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ARTICLE

Antioxidant/Lipoxygenase Inhibitory Activities and Chemical Compositions of Selected Essential Oils

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Twenty-five essential oils were tested for antioxidant activities using a conjugated diene assay, the aldehyde/carboxylic acid assay, the DPPH free radical scavenging assay, and the malonaldehyde/gas chromatography (MA/GC) assay. They were also tested for lipoxygenase inhibitory activities using the lipoxygenase inhibitor-screening assay. Thyme oil exhibited the greatest antioxidant effect in all assays (80−100%) except in the DPPH assay (60%). Clove leaf oil showed activities comparable to those of thyme oil (53−100%). Cinnamon leaf oil showed strong activities in the aldehyde/carboxylic acid assay (100%) and DPPH assay (84%), but only moderate activities in the conjugated diene assay (24%) and MA/GC assay (48%). Basil oil exhibited a strong effect in the DPPH assay (86%) and moderate activities in the MA/GC assay (35%). Bergamot oil exhibited 100% antioxidant activity in the aldehyde/carboxylic acid assay. Eucalyptus and chamomile oils showed appreciable activities only in the conjugated diene assay. Bitter orange oil exhibited moderate antioxidant activity (53%) only in the MA/GC assay. Aloe vera oil exhibited the greatest lipoxygenase inhibitory activity (96%), followed by thyme oil (86%) and bergamot oil (85%) at a concentration of 0.5 μg/mL. Chamomile oil showed slight lipoxygenase inhibitory activity at 0.5 μg/mL but strong lipoxygenase inducing activity at 5 μg/mL (−123%). Thyme and clove leaf oils contained high levels of thymol (23%) and eugenol (77%), respectively, as a principal of the antioxidant activity. The results obtained in the present study suggest that some essential oils possess strong medicinal activities, which can be utilized for treatment of certain diseases.

KEYWORDS: Lipoxygenase inhibitor; antioxidant; clove oil; essential oils; thyme oil

INTRODUCTION

Essential oils, which are odorous essences, are obtained from various natural plant materials including leaves, fruits, roots, flowers, and woods. They have been widely used in cosmetics, household products, and medicines, as well as foods and beverages. Approximately 3000 essential oils have been identified, and almost 300 are commercially important (*1*). In addition to the aforementioned uses, essential oils have been used for aromatherapy since ancient times in countries such as China, Egypt, Greece, and Turkey (*2*). The long history describing the use of essential oils for medicinal purposes strongly suggests that some pharmacologically active components are present in essential oils. Recent studies also suggest the medicinal activity of certain essential oils (*3*, *4*).

Therefore, evaluation of the pharmacological activities of plant essential oils commonly used in traditional medicine and aromatherapy has received considerable interest due to their presumed safe and therapeutic effects. In biological systems, studying the mechanisms by which essential oils demonstrate their activities (including anti-inflammatory and antioxidative) is complex and dependent on the overall composition of the essential oil. In vitro assays, such as the 15-lipoxygenase inhibitor screening assay for lipoxygenase inhibitory activity (*5*), and the conjugated diene assay and DPPH free radical scavenging assay for antioxidant activity (*6*) serve as models for preliminary observations in the evaluation of pharmacological activities. The knowledge gained from these assays can then be used in the verification of the presumed medicinal effects of these plant essential oils.

Because inflammation is commonly associated with oxidative damage, chemicals that can inhibit inflammation may also serve as suitable agents in inhibiting lipid oxidation. The topical application of plant remedies to prevent inflammation has been popular since ancient times. For example, the anti-inflammatory activities of aloe vera have been demonstrated in various assay systems, including carrageenan-induced edema in rats and inhibition of cyclooxygenase activity (*7*). Plant extracts and essential oils have been shown to possess numerous pharmacological activities, including anti-inflammatory (*7*−*10*) and antioxidative (*11*, *12*) activities. In the present study, 25 essential oils were tested for antioxidant and lipoxygenase inhibitory activity to investigate their possible use for pharmacological purposes.

