many popular remedies, there is a good possibility to develop medicinal and edible substances with competitive power.

Eating habits have been changed from eating food cooked at home to eating processed food in modern society. However, it is not easy to keep the original quality in processed food such as freshness, taste etc. Today, physical, chemical, and biological methods are used for increasing the safety from microbial contamination, and the most common method is to use food preservatives to prevent microbial contamination. Because of the increased public concern about healthy food, people do not want to eat food with high concentration of salt, sugar and chemical additives. Therefore, it is harder to prevent microbial contamination. On the other hand, alternative preservatives with no adverse effects are being sought after with the increased concern for the synthetic preservatives producing adverse side effects such as gastrointestinal diseases, carcinogenesis, and mutagenesis when accumulated in the body. Chemical preservatives in foods are highly restricted in some countries. The PL provision (product liability) issued recently also gives us an assignment for complete prevention of food born disease. According to the above trends, the food industry reevaluates the chemical preservatives and there is a need for non-toxic natural preservatives that could be used effectively in certain semi-processed and processed foods.

Many natural substances with antimicrobial activity exist in food, and the substances with antimicrobial activity known up to dateare conalbumin, avidin, and lysozyme in eggs; the protein substance including lactoferrin in milk; organic acids such as citric, succinic, benzoic, lactic, and propionic acids; fatty acids with the number of carbon between 12 and 18 contained in living tissue in small quantity; essential oil components of thyme, oregano, cinnamon, and cloves; and pigment related substances such as flavonols and proanthocyanins (tannins). Other substances including humulone, lupulone, hydrocinnamic derivative, caffeine, theophylline, theobromine, and phytoalexines are also known. The medium chain fatty acids with 8 to 12 carbon are known to have the most effective antimicrobial activity but most are reported to have the bacteriostatic property, and among many bacteriocins isolated from lactic acid bacteria, nisin, diplococcin, acidphilin, and colican are known to have the antimicrobial activity.

On the other hand, most common substances in plant extracts are spices and herbal medicines; and the antimicrobial activity is reported in many spices including garlic and onion, which are ones of the oldest spices, and in many herbal medicines including *Ganoderma lucidum* and *Lithospermum erythrorhizon*. Studies are present on phytoalexins such as flavonoids and alkaloids and on the relationship among the structure, antibacterial activity, and its mechanism of flavonoids. However, in the case of not so well-known grapefruit seed extract, many studies reported excellent effects in a wide variety of areas, but virtually no fundamental studies were done on determinating what are the active ingredients of grapefruit seed extract through which the antibacterial activity is expressed. Also, almost no studies exist on microorganisms related with the antibacterial effect and actual food preservative effect. It is hoped that this study provides the information about candidates with preservativeactivity from natural substances, and develop new preservatives satisfying safety concern. Thus, the result come from this study may cause to get patent and overcome very competitive market from OECD. The objectives of this study were to 1) develop food sources and medicinal herbs with preservative activity; and 2) examine the possiblitity to use natural substances as preservatives and apply to food industry.

MATERIALS AND METHODS

A. Materials

The medicinal herbs and food sources used in the present study were collected from all around the nation and shown in Table 2-3. According to the Food composition table published by RDA(Rural Development Administration) and MOHW(Ministry of Health and Welfare), and food materials such as cereals, potatoes and starches, pulses, seeds, vegetables, spice, seasonings, oils and fats, fishes and shellfishes, seaweeds, mushrooms, and fruits etc were classified. Total 360 kinds of medicinal herbs were purchased from domestic market of medicinal herbs and food sources. The samples were dried, cut into small pieces or grinded. And then the small pieces or grinded samples were extracted by 100% of methanol for 3 days. The extracts were separated by filter paper. The filtrate was concentrated in rotary evaporation at 40℃ and used as the crude extracts for preservative activity.

B. Methods

1. Screening of antimicrobial activity

1) Strains and culture media

The strains used in this study to exam the antimicrobial activity of medicinal herbs and food sources were shown in Table 1.

Table 1. List of strains and culture media tested

Microorganisms	Strains	Culture media	Incubation condition
Gram (+) bacteria	*Micrococcus luteus* ATCC 9341	Nutrient broth	37℃ for 3 days
	Bacillus subtilis ATCC 6633		
	Bacillus cereus KCCM 11774		
	Listeria monocytogenes ATCC 19111		
Gram (-) bacteria	*Pseudomonas aeruginosa* PAO 303		
	Salmonella typhimurium ATCC 29629		
	Escherichia coli ATCC 9637		
Yeasts	*Saccharomyces cerevisiae* KCTC 1552	Yeast-malt extract	28℃ for 3 days
	Candida albicans KCTC 1940		
Molds	*Penicilium citrinum* KCTC 1255	Potato dextrose	30℃ for 7 days
	Aspergillus flavus KCTC 1375		
	Aspergillus niger KCTC 2119		

*ATCC (America type culture collection); KCTC (Korean collection for type culture).

In order to measure the growth and antimicrobial activity of each strain, gram positive and negative strains were cultured in nutrient broth medium at 37℃ for 3 days with shaking and diluted to about $5x10^5$ CFU/ml, and used as test solution. Yeasts were cultured in yeast-malt extract medium at 28℃ for 3 days with shaking and diluted to about $5x10^5$ CFU/ml, and used as test solution. Fungi were cultured in potato dextrose agar medium at 30℃ for 7 days with shaking; spores were isolated and diluted to about $2x10^5$ spore/ml; and the diluted spore suspension was used as test solution.

2) Measurement of antimicrobial activity

The 170 extracts from food sources and total 190 extracts from medicinal herbs extracted with 100% methanol were prepared with two kinds of sample concentration (sample(Ⅰ): 100 mg/ml, sample(Ⅱ): 5,000 mg/ml), and antimicrobial activity of the extracts was measured by Paper disc and Macrobroth dilution methods. After culturing each microbe in suitable broth medium for 18~24 h, the degree of suspension was compared with 0.5-McFarland buffer turbidity standard to set the concentration of the culture medium at 10^9CFU/ml with a spectrophotometor. And then they were streaked on plates with the sterilized swabs soaked with culture. After streaking, the plates were dried for 5 min, 20 μl of the various food extracts examined was loaded on the paper discs (φ 8 mm Advantec, Toyo Roshi Kaisha, Japan) using strerilized micropippettes. And then the plates were incubated at proper temperature for target strains for 24 h and the diameters of the inhibition zones were measured. The smaller than 10 mm of the inhibition zone was determined as no antimicrobial activity, 10~15 mm was determined as moderate activity, and bigger than 15 mm was determined as strong activity. By examining extracts that are to have natural antimicrobial activity against target strains listed above, MIC (minimum inhibitory concentration), defined as the lowest concentration at which the growth of strains cannot be detected through the measurement of absorbance, was determined by dilution method after isolation and purification of selected extracts with antimicrobial activity. The culture medium was diluted again by 1000 folds to set the microbial concentration of the medium at about 10^6 CFU/ml, and diluted by two-fold serial dilution; 0.5 mL of final diluent was fraction into each medium, and 0.5 mL of each preservative was added into the medium. The mixture was culture for 24 h and the concentration that inhibits microbial growth was expressed as MIC. The natural extracts with strong antimicrobial activity were selected for further studies (18-19).

2. Separation of substances with the antimicrobial activity

1) *Salvia miltiorrhiza*

Methanol extract of *S. miltiorrhiza* was fractionated by solvents. The ethylacetate fraction which showed antimicrobial activity was adjusted to optimal pH and concentrated. Silicagel column chromatography was done with the ethylacetate-soluble fraction using chloroform-methanol (100:1) as the mobile phase. These fractions were divided largely into 5 fractions; and with the fractions showing antimicrobial activity, the second silicagel column chromatography was performed using hexane-ethylacetate(2:1) as the mobile phase. Preparative TLC was performed with chloroform-methanol(100:1) solvent, active bands were collected, and prep. HPLC was performed using reverse-phase column (μ-Bondapak C-18, 10x300 mm, Waters Co.) and mobile phase chloroform/water (30:70) to obtain pure compounds (Fig. 1). The single peak on HPLC chromatogram which showed antimicrobial activity was collected and designated for "compound T-1". The structure of T-1 was confirmed by data obtained from ^{1}H and ^{13}C-NMR spectra. ^{1}H and ^{13}C-NMR spectra were obtained using a NMR spectrometer.

2) *Sophora flavescens*

Methanol extract of *S. flavescens* was fractionated by solvents. The ethylacetate fraction which showed antimicrobial activity was adjusted to optimal pH and concentrated. Silicagel column chromatography was done with the ethylacetate-soluble fraction using chloroform-methanol (100:1) as the mobile phase. These fractions were divided largely into 5 fractions; and with the fractions showing antimicrobial activity, the second silicagel column chromatography was performed using hexane-ethylacetate(2:1) as the mobile phase. Preparative TLC was performed with chloroform-methanol(100:1) solvent, active bands were collected, and prep. HPLC was performed using reverse-phase column (μ-Bondapak C-18, 10x300 mm, Waters Co.) and mobile phase chloroform/water (35:65) to obtain pure compounds. The single peak on HPLC chromatogram which showed antimicrobial activity was collected and designated for "compound T-2". The structure of T-2 was confirmed by data obtained from ^{1}H and ^{13}C-NMR spectra. ^{1}H and ^{13}C-NMR spectra were obtained using a NMR spectrometer.

3) *Cinnamomum cassia*

In order to obtain solvent fractionation according to the polarity from *C. cassia*, methanol extract of *C. cassia* was fractionated by solvents. The ethylacetate fraction which showed antimicrobial activity was adjusted to optimal pH and concentrated. With chloroform-methanol(80:1→30:1) as the mobile phase for ethylacetate-soluble fraction, silicagel column chromatography was performed. These fractions were divided largely into 4 fractions, the fractions showing an antimicrobial activity were treated with hexane-ethylacetate (80:20) as the mobile phase. Preparative HPLC was performed to obtain pure compounds using normal-phase (Spherisorb Silica, 10x300 mm, Waters) and mobile phase (Fig. 2). The single peak on HPLC chromatogram which showed antimicrobial activity was collected and designated for "compound T-3". The structure of T-3 was confirmed by data obtained from ^{1}H and ^{13}C-NMR spectra. ^{1}H and ^{13}C-NMR spectra were obtained using a NMR spectrometer.

4) *Erythroniun japonicum*

In order to obtain solvent fractionation according to the polarity from *E. japonicum*, its methanol extract was fractionated by solvents. The ethylacetate fraction which showed antimicrobial activity was adjusted to optimal pH and concentrated. For ethylacetate-soluble fraction, silicagel column chromatography was done with chloroform-methanol (100:1→ 50:1) as the mobile phase. These fractions were divided largely into 4 fractions, and from the fractions showing an antimicrobial activity, pure compounds were obtained with prep. HPLC with hexane-ethylacetate (90:10) as the mobile phase using normal-phase (Spherisorb Silica, 10x300 mm, Waters) and mobile phase (Fig. 2). The single peak on HPLC chromatogram which showed antimicrobial activity was collected and designated for "compound T-4". The structure of T-4 was confirmed by data obtained from ^{1}H and ^{13}C-NMR spectra. ^{1}H and ^{13}C-NMR spectra were obtained using a NMR spectrometer.

Table 2. List of food materials collected nationwide for antimicrobial experiments

Name	Name
Acacia leaf	Crown daisy
Alabesque greenling	Dandelion
Alaska pollack, dried	Dog-tooth violet
Alaska pollack, fresh	Duchung tea
Alaska pollack immature, dried	Dunggulle tea
Alaska pollack,yellow	Egg plant
Allspice oleoresin(essential oil)	File fish,fillet,dried
Anchovy boiled-dried	Flounder
Anchovy, raw	Foxtail millet
Apple	Garlic
Asparagus	Geranium african oil(essential oil)
Banana	Ginger root
Banana bark	Gingko nuts
Beka squid	Gingko nuts leaf
Bergamot italian oil(essential oil)	Ginseng
Black soybean	Glutinous millet
Bois de rose(essential oil)	Granulated ark shell
Braken	Grape fruits seed
Broccoli	Grape seed
Buckwheat	Grape, large seed
Burdock	Green pepper
Butterbur	Hair tail
Cabbage	Hard-shelled mussel
Cabbage root	Hop, aroma
Cham Chwi	Hop, bitter
Charcoal	InJin-mugwort
Chard	Job's-tears
Chestnuts bark	Juda's ear
Chicory, greens	Jujube
Chinese quince	Kidney bean
Citron	Large green onion
Citrus fruit	Lavandin oil(essential oil)
Clavaria botrytis	Lavender absolute(essential oil)
Clove bud oil(essential oil)	Laver
Cocoa	Leek
Codonopsis lanceolate	Lemon
Codonopsis lanceolate chew	Lemon bark
Coffee	Lentinus edodes, dried
Coffee instant	Lettuce
Common sea squirt	Lettuce,Improved
Common squid	Little neck clam
Cowpeas	Mackerel
Crab	Mallow

Table 2. Continued

Name	Name
Mugwort	Seasame seeds (Korea)
Mungbean	Seasame seeds, black
Mushroom	Seaweed fusiform
Mustard leaf	Shepherd's purse
Neroli oil(essential oil)	Shining Ganodernia
Oakmoss absolute(essential oil)	Shrimp,dried
Onion	Small red bean
Oolong tea	Snail
Orange	Sorghum
Orange florida oil(essential oil)	Soybean
Orient clam	Soybean paste
Oyster	Soybean sprout
Oyster mushroom	Spanish mackerel
Pacific cod	Spinach
Pacific herring	Stem of taro
Pacific saury	Strawberry
Palmarosa madacascar oil(essential oil)	Sweet corn leaf
Peanuts	Sweet pepper, green
Pear	Sweet potato
Perilla leaf	Sweet potato stalk
Perilla seeds	Thyme white oil(essential oil)
Persimon	Tomato
Persimon bark, hard	Turban shell
Persimon leaf tea	Ulmin tea
Petitgrain limon tree(essential oil)	Unhulled Barley
Pimento leaf oil(essential oil)	Walnuts
Pine nuts	Walnuts bark
Potatoes	Warty sea squirt
Prosomillet	Water dropwort
Pumpkin seeds	Watermelon bark
Pumpkin, matured	Watermelon seed
Quail's egg	Whelk
Radish root	Wide rocambole stalk
Red pepper paste	Wild garlic
Rice	Winter fungus
Rice hull	Wintergreen oil(essential oil)
Royal fern	Wonduchung
Rye	Yellow croaker
Sea cucumber	Yellow tail
Sea lettuce	Zanthoxylum pipertitum
Sea mustard	
Sea staghom	
Sea tangle	
Seasame seeds (China)	

Table 3. List of medicinal herbs collected nationwide for antimicrobial experiments

Scientific name	Scientific name
Acanthopanax sessiliflorum	*Belamcanda chinensis*
Achyranthes japonica	*Betula platyphylla*
Aconitum pseudo-laeve	*Biota orientalis*
Acorus gramineus	*Bombyx mori*
Adenophora triphylla	*Brassica alba*
Agastache rugosa	*Bupleurum falcatum*
Akebia quinata	*Carthamus tinctoris*
Albizzia julibrissin	*Cassia obtusifolia*
Alisma orientale	*Catalpa ovata*
Allium tuberosum	*Celosia argentea*
Alpinia officinarum	*Chaenomeles sinensis*
Alpinia oxyphylla	*Chrysanthemum indicum*
Amomum cardamomum	*Chrysanthemum zawadskii*
Amomum tsao-ko Crevostet	*Cibotium barometz*
Amomum xanthioides	*Cimicifuga heracleifolia*
Amyda maakii	*Cinnamomum cassia(Cinnamomumi cortex spissus)*
Anemarrhena asphodeloides	*Cinnamomum cassia(Cinnamomumi ramulus)*
Angelica dahurica	*Cinnamomum cassia(Cinnamom bark)*
Angelica gigas	*Cistanche deserticola*
Angelica koreana	*Citrus aurantium*
Angelica tenuissima	*Citrus unshiu*
Angelica utilis	*Clematis mandshurica*
Anthriscus sylvestris	*Cnidium officinale*
Aralia cordata	*Codonopsis pilosula*
Arctium lappa	*Coix lacryma-jobi*
Areca catechu	*Commiphora molmol*
Arisaema amurense	*Coptis japonica*
Artemisia asiatica	*Cornus officinalis*
Asiasarum sieboldi	*Corydalis ternata*
Astragalus membranaceus	*Crataegus pinnatifida*
Atractylodes japonica	*Cryptotympana pustulata*
Atractylodes lancea	*Curcuma longa*

Table 3. Continued

Scientific name	Scientific name
Curcuma zedoaria	*Hordeum vulgare*
Cuscuta chinensi	*Houttuynia cordata*
Cynomorium songaricum	*Hovenia dulcis*
Cyperus rotundus	*Juniperus chinensis*
Dendrobium nobile	*Kalopanax pictus*
Dictamnus albus	*Kochia scoparia*
Dioscorea japonica	*Ledebouriella*
Dioscorea tokoro	*Leonurus sibiricus*
Dolichos lablab	*Ligustrum lucidum*
Drynaria fortunei	*Lindera strichnifolia*
Elsholtzia ciliata	*Lithospermum erythrorhizon*
Ephedra sinica	*Lonicera japonica thunberg(lonicerae flos)*
Epimedium koreanum	*Lonicera japonica thunberg(lonicerae folium)*
Equisetum hiemale	*Loranthus parasiticus*
Eriobotrya japonica	*Lycium chinense(lycii fructus)*
Eucommia ulmoides	*Lycium chinense(lycii radicis cortex)*
Eugenia caryophyllata	*Lycopus corenus*
Euryale ferox	*Magnolia officinalis*
Evodia officinalis	*Mahnolia denudata*
Foeniculum vulgare	*Medicata fementata*
Fossilia ossis	*Melia azedarach(meliae cortex)*
Gallus domesticus	*Melia azedarach(meliae fructus)*
Gardenia jasminoides	*Mentha arvensis*
Gastrodia elata	*Morinda officinalis*
Gentiana macrophylla	*Morus albal*
Gentiana scabra	*Myristica fragrans*
Geranium thunbergii	*Nardostachys chinensis*
Gleditsia japonica	*Nelumbo nucifera*
Glycyrrhiza uralensis fischer	*Ostrea gigas*
Gypsum	*Pachyma hoelen*
Haliotis gigantea	*Paeonia albiflora*
Halloysite	*Paeonia moutan*

Table 3. Continued

Scientific name	Scientific name
Patrinia villosa	*Rubus coreanus miquel*
Perilla frutescens	*Salvia miltiorrhiza*
Perilla sikokiana	*Sanguisorba officinalis*
Pharbitis nil choisy	*Saussurea lappa*
Phellodendron amurense	*Schizandra chinensis*
Phlomis umbrosa	*Scirpus flaviatilis*
Phyllostachys nigra	*Scrophularia vuergeriana*
Pinellia ternata breitenbach	*Scutellaria baicalensis*
Pinus densiflora	*Siegesbeckia orientalis*
Plantago asiatica	*Sinomenium acutum*
Platycodon grandiflorum	*Smilax china*
Polygala tenuifolia	*Sodium sulfate*
Polygonatum sibiricum	*Sophara japonica*
Polygonum aviculare	*Sophora flavescens*
Polygonum multiflorum	*Sophora subprostrata*
Polyporus umbellatus	*Spiradela polyrhiza*
Poria cocos wolf	*Stemona japonica*
Porsythia viridissima	*Talc*
Prunella vulgaris	*Tetrapanax papyriferus*
Prunus armeniaca	*Thuja orientalis*
Prunus mume	*Torreya nucifera*
Prunus nakaii	*Trichosanthes kirlowii*
Prunus persica	*Trogopterus xanthipes*
Psoralea corylifolia.	*Tussilago farfara*
Pueraria thunbergiana	*Typha orientalis*
Raphanus sativus	*Uncaria sinensis havil*
Rehmannia glutinosa	*Vitex rotundifolia*
Rheum palmatum	*Xanthium strumarium*
Rhus javanica	*Zanthoxylum schinifolium*
Rosa laevigata	*Zingiber officinale*
Rubia akane	*Zizyphus vulgaris*

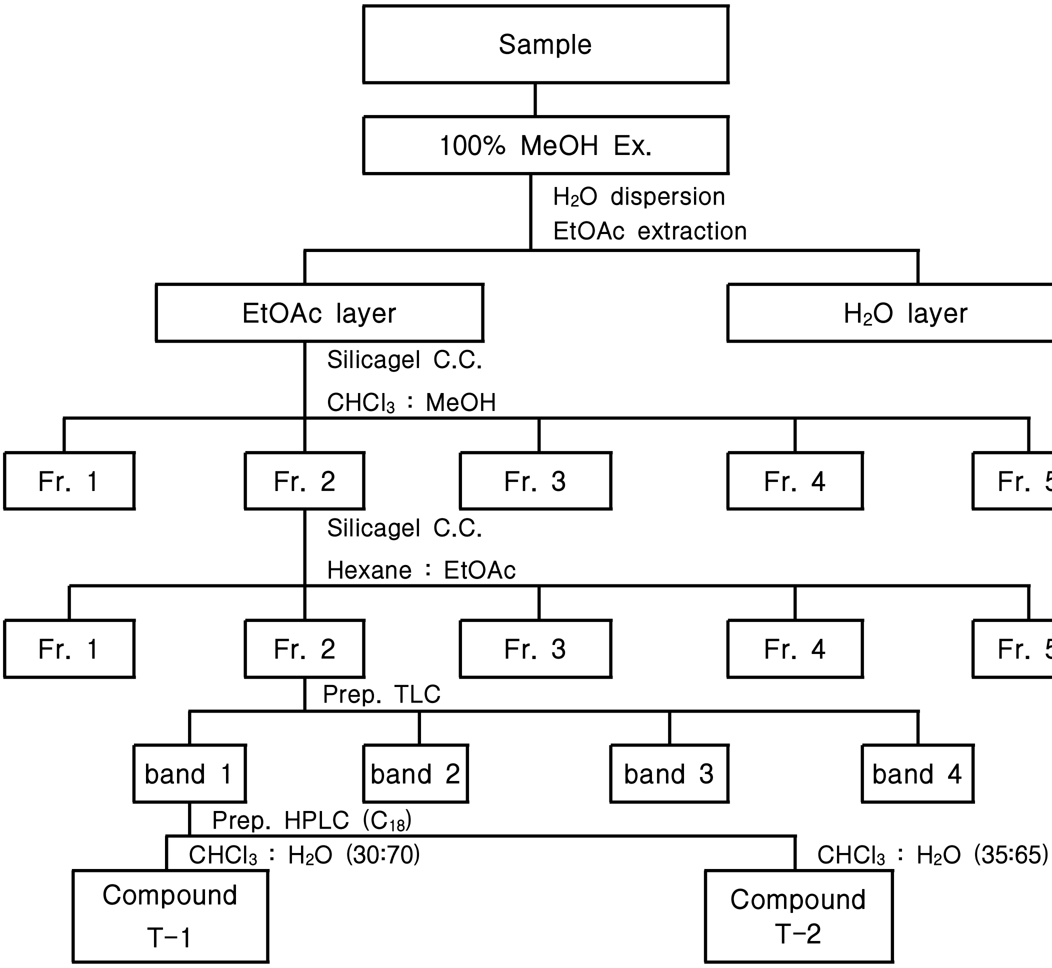

Fig. 1. Scheme of isolation of antimicrobial substances from *Salvia miltiorrhiza* and *Sophora flavescens.*

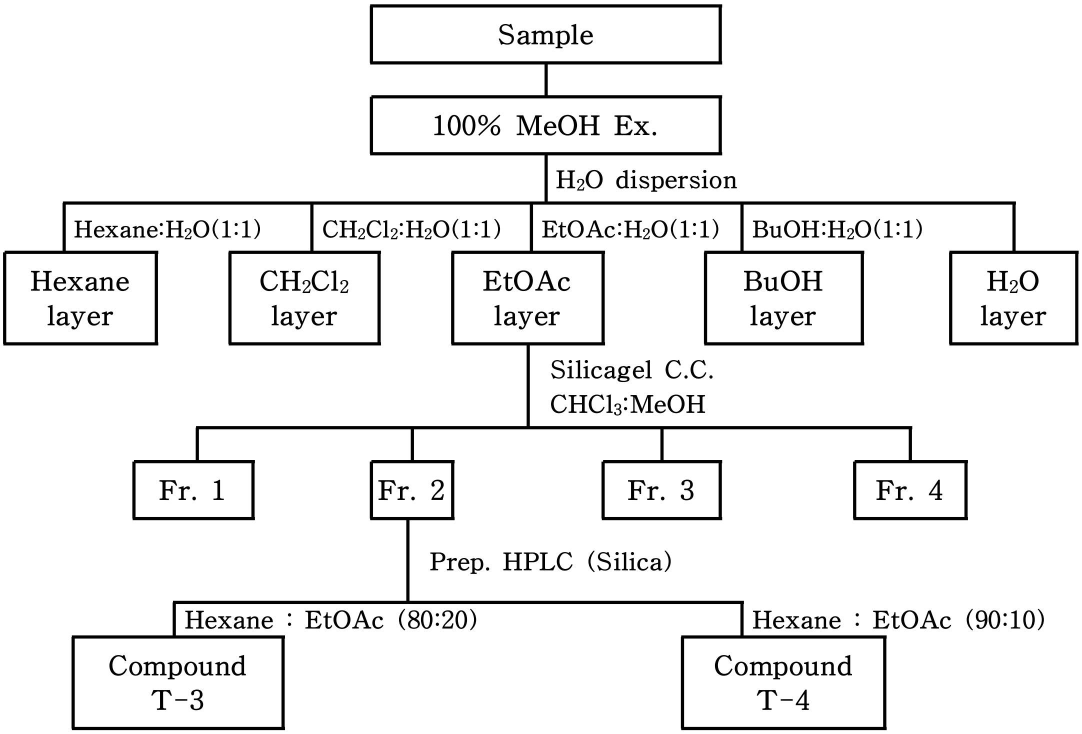

Fig. 2. Scheme of isolation of antimicrobial substances from *Cinnamomum cassia* and *Erythroniun japonicum*.

