J Food Sci Nutr
Vol 9, p 300~305 (2004)

J Food Science and Nutrition

# Antioxidative and Antimicrobial Activities of Cassia (*Cinnamomum cassia*) and Dill (*Anethum graveolens* L.) Essential Oils

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**Abstract**

**Antioxidative and antimicrobial activities of essential oils of cassia (*Cinnamomum cassia*) and dill (*Anethum graveolens* L.) were investigated. Essential oils used in this study were added to soybean oil and stored at 65°C for 9 days to examine their antioxidant activities using peroxide value (POV). The results showed that dill essential oil possessed a higher antioxidant activity than cassia essential oil. Strong antimicrobial activity was observed in cassia essential oil, whereas low activity was observed in dill essential oil against the test microorganisms. Heat stability and cell growth inhibitions were investigated with different concentrations of cassia oil. Results showed that cassia oil had thermal stability in a wide range of 70~160°C. Cassia inhibited cell growth of *Bacillus cereus* KCTC 1022, *Micrococcus luteus* ATCC 9341 and *Escherichia coli* ATCC 25922, but not great on *Salmonella typhimurium* ATCC 14028 at level of 200 ppm. In conclusion, the results indicate that dill essential oil could be a potential candidate for an antioxidative agent, while cassia essential oil could be suitable for use as an antimicrobial agent in the food industry.**

**Key words:** cassia, dill, essential oil, antioxidant, antimicrobial

## INTRODUCTION

Synthetic antioxidants and antimicrobial agents have been traditionally used in food processing. However, growing consumer's concern about potential side effect of synthetic chemicals and increased preferences for natural products have resulted in a reluctance to use synthetic additives in foods (1,2). Thus, an emerging research area is the screening and development of natural antioxidants or antimicrobial agents. The essential oils and extracts of natural plants have been extensively examined for this purpose (3-8).

Cassia (*Cinnamomum cassia*) is an aromatic bark which closely resembles cinnamon, but differs in that cassia is darker, coarser, thicker and more pungent. There are many varieties of cassia such as Chinese cassia, Indian cassia, Indonesian cassia, etc. Culinary use is in stewed fruits and spicy main dishes. The essential oil of cassia has medicinal properties similar to those of cinnamon and it has been used as folk remedy for diarrhea, nausea, vomiting and flatulence (9,10).

Dill (*Anethum graveolens* L.) is an annual of the parsley family, a native of the Mediterranean region and southern Russia. Dill is related to caraway, coriander and fennel and has been used as a spice from early times. Dill seed is used in pickles, meats and breads, while dillweed (dried leaves of dill) is used with fish and cheese. Essential oil of dill or dillweed has been used as traditional medicine to treat hiccups, stomach aches and flatulence (11,12). A number of studies on antioxidant and/or antimicrobial activities of essential oils have been reported, but little information is available for cassia and dill essential oils. The aim of the present study was to investigate the antimicrobial and antioxidant potential of the essential oils of cassia and dill.

## MATERIALS AND METHODS

### Materials

Cassia and dill essential oils were supplied by Kalsec Inc (Kalamazoo, MI, USA). 1,1-Diphenyl-2-picrylhydrazyl (DPPH), sodium thiosulfate were purchased from Sigma-Aldrich (St. Louis, MO, USA). All medium constituents such as beef extract and proteose peptone were purchased from Difco Lab (Michigan, IL, USA). All chemicals were of analytical grade commercially available.

### Measurement of electron donating ability

The antioxidant activity of essential oil was measured in terms of electron-donating ability (EDA) using the stable radical, DPPH. The 0.2 mL aliquots of different concentrations of cassia and dill essential oils were dissolved in 1.8 mL of $1.0\times10^{-4}$ M DPPH solutions

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(dissolved in 99.9% ethanol) to make final concentrations of 1.25, 2.50, 5.00, 7.50 and 10.00%, respectively. The mixtures were vortexed for 5 s and held at room temperature for 30 min. Absorbances were measured at 516 nm and EDA was calculated using the following equation: EDA (%) = $(1-A/B)\times 100$, where A is the absorbance of the test sample and B is the absorbance of the control. All samples were measured in triplicate.