MATERIALS AND METHODS

Chemicals and Reagents. HPLC-grade water, dichloromethane, methanol, ethyl acetate, and hexane were bought from Fisher Scientific Co., Ltd. (Fair Lawn, NJ). α-Tocopherol, 2,2,4-trimethylpentane (isooctane), hexanal, undecane, *N*-methylhydrazine, sodium dodecyl sulfate (SDS), and 2-methylpyrazine were purchased from Aldrich Chemical Co.

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(Milwaukee, WI). Nordihydroguaretic acid (NDGA) was bought from Cayman Chemical Co. (Ann Arbor, MI). 1,1-Diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$) was purchased from TCI Organic Chemicals (Portland, OR). Methyl linoleate was obtained from Nu-Chek Prep, Inc. (Elysian, MN). 1-Methylpyrazole was synthesized according to a previously reported method (*13*). Authentic volatile chemicals were a gift from Takata Koryo Co., Ltd. (Hyogo, Japan).

Essential Oils. Essential oils were received as a gift from International Flavors and Fragrances Inc. (Union Beach, NJ) and used without any pretreatment. The oils tested were aloe vera (*Barbadensis miller*), angelica seed (*Angelica archangelica*), anise star (*Illicium verum*), basil (*Ocimum basilicum*), bergamot (*Citrus bergamia*), bitter orange (*Citrus aurantium*), celery seed (*Apium graveolens*), chamomile (*Anthemis nobilis*), cinnamon leaf (*Cinnamomum zeylanicum*), clove leaf (*Eugenia caryophyllata*), eucalyptus (*Eucalyptus globulus*), ginger (*Zingiber officinale*), jasmine (*Jasminum officinale*), juniper berry (*Juniperus communis*), lavender (*Lavendula officinalis*), lemon (*Citrus limonum*), parsley seed (*Petroselinum sativum*), patchouli (*Pogostemon patchouli*), peppermint (*Mentha piperita*), rose (*Rosa damascena*), rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), sandalwood (*Santalum album*), thyme (*Thymus vulgaris*), and ylang-ylang (*Cananga odorata*).

Antioxidant Tests. Antioxidant tests were conducted using the conjugated diene assay, aldehyde/carboxylic acid assay, DPPH free radical scavenging assay, and malonaldehyde/gas chromatography (MA/GC) assay according to previously reported methods (*6*) with slight modifications.

Conjugated Diene Assay. This assay was performed according to the previously reported method with a slight modification (*14*). Stock solutions (1 mg/mL) of each essential oil were prepared. Angelica seed, celery seed, chamomile, ginger jasmine, juniper berry, lavender, parsley seed, patchouli, peppermint, rose, sandalwood, and ylang-ylang essential oils were dissolved in dichloromethane. Aloe vera, anise star, basil, bergamot, bitter orange, cinnamon leaf, clove leaf, eucalyptus, lemon, rosemary, sage, and thyme essential oils were dissolved in methanol. Various concentrations of each essential oil (0, 10, 20, 50, 100, and 200 μg/mL) were added to screw-capped amber vials (15 mL) containing 1 g of methyl linoleate. All sample vials for each essential oil were prepared in triplicate. All vials were then nitrogen purged for 20 s to remove the solvents. Oxidation of the samples was carried out at 40 °C in a shaker water bath in the dark. The hydroperoxides formed from methyl linoleate by oxidation were determined by a spectrophotometer (Hewlett-Packard 8452A diode array spectrophotometer). A reaction mixture (2 μL) from each vial was dissolved into a test tube containing 5 mL of 2,2,4-trimethylpentane. Each test tube was gently swirled, and then approximately 2.5 mL of the solution was poured into a quartz cuvette. Spectrophotometric readings at 0 h (blank) and after 24 h were taken at a wavelength of 234 nm (for conjugated diene absorption). The spectrophotometer was set to zero with 2,2,4-trimethylpentane. The amount of hydroperoxides (mmol/kg of methyl linoleate) was calculated using a molar absorptivity value of 26000. α-Tocopherol was used as a standard known natural antioxidant. The hydroperoxide levels were determined in a few selected essential oils up to 72 h. All experiments were repeated three times.