3. Comparing of antimicrobial activity betweein synthetic preservatives and isolated natural substances for antimicrobial activity

Among those initially screened samples according to the paper disk method, MIC of each sample was measured against each microbe to obtain more objective data with the substances showing antimicrobial activity (T-1, T-2, T-3, T-4) isolated from *Salvia miltiorrhiza*, *Sophora flavescens*, *Cinnamonum cassia*, and Dog-tooth violet and four types of most commonly used preservatives (benzoic acid, sorbic acid, propionic acid, dehydroacetic acid) in order to compare the degree of antimicrobial activity using the Macrobroth dilution method described above. MIC of each extract was determined against with target strains.

4. Experiment on the synergic effect of synthetic and natural preservatives

As for the experimental solution, 100 mg of natural preservatives purified from *Salvia miltiorrhiza*, *Sophora flavescens*, *Cinnamonum cassia*, and Dog-tooth violet, respectively were dissolved in 3 mL of 10% acetic acid and the volume was adjusted to 50 mL(0.2%) with distilled water. pH was adjusted to 6.0 using 0.1 M potassium phosphate (dibasic) to make 0.2% experimental solution. Benzoic acid, sorbic acid, propionic acid, and dehydroacetic acid experimental solutions were dissolved in distilled water to make 0.1% and 1% solutions. These were high-pressure steam sterilized at 121℃ for 15 min and kept cold until use. The cell number in the samples was counted according to the standard method of FDA, and the degree of proliferation in broth media was measured with absorbance at 540 nm using a UV-Visible spectrophotometer .

5. Experiment on food preservation using antimicrobial active substances

The target strains used in this study are bacteria causing food spoilage, food pathogens such as *E. coli*, *B. subtilis*, *S. typhimurium*, *L. monocytogenes* etc. Among the food used, seasoned common squid and dried slice of beef were used directly after purchase, and the ingredients of saengmyun, cream puffs, and potato salad were purchased and these foods were made at the laboratory adding the natural preservatives. As for the experiment against 4 preservatives, each experimental food treated with pressure and humidity and sterilized was diced at suitable size and placed in the petri dish where 50 µL of each cultured strain and 0.2% and 0.5% preservative per 1 g of food were mixed and incubated. The growth of each microbe in storage was measured with colony forming units (CFU).

RESULTS AND DISCUSSION

A. Preservative activity of food materials and medicinal herbs

The natural antimicrobial activity of 170 food material extracts was shown in Table 4-7 (Sample(I):100 mg/ml, Sample(II):5,000 mg/ml) against 4 types of Gram positive bacteria, 3 types of Gram negative bacteria, 2 types of yeasts and 3 types of molds. As shown in Table 4, Aroma hop, Bitter hop, Gingko nuts leaf, Chinese quince, and Dog-tooth violet

have antimicrobial activity against *M. luteus, B. subtilis, B. cereus, L. monocytogenes*. Especially, Aroma hop and Bitter hop have high activity against *B. subtilis* and *B. cereu* at low concentration. Mustard leaf, Leek, Grape large seed, Walnuts, and Coffee instant have strong preservative activity against *M. luteus, B. subtilis* and *B. cereus*, especially, Walnuts showed activity at low concentration of bacteria listed above. As shown in Table 5, Dog-tooth violet and Lemon have preservative activity against *P. aeruginosa, S. typhimurium* and *E. coli*. And Aroma hop, Bitter hop, and Soybean paste have strong preservative activity against *S. typhimurium* and *E. coli*. As shown in Table 6, Leek and Dog-tooth violet have preservative activity against *Sacch. cerevisiae* and *C. albicans*, Aroma hop, lemon, Oakmoss absolute have preservative activity against *Sacch. cerevisiae*, and Mustard leaf, Coffee instant, Coffee, and Wonduchung have preservative activity against *C. albicans*. As shown in Table 7, Dog-tooth violet, and Lemon showed preservative activity against *P. citrinum, Asp. flavus* and *Asp. niger*, Chinese quince has preservative activity against *Asp. flavus* and *Asp. niger*, Mustard leaf, and Aroma hop have preservative activity against *Asp. flavus*, Lemon bark has preservative activity against *P. citrinum*. Among spices, 5 spices have weak preservative activity against *Asp. niger*.

The natural preservative activity was shown in Table 8-11 by examining 190 extracts (Sample(I):100 mg/ml, Sample(II):5,000 mg/ml) from medicinal herbs that are to have natural antimicrobial activity against 4 types of Gram positive bacteria, 3 types of Gram negative bacteria, 2 types of yeasts and 3 types of molds. As shown in Table 8, *Sophora flavescens, Salvia miltiorrhiza, Commiphora molmol, Prunus mume, Cinnamomum cassia,* and *Siegesbeckia orientalis* have activity against 4 types of Gram positive bacteria (*M. luteus, B. subtilis, B. cereus, L. monocytogenes*). *Ostrea gigas, Chaenomeles sinensis, Angelica utilis, Acorus gramineus, Alpinia officinarum, Evodia officinalis, Sanguisorba officinalis,* and *Magnolia officinalis* have preservative activity against 3 of 4 Gram positive bacteria (*M. luteus, B. subtilis, B. cereus*). *Cornus officinalis, Zanthoxylum schinifolium, Schizandra chinensis,* and *Coptis japonica* have preservative activity against 3 of 4 Gram positive bacteria (*M. luteus, B. subtilis, L. monocytogenes*). *Phyllostachys nigra, Patrinia villosa, Geranium thunbergii,* and *Betula platyphylla* showed preservative activity against 3 of 4 Gram positive bacteria (*M. luteus, B. cereus, L. monocytogenes*).

As shown in Table 9, *Prunus mume,* and *Cinnamomum cassia* have preservative activity against all 3 types of Gram negative bacteria (*P. aeruginosa, S. typhimurium, E. coli*). *Rubus coreanus miquel* has preservative activity against 2 of 3 Gram negative bacteria (*P. aeruginosa, S. typhimurium*), and Eugenia caryophyllata has weak preservative activity against 2 of 3 Gram negative bacteria (*S. typhimurium, E. coli*). The medicinal herbs which have preservative activity against *E. coli* only were *Rehmannia glutinosa, Salvia miltiorrhiza, Vitex rotundifolia, Saussurea lappa, Acorus gramineus, Perilla sikokiana, Schizandra chinensis, Scrophularia vuergeriana, Geranium thunbergii*, and *Coptis japonica*. As shown in Table 10, the medicinal herbs with preservative activity against all 2 types of yeasts (*Sacch. cerevisiae, C. albicans*) were *Sophora flavescens, Cinnamomum cassia, Eugenia caryophyllata, Tetrapanax papyriferus, Elsholwzia ciliata*, and *Coptis japonica*. The medicinal herbs with preservative activity against *Sacch. cerevisiae* were *Nardostachys chinensis, Chrysanthemum indicum, Chrysanthemum zawadskii, Salvia miltiorrhiza, Amomum cardamomum, Sophora subprostrata, Cornus officinalis, Dendrobium nobile, Asiasarum sieboldi, Pinus densiflora, Artemisia asiatica, Evodia officinalis, Epimedium koreanum, Gentiana macrophylla, Cnidium officinale, Melia azedarach, Amomum tsao-ko Crevostet, Alisma orientale, Betula platyphylla, Geranium thunbergii, Corydalis ternata,* and *Polygonatum sibiricum.* The medicinal herbs with preservative activity against *C. albicans* were *Zingiber officinale, Cinnamomum cassia, Mahnolia denudata, Anemarrhena asphodeloides, Patrinia villosa,* and *Typha orientalis*. As shown in Table 11, the medicinal herbs with preservative activity against 2 of 3 types of molds (*Asp. flavus, Asp. niger*) were *Cornus officinalis, Rhus javanica, Cinnamomum cassia, and Eugenia caryophyllata.* The medicinal herbs with preservative activity against *Asp. niger* were *Cinnamomum cassia, Chrysanthemum zawadskii, Acorus gramineus, Asiasarum sieboldi, Foeniculum vulgare,* and *Amomum tsao-ko Crevostet.* This medicinal herbs had also preservative activity against *Asp. flavus* is *Prunus mume*. Among total 360 natural extracts from food sources and medicinal herbs, *Slvia miltiorrhizae, Sophora flavescens, Cinnamomum cassia*, and Dog-tooth violet were selected as the candidates for further study of preservative activity through stepwise solvent fractionation, considering the amount and condition of these substances with superior antimicrobial activity and extraction conditions.

Table 4. Antibacterial activity of food materials against Gram(+)bacteria

Unit : Inhibition zone diameter(mm)

Name	*M. luteus*[1)]		*B. subtilis*[2)]		*B. cereus*[3)]		*L. monocytogenes*[4)]	
	(Ⅰ)[5)]	(Ⅱ)[6)]	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Acacia leaf	–	–	–	–	–	–	–	–
Alabesque greenling	–	–	–	–	–	–	–	–
Alaska pollack,dried	–	–	–	–	–	–	–	–
Alaska pollack, fresh	–	–	–	–	–	–	–	–
Alaska pollack immature, dried	–	–	–	–	–	–	–	–
Alaska pollack,yellow	–	–	–	–	–	–	–	–
Allspice oleoresin(essential oil)	–	–	–	–	–	–	–	–
Anchovy boiled-dried	–	–	–	10	–	–	–	–
Anchovy, raw	–	–	–	–	–	–	–	–
Apple	–	–	–	–	–	12	–	–
Asparagus	–	–	–	–	–	–	–	–
Banana	–	–	–	–	–	–	–	–
Banana bark	–	–	–	–	–	–	–	–
Beka squid	–	–	–	–	–	–	–	–
Bergamot italian oil(essential oil)	–	–	–	–	–	–	–	–
Black soybean	–	–	–	–	–	–	–	–
Bois de rose(essential oil)	–	–	–	–	–	9	–	–
Braken	–	15	–	10	–	–	–	10
Broccoli	–	–	–	–	–	–	–	–
Buckwheat	–	–	–	–	–	22	–	–
Burdock	–	–	–	–		–	–	–
Butterbur	–	–	–	–	–	–	–	–
Cabbage	–	–	–	–	–	–	–	–
Cabbage root	–	–	–	–	–	–	–	–
Cham Chwi	–	–	–	–	–	–	–	–
Charcoal	–	–	–	–	–	–	–	–
Chard	–	–	–	–	–	–	–	–
Chestnuts bark	–	–	–	–	–	–	–	–
Chicory, greens	–	–	–	–	–	–	–	–
Chinese quince	–	22	–	14	–	30	–	12
Citron	–	–	–	12	–	13	–	10
Citrus fruit	–	–	–	–	–	–	–	–

1-4) See the Table 1.

5) Sample (Ⅰ) : 100mg/ml MeOH, 6) Sample (Ⅱ) : 5,000mg/ml MeOH

Table 4. Continued

Name	*M. luteus*		*B. subtilis*		*B. cereus*		*L. monocytogenes*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Clavaria botrytis	-	-	-	-	-	-	-	-
Clove bud oil(essential oil)	-	-	-	-	-	-	-	-
Cocoa	-	-	-	-	-	-	-	-
Codonopsis lanceoiate	-	-	-	-	-	-	-	-
Codonopsis lanceoiate chew	-	-	-	-	-	-	-	-
Coffee	-	-	-	-	-	16	-	-
Coffee instant	-	14	-	10	-	20	-	-
Common sea squirt	-	-	-	-	-	-	-	-
Common squid	-	-	-	-	-	-	-	-
Cowpeas	-	-	-	-	-	-	-	-
Crab	-	-	-	-	-	-	-	-
Crown daisy	-	-	-	-	-	-	-	-
Dandelion	-	-	-	-	-	-	-	-
Dog-tooth violet	-	14	-	15	-	23	-	17
Duchung tea	-	-	-	-	-	-	-	-
Dunggulle tea	-	-	-	-	-	-	-	-
Egg plant	-	-	-	-	-	-	-	-
File fish,fillet,dried	-	-	-	-	-	-	-	-
Flounder	-	-	-	-	-	-	-	-
Foxtail millet	-	-	-	-	-	-	-	10
Garlic	-	14	-	-	-	19	-	-
Geranium african oil(essential oil)	-	-	-	-	-	-	-	-
Ginger root	-	-	-	-	-	-	-	-
Gingko nuts	-	-	-	-	-	14	-	10
Gingko nuts leaf	-	15	-	12	-	27	-	13
Ginseng	-	-	-	-	-	-	-	-
Glutinous millet	-	-	-	-	-	-	-	-
Granulated ark shell	-	-	-	-	-	-	-	-
Grape fruits seed	-	-	-	10	-	-	-	-
Grape seed	-	15	-	15	-	-	-	13
Grape, large seed	-	10	-	17	-	10	-	-
Green pepper	-	-	-	-	-	-	-	-
Hair tail	-	-	-	-	-	-	-	-
Hard-shelled mussel	-	-	-	-	-	11	-	-
Hop, aroma	-	18(×10)	19	12(×10)	20	14(×10)	11	20
Hop, bitter	-	19(×10)	18	12(×10)	15	16(×10)	-	21
Injin mugwort	-	-	-	-	-	-	-	-

Table 4. Continued

Name	*M. luteus*		*B. subtilis*		*B. cereus*		*L. monocytogenes*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Job's-tears	–	–	–	–	–	–	–	–
Juda's ear	–	–	–	–	–	–	–	–
Jujube	–	–	–	–	–	–	–	–
Kidney bean	–	–	–	–	–	–	–	–
Large green onion	–	–	–	–	–	–	–	–
Lavandin oil(essential oil)	–	–	–	–	–	–	–	–
Lavender absolute(essential oil)	–	–	–	–	–	–	–	–
Laver	–	–	–	–	–	–	–	–
Leek	–	14	–	21	–	15	–	–
Lemon	–	21	–	–	–	17	–	10
Lemon bark	–	–	–	–	–	–	–	–
Lentinus edodes, dried	–	–	–	–	–	–	–	–
Lettuce	–	–	–	–	–	–	–	–
Lettuce,Improved	–	–	–	–	–	–	–	–
Little neck clam	–	–	–	–	–	–	–	–
Mackerel	–	–	–	–	–	–	–	–
Mallow	–	–	–	–	–	–	–	–
Mugwort	–	–	–	–	–	–	–	–
Mungbean	–	–	–	–	–	–	–	–
Mushroom	–	–	–	–	–	–	–	–
Mustard leaf	–	16	–	28	–	20	–	–
Neroli oil(essential oil)	–	–	–	–	–	13	–	–
Oakmoss absolute(essential oil)	–	–	–	–	–	–	–	–
Onion	–	–	–	–	–	–	–	–
Oolong tea	–	–	–	25	–	12	–	–
Orange	–	–	–	–	–	–	–	–
Orange florida oil(essential oil)	–	–	–	–	–	–	–	–
Orient clam	–	–	–	–	–	–	–	–
Oyster	–	–	–	–	–	–	–	–
Oyster mushroom	–	–	–	–	–	–	–	–
Pacific cod	–	–	–	–	–	–	–	–
Pacific herring	–	–	–	–	–	–	–	–
Pacific saury	–	–	–	–	–	–	–	–
Palmarosa madacascar oil(essential oil)	–	–	–	–	–	–	–	–
Peanuts	–	–	–	–	–	–	–	–
Pear	–	–	–	–	–	–	–	–
Perilla leaf	–	–	–	–	–	–	–	–

Table 4. Continued

Name	*M. luteus*		*B. subtilis*		*B. cereus*		*L. monocytogenes*	
	I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)
Perilla seeds	–	–	–	–	–	–	–	12
Persimon	–	–	–	–	–	9	–	–
Persimon bark, hard	–	–	–	–	–	–	–	–
Persimon leaf tea	–	–	–	–	–	–	–	–
Petitgrain limon tree(essential oil)	–	–	–	–	–	–	–	–
Pimento leaf oil(essential oil)	–	–	–	–	–	–	–	–
Pine nuts	–	–	–	–	–	–	–	–
Potatoes	–	–	–	–	–	–	–	–
Prosomillet	–	–	–	–	–	–	–	–
Pumpkin seeds	–	–	–	–	–	–	–	–
Pumpkin, matured	–	–	–	–	–	–	–	–
Quail's egg	–	–	–	–	–	–	–	–
Radish root	–	–	–	–	–	–	–	–
Red pepper paste	–	–	–	–	–	–	–	–
Rice	–	–	–	–	–	–	–	–
Rice hull	–	–	–	–	–	–	–	–
Royal fern	–	–	–	–	–	–	–	–
Rye	–	–	–	–	–	–	–	–
Sea cucumber	–		–	–	–	–	–	–
Sea lettuce	–	–	–	–	–	–	–	–
Sea mustard	–	–	–	–	–	–	–	–
Sea staghom	–	–	–	–	–	–	–	–
Sea tangle	–	–	–	–	–	–	–	–
Seasame seeds (China)	–	–	–	–	–	–	–	–
Seasame seeds (Korea)	–	–	–	–	–	–	–	–
Seasame seeds, black	–	–	–	–	–	–	–	–
Seaweed fusiform	–	–	–	–	–	–	–	–
Shepherd's purse	–	–	–	–	–	–	–	–
Shining Ganodernia	–	–	–	–	–	13	–	–
Shrimp,dried	–	–	–	–	–	10	–	–
Small red bean	–	–	–	–	–	–	–	–
Snail	–	–	–	–	–	–	–	–
Sorghum	–	–	–	–	–	–	–	–
Soybean	–	–	–	–	–	–	–	–
Soybean paste	–	–	–	–	–	–	–	–
Soybean sprout	–	–	–	–	–	–	–	–

Table 4. Continued

Name	*M. luteus*		*B. subtilis*		*B. cereus*		*L. monocytogenes*	
	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)
Spanish mackerel	-	-	-	-	-	-	-	-
Spinach	-	-	-	-	-	-	-	-
Stem of taro	-	-	-	-	-	-	-	-
Strawberry	-	-	-	-	-	-	-	-
Sweet corn leaf	-	-	-	-	-	-	-	-
Sweet pepper, green	-	-	-	-	-	-	-	-
Sweet potato	-	-	-	-	-	-	-	-
Sweet potato stalk	-	-	-	-	-	-	-	-
Thyme white oil(essential oil)	-	-	-	-	-	-	-	-
Tomato	-	-	-	-	-	-	-	-
Turban shell	-	-	-	-	-	-	-	-
Ulmin tea	-	-	-	-	-	-	-	-
Unhulled Barley	-	-	-	-	-	-	-	-
Walnuts	13	21	-	12	13	16	-	-
Walnuts bark	-	15	-	-	-	15	-	-
Warty sea squirt	-	-	-	-	-	-	-	-
Water dropwort	-	-	-	-	-	-	-	-
Watermelon bark	-	-	-	-	-	-	-	-
Watermelon seed	-	-	-	-	-	-	-	-
Whelk	-	-	-	-	-	-	-	-
Wide rocambole stalk	-	-	-	-	-	-	-	-
Wild garlic	-	-	-	-	-	-	-	-
Winter fungus	-	-	-	-	-	-	-	-
Wintergreen oil(essential oil)	-	-	-	-	-	-	-	-
Wonduchung	-	-	-	-	-	-	-	-
Yellow croaker	-	-	-	-	-	-	-	-
Yellow tail	-	-	-	-	-	-	-	-
Zanthoxylum pipertitum	-	20	-	10	-	-	-	-

Table 5. Antibacterial activity of food materials against Gram(-)bacteria

Unit : Inhibition zone diameter(mm)

Name	*P. aeruginosa*[1]		*S. typhimurium*[2]		*E. coli*[3]	
	(Ⅰ)[4]	(Ⅱ)[5]	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Acacia leaf	-	-	-	-	-	-
Alabesque greenling	-	-	-	-	-	-
Alaska pollack,dried	-	-	-	-	-	-
Alaska pollack, fresh	-	-	-	-	-	-
Alaska pollack,immature,dried	-	-	-	-	-	-
Alaska pollack,yellow	-	-	-	-	-	-
Allspice oleoresin(essential oil)	-	-	-	-	-	-
Anchovy boiled-dried	-	-	-	-	-	-
Anchovy, raw	-	-	-	-	-	-
Apple	-	-	-	-	-	-
Asparagus	-	-	-	-	-	-
Banana	-	-	-	-	-	-
Banana bark	-	-	-	-	-	-
Beka squid	-	-	-	-	-	-
Bergamot italian oil(essential oil)	-	-	-	-	-	-
Black soybean	-	-	-	-	-	-
Bois de rose(essential oil)	-	-	-	-	-	-
Braken	-	-	-	-	-	-
Broccoli	-	-	-	-	-	-
Buckwheat	-	-	-	-	-	-
Burdock	-	-	-	-	-	-
Butterbur	-	-	-	10	-	-
Cabbage	-	-	-	-	-	-
Cabbage root	-	-	-	-	-	-
Cham Chwi	-	-	-	-	-	-
Charcoal	-	-	-	-	-	-
Chard	-	-	-	-	-	-
Chestnuts bark	-	-	-	-	-	-
Chicory, greens	-	-	-	-	-	-
Chinese quince	-	-	-	-	-	-
Citron	-	-	-	-	-	-
Citrus fruit	-	-	-	-	-	-
Clavaria botrytis	-	-	-	-	-	-

1-3) See the Table 1.

4-5) See the Table 4.

Table 5. Continued

Name	*P. aeruginosa*		*S. typhimurium*		*E. coli*	
	(I)	(II)	(I)	(II)	(I)	(II)
Clove bud oil(essential oil)	-	-	-	-	-	-
Cocoa	-	-	-	-	-	-
Codonopsis lanceolate	-	-	-	-	-	-
Codonopsis lanceolate chew	-	-	-	-	-	-
Coffee	-	-	-	-	-	-
Coffee instant	-	-	-	-	-	-
Commom sea squirt	-	-	-	-	-	-
Common squid	-	-	-	-	-	-
Cowpeas	-	-	-	-	-	-
Crab	-	-	-	-	-	-
Crown daisy	-	-	-	-	-	-
Dandelion	-	-	-	-	-	-
Dog-tooth violet	-	16	-	17	-	17
Duchung tea	-	-	-	-	-	-
Dunggulle tea	-	-	-	-	-	-
Egg plant	-	-	-	-	-	-
File fish,fillet,dried	-	-	-	-	-	-
Flounder	-	-	-	-	-	-
Foxtail millet	-	-	-	-	-	-
Garlic	-	-	-	-	-	-
Geranium african oil(essential oil)	-	-	-	-	-	-
Ginger root	-	-	-	-	-	-
Gingko nuts	-	-	-	-	-	-
Gingko nuts leaf	-	-	-	-	-	-
Ginseng	-	-	-	-	-	-
Glutinous millet	-	-	-	-	-	-
Granulated ark shell	-	-	-	-	-	-
Grape fruits seed	-	-	-	-	-	-
Grape seed	-	-	-	-	-	-
Grape, large seed	-	-	-	-	-	-
Green pepper	-	-	-	-	-	-
Hair tail	-	-	-	-	-	-
Hard-shelled mussel	-	-	-	-	-	-
Hop, aroma	-	-	12	20	-	22
Hop, bitter	-	-	14	24	18	25
InJin-mugwort	-	-	-	-	-	-
Job's-tears	-	-	-	-	-	-
Juda's ear	-	-	-	-	-	-
Jujube	-	-	-	-	-	14

Table 5. Continued

Name	*P. aeruginosa* (Ⅰ)	*P. aeruginosa* (Ⅱ)	*S. typhimurium* (Ⅰ)	*S. typhimurium* (Ⅱ)	*E. coli* (Ⅰ)	*E. coli* (Ⅱ)
Kidney bean	-	-	-	-	-	-
Large green onion	-	-	-	-	-	-
Lavandin oil(essential oil)	-	-	-	-	-	-
Lavender absolute(essential oil)	-	-	-	-	-	-
Laver	-	-	-	-	-	-
Leek	-	-	-	-	-	-
Lemon	-	9	-	16	-	12
Lemon bark	-	-	-	-	-	-
Lentinus edodes, dried	-	-	-	-	-	-
Lettuce	-	-	-	-	-	-
Lettuce,Improved	-	-	-	-	-	-
Little neck clam	-	-	-	-	-	-
Mackerel	-	-	-	-	-	-
Mallow	-	-	-	-	-	-
Mugwort	-	-	-	12	-	-
Mungbean	-	-	-	-	-	-
Mushroom	-	-	-	-	-	-
Mustard leaf	-	-	-	-	-	-
Neroli oil(essential oil)	-	-	-	-	-	-
Oakmoss absolute(essential oil)	-	-	-	-	-	-
Onion	-	-	-	-	-	-
Oolong tea	-	-	-	-	-	-
Orange	-	-	-	-	-	-
Orange florida oil(essential oil)	-	-	-	-	-	-
Orient clam	-	-	-	-	-	-
Oyster	-	-	-	-	-	-
Oyster mushroom	-	-	-	-	-	-
Pacific cod	-	-	-	-	-	-
Pacific herring	-	-	-	-	-	-
Pacific saury	-	-	-	-	-	-
Palmarosa madacascar oil(essential oil)	-	-	-	-	-	-
Peanuts	-	-	-	-	-	-
Pear	-	-	-	-	-	-
Perilla leaf	-	-	-	-	-	-
Perilla seeds	-	-	-	-	-	-
Persimon	-	-	-	-	-	-
Persimon bark, hard	-	-	-	-	-	-
Persimon leaf tea	-	-	-	-	-	-
Petitgrain limon tree(essential oil)	-	-	-	-	-	-