**Measurement of antioxidative effect of essential oils on soybean oil**

Soybean oil was used as a substrate and peroxide values (POV) expressed in meq/kg oil were measured according to AOCS (13). Essential oils were appropriately diluted with ethanol and an aliquot was added to soybean oil to give final concentrations of 0, 3, 6 and 9% (v/w). After mixing for 2 min, fifteen grams of each sample was placed in a screw cap bottle and stored at 65°C for 9 days. A control was prepared by adding ethanol to soybean oil and BHT of 200 ppm was used for comparison. Samples were taken at a constant time interval and the degrees of oxidation were measured by determining the POV.

**Preparation of test microorganisms**

The essential oils of cassia and dill were tested against a panel of microorganisms including *Bacillus cereus* KCTC 1022, *Micrococcus luteus* ATCC 9341, *Staphylococcus aureus* IMSNU 11089, *Streptococcus mutans* KCTC 3300, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* ATCC 14028. Bacterial cultures ($O.D_{620}$=1.2~1.5) in nutrient broth were incubated overnight at 37°C at 150 rpm speed for seeding.

**Screening for antimicrobial activity**

Antimicrobial activity was measured using the paper disc (dia 8 mm, Advantec 27) method. Sterile paper discs were impregnated with 20 μL of cassia or dill essential oil diluted in ethanol (1:5). The paper discs were then placed onto the surface of the nutrient broth agar medium uniformly spread with 200 μL of each bacterial suspension and incubated at 37°C for 18 hrs. The results were recorded by measuring the clear zones (mm) of growth inhibition surrounding the disc, which indicates the presence of antimicrobial activity.

**Effects of concentration and temperature on antimicrobial activity**

The effect of concentration of cassia essential oil on the antimicrobial activity was determined with three microorganisms. The paper disc was saturated with different concentrations (0, 0.5, 1.0 and 2.0 μL/disc) of cassia essential oil and the same procedure as described above was used to determine antimicrobial activity. Thermal stability test was performed by heating cassia oil to 70, 120 and 160°C for 30 min. Then the paper disc was impregnated with 2 μL of each essential oil, placed on a paper disc, and the rest of the procedure was same as before.

**Effect of essential oil on the growth of bacteria**

The overnight culture ($O.D_{620}$=1.2~1.5) was inoculated (3 μL/mL) into the nutrient broth and essential oils of different concentrations (0, 50, 100, 150 and 200 ppm) were added. Bacterial growth of *Bacillus cereus*, *Micrococcus luteus*, *Escherichia coli* and *Salmonella typhimurium* in the presence of different levels of essential oils was examined every 4 hrs by monitoring the optical density at 620 nm during 24 hrs. Control sample was treated with ethanol without cassia essential oil.

**Statistical analysis**

Data were analyzed using SPSS 10.1 software (SPSS Inc., Chicago, IL, USA). Individual differences were evaluated by the Student's t-test or one way ANOVA. Statistically different significance was accepted when $p<0.05$.

## RESULTS AND DISCUSSION

**Electron donating ability**

Antioxidant activities of essential oils of cassia and dill were examined using DPPH and expressed as EDA. As shown in Fig. 1. EDA increased as the concentration of each essential oil was increased. At 1.25% concentration, the EDA of cassia and dill essential oil was 35.72 and 45.48%, respectively. At 10.0% concentration, EDA was increased to 75.29% for cassia and 89.1% for dill, indicating that dill has greater antioxidant activity than

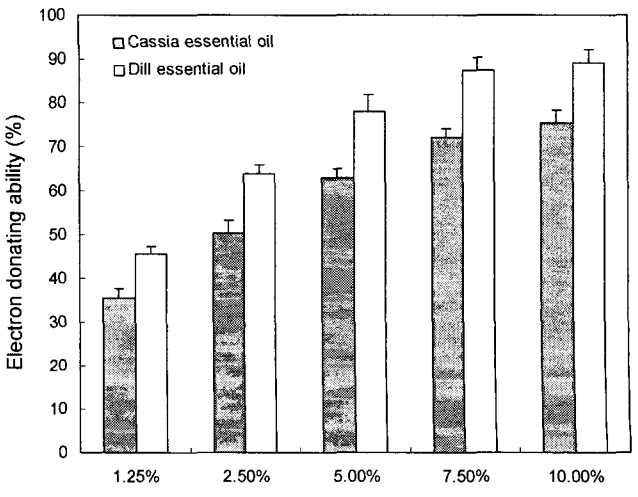


Fig. 1. Antioxidant activity of cassia and dill essential oils.