Aldehyde/Carboxylic Acid Assay. The abilities of the essential oils to inhibit the oxidation of hexanal to hexanoic acid were tested according to previously published methods (*15*). Various amounts of the essential oils were added to a 2 mL dichloromethane solution containing hexanal (3 mg/mL) and 0.2 mg/mL of undecane (gas chromatograph internal standard) to give concentrations of 0, 10, 20, 50, 100, 200, and 500 μg/mL. The sealed sample vials were then heated in a 60 °C water bath for 10 min to initiate sample oxidation. Vials were stored in the dark at room temperature for the duration of the study. The headspace of each vial was purged with pure air (0.9 L/min) for 3 s every 24 h during the first 10 days of the study. The decrease in hexanal was monitored at 5 day intervals for 40 days. The standard, α-tocopherol, was also monitored for validation of the assay. All sample concentrations were prepared in triplicate for testing of antioxidative activity. The quantitative analysis of hexanal levels was performed using an Agilent model 6890 gas chromatograph equipped with a 30 m × 0.25 mm i.d. (d_f = 1 μm) DB-1 bonded-phase fused silica capillary column (Agilent Technologies, Inc., Folsom, CA) and a flame ionization detector. The linear velocity of the helium carrier gas was 30 cm/s at a split ratio of 20:1. The oven temperature was programmed from 40 to 120 °C at 8 °C/min. Injector and detector temperatures were 300 and 280 °C, respectively.

DPPH Free Radical Scavenging Assay. The DPPH free radical scavenging abilities of the essential oils were measured. Various volumes (10, 20, 50, 100, or 200 μL) of the stock essential oil solutions (1 mg/mL) dissolved in methanol were removed and placed into vials to give the following concentrations (10, 20, 50, 100, and 200 μg/mL). Prior to absorbance measurements, a stock DPPH solution (7.6×10^{-5} M) in methanol was prepared. One milliliter of the DPPH$^{\bullet}$ solution was then added to each vial, the vials were vigorously shaken, and after 30 min in the dark (25 °C), the solutions were transferred to microcuvettes for spectrophotometric measurements. Blank samples containing the same amounts of methanol and DPPH$^{\bullet}$ solution were also prepared and measured daily. An Agilent 8452A diode array spectrophotometer (Agilent Technologies, Inc., Palo Alto, CA) set at 517 nm was used for measuring absorbance. α-Tocopherol, the standard, was also monitored for radical scavenging activity. All measurements were carried out in triplicate.

MA/GC Assay. Solutions containing squalene (160 μL) and various concentrations of each essential oil (0, 50, 100, or 500 μg/mL) were prepared in 25 mL of hexane. Reference standards containing the same concentrations of α-tocopherol were also prepared. Each sample solution (5 mL) was placed into quartz test tubes, placed under a gentle stream of nitrogen, and slowly turned to create a thin even coating of film on the surface of the test tube. All sample test tubes were prepared in triplicate. Test tubes were placed in a Rayonet RPR-100 (Southern New England Ultra Violet Co., Branford, CT) chamber reactor equipped with eight lamps (λ = 300 nm, 0.68 ± 0.2 mW/cm^2) and irradiated at a constant temperature of 31 °C for 12 h. Following irradiation, 0.20% SDS solution (5 mL) and *N*-methylhydrazine (100 μL) were added to each test tube. Test tubes were stirred for 1 h at 25 °C, and then the contents were poured into C-18 solid phase extraction cartridges (Varian Inc., Lake Forest, CA). Malonaldehyde, as 1-methylpyrazole, was recovered from the cartridges using ethyl acetate. The internal standard for the gas chromatograph, 2-methylpyrazine (150 μg), was then added. Extracts were then analyzed for malonaldehyde as 1-methylpyrazole by an Agilent model 6890 gas chromatograph equipped with a nitrogen phosphorus detector and a 30 m × 0.25 mm i.d. × 0.25 μm film thickness bonded phase DB-1 fused silica capillary column (Agilent Technologies, Inc., Folsom, CA). The linear velocity of the helium carrier gas was 30 cm/s at a split ratio of 20:1. The temperature of the oven was programmed from 70 °C (held for 3 min) to 180 °C at 8 °C/min. The injector and detector temperatures were 250 and 300 °C, respectively.