Table 5. Continued

Name	*P. aeruginosa*		*S. typhimurium*		*E. coli*	
	(I)	(II)	(I)	(II)	(I)	(II)
Pimento leaf oil(essential oil)	–	–	–	–	–	–
Pine nuts	–	–	–	–	–	–
Potatoes	–	–	–	–	–	–
Prosomillet	–	–	–	–	–	–
Pumpkin seeds	–	–	–	–	–	–
Pumpkin, matured	–	–	–	–	–	–
Quail's egg	–	–	–	–	–	–
Radish root	–	–	–	–	–	–
Red pepper paste	–	–	–	–	–	–
Rice	–	–	–	–	–	–
Rice hull	–	–	–	–	–	–
Royal fern	–	–	–	–	–	–
Rye	–	–	–	–	–	–
Sea cucumber	–	–	–	–	–	–
Sea lettuce	–	–	–	–	–	–
Sea mustard	–	–	–	–	–	–
Sea staghom	–	–	–	–	–	–
Sea tangle	–	–	–	–	–	–
Seasame seeds (China)	–	–	–	–	–	–
Seasame seeds (Korea)	–	–	–	–	–	–
Seasame seeds, black	–	–	–	–	–	–
Seaweed fusiform	–	–	–	–	–	–
Shepherd's purse	–	–	–	–	–	–
Shining Ganodernia	–	–	–	–	–	–
Shrimp,dried	–	–	–	–	–	–
Small red bean	–	–	–	–	–	–
Snail	–	–	–	–	–	–
Sorghum	–	–	–	–	–	–
Soybean	–	–	–	–	–	–
Soybean paste	–	–	–	10	–	10
Soybean sprout	–	–	–	–	–	–
Spanish mackerel	–	–	–	–	–	–
Spinach	–	–	–	–	–	–
Stem of taro	–	–	–	–	–	–
Strawberry	–	–	–	–	–	–
Sweet corn leaf	–	–	–	–	–	–
Sweet pepper, green	–	–	–	–	–	–
Sweet potato	–	–	–	–	–	–

Table 5. Continued

Name	*P. aeruginosa*		*S. typhimurium*		*E. coli*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Sweet potato stalk	–	–	–	–	–	–
Thyme white oil(essential oil)	–	–	–	–	–	–
Tomato	–	–	–	–	–	–
Turban shell	–	–	–	–	–	–
Ulmin tea	–	–	–	–	–	–
Unhulled Barley	–	–	–	–	–	–
Walnuts	–	–	–	–	–	–
Walnuts bark	–	–	–	–	–	–
Warty sea squirt	–	–	–	–	–	–
Water dropwort	–	–	–	–	–	–
Watermelon bark	–	–	–	–	–	–
Watermelon seed	–	–	–	–	–	–
Whelk	–	–	–	–	–	–
Wide rocambole stalk	–	–	–	–	–	–
Wild garlic	–	–	–	–	–	–
Winter fungus	–	–	–	–	–	–
Wintergreen oil(essential oil)	–	–	–	–	–	–
Wonduchung	–	–	–	–	–	–
Yellow croaker	–	–	–	–	–	–
Yellow tail	–	–	–	–	–	–
Zanthoxylum pipertitum	–	–	–	–	–	–

Table 6. Antimicrobial activity of food materials against yeasts

Unit : Inhibition zone diameter(mm)

Name	*Sacch. cerevisiae*[1)]		*C. albicans*[2)]	
	(Ⅰ)[3)]	(Ⅱ)[4)]	(Ⅰ)	(Ⅱ)
Acacia leaf	-	-	-	-
Alabesque greenling	-	-	-	-
Alaska pollack, dried	-	-	-	-
Alaska pollack, fresh	-	-	-	-
Alaska pollack,immature,dried	-	-	-	-
Alaska pollack,yellow	-	-	-	-
Allspice oleoresin(essential oil)	-	-	-	-
Anchovy boiled-dried	-	-	-	-
Anchovy, raw	-	-	-	-
Apple	-	-	-	-
Asparagus	-	-	-	-
Banana	-	-	-	-
Banana bark	-	-	-	-
Beka squid	-	-	-	-
Bergamot italian oil(essential oil)	-	-	-	-
Black soybean	-	-	-	-
Bois de rose(essential oil)	-	-	-	-
Braken	-	-	-	-
Broccoli	-	-	-	-
Buckwheat	-	-	-	-
Burdock	-	-	-	-
Butterbur	-	-	-	-
Cabbage	-	-	-	-
Cabbage root	-	-	-	-
Cham Chwi	-	-	-	-
Charcoal	-	-	-	-
Chard	-	-	-	-
Chestnuts bark	-	-	-	-
Chicory greens	-	-	-	-
Chinese quince	-	-	-	-
Citron	-	-	-	-
Citrus fruit	-	-	-	-
Clavaria botrytis	-	-	-	-

[1-2)] See the Table 1.

[3-4)] See the Table 4

Table 6. Continued

Name	*Sacch. cerevisiae*		*C. albicans*	
	(I)	(II)	(I)	(II)
Clove bud oil(essential oil)	-	-	-	-
Cocoa	-	-	-	-
Codonopsis lanceolate	-	-	-	-
Codonopsis lanceolate chew	-	-	-	-
Coffee	-	-	-	11
Coffee instant	-	-	-	16
Commom sea squirt	-	-	-	-
Common squid	-	-	-	-
Cowpeas	-	-	-	-
Crab	-	-	-	-
Crown daisy	-	-	-	-
Dandelion	-	-	-	-
Dog-tooth violet	-	15	-	14
Duchung tea	-	-	-	-
Dunggulle tea	-	-	-	-
Egg plant	-	-	-	-
File fish,fillet,dried	-	-	-	-
Flounder	-	-	-	-
Foxtail millet	-	-	-	-
Garlic	-	-	-	-
Geranium african oil(essential oil)	-	-	-	-
Ginger root	-	-	-	-
Gingko nuts	-	-	-	-
Gingko nuts leaf	-	-	-	-
Ginseng	-	-	-	-
Glutinous millet	-	-	-	-
Granulated ark shell	-	-	-	-
Grape fruits seed	-	-	-	-
Grape seed	-	-	-	-
Grape, large seed	-	-	-	-
Green pepper	-	-	-	-
Hair tail	-	-	-	-
Hard-shelled mussel	-	-	-	-
Hop, aroma	-	22	-	-
Hop, bitter	-	-	-	-
InJin-mugwort	-	-	-	-
Job's-tears	-	-	-	-
Juda's ear	-	-	-	-
Jujube	-	-	-	-

Table 6. Continued

Name	*Sacch. cerevisiae*		*C. albicans*	
	(I)	(II)	(I)	(II)
Kidney bean	-	-	-	-
Large green onion	-	-	-	-
Lavandin oil(essential oil)	-	-	-	-
Lavender absolute(essential oil)	-	-	-	-
Laver	-	-	-	-
Leek	-	10	-	33
Lemon	-	14	-	-
Lemon bark	-	-	-	-
Lentinus edodes, dried	-	-	-	-
Lettuce	-	-	-	-
Lettuce,Improved	-	-	-	-
Little neck clam	-	-	-	-
Mackerel	-	-	-	-
Mallow	-	-	-	-
Mugwort	-	-	-	-
Mungbean	-	-	-	-
Mushroom	-	-	-	-
Mustard leaf	-	-	-	10
Neroli oil(essential oil)	-	-	-	-
Oakmoss absolute(essential oil)	-	12	-	-
Onion	-	-	-	-
Oolong tea	-	-	-	-
Orange	-	-	-	-
Orange florida oil(essential oil)	-	-	-	-
Orient clam	-	-	-	-
Oyster	-	-	-	-
Oyster mushroom	-	-	-	-
Pacific cod	-	-	-	-
Pacific herring	-	-	-	-
Pacific saury	-	-	-	-
Palmarosa madacascar oil(essential oil)	-	-	-	-
Peanuts	-	-	-	-
Pear	-	-	-	-
Perilla leaf	-	-	-	-
Perilla seeds	-	-	-	-
Persimon	-	-	-	-
Persimon bark, hard	-	-	-	-
Persimon leaf tea	-	-	-	-
Petitgrain limon tree(essential oil)	-	-	-	-

Table 6. Continued

Name	*Sacch. cerevisiae*		*C. albicans*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Pimento leaf oil(essential oil)	-	-	-	-
Pine nuts	-	-	-	-
Potatoes	-	-	-	-
Prosomillet	-	-	-	-
Pumpkin seeds	-	-	-	-
Pumpkin, matured	-	-	-	-
Quail's egg	-	-	-	-
Radish root	-	-	-	-
Red pepper paste	-	-	-	-
Rice	-	-	-	-
Rice hull	-	-	-	-
Royal fern	-	-	-	-
Rye	-	-	-	-
Sea cucumber	-	-	-	-
Sea lettuce	-	-	-	-
Sea mustard	-	-	-	-
Sea staghom	-	-	-	-
Sea tangle	-	-	-	-
Seasame seeds(China)	-	-	-	-
Seasame seeds(Korea)	-	-	-	-
Seasame seeds, black	-	-	-	-
Seaweed fusiform	-	-	-	-
Shepherd's purse	-	-	-	-
Shining Ganodernia	-	-	-	-
Shrimp,dried	-	-	-	-
Small red bean	-	-	-	-
Snail	-	-	-	-
Sorghum	-	-	-	-
Soybean	-	-	-	-
Soybean paste	-	-	-	-
Soybean sprout	-	-	-	-
Spanish mackerel	-	-	-	-
Spinach	-	-	-	-
Stem of taro	-	-	-	-
Strawberry	-	-	-	-
Sweet corn leaf	-	-	-	-
Sweet pepper, green	-	-	-	-
Sweet potato	-	-	-	-

Table 6. Continued

Name	*Sacch. cerevisiae*		*C. albicans*	
	(I)	(II)	(I)	(II)
Sweet potato stalk	-	-	-	-
Thyme white oil(essential oil)	-	-	-	-
Tomato	-	-	-	-
Turban shell	-	-	-	-
Ulmin tea	-	-	-	-
Unhulled Barley	-	-	-	-
Walnuts	-	-	-	-
Walnuts bark	-	-	-	-
Warty sea squirt	-	-	-	-
Water dropwort	-	-	-	-
Watermelon bark	-	-	-	-
Watermelon seed	-	-	-	-
Whelk	-	-	-	-
Wide rocambole stalk	-	-	-	-
Wild garlic	-	-	-	-
Winter fungus	-	-	-	-
Wintergreen oil(essential oil)	-	-	-	-
Wonduchung	-	-	-	9
Yellow croaker	-	-	-	-
Yellow tail	-	-	-	-
Zanthoxylum pipertitum	-	-	-	-

Table 7. Antimicrobial activity of food materials against molds

Unit : Inhibition zone diameter(mm)

Name	*P. citrinum*[1)]		*Asp. flavus*[2)]		*Asp. niger*[3)]	
	(I)[4)]	(II)[5)]	(I)	(II)	(I)	(II)
Acacia leaf	-	-	-	-	-	-
Alabesque greenling	-	-	-	-	-	-
Alaska pollack, dried	-	-	-	-	-	-
Alaska pollack, fresh	-	-	-	-	-	-
Alaska pollack,immature,dried	-	-	-	-	-	-
Alaska pollack,yellow	-	-	-	-	-	-
Allspice oleoresin(essential oil)	-	-	-	-	-	-
Anchovy boiled-dried	-	-	-	-	-	-
Anchovy, raw	-	-	-	-	-	-
Apple	-	-	-	-	-	-
Asparagus	-	-	-	-	-	-
Banana	-	-	-	-	-	-
Banana bark	-	-	-	-	-	-
Beka squid	-	-	-	-	-	-
Bergamot italian oil(essential oil)	-	-	-	-	-	15
Black soybean	-	-	-	-	-	-
Bois de rose(essential oil)	-	-	-	-	-	-
Braken	-	-	-	-	-	-
Broccoli	-	-	-	-	-	-
Buckwheat	-	-	-	-	-	-
Burdock	-	-	-	-	-	-
Butterbur	-	-	-	-	-	-
Cabbage	-	-	-	-	-	-
Cabbage root	-	-	-	-	-	-
Cham Chwi	-	-	-	-	-	-
Charcoal	-	-	-	-	-	-
Chard	-	-	-	-	-	-
Chestnuts bark	-	-	-	-	-	-
Chicory greens	-	-	-	-	-	-
Chinese quince	-	-	-	13	-	11
Citron	-	-	-	-	-	-
Citrus fruit	-	-	-	-	-	-
Clavaria botrytis	-	-	-	-	-	-

[1-3)] See the Table 1.

[4-5)] See the Table 4.

Table 7. Continued

Name	*P. citrinum*		*Asp. flavus*		*Asp. niger*	
	(I)	(II)	(I)	(II)	(I)	(II)
Clove bud oil(essential oil)	-	-	-	-		
Cocoa	-	-	-	-	-	-
Codonopsis lanceolate	-	-	-	-		
Codonopsis lanceolate chew	-	-	-	-		
Coffee	-	-	-	-	-	-
Coffee instant	-	-	-	-	-	-
Commom sea squirt	-	-	-	-	-	-
Common squid	-	-	-	-	-	-
Cowpeas	-	-	-	-	-	-
Crab	-	-	-	-	-	-
Crown daisy	-	-	-	-	-	-
Dandelion	-	-	-	-	-	-
Dog-tooth violet	-	28	-	31	12	33
Duchung tea	-	-	-	-	-	-
Dunggulle tea	-	-	-	-	-	-
Egg plant	-	-	-	-	-	-
File fish,fillet,dried	-	-	-	-	-	-
Flounder	-	-	-	-	-	-
Foxtail millet	-	-	-	-	-	-
Garlic	-	-	-	-	-	-
Geranium african oil(essential oil)	-	-	-	-	-	-
Ginger root	-	-	-	-	-	-
Gingko nuts	-	-	-	-	-	-
Gingko nuts leaf	-	-	-	-	-	-
Ginseng	-	-	-	-	-	-
Glutinous millet	-	-	-	-	-	-
Granulated ark shell	-	-	-	-	-	-
Grape fruits seed	-	-	-	-	-	-
Grape seed	-	-	-	-	-	-
Grape, large seed	-	-	-	-	-	-
Green pepper	-	-	-	-	-	-
Hair tail	-	-	-	-	-	-
Hard-shelled mussel	-	-	-	-	-	-
Hop, aroma	-	-	-	12	-	-
Hop, bitter	-	-	-	-	-	-
InJin-mugwort	-	-	-	-	-	-
Job's-tears	-	-	-	-	-	-
Juda's ear	-	-	-	-	-	-
Jujube	-	-	-	-	-	-

Table 7. Continued

Name	*P. citrinum*		*Asp. flavus*		*Asp. niger*	
	(I)	(II)	(I)	(II)	(I)	(II)
Kidney bean	-	-	-	-	-	-
Large green onion	-	-	-	-	-	-
Lavandin oil(essential oil)	-	-	-	-	-	-
Lavender absolute(essential oil)	-	-	-	-	-	-
Laver	-	-	-	-	-	-
Leek	-	-	-	-	-	-
Lemon	-	21	-	26	-	20
Lemon bark	-	13	-	-	-	-
Lentinus edodes, dried	-	-	-	-	-	-
Lettuce	-	-	-	-	-	-
Lettuce,Improved	-	-	-	-	-	-
Little neck clam	-	-	-	-	-	-
Mackerel	-	-	-	-	-	-
Mallow	-	-	-	-	-	-
Mugwort	-	-	-	-	-	-
Mungbean	-	-	-	-	-	-
Mushroom	-	-	-	-	-	-
Mustard leaf	-	-	-	10	-	-
Neroli oil(essential oil)	-	-	-	-	-	10
Oakmoss absolute(essential oil)	-	-	-	-	-	12
Onion	-	-	-	-	-	-
Oolong tea	-	-	-	-	-	-
Orange	-	-	-	-	-	-
Orange florida oil(essential oil)	-	-	-	-	-	-
Orient clam	-	-	-	-	-	-
Oyster	-	-	-	-	-	-
Oyster mushroom	-	-	-	-	-	-
Pacific cod	-	-	-	-	-	-
Pacific herring	-	-	-	-	-	-
Pacific saury	-	-	-	-	-	-
Palmarosa madacascar oil(essential oil)	-	-	-	-	-	-
Peanuts	-	-	-	-	-	-
Pear	-	-	-	-	-	-
Perilla leaf	-	-	-	-	-	-
Perilla seeds	-	-	-	-	-	-
Persimon	-	-	-	-	-	-
Persimon bark, hard	-	-	-	-		
Persimon leaf tea	-	-	-	-	-	-
Petitgrain limon tree(essential oil)	-	-	-	-	-	-

Table 7. Continued

Name	*P. citrinum*		*Asp. flavus*		*Asp. niger*	
	(I)	(II)	(I)	(II)	(I)	(II)
Pimento leaf oil(essential oil)	–	–	–	–	–	12
Pine nuts	–	–	–	–	–	–
Potatoes	–	–	–	–	–	–
Prosomillet	–	–	–	–	–	–
Pumpkin seeds	–	–	–	–	–	–
Pumpkin, matured	–	–	–	–	–	–
Quail's egg	–	–	–	–	–	–
Radish root	–	–	–	–	–	–
Red pepper paste	–	–	–	–	–	–
Rice	–	–	–	–	–	–
Rice hull	–	–	–	–	–	–
Royal fern	–	–	–	–	–	–
Rye	–	–	–	–	–	–
Sea cucumber	–	–	–	–	–	–
Sea lettuce	–	–	–	–	–	–
Sea mustard	–	–	–	–	–	–
Sea staghom	–	–	–	–	–	–
Sea tangle	–	–	–	–		
Seasame seeds(China)	–	–	–	–	–	–
Seasame seeds(Korea)	–	–	–	–	–	–
Seasame seeds, black	–	–	–	–	–	–
Seaweed fusiform	–	–	–	–	–	–
Shepherd's purse	–	–	–	–	–	–
Shining Ganodernia	–	–	–	–	–	–
Shrimp,dried	–	–	–	–	–	–
Small red bean	–	–	–	–	–	–
Snail	–	–	–	–	–	–
Sorghum	–	–	–	–	–	–
Soybean	–	–	–	–	–	–
Soybean paste	–	–	–	–	–	–
Soybean sprout	–	–	–	–	–	–
Spanish mackerel	–	–	–	–	–	–
Spinach	–	–	–	–	–	–
Stem of taro	–	–	–	–	–	–
Strawberry	–	–	–	–	–	–
Sweet corn leaf	–	–	–	–	–	–
Sweet pepper, green	–	–	–	–	–	–
Sweet potato	–	–	–	–	–	–

Table 7. Continued

Name	*P. citrinum*		*Asp. flavus*		*Asp. niger*	
	(I)	(II)	(I)	(II)	(I)	(II)
Sweet potato stalk	-	-	-	-	-	-
Thyme white oil(essential oil)	-	-	-	-	-	12
Tomato	-	-	-	-	-	-
Turban shell	-	-	-	-	-	-
Ulmin tea	-	-	-	-	-	-
Unhulled Barley	-	-	-	-	-	-
Walnuts	-	-	-	-	-	-
Walnuts bark	-	-	-	-	-	-
Warty sea squirt	-	-	-	-	-	-
Water dropwort	-	-	-	-	-	-
Watermelon bark	-	-	-	-	-	-
Watermelon seed	-	-	-	-	-	-
Whelk	-	-	-	-	-	-
Wide rocambole stalk	-	-	-	-	-	-
Wild garlic	-	-	-	-		
Winter fungus	-	-	-	-	-	-
Wintergreen oil(essential oil)	-	-	-	-	-	-
Wonduchung	-	-	-	-	-	-
Yellow croaker	-	-	-	-	-	-
Yellow tail	-	-	-	-	-	-
Zanthoxylum pipertitum	-	-	-	-	-	-

Table 8. Antibacterial activity of medicinal herbs against Gram(+)bacteria

Unit : Inhibition zone diameter(mm)

Scientific name	*M. luteus*[1]		*B. subtilis*[2]		*B. cereus*[3]		*L. monocytogenes*[4]	
	(Ⅰ)[5]	(Ⅱ)[6]	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Acanthopanax sessiliflorum	-	-	-	-	-	-	-	-
Achyranthes japonica	-	-	-	-	-	-	-	-
Aconitum pseudo-laeve	-	-	-	-	-	11	-	-
Acorus gramineus	-	10	-	12	-	12	-	-
Adenophora triphylla	-	-	-	-	-	-	-	-
Agastache rugosa	-	-	-	-	-	22	-	-
Akebia quinata	-	-	-	-	-	-	-	-
Albizzia julibrissin	-	-	-	-	-	17	-	-
Alisma orientale	-	-	-	-	-	-	-	-
Allium tuberosum	-	-	-	-	-	-	-	-
Alpinia officinarum	-	16	-	12	14	18	-	-
Alpinia oxyphylla	-	-	-	-	-	-	-	-
Amomum cardamomum	-	-	-	-	-	13	-	12
Amomum tsao-ko Crevostet	-	-	-	-	-	-	-	-
Amomum xanthioides	-	-	-	14	-	-	-	-
Amyda maakii	-	-	-	-	-	-	-	-
Anemarrhena asphodeloides	-	-	-	-	-	14	-	-
Angelica dahurica	-	-	-	14	-	22	-	14
Angelica gigas	-	-	-	-	-	-	-	-
Angelica koreana	-	10	-	9	-	-	-	-
Angelica tenuissima	-	-	-	-	-	-	-	-
Angelica utilis	-	20	10	11	10	11	-	-
Anthriscus sylvestris	-	-	-	-	-	-	-	-
Aralia cordata	-	-	-	-	-	-	-	-
Arctium lappa	-	-	-	-	-	-	-	-
Areca catechu	-	-	-	-	-	-	-	11
Arisaema amurense	-	-	-	-	-	-	-	-
Artemisia asiatica	-	-	-	-	-	-	-	-
Asiasarum sieboldi	-	-	-	-	-	-	-	-
Astragalus membranaceus	-	-	-	-	-	21	-	-
Atractylodes japonica	-	-	-	-	-	-	-	+
Atractylodes lancea	-	-	-	-	-	17	-	-

[1-4] See the Table 1.

[5-6] See the Table 4.