cassia ($p<0.05$). Free radicals formed during various biological reactions can accelerate cellular injury and the aging process. Antioxidants have been identified as free radical scavengers that protect the human body from free radicals, thus retarding the progress of aging and chronic diseases (14,15). DPPH is currently used to evaluate antioxidant activity of single compounds. DPPH is a stable free radical and accepts an electron to become a stable molecule. It has a deep violet color with a strong absorption band at 516 nm, and becomes pale yellow when reacted with an antioxidant. Kulisic et al. (16) reported that the antioxidant activity of oregano essential oil was comparable with $\alpha$-tocopherol and BHT and the high antioxidant effect is related to the presence of thymol and carvacrol.

**Antioxidant effect of essential oils on soybean oil oxidation**

Soybean oil was mixed with different concentrations (0, 3, 6 and 9%, v/w) of essential oil and stored at 65°C and the POV determined. As shown in Fig. 2, POV increased as the storage time increased. Little differences were observed in POV between samples and control at the early stage of storage. However, at day 5 of storage, POV of the control sample was 34.29 meq/kg and significantly lower values were observed in soybean oils

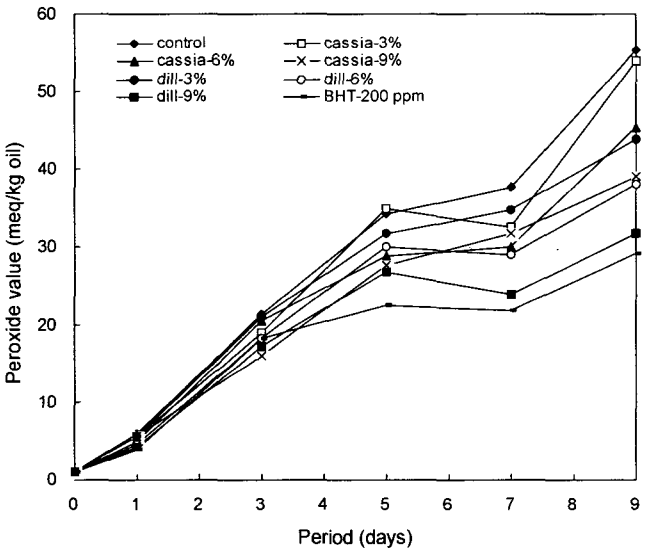


**Fig. 2.** Changes in peroxide value of soybean oils mixed with different concentrations of cassia and dill essential oils stored at 65°C.

treated with 6 and 9% of cassia or dill essential oil, respectively, ranging from 27 to 29 meq/kg ($p<0.05$). At day 9, dill oil exhibited a considerable inhibition of oxidation. POV of control sample was 55.20 meq/kg and those of soybean oils treated with 9% dill and cassia essential oil were 31.68 and 39.00 meq/kg, respectively, while soybean oil with BHT was 29.11 meq/kg. Dill essential oil had a greater antioxidative effect than cassia oil ($p<0.05$), which is in good agreement with the result shown in Fig. 1. Farag et al. (1) investigated the antioxidant activity of some spice essential oils (caraway, clove, cumin, rosemary, sage and thyme) on emulsified linoleic acid oxidation and found that caraway was most effective, followed by sage, cumin, rosemary, thyme and clove. Tepe et al. (3) reported that the essential oils of *Salvia cryptantha* and *Salvia multicaulis* exhibited greater antioxidative activities than BHT and ascorbic acid.