[7] Weak

Table 8. Continued

Scientific name	*M. luteus*		*B. subtilis*		*B. cereus*		*L. monocytogenes*	
	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)
Belamcanda chinensis	–	–	–	10	–	19	–	–
Betula platyphylla	–	12	–	–	–	12	–	12
Biota orientalis	–	–	–	11	–	–	–	–
Bombyx mori	–	–	–	–	–	–	–	–
Brassica alba	–	–	–	–	–	–	–	–
Bupleurum falcatum	–	–	–	–	–	10	–	–
Carthamus tinctoris	–	–	–	–	–	–	–	–
Cassia obtusifolia	–	–	–	–	–	–	–	–
Catalpa ovata	–	–	–	–	–	–	–	–
Celosia argentea	–	–	–	–	–	–	–	–
Chaenomeles sinensis	–	19	–	10	–	18	–	–
Chrysanthemum indicum	–	–	–	–	–	–	–	–
Chrysanthemum zawadskii	–	–	–	–	–	w13[7)]	–	–
Cibotium barometz	–	–	–	10	–	28	–	–
Cimicifuga heracleifolia	–	–	–	–	–	10	–	–
Cinnamomum cassia(Cinnamomumi cortex spissus)	–	11	–	15	–	w22[7)]	–	21
Cinnamomum cassia(Cinnamomi ramulus)	–	–	–	–	–	–	–	–
Cinnamomum cassia(Cinnamom bark)	–	–	–	13	–	–	–	–
Cistanche deserticola	–	–	–	–	–	–	–	–
Citrus aurantium	–	–	–	–	–	–	–	–
Citrus unshiu	–	–	–	–	–	–	–	–
Clematis mandshurica	–	–	–	–	–	13	–	–
Cnidium officinale	–	–	–	10	–	–	–	–
Codonopsis pilosula	–	–	–	–	–	14	–	–
Coix lacryma-jobi	–	–	–	–	–	–	–	–
commiphora molmol	–	11	–	18	–	14	–	11
Coptis japonica	16	27	14	21	–	–	–	12
Cornus officinalis	–	10	–	13	–	–	–	11
Corydalis ternata	–	–	–	–	–	–	–	13
Crataegus pinnatifida	–	–	–	14	19	43	–	–
Cryptotympana pustulata	–	–	–	–	–	–	–	–
Curcuma longa	–	–	–	–	–	–	–	–
Curcuma zedoaria	–	–	–	–	–	–	–	–
Cuscuta chinensi	–	–	–	–	–	–	–	–
Cynomorium songaricum	–	–	–	–	–	–	–	–
Cyperus rotundus	–	–	–	–	–	20	–	–

Table 8. Continued

Scientific name	*M. luteus*		*B. subtilis*		*B. cereus*		*L. monocytogenes*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Dendrobium nobile	–	–	–	–	–	–	–	–
Dictamnus albus	–	–	–	–	–	–	–	–
Dioscorea japonica	–	–	–	–	–	–	–	–
Dioscorea tokoro	–	–	–	–	–	–	–	–
Dolichos lablab	–	–	–	–	–	–	–	–
Drynaria fortunei	–	–	–	–	–	–	–	–
Elsholtzia ciliata	–	–	–	16	–	–	–	–
Ephedra sinica	–	–	–	–	–	–	–	–
Epimedium koreanum	–	–	–	–	–	–	–	–
Equisetum hiemale	–	–	–	–	–	19	–	–
Eriobotrya japonica	–	–	–	–	–	–	–	–
Eucommia ulmoides	–	–	–	10	–	–	–	–
Eugenia caryophyllata	–	–	–	–	–	17	–	w16[7)]
Euryale ferox	–	–	–	–	–	–	–	–
Evodia officinalis	–	11	–	15	12	19	–	–
Foeniculum vulgare	–	9	–	–	–	20	–	–
Fossilia ossis	–	–	–	–	–	–	–	–
Gallus domesticus	–	–	–	12	–	12	–	–
Gardenia jasminoides	–	–	–	–	–	22	–	–
Gastrodia elata	–	–	–	–	–	–	–	–
Gentiana macrophylla	–	–		–	–	–	–	–
Gentiana scabra	–	–	–	25	–	–	–	–
Geranium thunbergii	w16[7)]	w25[7)]		–	–	w18[7)]	–	w10[7)]
Gleditsia japonica	–	–	–	–	–	–	–	–
Glycyrrhiza uralensis fischer	11	20	–	18	–	23	12	14
Gypsum	–	–	–	–	–	–	–	–
Haliotis gigantea	–	–	–	–	–	–	–	–
Halloysite	–	–	–	–	–	–	–	–
Hordeum vulgare	–	–	–	–	–	–	–	–
Houttuynia cordata	–	–	–	–	–	26	–	–
Hovenia dulcis	–	–	–	–	–	–	–	–
Juniperus chinensis	–	–	–	–	–	–	–	12
Kalopanax pictus	–	–	–	–	–	13	–	–
Kochia scoparia	–	–	–	–	–	–	–	–
Ledebouriella	–	–	–	–	–	–	–	–

Table 8. Continued

Scientific name	*M. luteus*		*B. subtilis*		*B. cereus*		*L. monocytogenes*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Leonurus sibiricus	-	-	-	-	-	-	-	-
Ligustrum lucidum	-	-	-	-	-	10	-	-
Lindera strichnifolia	-	19	-	-	10	16	-	-
Lithospermum erythrorhizon	-	-	-	-	-	-	-	-
Lonicera japonica thunberg(lonicerae flos)	-	-	-	10	-	-	-	-
Lonicera japonica thunberg(lonicerae folium)	-	-	-	-	-	-	-	14
Loranthus parasiticus	-	-	-	-	-	-	-	-
Lycium chinense(lycii fructus)	-	12	-	-	-	22	-	-
Lycium chinense(lycii radicis cortex)	-	15	-	-	-	-	-	-
Lycopus corenus	-	-	-	-	-	-	-	-
Magnolia officinalis	-	12	-	10	-	21	-	-
Mahnolia denudata	-	11	-	-	-	13	-	-
Medicata fementata	-	-	-	-	-	-	-	-
Melia azedarach(meliae cortex)	-	-	-	-	-	-	-	-
Melia azedarach(meliae fructus)	-	-	-	-	-	-	-	-
Mentha arvensis	-	-	-	-	-	-	-	-
Morinda officinalis	-	-	-	-	-	17	-	-
Morus albal	-	-	-	-	-	-	-	10
Myristica fragrans	-	-	-	-	-	13	-	-
Nardostachys chinensis	-	-	-	-	-	11	-	-
Nelumbo nucifera	-	-	-	-	-	-	-	-
Ostrea gigas	-	15	-	19	-	13	-	-
Pachyma hoelen	-	-	-	-	-	-	-	-
Paeonia albiflora	-	-	-	-	-	21	-	-
Paeonia moutan	-	-	-	10	-	-	-	10
Patrinia villosa	-	12	-	-	9	16	9	13
Perilla frutescens	-	14	-	-	-	15	-	-
Perilla sikokiana	-	11	-	-	-	19	-	-
Pharbitis nil choisy	-	-	-	-	-	-	-	-
Phellodendron amurense	-	20	-	-	-	-	-	-
Phlomis umbrosa	-	-	-	21	-	13	-	-
Phyllostachys nigra	13	17	-	-	-	27	-	13
Pinellia ternata breitenbach	-	-	-	-	-	-	-	-
Pinus densiflora	-	-	-	-	-	-	-	9

Table 8. Continued

Scientific name	*M. luteus*		*B. subtilis*		*B. cereus*		*L. monocytogenes*	
	(I)	(II)	(I)	(II)	(I)	(II)	(I)	(II)
Plantago asiatica	-	-	-	-	-	-	-	11
Platycodon grandiflorum	-	-	-	-	-	-	-	-
Polygala tenuifolia	-	-	-	-	-	-	-	-
Polygonatum sibiricum	-	-	-	-	-	-	-	-
Polygonum aviculare	-	-	-	-	-	-	-	-
Polygonum multiflorum	-	-	-	-	-	-	-	-
Polyporus umbellatus	-	11	-	-	-	-	-	12
Poria cocos wolf	-	-	-	-	-	-	-	9
Porsythia viridissima	-	-	-	-	-	-	-	-
Prunella vulgaris	-	-	-	-	-	11	-	-
Prunus armeniaca	-	-	-	-	-	-	-	-
Prunus mume	-	34	-	22	14	29	-	19
Prunus nakaii	-	-	-	-	-	-	-	-
Prunus persica	-	-	-	-	-	-	-	-
Psoralea corylifolia	-	-	-	13	-	19	-	-
Pueraria thunbergiana	-	-	-	-	-	-	-	-
Raphanus sativus	-	-	-	-	-	-	-	-
Rehmannia glutinosa	-	-	-	-	-	-	-	-
Rheum palmatum	-	-	-	23	-	-	-	-
Rhus javanica	-	-	10	26	-	-	-	-
Rosa laevigata	-	-	-	-	-	20	-	-
Rubia akane	-	-	-	9	-	-	-	-
Rubus coreanus miquel	-	20	-	15	-	-	-	-
Salvia miltiorrhiza	14	25	-	13	-	20	10	13
Sanguisorba officinalis	11	15	-	9	-	12	-	-
Saussurea lappa	-	-	-	-	-	22	19	22
Schizandra chinensis	-	11	10	22	-	-	-	17
Scirpus flaviatilis	-	-	-	-	-	-	-	-
Scrophularia vuergeriana	-	-	-	-	-	22	-	-
Scutellaria baicalensis	-	17	-	-	-	16	-	-
Siegesbeckia orientalis	-	17	-	11	12	17	-	13
Sinomenium acutum	-	-	-	-	-	-	-	-
Smilax china	-	-	-	-	-	-	-	-
Sodium sulfate	-	-	-	-	-	-	-	-
Sophara japonica	-	-	-	-	-	12	-	-

Table 8. Continued

Scientific name	*M. luteus*		*B. subtilis*		*B. cereus*		*L. monocytogenes*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Sophora flavescens	19	25	16	17	17	20	-	13
Sophora subprostrata	-	-	-	-	-	-	-	11
Spiradela polyrhiza	-	-	-	-	-	12	-	-
Stemona japonica	-	-	-	-	-	-	-	-
Talc	-	-	-	-	-	-	-	-
Tetrapanax papyriferus	-	+	-	-	-	31	-	-
Thuja orientalis	-	-	-	-	-	13	-	-
Torreya nucifera	-	-	-	-	-	-	-	-
Trichosanthes kirlowii	-	-	-	-	-	-	-	-
Trogopterus xanthipes	-	-	-	-	-	-	-	-
Tussilago farfara	-	-	-	-	-	-	-	-
Typha orientalis	-	-	-	-	-	17	-	-
Uncaria sinensis havil	-	-	-	-	-	-	-	-
Vitex rotundifolia	-	-	-	16	-	-	-	-
Xanthium strumarium	-	-	-	-	-	-	-	-
Zanthoxylum schinifolium	-	11	-	9	-	-	-	10
Zingiber officinale	-	-	-	-	-	11	-	-
Zizyphus vulgaris	-	-	-	-	-	-	-	11

Table 9. Antibacterial activity of medicinal herbs against Gram(-)bacteria

Unit : Inhibition zone diameter(mm)

Scientific name	*P. aeruginosa*[1]		*S. typhimurium*[2]		*E. coli*[3]	
	(Ⅰ)[4]	(Ⅱ)[5]	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Acanthopanax sessiliflorum	-	-	-	-	-	-
Achyranthes japonica	-	-	-	-	-	-
Aconitum pseudo-laeve	-	-	-	-	-	-
Acorus gramineus	-	-	-	-	-	13
Adenophora triphylla	-	-	-	-	-	-
Agastache rugosa	-	-	-	-	-	-
Akebia quinata	-	-	-	-	-	-
Albizzia julibrissin	-	-	-	-	-	-
Alisma orientale	-	-	-	-	-	-
Allium tuberosum	-	-	-	-	-	-
Alpinia officinarum	-	-	-	-	-	-
Alpinia oxyphylla	-	-	-	-	-	-
Amomum cardamomum	-	-	-	-	-	-
Amomum tsao-ko Crevostet	-	-	-	-	-	-
Amomum xanthioides	-	-	-	-	-	-
Amyda maakii	-	-	-	-	-	-
Anemarrhena asphodeloides	-	-	-	-	-	-
Angelica dahurica	-	-	-	-	-	-
Angelica gigas	-	-	-	-	-	-
Angelica koreana	-	-	-	-	-	-
Angelica tenuissima	-	-	-	-	-	-
Angelica utilis	-	-	-	-	-	-
Anthriscus sylvestris	-	-	-	-	-	-
Aralia cordata	-	-	-	12	-	-
Arctium lappa	-	-	-	-	-	-
Areca catechu	-	-	-	-	-	-
Arisaema amurense	-	-	-	-	-	-
Artemisia asiatica	-	-	-	-	-	-
Asiasarum sieboldi	-	-	-	-	-	-
Astragalus membranaceus	-	-	-	-	-	-
Atractylodes japonica	-	-	-	-	-	-
Atractylodes lancea	-	-	-	-	-	-

[1-3] See the Table 1.

[4-5] See the Table 4.

[6] See the Table 8.

Table 9. Continued

Scientific name	*P. aeruginosa*		*S. typhimurium*		*E. coli*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Belamcanda chinensis	-	-	-	-	-	-
Betula platyphylla	-	-	-	-	-	-
Biota orientalis	-	-	-	-	-	-
Bombyx mori	-	-	-	-	-	-
Brassica alba	-	-	-	-	-	-
Bupleurum falcatum	-	-	-	-	-	-
Carthamus tinctoris	-	-	-	-	-	-
Cassia obtusifolia	-	-	-	-	-	-
Catalpa ovata	-	-	-	-	-	-
Celosia argentea	-	-	-	-	-	-
Chaenomeles sinensis	-	-	-	-	-	-
Chrysanthemum indicum	-	-	-	-	-	-
Chrysanthemum zawadskii	-	-	-	-	-	-
Cibotium barometz	-	-	-	-	-	-
Cimicifuga heracleifolia	-	-	-	-	-	-
Cinnamomum cassia(Cinnamomumi cortex spissus)	-	34	-	23	-	30
Cinnamomum cassia(Cinnamomumi ramulus)	-	-	-	-	-	-
Cinnamomum cassia(Cinnamom bark)	-	-	-	-	-	-
Cistanche deserticola	-	-	-	-	-	-
Citrus aurantium	-	-	-	-	-	-
Citrus unshiu	-	-	-	-	-	-
Clematis mandshurica	-	-	-	-	-	-
Cnidium officinale	-	-	-	-	-	-
Codonopsis pilosula	-	-	-	-	-	-
Coix lacryma-jobi	-	-	-	-	-	-
Commiphora molmol	-	-	-	-	-	-
Coptis japonica	-	-	-	-	-	13
Cornus officinalis	-	-	-	-	-	-
Corydalis ternata	-	-	-	-	-	-
Crataegus pinnatifida	-	-	-	-	-	-
Cryptotympana pustulata	-	-	-	-	-	-
Curcuma longa	-	-	-	-	-	-
Curcuma zedoaria	-	-	-	-	-	-
Cuscuta chinensi	-	-	-	-	-	-
Cynomorium songaricum	-	-	-	-	-	-

Table 9. Continued

Scientific name	*P. aeruginosa*		*S. typhimurium*		*E. coli*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Cyperus rotundus	–	–	–	–	–	–
Dendrobium nobile	–	–	–	–	–	–
Dictamnus albus	–	–	–	–	–	–
Dioscorea japonica	–	–	–	–	–	–
Dioscorea tokoro	–	–	–	–	–	–
Dolichos lablab	–	–	–	–	–	–
Drynaria fortunei	–	–	–	–	–	–
Elsholtzia ciliata	–	–	–	–	–	–
Ephedra sinica	–	11	–	–	–	–
Epimedium koreanum	–	–	–	–	–	–
Equisetum hiemale	–	–	–	–	–	–
Eriobotrya japonica	–	–	–	–	–	–
Eucommia ulmoides	–	–	–	–	–	–
Eugenia caryophyllata	–	–	–	w14[6)]	–	w23[6)]
Euryale ferox	–	–	–	–	–	–
Evodia officinalis	–	–	–	–	–	–
Foeniculum vulgare	–	–	–	–	–	–
Fossilia ossis	–	–	–	–	–	–
Gallus domesticus	–	–	–	–	–	–
Gardenia jasminoides	–	–	–	–	–	–
Gastrodia elata	–	–	–	–	–	–
Gentiana macrophylla	–	–	–	–	–	–
Gentiana scabra	–	–	–	–	–	–
Geranium thunbergii	–	–	–	–	–	12
Gleditsia japonica	–	–	–	–	–	–
Glycyrrhiza uralensis fischer	–	–	–	–	–	–
Gypsum	–	–	–	–	–	–
Haliotis gigantea	–	–	–	–	–	–
Halloysite	–	–	–	–	–	–
Hordeum vulgare	–	–	–	–	–	–
Houttuynia cordata	–	–	–	–	–	–
Hovenia dulcis	–	–	–	–	–	–
Juniperus chinensis	–	–	–	–	–	–
Kalopanax pictus	–	–	–	–	–	–
Kochia scoparia	–	–	–	–	–	–

Table 9. Continued

Scientific name	*P. aeruginosa*		*S. typhimurium*		*E. coli*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Ledebouriella	-	-	-	-	-	-
Leonurus sibiricus	-	-	-	-	-	-
Ligustrum lucidum	-	-	-	-	-	-
Lindera strichnifolia	-	-	-	-	-	-
Lithospermum erythrorhizon	-	-	-	-	-	-
Lonicera japonica thunberg(lonicerae flos)	-	-	-	-	-	-
Lonicera japonica thunberg(lonicerae folium)	-	-	-	-	-	-
Loranthus parasiticus	-	-	-	-	-	-
Lycium chinense(lycii fructus)	-	-	-	-	-	-
Lycium chinense(lycii radicis cortex)	-	-	-	-	-	-
Lycopus corenus	-	-	-	-	-	-
Magnolia officinalis	-	-	-	-	-	-
Mahnolia denudata	-	-	-	-	-	-
Medicata fementata	-	-	-	-	-	-
Melia azedarach(meliae cortex)	-	-	-	-	-	-
Melia azedarach(meliae fructus)	-	-	-	-	-	-
Mentha arvensis	-	-	-	-	-	-
Morinda officinalis	-	-	-	-	-	-
Morus albal	-	-	-	-	-	-
Myristica fragrans	-	-	-	-	-	-
Nardostachys chinensis	-	-	-	-	-	-
Nelumbo nucifera	-	-	-	-	-	-
Ostrea gigas	-	-	-	-	-	-
Pachyma hoelen	-	-	-	-	-	-
Paeonia albiflora	-	-	-	-	-	-
Paeonia moutan	-	-	-	-	-	-
Patrinia villosa	-	-	-	-	-	-
Perilla frutescens	-	-	-	-	-	-
Perilla sikokiana	-	-	-	-	-	13
Pharbitis nil choisy	-	-	-	-	-	-
Phellodendron amurense	-	-	-	-	-	-
Phlomis umbrosa	-	-	-	10	-	-
Phyllostachys nigra	-	-	-	-	-	-
Pinellia ternata breitenbach	-	-	-	-	-	-

Table 9. Continued

Scientific name	*P. aeruginosa*		*S. typhimurium*		*E. coli*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Pinus densiflora	–	–	–	–	–	–
Plantago asiatica	–	–	–	–	–	–
Platycodon grandiflorum	–	–	–	–	–	–
Polygala tenuifolia	–	–	–	–	–	–
Polygonatum sibiricum	–	–	–	–	–	–
Polygonum aviculare	–	–	–	–	–	–
Polygonum multiflorum	–	–	–	–	–	–
Polyporus umbellatus	–	–	–	13	–	–
Poria cocos wolf	–	–	–	–	–	–
Porsythia viridissima	–	–	–	–	–	–
Prunella vulgaris	–	–	–	–	–	–
Prunus armeniaca	–	–	–	–	–	–
Prunus mume	–	10	–	16	–	18
Prunus nakaii	–	–	–	–	–	–
Prunus persica	–	–	–	–	–	–
Psoralea corylifolia	–	–	–	–	–	–
Pueraria thunbergiana	–	–	–	–	–	–
Raphanus sativus	–	–	–	–	–	–
Rehmannia glutinosa	–	–	–	–	–	w25[6)]
Rheum palmatum	–	–	–	–	–	–
Rhus javanica	–	–	–	–	–	–
Rosa laevigata	–	–	–	–	–	–
Rubia akane	–	–	–	–	–	–
Rubus coreanus miquel	–	12	–	13	–	–
Salvia miltiorrhiza	–	–	–	–	–	14
Sanguisorba officinalis	–	–	–	14	–	–
Saussurea lappa	–	–	–	–	–	14
Schizandra chinensis	–	–	–	–	–	20
Scirpus flaviatilis	–	–	–	–	–	–
Scrophularia vuergeriana	–	–	–	–	–	w15[6)]
Scutellaria baicalensis	–	–	–	–	–	–
Siegesbeckia orientalis	–	–	–	–	–	–
Sinomenium acutum	–	–	–	–	–	–
Smilax china	–	–	–	–	–	–
Sodium sulfate	–	–	–	–	–	–

Table 9. Continued

Scientific name	*P. aeruginosa*		*S. typhimurium*		*E. coli*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Sophara japonica	−	−	−	−	−	−
Sophora flavescens	−	−	−	−	−	−
Sophora subprostrata	−	−	−	−	−	−
Spiradela polyrhiza	−	−	−	−	−	−
Stemona japonica	−	−	−	−	−	−
Talc	−	−	−	−	−	−
Tetrapanax papyriferus	−	−	−	−	−	−
Thuja orientalis	−	−	−	−	−	−
Torreya nucifera	−	−	−	−	−	−
Trichosanthes kirlowii	−	−	−	−	−	−
Trogopterus xanthipes	−	−	−	−	−	−
Tussilago farfara	−	−	−	−	−	−
Typha orientalis	−	−	−	−	−	−
Uncaria sinensis havil	−	−	−	−	−	−
Vitex rotundifolia	−	−	−	−	−	w18[6)]
Xanthium strumarium	−	−	−	−	−	−
Zanthoxylum schinifolium	−	−	−	−	−	−
Zingiber officinale	−	−	−	−	−	−
Zizyphus vulgaris	−	−	−	−	−	−

Table 10. Antimicrobial activity of medicinal herbs against yeasts

Unit : Inhibition zone diameter(mm)

Scientific name	*Sacch. cerevisiae*[1)]		*C. albicans*[2)]	
	(Ⅰ)[3)]	(Ⅱ)[4)]	(Ⅰ)	(Ⅱ)
Acanthopanax sessiliflorum	-	-	-	-
Achyranthes japonica	-	-	-	-
Aconitum pseudo-laeve	-	-	-	-
Acorus gramineus	-	-	-	-
Adenophora triphylla	-	-	-	-
Agastache rugosa	-	-	-	-
Akebia quinata	-	-	-	-
Albizzia julibrissin	-	-	-	-
Alisma orientale	-	14	-	-
Allium tuberosum	-	-	-	-
Alpinia officinarum	-	-	-	-
Alpinia oxyphylla	-	-	-	-
Amomum cardamomum	-	12	-	-
Amomum tsao-ko Crevostet	-	12	-	-
Amomum xanthioides	-	-	-	-
Amyda maakii	-	-	-	-
Anemarrhena asphodeloides	-	-	-	15
Angelica dahurica	-	-	-	-
Angelica gigas	-	-	-	-
Angelica koreana	-	-	-	-
Angelica tenuissima	-	-	-	-
Angelica utilis	-	-	-	-
Anthriscus sylvestris	-	-	-	-
Aralia cordata	-	-	-	-
Arctium lappa	-	-	-	-
Areca catechu	-	-	-	-
Arisaema amurense	-	-	-	-
Artemisia asiatica	-	13	-	-
Asiasarum sieboldi	12	20	-	-
Astragalus membranaceus	-	-	-	-
Atractylodes japonica	-	-	-	-

1-2) See the Table 1.

3-4) See the Table 4.

5) See the Table 8.

Table 10. Continued

Scientific name	*Sacch. cerevisiae*		*C. albicans*	
	(I)	(II)	(I)	(II)
Atractylodes lancea	-	-	-	-
Belamcanda chinensis	-	-	-	-
Betula platyphylla	-	13	-	-
Biota orientalis	-	-	-	-
Bombyx mori	-	-	-	-
Brassica alba	-	-	-	-
Bupleurum falcatum	-	-	-	-
Carthamus tinctoris	-	-	-	-
Cassia obtusifolia	-	-	-	-
Catalpa ovata	-	-	-	-
Celosia argentea	-	-	-	-
Chaenomeles sinensis	-	-	-	-
Chrysanthemum indicum	-	-	-	-
Chrysanthemum zawadskii	-	13	-	-
Cibotium barometz	-	-	-	-
Cimicifuga heracleifolia	-	-	-	-
Cinnamomum cassia(Cinnamomumi cortex spissus)	-	40	-	w20[5)]
Cinnamomum cassia(Cinnamomumi ramulus)	-	-	-	26
Cinnamomum cassia(Cinnamom bark)	-	-	-	-
Cistanche deserticola	-	-	-	-
Citrus aurantium	-	-	-	-
Citrus unshiu	-	-	-	-
Clematis mandshurica	-	-	-	-
Cnidium officinale	-	10	-	-
Codonopsis pilosula	-	-	-	-
Coix lacryma-jobi	-	-	-	-
Commiphora molmol	-	-	-	-
Coptis japonica	-	15	-	15
Cornus officinalis	-	10	-	-
Corydalis ternata	-	15	-	-
Crataegus pinnatifida	-	-	-	-
Cryptotympana pustulata	-	-	-	-
Curcuma longa	-	-	-	-
Curcuma zedoaria	-	-	-	-
Cuscuta chinensi	-	-	-	-

Table 10. Continued

Scientific name	*Sacch. cerevisiae*		*C. albicans*	
	(I)	(II)	(I)	(II)
Cynomorium songaricum	–	–	–	–
Cyperus rotundus	–	–	–	–
Dendrobium nobile	–	13	–	–
Dictamnus albus	–	–	–	–
Dioscorea japonica	–	–	–	–
Dioscorea tokoro	–	–	–	–
Dolichos lablab	–	–	–	–
Drynaria fortunei	–	–	–	–
Elsholwzia ciliata	–	11	–	12
Ephedra sinica	–	–	–	–
Epimedium koreanum	–	13	–	–
Equisetum hiemale	–	–	–	–
Eriobotrya japonica	–	–	–	–
Eucommia ulmoides	–	–	–	–
Eugenia caryophyllata	–	20	–	w18
Euryale ferox	–	–	–	–
Evodia officinalis	–	20	–	–
Foeniculum vulgare	–	–	–	–
Fossilia ossis	–	–	–	–
Gallus domesticus	–	–	–	–
Gardenia jasminoides	–	–	–	–
Gastrodia elata	–	–	–	–
Gentiana macrophylla	–	18	–	–
Gentiana scabra	–	–	–	–
Geranium thunbergii	–	13	–	–
Gleditsia japonica	–	–	–	–
Glycyrrhiza uralensis fischer	–	10	–	–
Gypsum	–	–	–	–
Haliotis gigantea	–	–	–	–
Halloysite	–	–	–	–
Hordeum vulgare	–	–	–	–
Houttuynia cordata	–	–	–	–
Hovenia dulcis	–	–	–	–
Juniperus chinensis	–	–	–	–
Kalopanax pictus	–	–	–	–

Table 10. Continued

Scientific name	*Sacch. cerevisiae*		*C. albicans*	
	(I)	(II)	(I)	(II)
Kochia scoparia	-	-	-	-
Ledebouriella	-	-	-	-
Leonurus sibiricus	-	-	-	-
Ligustrum lucidum	-	-	-	-
Lindera strichnifolia	-	-	-	-
Lithospermum erythrorhizon	-	-	-	-
Lonicera japonica thunberg(lonicerae flos)	-	-	-	-
Lonicera japonica thunberg(lonicerae folium)	-	-	-	-
Loranthus parasiticus	-	-	-	-
Lycium chinense(lycii fructus)	-	-	-	-
Lycium chinense(lycii radicis cortex)	-	-	-	-
Lycopus corenus	-	-	-	-
Magnolia officinalis	-	-	-	-
Mahnolia denudata	-	-	-	10
Medicata fementata	-	-	-	-
Melia azedarach(meliae cortex)	-	-	-	-
Melia azedarach(meliae fructus)	-	13	-	-
Mentha arvensis	-	-	-	-
Morinda officinalis	-	-	-	-
Morus albal	-	-	-	-
Myristica fragrans	-	-	-	-
Nardostachys chinensis	-	15	-	-
Nelumbo nucifera	-	-	-	-
Ostrea gigas	-	-	-	-
Pachyma hoelen	-	-	-	-
Paeonia albiflora	-	-	-	-
Paeonia moutan	-	-	-	-
Patrinia villosa	-	-	-	11
Perilla frutescens	-	-	-	-
Perilla sikokiana	-	-	-	-
Pharbitis nil choisy	-	-	-	-
Phellodendron amurense	-	-	-	-
Phlomis umbrosa	-	-	-	-
Phyllostachys nigra	-	-	-	-

Table 10. Continued

Scientific name	*Sacch. cerevisiae*		*C. albicans*	
	(I)	(II)	(I)	(II)
Pinellia ternata breitenbach	-	-	-	-
Pinus densiflora	-	13	-	-
Plantago asiatica	-	-	-	-
Platycodon grandiflorum	-	-	-	-
Polygala tenuifolia	-	-	-	-
Polygonatum sibiricum	-	18	-	-
Polygonum aviculare	-	-	-	-
Polygonum multiflorum	-	-	-	-
Polyporus umbellatus	-	-	-	-
Poria cocos wolf	-	-	-	-
Porsythia viridissima	-	-	-	-
Prunella vulgaris	-	-	-	-
Prunus armeniaca	-	-	-	-
Prunus mume	-	-	-	-
Prunus nakaii	-	-	-	-
Prunus persica	-	-	-	-
Psoralea corylifolia	-	-	-	-
Pueraria thunbergiana	-	-	-	-
Raphanus sativus	-	-	-	-
Rehmannia glutinosa	-	-	-	-
Rheum palmatum	-	-	-	-
Rhus javanica	-	-	-	-
Rosa laevigata	-	-	-	-
Rubia akane	-	-	-	-
Rubus coreanus miquel	-	-	-	-
Salvia miltiorrhiza	-	15	-	-
Sanguisorba officinalis	-	-	-	-
Saussurea lappa	-	-	-	-
Schizandra chinensis	-	-	-	-
Scirpus flaviatilis	-	-	-	-
Scrophularia vuergeriana	-	-	-	-
Scutellaria baicalensis	-	-	-	-
Siegesbeckia orientalis	-	-	-	-
Sinomenium acutum	-	-	-	-
Smilax china	-	-	-	-
Sodium sulfate	-	-	-	-
Sophara japonica	-	-	-	-
Sophora flavescens	-	12	20	18

Table 10. Continued

Scientific name	*Sacch. cerevisiae*		*C. albicans*	
	(I)	(II)	(I)	(II)
Sophora subprostrata	–	20	–	–
Spiradela polyrhiza	–	–	–	–
Stemona japonica	–	–	–	–
Talc	–	–	–	–
Tetrapanax papyriferus	–	26	–	11
Thuja orientalis	–	–	–	–
Torreya nucifera	–	–	–	–
Trichosanthes kirlowii	–	–	–	–
Trogopterus xanthipes	–	–	–	–
Tussilago farfara	–	–	–	–
Typha orientalis	–	–	–	10
Uncaria sinensis	–	–	–	–
Vitex rotundifolia	–	–	–	–
Xanthium strumarium	–	–	–	–
Zanthoxylum schinifolium	–	–	–	–
Zingiber officinale	–	–	–	9
Zizyphus vulgaris	–	–	–	–

Table 11. Antimicrobial activity of medicinal herbs against molds

Unit : Inhibition zone diameter(mm)

Scientific name	*P. citrinum*[1]		*Asp. flavus*[2]		*Asp. niger*[3]	
	(I)[4]	(II)[5]	(I)	(II)	(I)	(II)
Acanthopanax sessiliflorum	-	-	-	-	-	-
Achyranthes japonica	-	-	-	-	-	-
Aconitum pseudo-laeve	-	-	-	-	-	-
Acorus gramineus	-	-	-	-	-	w12[6]
Adenophora triphylla	-	-	-	-	-	-
Agastache rugosa	-	-	-	-	-	-
Akebia quinata	-	-	-	-	-	-
Albizzia julibrissin	-	-	-	-	-	-
Alisma orientale	-	-	-	-	-	-
Allium tuberosum	-	-	-	-	-	-
Alpinia officinarum	-	-	-	-	-	+
Alpinia oxyphylla	-	-	-	-	-	-
Amomum cardamomum	-	-	-	-	-	-
Amomum tsao-ko Crevostet	-	-	-	-	-	18
Amomum xanthioides	-	-	-	-	-	-
Amyda maakii	-	-	-	-	-	-
Anemarrhena asphodeloides	-	-	-	-	-	-
Angelica dahurica	-	-	-	-	-	-
Angelica gigas	-	-	-	-	-	-
Angelica koreana	-	-	-	-	-	-
Angelica tenuissima	-	-	-	-	-	-
Angelica utilis	-	-	-	-	-	-
Anthriscus sylvestris	-	-	-	-	-	-
Aralia cordata	-	-	-	-	-	-
Arctium lappa	-	-	-	-	-	-
Areca catechu	-	-	-	-	-	-
Arisaema amurense	-	-	-	-	-	-
Artemisia asiatica	-	-	-	-	-	-
Asiasarum sieboldi	-	-	-	-	-	13
Astragalus membranaceus	-	-	-	-	-	-
Atractylodes japonica	-	-	-	-	-	-
Atractylodes lancea	-	-	-	-	-	-

1-3) See the Table 1.

4-5) See the Table 4.

6) See the Table 8.

Table 11. Continued

Scientific name	*P. citrinum*		*Asp. flavus*		*Asp. niger*	
	(I)	(II)	(I)	(II)	(I)	(II)
Belamcanda chinensis	–	–	–	–	–	–
Betula platyphylla	–	–	–	–	–	–
Biota orientalis	–	–	–	–	–	–
Bombyx mori	–	–	–	–	–	–
Brassica alba	–	–	–	–	–	–
Bupleurum falcatum	–	–	–	–	–	–
Carthamus tinctoris	–	–	–	–	–	–
Cassia obtusifolia	–	–	–	–	–	–
Catalpa ovata	–	–	–	–	–	–
Celosia argentea	–	–	–	–	–	–
Chaenomeles sinensis	–	–	–	–	–	–
Chrysanthemum indicum	–	–	–	–	–	–
Chrysanthemum zawadskii	–	–	–	–	–	w15[6)]
Cibotium barometz	–	–	–	–	–	–
Cimicifuga heracleifolia	–	–	–	–	–	–
Cinnamomum cassia(Cinnamomumi cortex spissus)	–	–	–	19	–	42
Cinnamomum cassia(Cinnamomi ramulus)	–	–	–	–	–	20
Cinnamomum cassia(Cinnamom bark)	–	–	–	–	–	–
Cistanche deserticola	–	–	–	–	–	–
Citrus aurantium	–	–	–	–	–	–
Citrus unshiu	–	–	–	–	–	–
Clematis mandshurica	–	–	–	–	–	–
Cnidium officinale	–	–	–	–	–	–
Codonopsis pilosula	–	–	–	–	–	–
Coix lacryma-jobi	–	–	–	–	–	–
Commiphora molmol	–	–	–	–	–	–
Coptis japonica	–	–	–	–	–	–
Cornus officinalis	–	–	–	14	–	10
Corydalis ternata	–	–	–	–	–	–
Crataegus pinnatifida	–	–	–	–	–	–
Cryptotympana pustulata	–	–	–	–	–	–
Curcuma longa	–	–	–	–	–	–
Curcuma zedoaria	–	–	–	–	–	–
Cuscuta chinensi	–	–	–	–	–	–
Cynomorium songaricum	–	–	–	–	–	–

Table 11. Continued

Scientific name	*P. citrinum*		*Asp. flavus*		*Asp. niger*	
	(I)	(II)	(I)	(II)	(I)	(II)
Cyperus rotundus	–	–	–	–	–	–
Dendrobium nobile	–	–	–	–	–	–
Dictamnus albus	–	–	–	–	–	–
Dioscorea japonica	–	–	–	–	–	–
Dioscorea tokoro	–	–	–	–	–	–
Dolichos lablab	–	–	–	–	–	–
Drynaria fortunei	–	–	–	–	–	–
Elsholwzia ciliata	–	–	–	–	–	–
Ephedra sinica	–	–	–	–	–	–
Epimedium koreanum	–	–	–	–	–	–
Equisetum hiemale	–	–	–	–	–	–
Eriobotrya japonica	–	–	–	–	–	–
Eucommia ulmoides	–	–	–	–	–	–
Eugenia caryophyllata	–	–	–	14	–	26
Euryale ferox	–	–	–	–	–	–
Evodia officinalis	–	–	–	–	–	–
Foeniculum vulgare	–	–	–	–	–	10
Fossilia ossis	–	–	–	–	–	–
Gallus domesticus	–	–	–	–	–	–
Gardenia jasminoides	–	–	–	–	–	–
Gastrodia elata	–	–	–	–	–	–
Gentiana macrophylla	–	–	–	–	–	–
Gentiana scabra	–	–	–	–	–	–
Geranium thunbergii	–	–	–	–	–	–
Gleditsia japonica	–	–	–	–	–	–
Glycyrrhiza uralensis fischer	–	–	–	–	–	–
Gypsum	–	–	–	–	–	–
Haliotis gigantea	–	–	–	–	–	–
Halloysite	–	–	–	–	–	–
Hordeum vulgare	–	–	–	–	–	–
Houttuynia cordata	–	–	–	–	–	–
Hovenia dulcis	–	–	–	–	–	–
Juniperus chinensis	–	–	–	–	–	–
Kalopanax pictus	–	–	–	–	–	–
Kochia scoparia	–	–	–	–	–	–

Table 11. Continued

Scientific name	*P. citrinum*		*Asp. flavus*		*Asp. niger*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Ledebouriella	-	-	-	-	-	-
Leonurus sibiricus	-	-	-	-	-	-
Ligustrum lucidum	-	-	-	-	-	-
Lindera strichnifolia	-	-	-	-	-	-
Lithospermum erythrorhizon	-	-	-	-	-	-
Lonicera japonica thunberg(lonicerae flos)	-	-	-	-	-	-
Lonicera japonica thunberg(lonicerae folium)	-	-	-	-	-	-
Loranthus parasiticus	-	-	-	-	-	-
Lycium chinense(lycii fructus)	-	-	-	-	-	-
Lycium chinense(lycii radicis cortex)	-	-	-	-	-	-
Lycopus corenus	-	-	-	-	-	-
Magnolia officinalis	-	-	-	-	-	-
Mahnolia denudata	-	-	-	-	-	-
Medicata fementata	-	-	-	-	-	-
Melia azedarach(meliae cortex)	-	-	-	-	-	-
Melia azedarach(meliae fructus)	-	-	-	-	-	-
Mentha arvensis	-	-	-	-	-	-
Morinda officinalis	-	-	-	-	-	-
Morus albal	-	-	-	-	-	-
Myristica fragrans	-	-	-	-	-	-
Nardostachys chinensis	-	-	-	-	-	-
Nelumbo nucifera	-	-	-	-	-	-
Ostrea gigas	-	-	-	-	-	-
Pachyma hoelen	-	-	-	-	-	-
Paeonia albiflora	-	-	-	-	-	-
Paeonia moutan	-	-	-	-	-	-
Patrinia villosa	-	-	-	-	-	-
Perilla frutescens	-	-	-	-	-	-
Perilla sikokiana	-	-	-	-	-	-
Pharbitis nil choisy	-	-	-	-	-	-
Phellodendron amurense	-	-	-	-	-	-
Phlomis umbrosa	-	-	-	-	-	-
Phyllostachys nigra	-	-	-	-	-	-
Pinellia ternata breitenbach	-	-	-	-	-	-

Table 11. Continued

Scientific name	*P. citrinum*		*Asp. flavus*		*Asp. niger*	
	(I)	(II)	(I)	(II)	(I)	(II)
Pinus densiflora	–	–	–	–	–	–
Plantago asiatica	–	–	–	–	–	–
Platycodon grandiflorum	–	–	–	–	–	–
Polygala tenuifolia	–	–	–	–	–	–
Polygonatum sibiricum	–	–	–	–	–	–
Polygonum aviculare	–	–	–	–	–	–
Polygonum multiflorum	–	–	–	–	–	–
Polyporus umbellatus	–	–	–	–	–	–
Poria cocos wolf	–	–	–	–	–	–
Porsythia viridissima	–	–	–	–	–	–
Prunella vulgaris	–	–	–	–	–	–
Prunus armeniaca	–	–	–	–	–	–
Prunus mume	–	–	–	20	–	–
Prunus nakaii	–	–	–	–	–	–
Prunus persica	–	–	–	–	–	–
Psoralea corylifolia	–	–	–	–	–	–
Pueraria thunbergiana	–	–	–	–	–	–
Raphanus sativus	–	–	–	–	–	–
Rehmannia glutinosa	–	–	–	–	–	–
Rheum palmatum	–	–	–	–	–	–
Rhus javanica	–	–	–	24	–	22
Rosa laevigata	–	–	–	–	–	–
Rubia akane	–	–	–	–	–	–
Rubus coreanus miquel	–	–	–	–	–	–
Salvia miltiorrhiza	–	–	–	–	–	–
Sanguisorba officinalis	–	–	–	–	–	–
Saussurea lappa	–	–	–	–	–	–
Schizandra chinensis	–	–	–	–	–	–
Scirpus flaviatilis	–	–	–	–	–	–
Scrophularia vuergeriana	–	–	–	–	–	–
Scutellaria baicalensis	–	–	–	–	–	–
Siegesbeckia orientalis	–	–	–	–	–	–
Sinomenium acutum	–	–	–	–	–	–
Smilax china	–	–	–	–	–	–
Sodium sulfate	–	–	–	–	–	–

Table 11. Continued

Scientific name	*P. citrinum*		*Asp. flavus*		*Asp. niger*	
	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)	(Ⅰ)	(Ⅱ)
Sophara japonica	–	–	–	–	–	–
Sophora flavescens	–	–	–	–	–	–
Sophora subprostrata	–	–	–	–	–	–
Spiradela polyrhiza	–	–	–	–	–	–
Stemona japonica	–	–	–	–	–	–
Talc	–	–	–	–	–	–
Tetrapanax papyriferus	–	–	–	–	–	–
Thuja orientalis	–	–	–	–	–	–
Torreya nucifera	–	–	–	–	–	–
Trichosanthes kirlowii	–	–	–	–	–	–
Trogopterus xanthipes	–	–	–	–	–	–
Tussilago farfara	–	–	–	–	–	–
Typha orientalis	–	–	–	–	–	–
Uncaria sinensis havil	–	–	–	–	–	–
Vitex rotundifolia	–	–	–	–	–	–
Xanthium strumarium	–	–	–	–	–	–
Zanthoxylum schinifolium	–	–	–	–	–	–
Zingiber officinale	–	–	–	–	–	–
Zizyphus vulgaris	–	–	–	–	–	–

B. Isolation of compounds with preservative activity

The ethylacetate-soluble fractions of *Solvia miltiorrhiza*, *Sophora flavescens*, *Cinamomum cassia* and *Erythronium japonicum*(Dog-tooth violet), which showed strong antimicrobial activity, were concentrated and performed silicagel column chromatography, pure compounds were isolated using prep. HPLC and HPLC. Purity was evaluated with HPLC.

Compound T-1 isolated from *Solvia miltiorrhiza* was dark blue crystal in plate shape with the [M^+] molecular weight of 296 confirmed by the results of MS spectrum. The compound T-1 showed benzene ring from aromatic proton at 7.89(1H, t) ppm and 7.42(2H, ABq) ppm on ^{1}H-NMR spectrum; methine proton at 4.38(1H, t) ppm, 4.31(1H, dd) ppm and 3.55(1H, m) ppm; methylene proton at 3.17(2H, brt) ppm, 1.65(4H, m) ppm; methyl proton signal at 1.40(3H, d) ppm and 1.28(6H, s) ppm. A total of 19 carbon peak was observed on ^{13}C-NMR spectrum with 2 carbonyl carbon (184.3, 175.7 ppm), 6 aromatic quarternary carbon, 2 aromatic methine carbon (150-100 ppm), 1 methine carbon, 4 methylene carbon, 1 quarternary carbon, 3 methyl carbon signals. Therefore, compound T-1 was identified as cryptotanshinone with flavanone skeleton combined with dihydrofuran ring and 2 methyl group. These results agreed with the results of the study by Honda *et. al.*(20) and the data from TradiMed (21) (Fig. 3).

Compound T-1

^{1}H-NMR(500MHz, $CDCl_3$) : δ7.89(1H, t, J=9.0Hz, H-16α), δ7.42(2H, ABq, J=8.0Hz, H-6, 7), δ4.83(1H, t), δ4.31(1H, dd, J=6.0, 9.0Hz, H-16β), δ3.55(1H, m, H-15α), δ3.17(2H, brt, H-3), δ1.65(4H, m, H-1, 2), δ1.40(3H, d, J=6.8Hz. H-17), δ1.28(6H, s, H-18, 19)

^{13}C-NMR(100MHz, $CDCl_3$) : δ29.7(C-1), δ19.1(C-2), δ37.9(C-3), δ34.9(C-4), δ143.7(C-5), δ132.5(C-6), δ122.5(C-7), δ128.5(C-8), δ126.3(C-9), δ152.4(C-10), δ184.3(C-11), δ175.7(C-12), δ118.3(C-13), δ170.7(C-14), δ34.7(C-15), δ81.5(C-16), δ18.8(C-17a), δ31.9(C-18), δ31.9(C-19)

Compound T-2 isolated from *Sophora flavescens* showed aromatic proton at 7.28(2H, d) ppm on ^{1}H-NMR spectrum; olefin protonat 6.80(2H, dd) ppm, 6.33(1H, d) ppm, and 6.09(1H, d) ppm; methine proton at 5.26(1H, dd) ppm, 5.12(1H, t) ppm, 3.78(3H, d) ppm, and 3.20(2H, m) ppm; methylene proton at 2.97(1H, m) ppm, 2.65(2H, m) ppm, and 1.42(2H, d) ppm; methyl proton signal at 1.61(3H, d) ppm, 1.54(3H, d) ppm, and 1.10(3H, m). A total of 25 carbon peak was observed on ^{13}C-NMR spectrum with 2 carbonyl carbon (193.8, 192.8 ppm), 7 aromatic quarternary carbon, 10 aromatic methine carbon (130-125 ppm), 5 methine carbon, 4 methylene carbon, 1 quarternary carbon, 3 methyl carbon signals. Therefore, the results of DEPT(Distortionless Enhancement by Polarization Transfer) 90 and 135 FAB-MS(Fast Atom Bombardment-Mass Spectrometer) spectrum identified compound T-2 as kushenol H with the molecular weight of 472 flavanone skeleton combined with the compound of isoprenoid. These results agreed with the results of the studies by Ryu *et. al.*(22) and Wu *et. al.* (23) (Fig. 4).

Compound T-2

^{1}H-NMR(400MHz, $CDCl_3$) : δ7.28(2H, d, J=8.4Hz, H-6'), δ6.80(2H, dd, J=2.0Hz, H-3'), δ6.33(1H, d, J=2.0, 8.4Hz, H-5'), δ6.09(1H, d, H-6), δ 5.26(1H, dd, J=11.0Hz, H-2), δ5.12(1H, t), δ4.41(1H, brs, H-9a), δ 4.36(1H, dd, J=1.0, 11.0Hz, H-3), δ3.97(1H, brs, D_2O exchangeable, 5a-OH), δ3.78(3H, d, 5OMe), δ3.20(2H, m, H-1a), δ2.97(1H, m, H-2a), δ2.65(2H, m), δ1.61(3H, d), δ1.54(3H, d, H-10a), δ1.42(2H, d), δ 1.14(1H, m, H-4a), δ1.10(3H, m), δ0.97(6H, s, H-6a, H-7a)

^{13}C-NMR(100MHz, $CDCl_3$) : δ77.0(C-2), δ71.5(C-3), δ193.8(C-4), δ 192.8(C-5), δ92.4(C-6), δ162.5(C-7), δ107.0(C-8), δ162.0(C-9), δ 102.4(C-10), δ114.2(C-1'), δ157.0(C-2'), δ102.4(C-3'), δ158.4(C-4'), δ 106.2(C-5'), δ129.3(C-6'), δ27.3(C-1a), δ46.5(C-2a), δ26.8(C-3a), δ 41.6(C-4a), δ68.8(C-5a), δ29.5(C-6a), δ29.1(C-7a), δ148.0(C-8a), δ 111.0(C-9a), δ17.9(C-10a)

Compound T-3 isolated from *Cinnamomum cassia* showed 6 proton peaks due to benzene ring at δ7.52～7.34 on ^{1}H-NMR spectrum and proton peak at 9.65～9.63(-CHO, d, J=8 Hz), 6.69～6.63(=CH, t), and 6.95～6.87(-CH, d, J=8 Hz). 6 carbon peaks were observed on ^{13}C-NMR spectrum; IR spectrum showed the absorbance band due to C=O group at 1727 cm^{-1}, =C-H at 3029 cm^{-1}, and =CH at 3047 cm^{-1}; and as for MS spectrum, a strong peak equivalent to molecular ion peak at m/z 132 and $(M\text{-}CHO)^+$ with the aldehyde separated from this molecule at m/z 103. This compound was identified as cinnamaldehyde using data base of traditional Oriental medicines (21) as the reference (Fig. 5).

Compound T-3

^{1}H-NMR(400MHz, $CDCl_3$) : δ7.52～7.34(5H, m, H-A), δ6.95～6.87(1H, d, J=8Hz, H-B), δ6.69～6.63(1H, t, J=7.8Hz, H-C), δ9.65～9.63(1H, d, J=8Hz, H-D)

^{13}C-NMR(100MHz, $CDCl_3$) : δ132.67(C-1), δ120.89(C-2), δ116.67(C-3), δ128.62(C-4), δ128.30(C-5), δ131.28(C-6)

IR(KBr, cm^{-1}) : 3047, 3029, 1727(C=O), 1496, 1328, 1204, 1029, 606, 493

MS(EI. m/z. rel. int) : 132[M^+], 131(100), 103(56), 104(29), 77(42), 78(31), 51(31)

Compound T-4 isolated from Dog-tooth violet showed *cis* olefinic proton at 6.43(1H, d, J=9.65 Hz, H-3) and 7.73～7.70(1H, t, J=9.5 Hz, H-4) on ^{1}H-NMR spectrum, and 4 protons distributed in a benzene ring were observed at δ7.29-7.23(1H, m, H-6). A total of 9 carbon peaks including one characteristical carbonyl carbon was observed on ^{13}C-NMR spectrum, and benzene ring (1671 cm^{-1}) and C=O group (1786 cm^{-1}), lactone (1622 cm^{-1}) were observed on IR(KBr, cm^{-1}) spectrum. A strong ion peak equivalent to m/z 146 on MS spectrum, and $(M\text{-}CO)^+$ group without CO was shown at m/z 118. With these results, this compound was identified as courmarin compared to the results of the study by Nakazima *et. al.* (24) and data from EPA/NIH Mass spectral Data base (25) (Fig. 6).