**Antimicrobial activity**

Four gram-positive and three gram-negative bacteria were used for testing antimicrobial activity of cassia and dill essential oils (Table 1). Cassia oil showed broad and remarkable antimicrobial spectrum against both gram-positive and gram-negative bacteria, whereas dill showed limited activities. Greatest activity was observed with cassia oil toward *Bacillus cereus*, followed by *Streptococcus mutans*, *Escherichia coli* and *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Salmonella typhimurium* and *Staphylococcus aureus*. Delaquis et al. (17) reported that dill essential oil had a low antimicrobial activity against microorganisms such as *Escherichia coli*, *Salmonella typhimurium*, *Listeria monocytogenes* and *Staphylococcus aureus*. This finding is in agreement with the results of ours. The antimicrobial activity of essential oils from *Mentha arvensis* and *Agastache rugosa* against *E. coli* O157:H7 and *Salmonella typhimurium* was investigated using paper discs and potent inhibition activities were observed from their clear zone ranging from 9~20 mm (5).

**Effect of concentration and temperature on antimicrobial activity**

Concentration dependence (0.5, 1.0 and 2.0 μL/disc) of cassia oil on antimicrobial activity was examined with *Bacillus cereus*, *Escherichia coli* and *Micrococcus luteus* and the result is shown in Fig. 3. Antimicrobial activity was observed at concentrations as low as 0.5 μL/disc

**Table 1.** Antimicrobial activity of cassia and dill essential oils (clear zone: mm)

| | *Bacillus cereus* | *Micrococcus luteus* | *Staphylococcus aureus* | *Streptococcus mutans* | *Escherichia coli* | *Pseudomonas aeruginosa* | *Salmonella typhimurium* |
|---|---|---|---|---|---|---|---|
| Cassia oil | 40 | 35 | 29 | 39 | 39 | 30 | 30 |
| Dill oil | 10 | 10 | 9 | 9 | 9 | 20 | 15 |

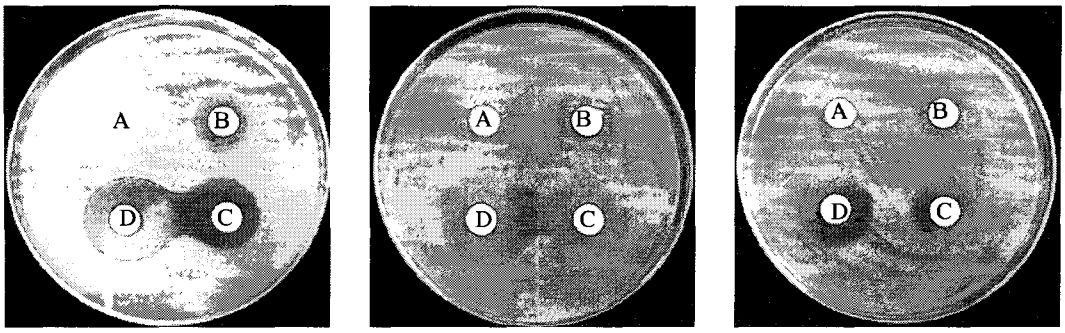


**Fig. 3.** Effect of concentration of cassia essential oil on the growth of microorganisms. left: *Bacillus cereus,* middle: *Escherichia coli* and right: *Micrococcus luteus.* A: 0 μL/disc, B: 0.5 μL/disc, C: 1.0 μL/disc and D: 2.0 μL/disc.

in *Bacillus cereus* and *Escherichia coli* and at concentration of 1.0 μL/disc in *Micrococcus luteus.* Antimicrobial activity of cassia essential oil was increased as its concentration was increased. Cassia oil was particularly effective against *Bacillus cereus* and *Escherichia coli.* The antimicrobial activity of essential oils is often related to the presence of phenolic compounds. The major constituent of cassia oil is cinnamic aldehyde which is believed to contribute to the strong antimicrobial effects (18).

The mechanism of the antimicrobial action of essential oils has been well documented. Inhibition is caused by the phenolic components in essential oils which sensitize the phospholipid bilayer of the cell membrane, thus increasing the permeability which results in a leakage of the intracellular constituents (19).

Thermal stability of cassia essential oil was examined by heating the essential oils to 70°C, 120°C and 160°C for 30 min and the antimicrobial activity was measured against *Bacillus cereus* and *Escherichia coli.* As shown in Fig. 4, cassia essential oil was stable at high temperatures, although the activity was slightly reduced with the increasing temperature. But the inhibitory effect of cassia essential oil was not destroyed by heat treatment and the components in the cassia oil were very heat-stable. Antimicrobial activity of ethanol extracts of agrimoniae herb was decreased by heat treatment at 80, 100 and 160°C, but still showed a strong inhibitory effect (20). Natural antimicrobial agents are potentially useful as food additives to extend the shelf life of foods. Because cassia essential oil has thermal stability, it could be applied in a variety of processed foods.

**Inhibition of bacterial growth**

Five different levels (0, 0.5, 0.75, 1.0 and 1.5 μL/mL) of cassia essential oil was added to bacterial culture and growth inhibition was examined every 4 hours by monitoring optical density at 620 nm during 24 hrs of incubation and the results are shown in Fig. 5 and 6. *Bacillus cereus* was found to be the most susceptible to cassia oil, whereas *Salmonella typhimurium* was the least susceptible. For gram-positive bacteria, 50 ppm of cassia essential oil exhibited no growth inhibition of *Bacillus cereus,* but the addition of 200 ppm completely inhibited the growth. The growth of *Micrococcus luteus* slowed down with the addition of 50 and 100 ppm of cassia oil. Noticeable suppression was observed at 150 ppm and complete depletion was observed over 200 ppm. For gram-negative bacteria, *Escherichia coli* survived at 150 ppm of cassia oil and the growth was almost completely inhibited with 200 ppm of cassia oil. *Salmonella typhimurium* was more resistant than any other strains, although the growth was suppressed by the cassia oil, but not completely inhibited at the level of 200 ppm as

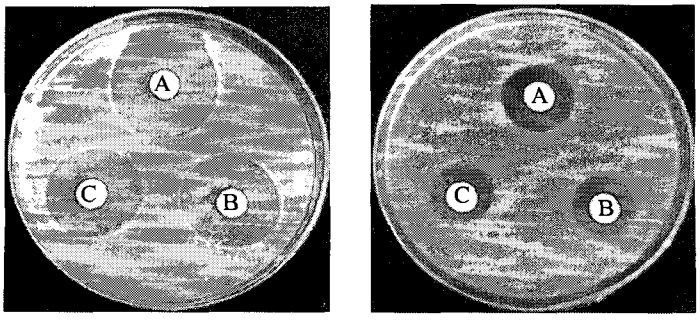


**Fig. 4.** Thermal stability of cassia essential oil on the growth of microorganisms. left: *Bacillus cereus* and right: *Escherichia coli,* A: 70°C, B: 120°C and C: 160°C.

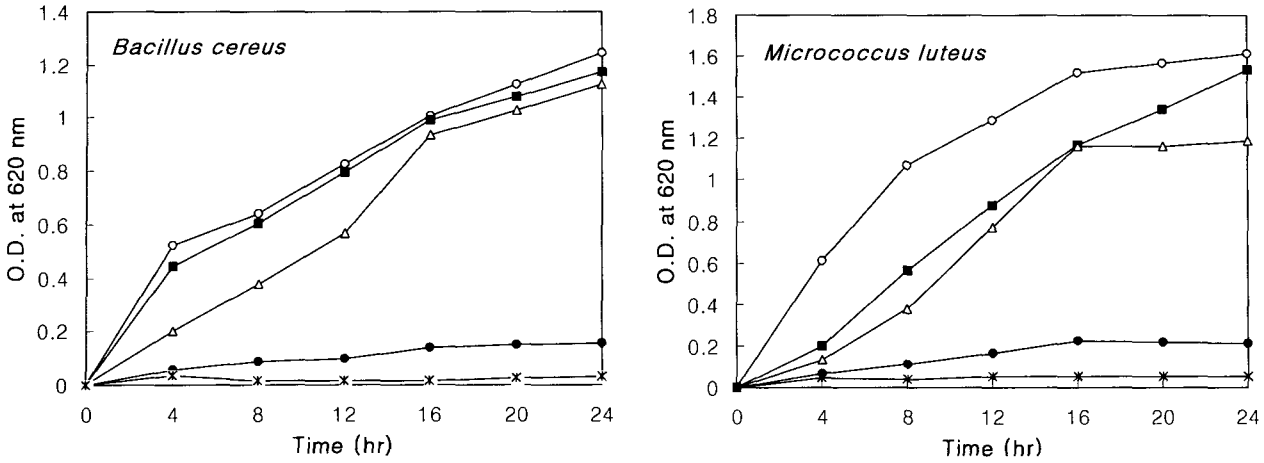


**Fig. 5.** Effect of cassia essential oil on the growth of gram-positive bacteria.
—○— control —■— 50 ppm —△— 100 ppm —●— 150 ppm —✳— 200 ppm