Compound T-4

^{1}H-NMR(400MH$_Z$, $CDCl_3$) : δ7.73～7.70(1H, t, J=9.5Hz, H-4), δ7.56～7.50(1H, m, H-7), δ7.48(1H, m, H-5), δ7.35～7.30(1H, m, H-8), δ7.29～7.23(1H, m, H-6), δ6.43(1H, d, J=9.5Hz, H-3)

^{13}C-NMR(100MH$_Z$, $CDCl_3$) : δ160.79(C-1), δ154.07(C-2), δ143.44(C-3), δ131.84(C-4), δ127.86(C-5), 124.43(C-6), 118.85(C-7), 116.93(C-8), 116.73(C-9)

IR(KBr, cm^{-1}) : 1786(C=O), 1671, 1622(lacton), 1488, 1381, 1155, 995, 528

MS(EI. m/z. rel. int) : 146.0[M^+](62), 118(100), 90.0(45), 89.0(37), 63.0(28), 64.0(12), 51.0(11), 39.0(13)

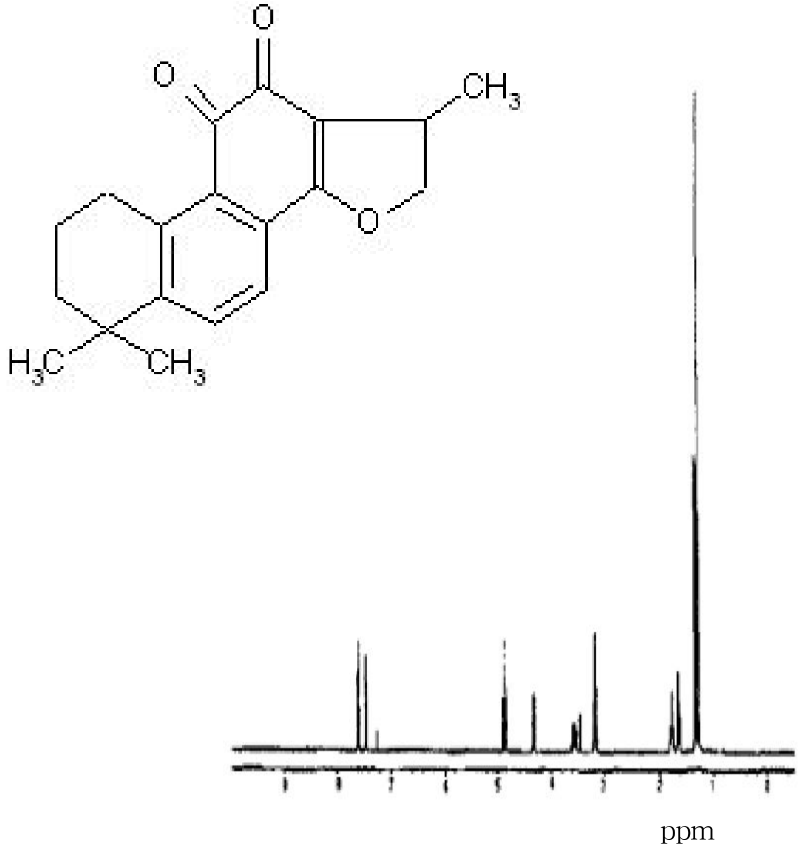

Fig. 3. Structure and [1]H-NMR Spectrum (500MHz, $CDCl_3$) of T-1 compound from *Salvia miltiorrhiza.*

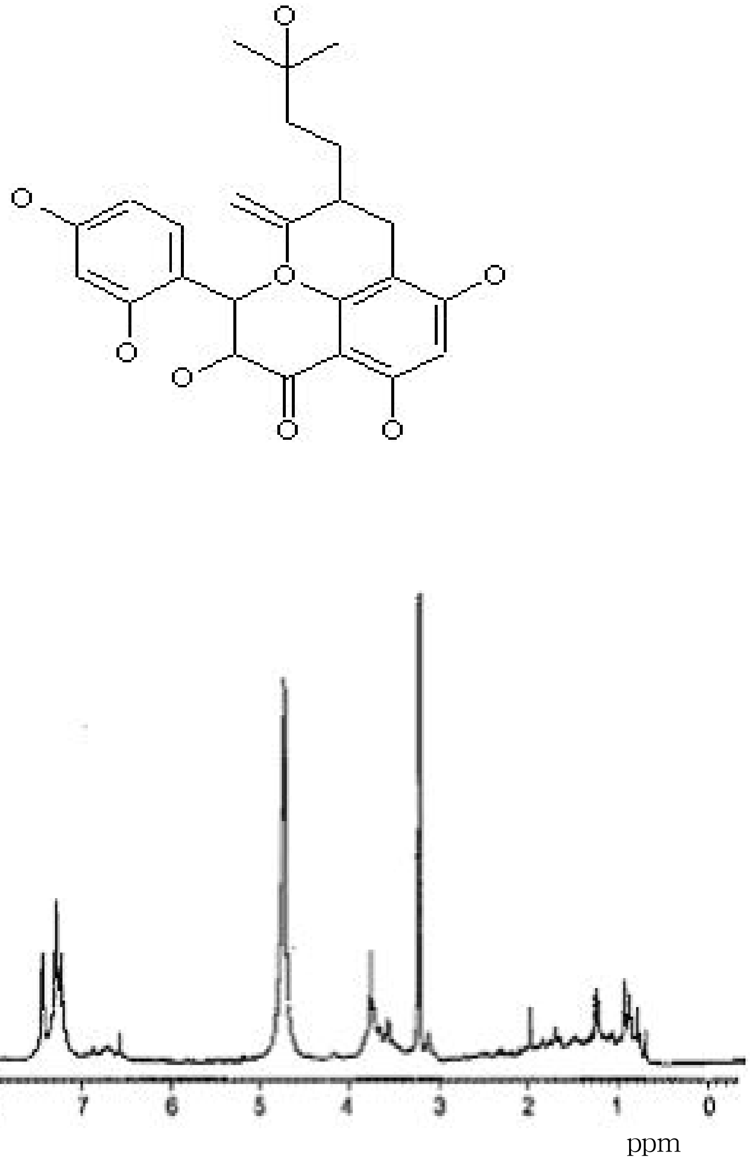

Fig. 4. Structure and [1]H-NMR spectrum (400MHz, $CDCl_3$) of T-2 compound from *Sophora flavescens.*

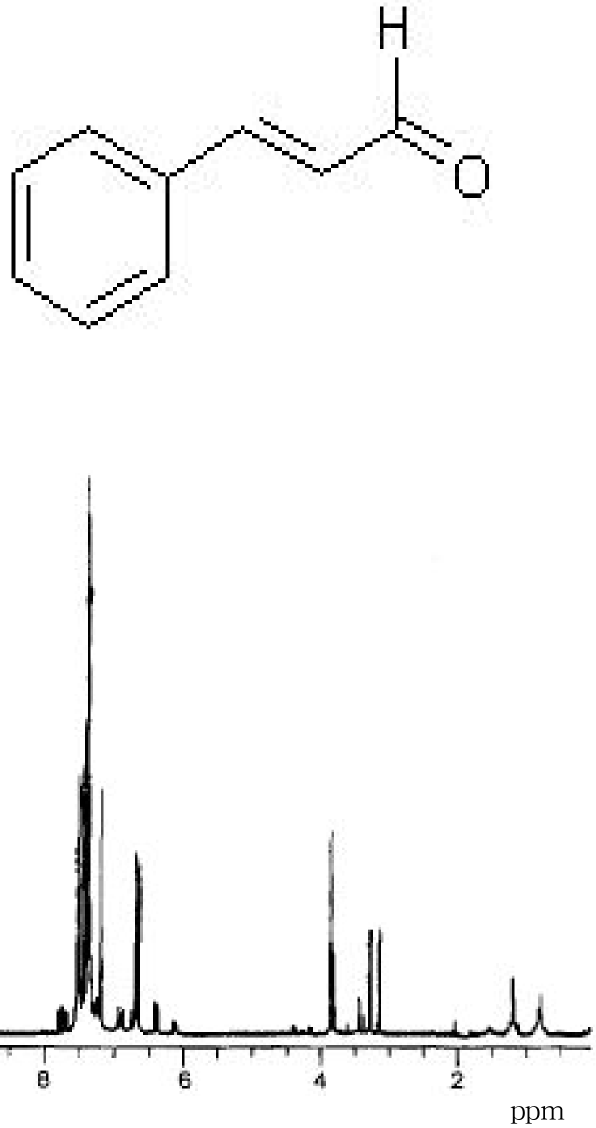

Fig. 5. Structure and ¹H-NMR spectrum (400MHz, $CDCl_3$) of T-3 compound from *Cinnamomum cassia.*

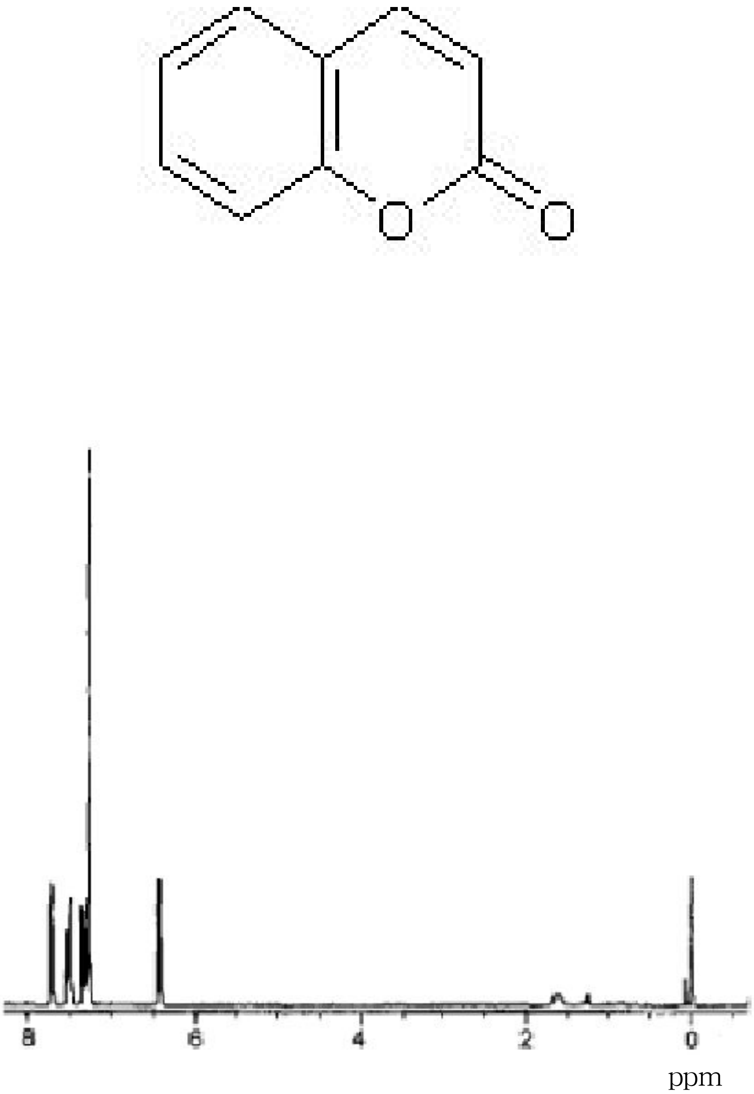

Fig. 6. Structure and ¹H-NMR spectrum (400MHz, $CDCl_3$) of T-4 compound from *Erythroniun japonicum.*

C. Antimicrobial property of synthetic preservatives and the isolated compounds

The antimicrobial property of most commonly used synthetic preservatives, i.e., benzoic, sorbic, propionic and dehydroacetic acids and that of the isolated compounds are shown in Table 12. Benzoic acid is used widely throughout the world as a preservative and is known to increase the preservation property of processed food by having the antimicrobial property against harmful microbes including bacteria, yeasts, and fungi. Generally, carboxylic acids with low molecular weight including especially benzoic acid, and sorbic acid and their salt forms alone or together are used extensively as preservatives in foods, beverages, and cosmetics throughout the world including Korea where these preservatives are allowed to be used in margarine, aloe gel, fermented soybean pastes and salted fish (8). Also, benzoic acid has an excellent antimicrobial property for food with pH between 2.5 to 4.0, especially effective for yeasts and bacteria, and is effective against fungi when combined with sorbic acid at the concentration around 0.05~0.1%. With the molecular formula C_5H_7COOH, sorbic acid shows increased antimicrobial activity in pH over 6.5 and is known to suppress growth of fungi and yeasts by preventing the α-unsaturated double bonding in the aliphatic chain in fungi and yeasts.

Cryptotanshinone isolated from *Salvia miltiorrhizae* showed the strongest antibacterial activity with MIC of 3.91 μg/ml against the Gram positive rode such as *B. subtilis* and *B. cereus* which are known to be a food decaying bacterium. According to Seiya(26), the food preservative benzoic acid showed MIC of 1,000 ppm against *B. subtilis*, showing the antibacterial activity of about 256 times weaker than that of cryptotanshinone. Also, when natural extracts showing the antibacterial activity against *B. subtilis* were examined, Ahn (27) reported that MIC of moxa oil extracted with ester from reextracting the moxa leaf extract was 25 μg/ml, Lee *et. al.* (28) reported MIC of water-soluble extract of scute decoction was 10,000 μg/ml, and Kang *et. al.* (29) reported MIC of ethanol extract of leaf mustard was 10,000 μg/ml. It was concluded that cryptotanshinone showed much stronger antimicrobial effect than that of the natural extracts mentioned above. *L. monocytogenes,* Gram positive rod like *B. subtilis* and *B. cereus*, known to grow at low temperature can contaminate frozen or refrigerated foods such as ice-creams. The antibacterial activity against *L. monocytogenes* with MIC of cryptotanshinone was 7.82 μg/ml which was the second strongest among the strains tested. Seiya (26) reported that MIC of the food preservative benzoic acid was 125-4,000 ppm, showing that cryptotanshinone has the antibacterial activity 16~512 times stronger than that of benzoic acid. Also, there are many reports for MIC against the genus *Staphylococcus*; MIC of water-soluble extract of scute decoction reported by Lee *et. al.* (28) was 1,000 μg/ml, that of leaf mustard ethanol extract reported by Kang *et. al.* (29) was 20,000 μg/ml, and that of *Lithospermum erythrorhizon* ethanol extract reported by Park *et. al.* (30) was 1,000 μg/ml, showing cryptotanshinone having more than 100 fold superior antibacterial activity against Gram positive bacteria than these natural extracts. MIC of cryptotanshinone against the Gram negative coccus *E. coli* was 62.50 μg/ml, and MICs against the yeasts *Sacch. cerevisiae* and *C. albicans* were 500 and 800μg/ml, respectively (29, 30). However, Park *et. al.* (30) reported that MIC of *Lithospermum erythrorhizon* ethanol extract against *E. coli* was more than 1,500 μg/ml and against *Sacch. cereviae* was 1,000 μg/ml, and Kang *et al.* (29) reported that MIC of leaf mustard ethanol extract against *E. coli* was 20,000 μg/ml, showing superior antibacterial effect of cryptotanshinone than that of *Lithospermum erythrorhizon* or leaf mustard ethanol extract. Honda *et al.* (20) reported that MIC of cryptotanshinone against Gram positive bacteria was 0.78-12.5 μg/ml, showing a lower MIC than this study result, 3.91-31.25 μg/ml; however, the results of their studies were similar to the results of this study.

Thus, cryptotanshinone showed a superior antibacterial effect against Gram positive bacteria, but a weak effect against Gram negative bacteria or yeasts, due to the fact that peptidoglycan on the cell membrane of Gram positive bacteria is exposed to surface, easily attacked by the substances showing antibacterial effect but Gram negative bacteria have an outer wall mainly composed with lipopolysaccharide protecting peptidoglycan (31). Thus cryptotanshinone, showing strong antimicrobial activity against Gram positive bacteria, is thought to prevent food poisoning or food decay since it has a very strong antibacterial effect against gram positive bacteria when added to food.

Kushenol H isolated from *Sophora flavescens* showed a similar antibacterial effect compared with synthetic preservatives, a definite suppressing effect against the growth of *B. subtilis* at the concentration of 50 ppm and of *L. monocytogenes* at the concentration of 50 ppm, but no suppressing effect against *E. coli* even at the concentration of 100 ppm. This compound also showed no definite antibacterial effect against yeasts and fungi as in the case of cryptotanshinone, but the synthetic preservatives benzoic acid and sorbic acid showed MIC of more than 500 ppm against *L. monocytogenes*, and 100 ppm against *E. coli* (32), indicating that the antibacterial substance kushenol H isolated from *Sophora flavescens* has the sufficient value as a natural preservative.

Cinnamaldehyde isolated from *Cinnamomum cassia* had MIC of 50µg/ml against *B. subtilis* and *B. cereus*, and Islam *et al.* (33) reported that MIC of the natural antibacterial substance protamine against *B. subtilis* was 200 µg/ml, showing 4 times higher antibacterial effect than that of protamine. MIC against *S. typhimurium* was 200 µg/ml, and Kota *et al.* (34) reported that MICs of epicatechin gallate (ECg) and epigallocatechin gallate (EGCg) with superior antibacterial effect isolated from green tea were 160 µg/ml and 320 µg/ml, respectively, showing similar MICs compared with that of cinnamaldehyde. Also, MIC of cinnamaldehyde against *L. monocytogenes* was 200 µg/ml, and MICs against the Gram negative bacterial *E. coli, P. aeruginosa,* and *S. typhimurium* were all 200 μg/ml. Kang *et al.* (29) had reported that MIC of leaf mustard extract against Gram negative bacteria was 20,000 μg/ml, showing about 100 fold higher antibacterial effect than that of cinnamaldehyde. As for MIC of cinnamaldehyde against yeasts, MIC against *C. albicans* and *S. cerevisiae* was 400 μg/ml, and in the case of fungi, the antimicrobial effect appeared at the concentration of 2,000 μg/ml against *Penicillium* spp., *A. niger*, and *A. flavus*.

Thus, MIC of cinnamaldeghyde was different according to the type of microbe but showed antimicrobial property against bacteria and true fungi, and showed especially strong antibacterial effect against *Bacillus* spp.

Table 12. Minimum inhibitory concentration of isolated compounds and commercial preservatives

Micro organism	MIC(μg/ml)							
	Crypto-tanshinone	Kushenol H	Cinnam aldehyde	Coumarin	Benzoic acid	Sorbic acid	Dehydro - acetic acid	Propionic acid
M. L.	31.25	100	100	250	50	50	1,000	2,000
B. S.	3.91	50	50	500	50	50	1,000	2,000
B. C.	3.91	50	50	250	50	50	1,000	2,000
L. M	7.82	50	200	125	50	50	1,000	2,000
P. A.	100	400	200	125	200	100	20,000	3,000
S. T.	15.63	400	200	125	200	100	20,000	3,000
E. C.	62.50	100	200	250	200	100	20,000	3,000
S. C.	500	500	400	500	25	25	200	2,000
C. A.	800	500	400	500	25	25	200	2,000
A. N.	1,000	>1,000	2,000	500	250	100	2,000	2,000
A. F.	>1,000	>1,000	2,000	>1,000	250	100	2,000	2,000
P. C.	>1,000	>1,000	2,000	>1,000	250	100	2,000	2,000

M.L. : *M. luteus* ATCC 9341, B.S. : *B. subtilis* ATCC 6633, B.C. : *B. cereus* ATCC 11774, L.M. : *L. monocytogenes* ATCC 19111, P.A. : *P. aeruginosa* PAO 303, S.T. : *S. typhimurium* ATCC 29629, E.C. : *E. coli* ATCC 9637, S.C. : *Sacch. cerevisiae* KCTC 1552, C.A. : *C. albicans* KCTC 1940, A.N. : *Asp. niger* KCTC 2119, A.F. : *Asp. flavus* KCTC 1375, P.C. : *P. citrinum* KCTC 2119

D. Synergic effect of synthetic preservatives and isolated compounds

The results of the effects on the antimicrobial activity of synthetic and natural preservatives are shown in Tables 13-28. When cryptotanshinone was used with synthetic preservatives, the antimicrobial property increased to be compared to when each was used alone, and when 0.2% cryptotanshinone and 1.0% of the four types of synthetic preservatives were used together, bacterial proliferation was completely suppressed until three days. Since cryptotanshinone showed a very strong effect of lowering proliferation of Gram positive bacteria such as *B. subtilis* and *L. monocytogenes*, the antimicrobial property increased when this substance was used together with synthetic preservatives. When benzoic acid, sorbic acid, propionic acid or dehydroacetic acid was used together, although a difference existed in the degree of antimicrobial property compared with when cryptotanshinone was used alone, the antimicrobial property increased significantly with increased concentration of these preservatives. Also, kushenol H as in the case of cryptotanshinone, when synthetic preservatives were used together, the antimicrobial property increased compared with when used alone. However, kushenol H especially showed a strong antimicrobial property against the bacteria inducing food-borne pathogens such as *L. monocytogenes* and *S. typhimurium* with the especially strong antimicrobial property against *L. monocytogenes*. As the most typical agent causing food poisoning that posed serious problems with food safety after 1980's in Europe and America, *L. monocytogenes* can proliferate in low temperature, is heat resistant, acid resistant, and poses special problems in frozen food. When infected, this strain causes cerebral meningitis or sepsis in pregnant women, children and immunocompromised people, and is known to result in the highest mortality among those food poisoning bacteria known today. Many studies are reported recently on suppressing growth or sterilization of this bacterium with natural sources including bacteriocin, monolaurin, lactofericin, and plant oil. In the case of cinnamaldehyde or coumarin, the antimicrobial property increased compared with when synthetic preservatives were used alone. Nowadays, many studies have been conducted on increasing preservative activity of food using preservatives at low concentrations by combining sorbic acid and other food additives considering food flavor, safety and practicality. Glass *et al.* (35) investigated the antimicrobial property when BHA and potassium sorbate are used alone or used together, reported that using BHA and potassium sorbate together has more suppressing effect against Gram negative bacteria compared with when used alone and reported effective suppression of *S. typhimurium* when these were used together.

Table 13. Suppression of *B. subtilis* ATCC 6633 growth caused by various artificial additives with cryptotanshinone (T-1)

unit : O.D.

T-1 / Artificial additives		Nutrient broth					
		0%			0.2%		
		24 hr[1]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.81[2]	1.11	1.75	0.53	0.69	0.90
Sorbic acid	0.1%	0.73	1.00	1.21	0.24	0.44	0.88
	1.0%	0.35	0.80	0.92	0.00	0.00	0.00
Benzoic acid	0.1%	0.36	0.92	1.13	0.00	0.03	0.22
	1.0%	0.18	0.89	0.95	0.00	0.00	0.08
Propionic acid	0.1%	0.33	0.99	1.16	0.00	0.04	0.25
	1.0%	0.20	0.87	0.90	0.00	0.00	0.06
Dehydroacetic acid	0.1%	0.31	0.91	1.12	0.00	0.04	0.27
	1.0%	0.17	0.85	0.91	0.00	0.00	0.07

[1] Incubation time

[2] O.D. at 540 nm

Table 14. Suppression of *L. monocytogenes* ATCC 19111 growth caused by various artificial additives with cryptotanshinone (T-1)

unit : O.D.

T-1 / Artificial additives		Nutrient broth					
		0%			0.2%		
		24 hr[1]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.85[2]	1.11	1.88	0.59	0.73	0.98
Sorbic acid	0.1%	0.82	1.03	1.28	0.24	0.46	0.81
	1.0%	0.41	0.84	0.96	0.00	0.00	0.00
Benzoic acid	0.1%	0.83	0.99	1.38	0.00	0.03	0.22
	1.0%	0.28	0.92	0.98	0.00	0.00	0.00
Propionic acid	0.1%	0.85	0.91	1.25	0.00	0.05	0.20
	1.0%	0.24	0.96	0.97	0.00	0.00	0.00
Dehydroacetic acid	0.1%	0.81	0.95	1.27	0.00	0.03	0.25
	1.0%	0.23	0.92	0.96	0.00	0.00	0.00

[1-2] See the Table 13

Table 15. Suppression of *S. typhimurium* ATCC 29629 growth caused by various artificial additives with cryptotanshinone (T-1)

unit : O.D.

T-1 / Artificial additives		Nutrient broth					
		0%			0.2%		
		24 hr[1)]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.83[2)]	1.01	1.59	0.69	0.79	1.02
Sorbic acid	0.1%	0.82	1.03	1.38	0.34	0.41	0.92
	1.0%	0.41	0.94	1.06	0.00	0.00	0.00
Benzoic acid	0.1%	0.80	0.99	1.25	0.00	0.03	0.20
	1.0%	0.25	0.91	0.96	0.00	0.00	0.00
Propionic acid	0.1%	0.82	1.11	1.28	0.00	0.05	0.28
	1.0%	0.26	0.89	0.97	0.00	0.00	0.05
Dehydroacetic acid	0.1%	0.79	1.03	1.27	0.00	0.03	0.20
	1.0%	0.29	0.38	0.56	0.00	0.00	0.04

[1-2)] See the Table 13

Table 16. Suppression of *E. coli* ATCC 9637 growth caused by various artificial additives with cryptotanshinone (T-1)

unit : O.D.

Artificial additives \ T-1		Nutrient broth					
		0%			0.2%		
Artificial additives		24 hr[1)]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.79[2)]	1.17	1.18	0.59	0.78	0.98
Sorbic acid	0.1%	0.79	1.03	1.28	0.24	0.45	1.00
	1.0%	0.42	0.54	0.96	0.00	0.00	0.00
Benzoic acid	0.1%	0.81	0.99	1.25	0.00	0.13	0.30
	1.0%	0.36	0.62	0.95	0.00	0.00	0.00
Propionic acid	0.1%	0.79	0.95	1.28	0.00	0.08	0.20
	1.0%	0.33	0.61	0.94	0.00	0.00	0.00
Dehydroacetic acid	0.1%	0.75	0.89	1.14	0.00	0.07	0.24
	1.0%	0.37	0.67	0.96	0.00	0.00	0.05

[1-2)] See the Table 13

Table 17. Suppression of *B. subtilis* ATCC 6633 growth caused by various artificial additives with kushenol H (T-2)

unit : O.D.

T-2 / Artificial additives		Nutrient broth					
		0%			0.2%		
		24 hr[1)]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.97[2)]	1.26	1.77	0.58	0.79	0.96
Sorbic acid	0.1%	0.79	1.08	1.08	0.24	0.51	0.81
	1.0%	0.42	0.86	0.86	0.08	0.08	0.03
Benzoic acid	0.1%	0.36	1.01	1.18	0.00	0.03	0.19
	1.0%	0.24	0.79	0.88	0.00	0.00	0.08
Propionic acid	0.1%	0.36	1.04	1.11	0.00	0.05	0.29
	1.0%	0.16	0.85	0.87	0.00	0.00	0.05
Dehydroacetic acid	0.1%	0.32	0.92	0.94	0.00	0.06	0.31
	1.0%	0.15	0.89	0.90	0.00	0.00	0.07

[1-2)] See the Table 13

Table 18. Suppression of *L. monocytogenes* ATCC 19111 growth caused by various artificial additives with kushenol H (T-2)

unit : O.D.

T-2 / Artificial additives		Nutrient broth					
		0%			0.2%		
		24 hr[1]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.94[2]	1.16	1.77	0.32	0.49	0.76
Sorbic acid	0.1%	0.79	1.08	1.08	0.12	0.51	0.98
	1.0%	0.42	0.86	0.87	0.00	0.00	0.00
Benzoic acid	0.1%	0.36	0.99	1.17	0.00	0.03	0.12
	1.0%	0.24	0.81	0.89	0.00	0.00	0.00
Propionic acid	0.1%	0.36	1.01	1.02	0.00	0.04	0.16
	1.0%	0.16	0.79	0.85	0.00	0.00	0.00
Dehydroacetic acid	0.1%	0.32	0.92	0.94	0.00	0.03	0.15
	1.0%	0.16	0.89	0.89	0.00	0.00	0.00

[1-2] See the Table 13

Table 19. Suppression of *S. typhimurium* ATCC 29629 growth caused by various artificial additives with kushenol H (T-2)

unit : O.D.