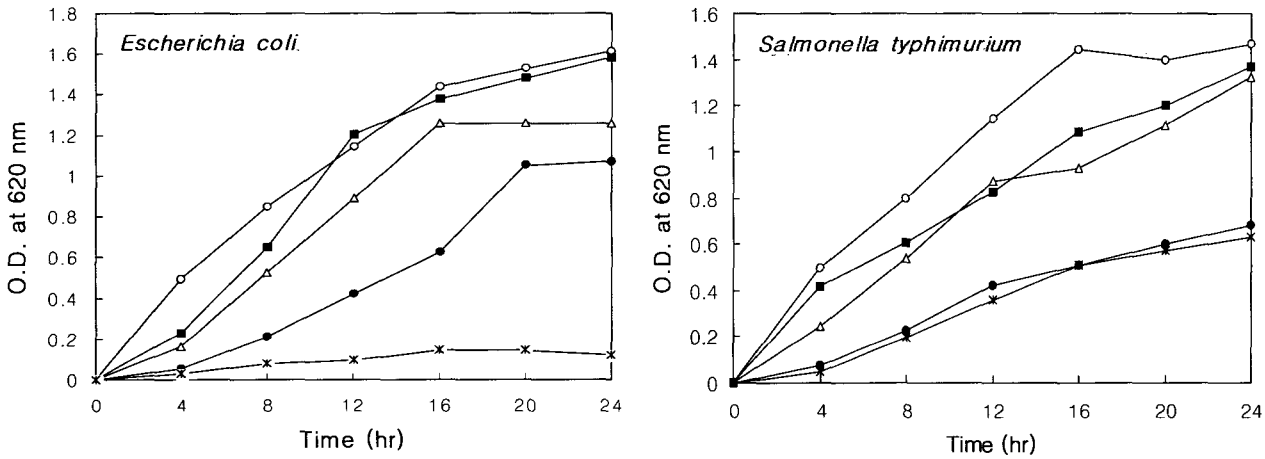


**Fig. 6.** Effect of cassia essential oil on the growth of gram-negative bacteria.
—○— control —■— 50 ppm —△— 100 ppm —●— 150 ppm —✳— 200 ppm

shown in Fig. 6. Chung et al. (21) reported that the growth of *Bacillus cereus*, *Pseudomonas syringae* and *Corynebacterium xerosis* was completely inhibited by chopi at concentrations of 500 ppm. Woo et al. (22) reported that vinegars had potent inhibition activity against *Staphylococcus aureus* and *Escherichia coli* at 25 μL/mL concentration. Our result showed that gram-negative bacteria were more resistant than gram-positive ones to cassia essential oil, which is in agreement with the results of others (23-25) and this resistance is attributed to the presence of cell wall polysaccharides (18). However, Deans and Ritchie (26) could not find differences according to the gram staining reaction.

## ACKNOWLEDGEMENT

This work was supported by the Daejin University Research Grants in 2004.

## REFERENCES

1. Farag RS, Badel AZMA, Hewedl FM, El-Baroty GSA. 1989. Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. *J Am Oil Chem Soc* 66: 792-799.
2. Namiki M. 1990. Antioxidants/antimutagens in food. *Crit Rev Food Sci Nutr* 29: 273-300.
3. Tepe B, Donmez E, Unlu M, Candan F, Daferera D, Vardar-Unlu G, Polissiou M, Sokmen A. 2004. Antimicrobial and antioxidative activities of the essential oils and methanol extracts of *Salvia cryptantha* (Montbret et Aucher ex Benth.) and *Salvia multicaulis* (Vahl). *Food Chem* 84: 519-525.
4. Tepe B, Daferera D, Sokmen A, Sokmen M, Polissiou M. 2005. Antimicrobial and antioxidant activities of the essential oil and various extracts of *Salvia tomentosa* Miller (Lamiaceae). *Food Chem* 90: 333-340.
5. Lee SE, Park CG, Cha MS, Kim JK, Seong NS, Bang KH, Bang JK. 2002. Antimicrobial activity of essential oils from *Mentha arvensis* L. var. *Piperascens Malivaud* and *Agastache rugosa* O. Kuntze on *Escherichia coli* and *Salmonella typhimurium*. *Korean J Medicinal Crop Sci* 10: 206-211.
6. Kulisic T, Radonic A, Katalinic V, Milos M. 2004. Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chem* 85: 633-640.
7. Economou KD, Orepoulou V, Thomopoulos CD. 1991.

Antioxidant properties of some plant extracts of the Labiate family. *J Amer Oil Chem Soc* 8: 109-113.
8. Chang SS, Ostric-Matijasevic B, Hsieh OAL, Huang CL. 1977. Natural antioxidants from rosemary and sage. *J Food Sci* 42: 1102-1106.
9. Mok JS, Song KC, Choi NJ, Yang HS. 2001. Antibacterial effect of cinnamon (*Cinnamomum cassia*) bark extract against fish pathogenic bacteria. *J Korean Fish Soc* 34: 545-549.
10. Park SH, Kang BS. 2001. Studies on the effects of the parts of *Cinamomum cassia* Presl on the antioxidation (II). *Kor J Herbology* 16: 159-172.
11. Han SI, Sung JD, Kim HT. 2000. Effects of seeding date and method on growth and yield in dill (*Anethum graveolens* L.). *Korean J Medicinal Crop Sci* 8: 64-68.
12. http://botanical.com/botanical/mgmh/d/dill--13.html.
13. AOCS. 1990. *Official methods and recommended practices of the American Oil Chemistry Society*. 4th ed. AOCS press, Champaign. Cd 8-53.
14. Kinsella JE, Frankel E, German B, Kanner J. 1993. Possible mechanism for the protective role of the antioxidant in wine and plant foods. *Food Technol* 47: 85-89.
15. Pryor WA. 1991. The antioxidant nutrient and disease prevention-what do we know and what do we need to find out? *Amer J Clin Nutr* 53: 391-393.
16. Kulisic T, Radonic A, Katalinic V, Milos M. 2004. Use of different methods for testing antioxidative activity of oregano essential oil. *Food Chem* 85: 633-640.
17. Delaquis PJ, Stanich K, Girard B, Mazza G. 2002. Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *Int J Food Micro* 74: 101-109.
18. Ouattara B, Simard RE, Holley RA, Piette GJP, Begin A. 1997. Antibacterial activity of selected fatty acids and essential oils against six meat spoilage organisms. *Int J Food Mic* 37: 155-162.
19. Kim J, Marshall MR, Wei C. 1995. Antibacterial activity of some essential oils components against five foodborne pathogens. *J Agric Food Chem* 43: 2839-2845.
20. Park NY, Park KN, Lee SH. 2004. Antimicrobial activities and food preservative effects of agrimoniae herba. *J Korean Soc Food Sci Nutr* 33: 244-248.
21. Chung SK, Jung JD, Cho SH. 1999. Antimicrobial acitivities of Chopi (*Zanthoxylum peperitum* DC.) extract. *J Korean Soc Food Sci Nutr* 28: 371-377.
22. Woo SM, Jang SY, Kim OM, Youn KS, Jeong YJ. 2004. Antimicrobial effects of vinegar on the harmful food-borne organisms. *Korean J Food Preserv* 11: 117-121.
23. Baek JW, Chung SH, Moon GS. 2002. Antimicrobial activities of ethanol extracts from Korean bamboo culms and leaves. *Korean J Food Sci Technol* 34: 1073-1078.
24. Chung HJ. 2000. Antioxidative and antimicrobial activities of *Opuntia ficus indica* var. saboten. *Korean J Soc Food Sci* 16: 160-166.
25. Shelef LA, Naglik OA, Bogen DW. 1980. Sensitivity of some common food-borne bacteria to the spices sage, rosemary, and allspice. *J Food Sci* 45: 1042-1044.
26. Deans SG, Ritchie G. 1987. Antibacterial properties of plant essential oils. *Int J Food Microbiol* 5: 165-180.

(Received October 5, 2004; Accepted November 26, 2004)