T-2 / Artificial additives		Nutrient broth					
		0%			0.2%		
		24 hr[1]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.92[2]	1.16	1.66	0.58	0.79	0.96
Sorbic acid	0.1%	0.82	1.08	1.18	0.24	0.44	0.93
	1.0%	0.42	0.86	0.98	0.04	0.04	0.03
Benzoic acid	0.1%	0.36	1.11	1.82	0.00	0.03	0.12
	1.0%	0.24	0.89	0.88	0.00	0.03	0.08
Propionic acid	0.1%	0.38	1.07	1.89	0.00	0.03	0.20
	1.0%	0.16	0.86	0.82	0.00	0.00	0.09
Dehydroacetic acid	0.1%	0.32	0.92	1.23	0.00	0.03	0.11
	1.0%	0.16	0.89	0.96	0.00	0.00	0.00

[1-2] See the Table 13

Table 20. Suppression of *E. coli* ATCC 9637 growth caused by various artificial additives with kushenol H (T-2)

unit : O.D.

T-2 / Artificial additives		Nutrient broth					
		0%			0.2%		
		24 hr[1)]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.85[2)]	1.11	1.73	0.53	0.69	0.90
Sorbic acid	0.1%	0.79	1.00	1.18	0.24	0.45	0.97
	1.0%	0.35	0.80	0.98	0.00	0.00	0.00
Benzoic acid	0.1%	0.36	0.92	2.08	0.00	0.03	0.29
	1.0%	0.18	0.88	0.86	0.00	0.00	0.11
Propionic acid	0.1%	0.29	0.89	1.92	0.00	0.04	0.21
	1.0%	0.14	0.78	0.86	0.00	0.00	0.08
Dehydroacetic acid	0.1%	0.31	0.92	1.23	0.00	0.07	0.19
	1.0%	0.17	0.89	0.96	0.00	0.00	0.09

1-2) See the Table 13

Table 21. Suppression of *B. subtilis* ATCC 6633 growth caused by various artificial additives with cinnamaldehyde (T-3)

unit : O.D.

T-3 / Artificial additives		Nutrient broth					
		0%			0.2%		
Artificial additives		24 hr[1)]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.95[2)]	1.17	1.95	0.53	0.69	0.90
Sorbic acid	0.1%	0.89	1.00	1.18	0.24	0.47	0.68
	1.0%	0.45	0.80	0.98	0.00	0.00	0.00
Benzoic acid	0.1%	0.46	0.92	1.08	0.00	0.08	0.25
	1.0%	0.28	0.89	0.86	0.00	0.00	0.08
Propionic acid	0.1%	0.44	0.89	1.15	0.00	0.03	0.20
	1.0%	0.21	0.81	0.89	0.00	0.07	0.12
Dehydroacetic acid	0.1%	0.47	0.92	1.23	0.00	0.04	0.23
	1.0%	0.27	0.89	0.96	0.00	0.08	0.11

[1-2)] See the Table 13

Table 22. Suppression of *L. monocytogenes* ATCC 19111 growth caused by various artificial additives with cinnamaldehyde (T-3)

unit : O.D.

T-3		Nutrient broth					
		0%			0.2%		
Artificial additives		24 hr[1)]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.96[2)]	1.27	1.95	0.58	076	0.90
Sorbic acid	0.1%	0.99	1.10	1.18	0.24	0.41	0.69
	1.0%	0.48	0.86	0.98	0.00	0.00	0.00
Benzoic acid	0.1%	0.49	0.96	1.08	0.00	0.09	0.18
	1.0%	0.28	0.39	0.86	0.00	0.00	0.00
Propionic acid	0.1%	0.46	0.99	1.05	0.00	0.03	0.11
	1.0%	0.38	0.49	0.88	0.00	0.00	0.00
Dehydroacetic acid	0.1%	0.37	0.92	1.23	0.00	0.03	0.08
	1.0%	0.27	0.79	0.96	0.00	0.04	0.08

[1-2)] See the Table 13

Table 23. Suppression of *S. typhimurium* ATCC 29629 growth caused by various artificial additives with cinnamaldehyde (T-3)

unit : O.D.

T-3 / Artificial additives		Nutrient broth					
		0%			0.2%		
		24 hr[1]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.96[2]	1.27	1.95	0.62	0.84	0.95
Sorbic acid	0.1%	0.12	1.23	2.18	0.24	0.52	0.70
	1.0%	0.48	0.86	1.21	0.00	0.08	0.04
Benzoic acid	0.1%	0.49	0.96	1.08	0.00	0.08	0.18
	1.0%	0.28	0.39	0.86	0.00	0.00	0.00
Propionic acid	0.1%	0.46	0.99	1.18	0.00	0.03	0.17
	1.0%	0.38	0.49	0.76	0.00	0.02	0.00
Dehydroacetic acid	0.1%	0.37	0.92	1.23	0.00	0.04	0.08
	1.0%	0.27	0.79	0.96	0.00	0.08	0.04

[1-2] See the Table 13

Table 24. Suppression of *E. coli* ATCC 9637 growth caused by various artificial additives with cinnamaldehyde (T-3)

unit : O.D.

T-3		Nutrient broth					
		0%			0.2%		
Artificial additives		24 hr[1)]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.96[2)]	1.27	1.95	0.61	0.83	0.99
Sorbic acid	0.1%	0.12	1.23	2.18	0.24	0.44	0.71
	1.0%	0.48	0.86	1.21	0.00	0.08	0.04
Benzoic acid	0.1%	0.49	0.96	1.08	0.00	0.06	0.19
	1.0%	0.28	0.39	0.86	0.00	0.00	0.00
Propionic acid	0.1%	0.46	0.99	1.18	0.00	0.03	0.09
	1.0%	0.38	0.49	0.76	0.00	0.00	0.00
Dehydroacetic acid	0.1%	0.37	0.92	1.23	0.00	0.03	0.09
	1.0%	0.27	0.79	0.96	0.00	0.07	0.04

[1-2)] See the Table 13

Table 25. Suppression of *B. subtilis* ATCC 6633 growth caused by various artificial additives with coumarin (T-4)

unit : O.D.

T-4 / Artificial additives		Nutrient broth					
		0%			0.2%		
		24 hr[1)]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.12[2)]	1.38	1.75	0.72	0.86	1.23
Sorbic acid	0.1%	0.12	1.12	2.18	0.24	0.50	0.71
	1.0%	0.48	0.86	1.21	0.00	0.08	0.04
Benzoic acid	0.1%	0.49	0.96	1.08	0.00	0.09	0.16
	1.0%	0.28	0.58	0.86	0.00	0.00	0.00
Propionic acid	0.1%	0.46	0.99	1.18	0.00	0.03	0.13
	1.0%	0.38	0.49	0.76	0.00	0.02	0.00
Dehydroacetic acid	0.1%	0.37	0.62	1.23	0.00	0.04	0.07
	1.0%	0.27	0.79	0.96	0.00	0.08	0.05

[1-2)] See the Table 13

Table 26. Suppression of *L. monocytogenes* ATCC 19111 growth caused by various artificial additives with coumarin (T-4)

unit : O.D.

T-4 / Artificial additives		Nutrient broth					
		0%			0.2%		
		24 hr[1)]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.18[2)]	1.48	1.78	0.82	0.89	1.22
Sorbic acid	0.1%	0.12	1.12	2.18	0.24	0.53	0.72
	1.0%	0.48	0.86	1.21	0.00	0.08	0.04
Benzoic acid	0.1%	0.49	0.96	1.08	0.00	0.15	0.17
	1.0%	0.28	0.58	0.86	0.00	0.00	0.00
Propionic acid	0.1%	0.46	0.99	1.18	0.00	0.03	0.13
	1.0%	0.38	0.49	0.76	0.00	0.02	0.00
Dehydroacetic acid	0.1%	0.37	0.62	1.23	0.00	0.03	0.09
	1.0%	0.27	0.79	0.96	0.00	0.09	0.05

1-2) See the Table 13

Table 27. Suppression of *S. typhimurium* ATCC 29629 growth caused by various artificial additives with coumarin (T-4)

unit : O.D.

T-4 / Artificial additives		Nutrient broth					
		0%			0.2%		
		24 hr[1)]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.28[2)]	1.52	1.82	0.82	0.98	1.25
Sorbic acid	0.1%	0.12	1.12	2.18	0.24	0.47	0.88
	1.0%	0.48	0.86	1.21	0.00	0.00	0.00
Benzoic acid	0.1%	0.49	0.96	1.08	0.00	0.12	0.19
	1.0%	0.28	0.58	0.86	0.00	0.00	0.00
Propionic acid	0.1%	0.46	0.69	1.18	0.00	0.03	0.18
	1.0%	0.38	0.49	0.76	0.00	0.02	0.00
Dehydroacetic acid	0.1%	0.37	0.62	1.23	0.00	0.03	0.09
	1.0%	0.27	0.59	0.96	0.00	0.04	0.00

[1-2)] See the Table 13

Table 28. Suppression of *E. coli* ATCC 9637 growth caused by various artificial additives with coumarin (T-4)

unit : O.D.

T-4 / Artificial additives		Nutrient broth					
		0%			0.2%		
		24 hr[1)]	48 hr	72 hr	24 hr	48 hr	72 hr
Control		0.48[2)]	1.32	1.82	0.82	0.99	1.23
Sorbic acid	0.1%	0.12	1.12	2.18	0.24	0.41	0.72
	1.0%	0.48	0.86	1.21	0.00	0.00	0.00
Benzoic acid	0.1%	0.49	0.96	1.08	0.00	0.14	0.19
	1.0%	0.28	0.58	0.86	0.00	0.00	0.00
Propionic acid	0.1%	0.46	0.69	1.18	0.00	0.04	0.18
	1.0%	0.38	0.49	0.76	0.00	0.03	0.00
Dehydroacetic acid	0.1%	0.37	0.62	1.23	0.00	0.03	0.08
	1.0%	0.27	0.59	0.96	0.00	0.04	0.00

[1-2)] See the Table 13

E. Effect of food preservation of substances with antimicrobial activity

In order to determine the optimal concentration of sterilization for each microbe using the isolated compounds, precultured strains were inoculated into the medium containing 0%, 0.2%, and 0.5% of each isolated compound and incubated at 37℃ for 5 days. Absorbance was measured each hour to measure the antimicrobial property, and the results of antimicrobial property of each strain are shown in Tables 29-48. *E. coli* causes frequent problems by inducing food poisoning, and *B. substilis* is widely distributed in nature with its spores scattered in soil, frequently mixed with food, and especially poses problems in canned food. *S. typhimurium* and *L. monocytogenes* frequently cause food poisoning. When these strains are not treated with preservatives, their growth continued, but the cell counts decreased significantly compared with the control group when 0.2% preservative was added. After that, the cell counts decreased gradually, and increased slightly on day 5. On the other hand, when the preservatives were added at 0.5%, no bacterial growth was seen from the beginning. As shown in the experimental results, all strains were killed on day 1 and showed no proliferation even after 5 days although a difference existed on the degree of decrease. No difference was seen in the effect of the preservatives, but in the case of cryptotanshinone isolated from *Salvia miltiorrhiza*, the antimicrobial property was significant in Gram positive strains and Gram negative strains.

With these results, I think that the treatment with 0.5% preservative would sterilize and function as a bacteriostatic agent by suppressing the proliferation of those microbes causing food poisoning and decay, suggest the possibility of these substances as effective antimicrobial agents for food storage and safety.

Table 29. Effect of concentration of cryptotanshinone in the saengmyon against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Cryptotanshinone (%)		Gram(+)bacteria		Gram(-)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.2×10^{4}	6.7×10^{3}	8.2×10^{3}	8.7×10^{4}
	3day	4.2×10^{4}	6.9×10^{3}	7.7×10^{3}	3.0×10^{5}
	5day	8.5×10^{4}	7.8×10^{3}	9.3×10^{3}	3.5×10^{5}
0.2	1day	3.6×10^{2}	3.5×10^{1}	2.7×10^{2}	8.0×10^{1}
	3day	4.4×10^{2}	$<1.0\times10^{1}$	1.9×10^{2}	1.5×10^{2}
	5day	3.0×10^{1}	$<1.0\times10^{1}$	1.1×10^{2}	1.8×10^{2}
0.5	1day	2.4×10^{2}	1.2×10^{1}	2.1×10^{2}	5.1×10^{1}
	3day	1.2×10^{2}	$<1.0\times10^{1}$	1.5×10^{2}	9.1×10^{1}
	5day	$<1.0\times10^{1}$	$<1.0\times10^{1}$	8.5×10^{1}	1.2×10^{2}

Table 30. Effect of concentration of cryptotanshinone in the seasoned common squid against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Cryptotanshinone (%)		Gram(+)bacteria		Gram(-)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.4×10^4	7.7×10^3	8.6×10^3	8.2×10^4
	3day	4.8×10^4	6.5×10^3	8.5×10^3	2.8×10^5
	5day	9.5×10^4	8.9×10^3	1.1×10^4	3.3×10^5
0.2	1day	3.2×10^2	5.0×10^1	5.1×10^2	9.5×10^1
	3day	4.7×10^2	$<1.0\times10^1$	2.1×10^2	1.4×10^2
	5day	2.0×10^1	$<1.0\times10^1$	1.3×10^2	1.6×10^2
0.5	1day	2.2×10^2	2.4×10^1	3.1×10^2	7.1×10^1
	3day	1.5×10^2	$<1.0\times10^1$	1.6×10^2	1.0×10^2
	5day	$<1.0\times10^1$	$<1.0\times10^1$	7.7×10^1	1.3×10^2

Table 31. Effect of concentration of cryptotanshinone in the dried slices of beef against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Cryptotanshinone (%)		Gram(+)bacteria		Gram(-)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.9×10^{4}	6.2×10^{3}	8.1×10^{3}	8.8×10^{4}
	3day	5.1×10^{4}	6.3×10^{3}	7.7×10^{3}	2.5×10^{5}
	5day	7.8×10^{4}	7.5×10^{3}	8.3×10^{3}	3.8×10^{5}
0.2	1day	3.3×10^{2}	4.4×10^{1}	3.5×10^{2}	9.0×10^{1}
	3day	4.8×10^{2}	$<1.0\times10^{1}$	2.2×10^{2}	1.9×10^{2}
	5day	2.0×10^{1}	$<1.0\times10^{1}$	8.0×10^{1}	1.7×10^{2}
0.5	1day	2.6×10^{2}	3.8×10^{1}	2.3×10^{2}	6.1×10^{1}
	3day	1.5×10^{2}	$<1.0\times10^{1}$	1.4×10^{2}	1.0×10^{2}
	5day	$<1.0\times10^{1}$	$<1.0\times10^{1}$	7.5×10^{1}	1.1×10^{2}

Table 32. Effect of concentration of cryptotanshinone in the cream puff against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Cryptotanshinone (%)		Gram(+)bacteria		Gram(-)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.1×10^{4}	6.5×10^{3}	8.8×10^{3}	8.1×10^{4}
	3day	4.4×10^{4}	6.6×10^{3}	8.5×10^{3}	2.9×10^{5}
	5day	8.1×10^{4}	8.5×10^{3}	1.0×10^{4}	3.8×10^{5}
0.2	1day	4.6×10^{2}	4.5×10^{1}	4.5×10^{2}	6.4×10^{1}
	3day	2.6×10^{2}	$<1.0\times10^{1}$	2.6×10^{2}	5.8×10^{1}
	5day	5.0×10^{1}	$<1.0\times10^{1}$	1.1×10^{2}	7.5×10^{1}
0.5	1day	2.6×10^{2}	1.6×10^{1}	3.1×10^{2}	7.1×10^{1}
	3day	1.3×10^{2}	$<1.0\times10^{1}$	1.6×10^{2}	7.5×10^{1}
	5day	$<1.0\times10^{1}$	$<1.0\times10^{1}$	7.5×10^{1}	1.1×10^{2}

Table 33. Effect of concentration of cryptotanshinone in the potato salad against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Cryptotanshinone (%)		Gram(+)bacteria		Gram(−)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.6×10^4	6.2×10^3	8.8×10^3	7.5×10^4
	3day	5.5×10^4	7.4×10^3	7.5×10^3	2.5×10^5
	5day	7.7×10^4	8.7×10^3	9.5×10^3	2.8×10^5
0.2	1day	5.6×10^2	4.5×10^1	3.6×10^2	7.0×10^1
	3day	3.2×10^2	$<1.0\times10^1$	1.7×10^2	1.3×10^2
	5day	2.0×10	$<1.0\times10^1$	1.1×10^2	1.5×10^2
0.5	1day	2.5×10^2	2.4×10^1	2.6×10^2	4.5×10^1
	3day	1.4×10^2	$<1.0\times10^1$	1.2×10^2	7.1×10^1
	5day	$<1.0\times10^1$	$<1.0\times10^1$	6.5×10^1	1.2×10^2

Table 34. Effect of concentration of kushenol H in the saengmyon against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Kushenol H (%)		Gram(+)bacteria		Gram(-)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.8×10^{4}	6.6×10^{3}	8.8×10^{3}	9.6×10^{4}
	3day	4.8×10^{4}	7.2×10^{3}	9.7×10^{3}	3.5×10^{5}
	5day	7.7×10^{4}	8.8×10^{3}	1.2×10^{4}	3.6×10^{5}
0.2	1day	2.5×10^{4}	6.0×10^{3}	5.2×10^{3}	1.5×10^{5}
	3day	2.2×10^{3}	5.5×10^{2}	4.7×10^{2}	1.2×10^{4}
	5day	1.5×10^{2}	3.2×10^{1}	3.1×10^{1}	1.5×10^{3}
0.5	1day	1.9×10^{4}	4.2×10^{3}	2.1×10^{3}	1.6×10^{5}
	3day	1.2×10^{2}	3.1×10^{1}	1.2×10^{2}	1.2×10^{2}
	5day	$<1.0\times10^{1}$	1.2×10^{1}	$<1.0\times10^{1}$	$<1.0\times10^{1}$

Table 35. Effect of concentration of kushenol H in the seasoned common squid against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Kushenol H (%)		Gram(+)bacteria		Gram(-)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.5×10^{4}	6.7×10^{3}	8.8×10^{3}	8.5×10^{4}
	3day	3.9×10^{4}	7.7×10^{3}	8.9×10^{3}	2.8×10^{5}
	5day	7.7×10^{4}	8.5×10^{3}	9.9×10^{3}	3.7×10^{5}
0.2	1day	2.4×10^{4}	6.6×10^{3}	5.2×10^{3}	1.5×10^{5}
	3day	1.4×10^{3}	7.2×10^{2}	6.1×10^{2}	1.8×10^{4}
	5day	1.2×10^{2}	4.3×10^{1}	4.2×10^{1}	2.0×10^{3}
0.5	1day	2.2×10^{4}	5.3×10^{3}	4.1×10^{3}	9.5×10^{4}
	3day	2.2×10^{2}	3.4×10^{1}	3.7×10^{2}	1.2×10^{4}
	5day	1.1×10^{1}	2.1×10^{1}	$>2.9\times10^{1}$	1.5×10^{2}

Table 36. Effect of concentration of kushenol H in the dried slices of beef against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Kushenol H (%)		Gram(+)bacteria		Gram(−)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.4×10^{4}	6.5×10^{3}	8.8×10^{3}	8.7×10^{4}
	3day	4.4×10^{4}	7.5×10^{3}	7.5×10^{3}	2.9×10^{5}
	5day	8.6×10^{4}	8.7×10^{3}	1.0×10^{4}	35×10^{4}
0.2	1day	2.5×10^{4}	4.1×10^{3}	4.1×10^{3}	1.5×10^{4}
	3day	2.1×10^{3}	2.5×10^{2}	2.9×10^{2}	8.0×10^{2}
	5day	1.4×10^{2}	1.4×10^{1}	1.4×10^{1}	2.1×10^{2}
0.5	1day	2.2×10^{4}	3.3×10^{3}	2.7×10^{3}	1.9×10^{4}
	3day	1.7×10^{2}	1.3×10^{1}	2.4×10^{2}	7.0×10^{1}
	5day	$<1.0\times10^{1}$	$<1.0\times10^{1}$	$<1.0\times10^{1}$	3.1×10^{1}

Table 37. Effect of concentration of kushenol H in the cream puff against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Kushenol H (%)		Gram(+)bacteria		Gram(-)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.4×10^{4}	5.6×10^{3}	8.5×10^{3}	8.1×10^{4}
	3day	5.2×10^{4}	6.9×10^{3}	7.9×10^{3}	2.9×10^{5}
	5day	9.6×10^{4}	8.5×10^{3}	1.1×10^{4}	3.7×10^{5}
0.2	1day	1.9×10^{4}	4.1×10^{3}	1.2×10^{3}	4.4×10^{4}
	3day	1.4×10^{3}	2.2×10^{2}	3.1×10^{2}	3.3×10^{3}
	5day	3.0×10^{2}	7.0×10^{1}	2.2×10^{1}	2.1×10^{2}
0.5	1day	1.8×10^{4}	3.1×10^{3}	8.2×10^{2}	2.2×10^{5}
	3day	3.5×10^{2}	5.1×10^{1}	4.0×10^{2}	2.1×10^{2}
	5day	2.1×10^{1}	5.1×10^{1}	1.4×10^{1}	1.5×10^{1}

Table 38. Effect of concentration of kushenol H in the potato salad against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Kushenol H (%)		Gram(+)bacteria		Gram(−)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.4×10^{4}	7.7×10^{3}	8.3×10^{3}	8.2×10^{4}
	3day	4.8×10^{4}	7.2×10^{3}	7.2×10^{3}	2.8×10^{5}
	5day	9.2×10^{4}	8.0×10^{3}	1.1×10^{4}	3.7×10^{5}
0.2	1day	1.1×10^{4}	5.3×10^{2}	7.4×10^{3}	4.4×10^{4}
	3day	3.7×10^{3}	1.5×10^{2}	5.4×10^{2}	3.5×10^{4}
	5day	4.1×10^{2}	4.6×110^{1}	4.5×10^{1}	1.5×10^{3}
0.5	1day	1.6×10^{4}	4.1×10^{3}	6.4×10^{4}	3.2×10^{5}
	3day	1.5×10^{2}	1.2×10^{1}	4.7×10^{2}	2.1×10^{3}
	5day	2.3×10^{1}	3.1×10^{1}	3.1×10^{1}	1.2×10^{1}

Table 39. Effect of concentration of cinnamaldehyde in the saengmyon against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Cinnamaldehyde (%)		Gram(+)bacteria		Gram(−)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.5×10^{4}	6.5×10^{3}	8.8×10^{3}	8.4×10^{5}
	3day	7.5×10^{4}	6.5×10^{3}	8.3×10^{3}	3.1×10^{5}
	5day	9.6×10^{4}	8.0×10^{3}	1.1×10^{4}	3.3×10^{5}
0.2	1day	2.8×10^{4}	3.2×10	5.4×10^{3}	1.6×10^{5}
	3day	2.0×10^{3}	4.5×10^{2}	4.5×10^{2}	1.4×10^{4}
	5day	1.4×10^{2}	3.8×10^{1}	4.1×10^{1}	1.0×10^{3}
0.5	1day	2.1×10^{4}	4.0×10^{3}	2.8×10^{3}	1.5×10^{5}
	3day	1.3×10^{2}	2.7×10^{1}	1.4×10^{2}	2.0×10^{2}
	5day	4.0×10^{1}	1.9×10^{1}	$<1.0\times10^{1}$	$<1.0\times10^{1}$

Table 40. Effect of concentration of cinnamaldehyde in the seasoned common squid against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Cinnamaldehyde (%)		Gram(+)bacteria		Gram(-)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.5×10^{4}	7.5×10^{3}	8.1×10^{3}	9.7×10^{4}
	3day	5.2×10^{4}	7.8×10^{3}	8.5×10^{3}	3.2×10^{5}
	5day	7.5×10^{4}	8.5×10^{3}	1.1×10^{4}	4.0×10^{5}
0.2	1day	2.2×10^{4}	6.6×10^{3}	5.2×10^{3}	1.7×10^{5}
	3day	2.0×10^{3}	6.4×10^{2}	5.7×10^{2}	1.8×10^{4}
	5day	1.4×10^{2}	4.8×10^{1}	4.9×10^{1}	2.1×10^{3}
0.5	1day	2.7×10^{4}	6.0×10^{3}	4.4×10^{3}	1.0×10^{5}
	3day	2.4×10^{2}	4.3×10^{1}	3.9×10^{2}	13.1×10^{2}
	5day	1.0×10^{1}	3.2×10^{1}	2.7×10^{1}	1.5×10^{2}

Table 41. Effect of concentration of cinnamaldehyde in the dried slices of beef against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Cinnamaldehyde (%)		Gram(+)bacteria		Gram(−)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.4×10^{4}	4.5×10^{3}	8.6×10^{3}	9.9×10^{4}
	3day	5.5×10^{4}	5.5×10^{3}	7.9×10^{3}	3.1×10^{5}
	5day	8.6×10^{4}	8.8×10^{3}	1.1×10^{4}	3.9×10^{5}
0.2	1day	2.7×10^{4}	3.2×10^{3}	4.3×10^{3}	2.6×10^{4}
	3day	2.4×10^{3}	2.6×10^{2}	2.8×10^{2}	1.5×10^{3}
	5day	1.2×10^{2}	1.7×10^{1}	1.5×10^{1}	3.1×10^{2}
0.5	1day	2.4×10^{4}	3.0×10^{3}	2.9×10^{3}	1.4×10^{4}
	3day	1.1×10^{2}	1.6×10^{1}	2.1×10^{2}	9.0×10^{1}
	5day	4.0×10^{1}	$<1.0\times10^{1}$	$<1.0\times10^{1}$	2.2×10^{1}

Table 42. Effect of concentration of cinnamaldehyde in the cream puff against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Cinnamaldehyde (%)		Gram(+)bacteria		Gram(−)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.7×10^{4}	6.8×10^{3}	8.2×10^{3}	8.5×10^{4}
	3day	4.5×10^{4}	7.4×10^{3}	7.9×10^{3}	3.2×10^{5}
	5day	8.7×10^{4}	8.5×10^{3}	9.4×10^{3}	3.9×10^{5}
0.2	1day	1.7×10^{4}	5.1×10^{3}	2.2×10^{3}	3.3×10^{4}
	3day	1.9×10^{3}	3.3×10^{2}	4.1×10^{2}	4.6×10^{3}
	5day	3.7×10^{2}	7.4×10^{1}	3.2×10^{1}	2.1×10^{2}
0.5	1day	1.8×10^{4}	4.1×10^{3}	1.8×10^{3}	2.0×10^{5}
	3day	3.9×10^{2}	6.2×10^{1}	5.1×10^{2}	3.2×10^{2}
	5day	3.6×10^{1}	6.2×10^{1}	1.5×10^{1}	1.9×10^{1}

Table 43. Effect of concentration of cinnamaldehyde in the potato salad against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Cinnamaldehyde (%)		Gram(+)bacteria		Gram(-)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.5×10^{4}	5.5×10^{3}	8.5×10^{3}	8.1×10^{4}
	3day	4.9×10^{4}	6.7×10^{3}	7.5×10^{3}	3.4×10^{5}
	5day	8.5×10^{4}	8.8×10^{3}	1.0×10^{4}	4.0×10^{5}
0.2	1day	1.6×10^{4}	3.5×10^{3}	7.5×10^{3}	5.2×10^{4}
	3day	2.9×10^{3}	4.7×10^{2}	5.4×10^{2}	2.9×10^{4}
	5day	3.1×10^{2}	1.5×10^{1}	4.0×10^{1}	1.4×10^{3}
0.5	1day	1.1×10^{4}	3.2×10^{3}	2.2×10^{3}	3.6×10^{5}
	3day	2.0×10^{2}	1.6×10^{1}	4.9×10^{2}	1.9×10^{3}
	5day	2.4×10^{1}	2.1×10^{1}	2.8×10^{1}	1.3×10^{2}

Table 44. Effect of concentration of coumarin in the saengmyon against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Coumarin (%)		Gram(+)bacteria		Gram(-)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.4×10^4	6.4×10^3	8.4×10^3	9.1×10^4
	3day	4.5×10^4	7.3×10^3	7.9×10^3	2.9×10^5
	5day	8.7×10^4	8.5×10^3	1.1×10^4	3.8×10^5
0.2	1day	2.6×10^4	1.0×10^3	5.6×10^3	1.8×10^5
	3day	2.1×10^3	5.7×10^2	4.3×10^2	1.3×10^4
	5day	1.8×10^2	3.0×10^1	2.9×10^1	1.5×10^3
0.5	1day	1.7×10^4	4.6×10^3	2.8×10^3	1.6×10^5
	3day	1.1×10^2	3.0×10^1	1.9×10^2	1.6×10^2
	5day	$<1.0\times10^1$	1.3×10^1	1.1×10^1	$<1.0\times10^1$

Table 45. Effect of concentration of coumarin in the seasoned common squid against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Coumarin (%)		Gram(+)bacteria		Gram(-)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.1×10^{4}	7.7×10^{3}	8.8×10^{3}	8.8×10^{4}
	3day	4.0×10^{4}	7.4×10^{3}	7.9×10^{3}	2.8×10^{5}
	5day	9.4×10^{4}	8.2×10^{3}	9.1×10^{3}	3.6×10^{5}
0.2	1day	2.6×10^{4}	6.5×10^{2}	5.4×10^{3}	1.6×10^{5}
	3day	1.6×10^{3}	6.8×10^{2}	5.9×10^{2}	1.7×10^{5}
	5day	1.1×10^{2}	4.0×10^{1}	3.9×10^{1}	1.9×10^{3}
0.5	1day	2.4×10^{4}	5.2×10^{3}	4.9×10^{3}	1.1×10^{5}
	3day	1.8×10^{2}	3.1×10^{1}	3.2×10^{2}	1.4×10^{3}
	5day	$<1.0\times10^{1}$	2.6×10^{1}	2.4×10^{1}	1.7×10^{1}

Table 46. Effect of concentration of coumarin in the dried slices of beef against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Coumarin (%)		Gram(+)bacteria		Gram(-)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.4×10^{4}	6.0×10^{3}	8.0×10^{3}	7.7×10^{4}
	3day	4.5×10^{4}	6.8×10^{3}	7.1×10^{3}	3.3×10^{5}
	5day	7.9×10^{4}	7.5×10^{3}	9.3×10^{3}	3.7×10^{5}
0.2	1day	2.7×10^{4}	4.0×10^{3}	4.6×10^{3}	2.5×10^{4}
	3day	2.2×10^{3}	2.6×10^{2}	2.9×10^{2}	1.1×10^{3}
	5day	1.6×10^{2}	1.7×10^{1}	1.7×10^{1}	2.4×10^{2}
0.5	1day	2.4×10^{4}	3.2×10^{3}	3.5×10^{3}	1.1×10^{4}
	3day	2.0×10^{2}	1.7×10^{1}	3.4×10^{2}	9.0×10^{1}
	5day	$<1.0\times10^{1}$	$<1.0\times10^{1}$	$<1.0\times10^{1}$	2.8×10^{1}

Table 47. Effect of concentration of coumarin in the cream puff against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Coumarin (%)		Gram(+)bacteria		Gram(−)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	3.4×10^{4}	6.4×10^{3}	8.4×10^{3}	9.0×10^{4}
	3day	4.4×10^{4}	6.4×10^{3}	9.0×10^{3}	3.5×10^{5}
	5day	8.1×10^{4}	8.5×10^{3}	9.8×10^{3}	4.1×10^{5}
0.2	1day	1.9×10^{4}	3.9×10^{2}	1.6×10^{3}	0.4×10^{4}
	3day	1.5×10^{3}	2.4×10^{2}	3.2×10^{2}	4.2×10^{3}
	5day	4.1×10^{2}	6.3×10^{1}	3.1×10^{1}	3.1×10^{2}
0.5	1day	2.1×10^{4}	3.6×10^{3}	0.8×10^{3}	13.5×10^{4}
	3day	3.2×10^{2}	4.9×10^{1}	3.9×10^{2}	1.9×10^{2}
	5day	2.4×10^{1}	4.7×10^{1}	1.2×10^{1}	1.8×10^{1}

Table 48. Effect of concentration of coumarin in the potato salad against various bacteria at 37℃ for 5 days

Unit : CFU/ml

Coumarin (%)		Gram(+)bacteria		Gram(-)bacteria	
		B. subtilis	*L. monocytogenes*	*S. typhimurium*	*E. coli*
0	1day	4.8×10^4	7.7×10^3	9.3×10^3	1.1×10^5
	3day	5.2×10^4	8.0×10^3	8.5×10^3	3.3×10^5
	5day	9.5×10^4	9.9×10^3	1.1×10^4	4.3×10^5
0.2	1day	1.8×10^4	6.3×10^3	7.8×10^3	4.6×10^4
	3day	3.8×10^3	4.2×10^2	5.7×10^2	3.5×10^4
	5day	4.7×10^2	4.8×10^1	4.3×10^1	1.6×10^3
0.5	1day	2.0×10^4	4.6×10^3	4.4×10^4	2.4×10^5
	3day	1.9×10^2	1.2×10^1	4.7×10^2	2.5×10^3
	5day	2.7×10^1	3.8×10^1	3.3×10^1	1.4×10^2

F. Investigation on the screening of new substances with antimicrobial activity from food materials and medicinal herbs

With mass production and distribution of food with the recent development in the food industry, the use of preservatives increased to preserve food safely and for a longer period. However, substitute preservatives with no adverse affects are being sought after with the increased concern for the synthetic preservatives producing adverse side effects such as gastrointestinal dioterss, carcinogenesis, and mutagenesis when accumulated in the body. Therefore, studies are active in producing natural preservatives from natural foods and herbal medicines (36). Since plants are known to contain various effective ingredients and produce antimicrobial substances as a means of self-defense, attempts have been made to discover the active ingredients of antimicrobial activity in plant sources. Many natural substances with antimicrobial activity exist from food, and the substances with antimicrobial activity know until today are conalbumin, avidin, and lysozyme (37-38) in eggs; the protein substance including lactoferrin (39) in milk; organic acids such as citric, succinic, benzoic, lactic, and propionic acids (40-42); fatty acids (43-44) with the number of carbon between 12 and 18 contained in living tissue in small quantity; essential oil components of thyme, oregano, cinnamon, and cloves; and pigment related substances such as flavonols and proanthocyanins (tannins) (45-48). Other substances including humulone, lupulone, hydrocinnamic derivative, caffeine, theophylline, theobromine, and phytoalexines are also known

(49-52). Among these, the medium chain fatty acids with 8 to 12 carbon are known to have the most effective antimicrobial activity but most are reported to have the bacteriostatic property, and fatty acid ester from polyhydric alcohol also has antimicrobial activity. Among many bacteriocins isolated from lactic acid bacteria, nisin, diplococcin, acidphilin, and colican are known to have the antimicrobial activity (53).

On the other hand, most common substances in plant extracts are spices and herbal medicines; the antimicrobial activities were reported in many spices including garlic and onion, which are ones of the oldest spices, and in many herbal medicines including *Ganoderma lucidum* and *Lithospermum erythrorhizon* (52-54). Although studies to investigate the substances with antimicrobial activity in plants found in Korea and researches on the use of these substances were conducted, studies on the identification of the active ingredients with the antimicrobial activity are still lacking (57-61).

On the other hand, the antimicrobial activities were showed in many medicinal herbs or plants such as Mugwort, *Sinomenium acutum, Chrysanthemum indicum, Houttuynia cordata,* and *Sophora flavescens* and according to Kim *et. al.* (62) coumarin and other compounds from Mugwort have antimicrobial activity. And also, *Houttuynia cordata* recently used in nutraceutical foods was reported to have strong antimicrobial activity against *P. aeruginosa, M. leteus,* and *B. subtilis*; *Bletilla striata, Salvia miltiorrhiza,* and *Magnolia officinalis* have strong antimicrobial activity against Gram positive bacteria; *Carpesium macrocephalum, Curcuma longa, Porsythiaviridissima,* and *Gardenia jasminoides* have antimicrobial activity against some bacteria and fungi. Also, among those components that show the antibacterial activity, studies are present on phytoalexins such as flavonoids and alkaloids (10) and on the relationship among the structure, antibacterial activity, and its mechanism of flavonoids. However, in the case of well-known grapefruit seed extract, many studies reported excellent effects in a wide variety of areas, but virtually no fundamental studies were done on determinating what is the active ingredients of grapefruit seed extract through which the antibacterial activity is expressed. There are many substances which are unknown or known but their characteristics are still unknown. Thus, the substances have not properly applied to industry or been in practical uses. Also, there are some studies about preservative activity but almost no studies exist on microorganisms related with the antibacterial effect and actual food preservative effect. Therefore, there is a need for studies about safety of food preservatives induced from natural substances and medicinal herbs. *S. flavescens* are not allowed to be used as food materials by Korea Food Sanitation Act (7), but *S. miltiorrhiza* could be used in food. And also, *E. japonicum* and *C. cassia* could be used in food; however, the single compound extracted from both herbs should be tested for safety before using the extracts as a source of nutraceutical food or food additives. Therefore, the safety test should be done for the compounds tested in this study before industrial application.

CONCLUSION

The medicinal herbs and food sources used in the present study were collected from all around the nation. According to the Food composition table published by RDA(Rural Development Administration) and MOHW(Ministry of Health and Welfare), food materials such as cereals, potatoes and starches, pulses, seeds, vegetables, spice, seasonings, oils and fats, fishes and shellfishes, seaweeds, mushrooms, and fruits etc., 170 kinds of food materials and 190 kinds of medicinal herbs were examined for developing new preservatives. The antimicrobial activity test was done twice for screening of new preservatives. The result showed that the methanol extracts of *S. miltiorrhizae, S. flavescens, C. cassia*, and *E. japonicum* exhibited a strong antimicrobial activity. The extracts from natural substances that demonstrated high antimicrobial activities were selected and purified on a column chromatography, HPLC etc., and the chemical structures of these four compounds were identified as cryptotanshinone, kushenol H, cinnamaldehyde, and coumarin from *S. miltiorrhizae, S. flavescens, C. cassia*, and *E. japonicum*, respectively through instrumental analysis using ^{1}H-NMR, ^{13}C-NMR. MICs against bacteria, yeasts, and molds were measured using macrobroth dilution method. MICs of cryptotanshine against 4 types of Gram positive bacteria (*Micrococcus luteus, Bacillus subtilis, B. cereus, Listeria monocytogenes*) were 3.91~31.25 ppm, MICs against 3 types of Gram negative bacteria (*Pseudomonas aeruginosa,* *Salmonella typhimurium, Escherichia coli*) were 15.63~100 ppm. And also, MICs against 2 types of yeasts (*Saccharomyces cerevisiae, Candida albicans*) and 3 types of molds (*Aspergillus niger, Asp. flavus, Penicillium citrinum*) were 500 and 1,000 ppm, respectively. MICs of kushenol H against 4 types of Gram positive bacteria were 50~100 ppm, MICs against 3 types of Gram negative, 2 types of yeasts, and 3 types of molds were 100~400, 500, and ≤1,000 ppm, respectively. MICs of cinnamaldehyde against 4 types of Gram positive bacteria were 50~200 ppm, MICs against 3 types of Gram negative, 2 types of yeasts, and 3 types of molds were 200, 400, and ≤1,000 ppm, respectively. MICs of coumarin against 4 types of Gram positive bacteria, 3 types of Gram negative, 2 types of yeasts, and 3 types of molds were 125~500, 125~250, 500, and 500~1,000 ppm, respectively. According to the results, the antimicrobial activities of these 4 extracts were higher than those of benzoic acid, sorbic acid, propionic acid, and dehydroacetic acid which are commonly used synthetic preservatives. Especially, in the case of cryptotanshinone isolated from *Salvia miltiorrhiza*, the antimicrobial property was significant in Gram positive bacteria, so cryptotanshinone has possibility to be developed as a preservatives which could be applied to the foods spoiled by Gram positive bacteria. For the purpose of increasing the antimicrobial activity, the synergistic effect of 4 synthetic and 4 purified natural preservatives was examed. The synergistic effect of combining benzoic acid, sorbic acid and natural preservatives on foodborne pathogens was especially high. Addition of purified natural preservatives to seasoned common squid, dried slice of beef, saengmyon, cream puffs, and potato salad resulted in the prolonged shelf life with a significantly decreased number of viable cells compared with control. When the strains tested are not treated with preservatives, their growth continued, but the cell counts decreased significantly compared with the control group when 0.2% preservative was added. On the other hand, when the preservatives were added at 0.5%, no bacterial growth was seen from the beginning. As shown in the experimental results, all strains were killed on day 1 and showed no proliferation even after 5 days although a difference existed on the degree of decrease. In the case of cryptotanshinone isolated from *Salvia miltiorrhiza*, the antimicrobial property was significant in Gram positive strains and Gram negative strains.

Abstract in Korean

전국 각지에서 수집한 시료는 보건복지부 및 농촌진흥청에서 발행된 한국 식품성분표의 분류방법에 따라 곡류, 서류, 전분류, 두류, 종실류, 채소류, 향신료, 조미류, 유지류, 어패류, 해조류, 버섯류 및 과실류 등으로 분류하여 식품원료 170종 및 생약재 190종으로부터 신규 보존활성물질 탐색 목적으로 항미생물검색법을 채택하여 2차에 걸쳐 보존활성을 검색하였으며, 그 결과 단삼, 고삼, 육계 및 얼레지의 메탄올추출물이 강한 보존활성을 나타내었다. 선정된 자연산물을 대상으로 항균활성물질의 최적 추출조건과 분리, 정제 등의 조건을 통해 column chromatography, HPLC 등을 이용하여 항균활성물질을 정제한 후 ^{1}H-NMR, ^{13}C-NMR 등 기기분석을 통해 4개의 활성물질의 구조를 규명한 결과, 분리 정제된 보존 활성물질은 단삼에서는 cryptotanshinone, 고삼에서는 kushenol H, 육계에서는 cinnamaldehyde, 얼레지에서는 coumarin 이었다. 이들 각각의 물질을 macrobroth dilution법으로 세균, 효모, 곰팡이에 대해 최소저해농도(MIC)를 측정한 결과, cryptotanshine는 4종류의 그람 양성 세균(*Micrococcus luteus, Bacillus subtilis, B. cereus, Listeria monocytogenes*)에서는 3.91 ~ 31.25이었고, 3종류의 그람음성세균 (*Pseudomonas aeruginosa, Salmonella typhimurium, Escherichia coli*) 에서는 15.63 ~ 100이었다. 또한, 2종류의 효모(*Saccharomyces cerevisiae, Candida albicans*) 및 3종류의 곰팡이(*Aspergillus niger, Asp. flavus, Penicillium citrinum*)에서도 각각 500 및 1,000이하로 나타났다. Kushenol H에서도 cryptotanshinone과 마찬가지로 4종류의 그람양성세균에서 50 ~ 100이었고, 3종류의 그람음성세균, 2종류의 효모 및 3종류의 곰팡이에서는 각각 100 ~ 400, 500 및 1,000이하로 나타났다. Cinnamaldehyde도 역시 4종류의 그람양성 세균에서는 50 ~ 200이였고, 3종류의 그람음성세균; 2종류의 효모 및 3종류의 곰팡이에서는 각각 50 ~ 200, 200, 400 및 1,000이하로 나타났다. Coumarin도 마찬가지로 4종류의 그람양성세균, 3종류의 그람음성세균, 2종류의 효모 및 3

종류의 곰팡이에서도 각각 125 ~ 500, 125 ~ 250, 500 및 500 ~ 1,000으로 나타났다. 그 결과를 볼 때, 기존에 허가된 대표적인 합성보존료인 benzoic acid, sorbic acid, propionic acid, dehydroacetic acid보다 높은 활성을 나타내었다. 특히 단삼에서 분리한 cryptotanshinone의 경우에는 그람양성세균에 대해서 매우 높은 활성을 나타내어 그람양성세균이 문제시되는 식품에 천연 보존제로서 개발 가능성을 시사하였다. 기존에 허가된 합성보존료 4품목과 분리한 보존물질을 병용하여 보존효과를 높이려는 실험을 수행한 결과, 단독 처리한 경우보다는 합성보존료와 분리한 보존물질을 병용하였을 때의 기대했던 것과 같이 항균효과가 증가하였으며, 특히 안식향산과 소르빈산의 항균물질 첨가에 의해 항균효과가 뚜렷하게 증가하였다. 또한, 기존 보존료인 benzoic acid는 4종류의 그람 양성 세균에서 50이였고, 3종류의 그람 음성 세균에서는 200이었다. 2종류의 효모에서는 25이였고, 3종류의 곰팡이세균에서는 250으로 나타내었다. 역시 sorbic acid도 각각 50, 100, 25 및 100을 나타내었고, dehydroacetic acid도 마찬가지로 각각 1,000, 20,000, 200 및 2,000을 나타내었고, propionic acid 역시 각각 2,000, 3,000, 2,000 및 2,000을 나타내었다. 또한 5가지 대상식품인 조미오징어, 건조육포, 생면, 슈크림빵 및 감자샐러드에 첨가하여 보존효과를 조사한 결과, 시료간의 차이와 농도에 의한 차이는 있었지만 무처리구에 비해 항균효과가 나타남을 알 수 있었다. 모든 균들에 항균활성 물질을 처리하지 않았을 때에는 균의 생장이 계속 증가하였으나, 0.2% 넣었을 때는 대조군에 비해 현저히 감소되었다. 반면에 0.5% 넣었을 때에는 초기부터 세균의 생육은 거의 보이지 않았다. 또한, 모든 균주에 각기 적용된 식품들이 1일째 사멸되거나 활성을 잃은 균들이 감소되나 정도의 차이는 있었으며, 5일째에는 더 이상 증식하지 못함을 보였다. 단삼에서 분리한 cryptotanshinone은 그람양성세균 및 그람음성세균에 탁월한 항균력을 나타내었다.

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사　　사

늦은 나이에 만학을 할 수 있는 학문에 대한 도전의 용기와 기회를 주시고, 끊임없는 애정으로 세심한 지도와 격려를 아끼지 않으셨던 이명렬 지도교수님께 머리 숙여 진심으로 감사드립니다. 박사과정이 진행되는 지난 4년 동안 열정적인 지도와 검토는 물론, 저에게 학문적 역량을 크게 넓혀주신 장해춘 교수님을 비롯하여, 늘 관심을 가지고 따뜻한 마음으로 격려와 조언을 아끼지 않으셨던 최준식 부총장님과 자연과학대 및 약학대학의 여러 교수님들, 바쁘신 중에서도 본 논문에 대한 체계적 지도와 지식으로 논문의 질과 품위를 높여주신 목포대학교 정순택 교수님과 김인철 교수님의 완성도 높은 심사에도 감사드립니다.

또한, 이 논문의 주제선정에서부터 논문이 완성되기까지 각종 실험과 자료준비 등 연구수행에 모든 것을 아낌없이 지원, 검토해 주시고, 세심한 배려와 도움으로 좋은 논문이 나올 수 있도록 지도해 주신 식품의약품안전청 김희연 과장님과 시험분석센터 허 석 박사님, 최우정 박사님 그리고 직원 여러분들께도 진심으로 다시 한 번 감사들 드립니다.

박사과정동안 항상 따뜻한 관심과 격려로 저에게 많은 정신적 도움을 주셨던 한양대학교 오웅탁 교수님과 대구미래대학 하상철 교수님, 건국대학교 서건호 교수님을 비롯하여 학업을 계속할 수 있도록 적극적으로 힘이 되어주신 경인지방식품의약품안전청 이계융 청장님과 식품본부장 최성락 국장님, 이건호 과장님, 김형중, 김영선 과장님, 그리고 힘들고 어려운 과정을 함께 하면서 항상 커다란 활력으로 조력자가 되어준 아우 안영순 선생님과 전남도청의 강영구 박사님, 광주시 보건환경연구원의 백계진 부장님과 하동룡 과장님, 조배식 박사님, 박정휘 선생님 그리고 조선대학교 생화학교실의 최현숙 박사님을 비롯한 실험실 여러분들과 그 밖에 물심양면으로 도움을 주신 여러분께 마음

깊이 감사와 경의를 표합니다.

생전에 의사로서 평생 오지에서 인술을 펴시다 일찍이 돌아가신 아버님과 10남매 모두를 훌륭한 사회의 역군으로 꿋꿋이 홀로 길러내시고 이제는 아버님 곁으로 떠나가신 어머님의 영전에 이 논문을 바치고자 합니다. 또한, 한없는 사랑과 믿음으로 나를 이끌어 주신 큰형님을 비롯한 자랑스럽고 존경하는 형님들과 누나들 그리고 매형들과 형수님들, 특히 저에게 학문의 기회와 삶의 지표를 제시해 주셨을 뿐만 아니라, 부모님을 대신하여 언제나 제게 든든한 후원자가 되어주시고, 지켜주시는 약사이자 행정학 박사이신 준표형님과 형수님께 다시 한 번 감사들 드립니다. 끊임없는 사랑과 격려 속에 오늘의 나를 있게 만들어주신 장인 어르신과 장모님, 그리고 처남과 처제들께도 감사의 말씀을 올리며 무엇보다도 오늘의 결실이 있기까지 많은 어려움 속에서도 지극정성과 사랑으로 묵묵히 내조해주는 내 삶의 가장 커다란 힘의 원천인 사랑하는 아내 박은숙과 큰 아들 성혁이, 그리고 성윤이와 함께 이 모든 영광과 명예와 기쁨을 함께 하고자 합니다. 이 영광이 있기까지 저에게 은혜를 베풀어주신 모든 분들께 소원하시는 일들이 모두 이루어지시고, 건강과 사랑과 행복이 풍족하게 함께 하시기를 기도합니다.

2007년 잠실에서 홍 영표 드림

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저작물 이용 허락서

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논문제목	한글 : **식품원료 및 생약재로부터 신규 보존활성물질 탐색에 관한 연구** 영문 : New Substances with Preservative Activity from Food Materials and Medicinal Herbs				

본인이 저작한 위의 저작물에 대하여 다음과 같은 조건아래 조선대학교가 저작물을 이용할 수 있도록 허락하고 동의합니다.

- 다 음 -

1. 저작물의 DB구축 및 인터넷을 포함한 정보통신망에의 공개를 위한 저작물의 복제, 기억장치에의 저장, 전송 등을 허락함
2. 위의 목적을 위하여 필요한 범위 내에서의 편집・형식상의 변경을 허락함. 다만, 저작물의 내용변경은 금지함.
3. 배포・전송된 저작물의 영리적 목적을 위한 복제, 저장, 전송 등은 금지함.
4. 저작물에 대한 이용기간은 5년으로 하고, 기간종료 3개월 이내에 별도의 의사 표시가 없을 경우에는 저작물의 이용기간을 계속 연장함.
5. 해당 저작물의 저작권을 타인에게 양도하거나 또는 출판을 허락을 하였을 경우에는 1개월 이내에 대학에 이를 통보함.
6. 조선대학교는 저작물의 이용허락 이후 해당 저작물로 인하여 발생하는 타인에 의한 권리 침해에 대하여 일체의 법적 책임을 지지 않음
7. 소속대학의 협정기관에 저작물의 제공 및 인터넷 등 정보통신망을 이용한 저작물의 전송・출력을 허락함.

동의여부 : 동의(0) 조건부 동의( ) 반대( )

2007 년 8월 일

저작자: 홍 영 표 (서명 또는 인)

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