15-Lipoxygenase Inhibitory Test. Lipoxygenase inhibitory tests were conducted using the Lipoxygenase Inhibitor Screening Assay (LISA) Kit (Cayman Chemical Co.) according to a previously reported method (*5*). The solutions provided commercially in the LISA kit were 0.1 M Tris-HCl assay buffer (pH 7.4), developing agents 1 and 2 (chromogen), soybean enzyme 15-lipoxygenase (15-LOX) standard, arachidonic acid, and KOH. An assay buffer was diluted 10-fold with HPLC-grade water before use. Chromogen, which was used within 1 h, was prepared by mixing equal amounts of developing agents 1 and 2. A blank well was prepared by adding assay buffer solution (100 μL) to a well in a 96-well plate supplied by the LISA kit. A positive control well was made by mixing 10 μL of 15-LOX solution and 990 μL of assay buffer. A substrate solution was prepared by mixing 25 μL of arachidonic acid and 25 μL of KOH in ethanol. After the substrate solution was vortexed, it was diluted with 950 μL of HPLC-grade water. The substrate solution was used within 30 min to prevent degradation. The solution prepared was stored at 0 °C until used.

A 15-LOX solution (10 μL), a test sample (10 μL), and assay buffer (980 μL) were placed in the testing well. The reaction was initiated by adding 10 μL of substrate solution to a positive control well (blank sample) and the test sample well. All testing wells were covered and placed on a shaker (Bellco Biotechnology, Vineland, NJ) for 5 min. Chromogen (100 μL) was added to the reaction wells to stop the enzyme catalysis and prevent further development of the reaction. The levels of hydroperoxides (HP) produced by 15-lipoxygenase from arachidonic acid were measured at UV absorbance of 490 nm. The inhibitory effect (%) was calculated using the following equation:

$$[(\text{amount of HP produced in the blank sample} - \text{amount of HP produced in a sample})/\text{amount of HP produced in the blank sample}] \times 100$$

Three concentrations (0.5, 1, and 5 μg/mL) of each essential oil as well as a standard lipoxygenase inhibitory chemical, NDGA, were tested for

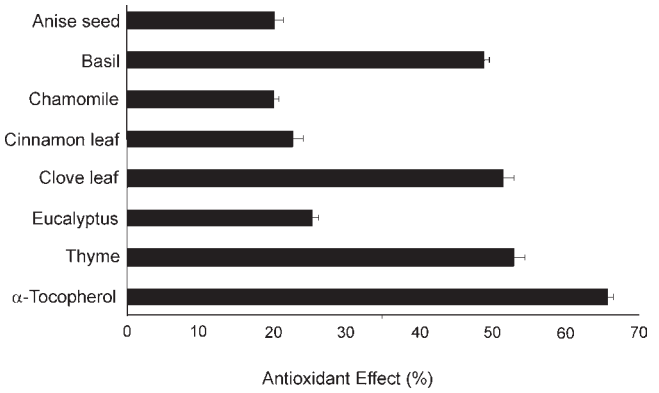

Figure 1. Results for the essential oils exhibiting appreciable effects at 200 μg/mL after 24 h in the conjugated diene assay.

lipoxygenase inhibitory activity. The entire assay was performed in duplicate.

Analysis of the Essential Oils. Chemicals in the selected essential oils were identified by comparison with the Kovats gas chromatographic retention index *I*, and by comparison to the MS fragmentation patterns of authentic chemicals. An Agilent model 6890 gas chromatograph equipped with a flame ionization detector and a 60 m × 0.25 mm i.d. × 0.25 μm DB-WAX bonded phase fused silica capillary column (Agilent Technologies, Inc., Folsom, CA) was used for the quantitative analysis of the major volatile constituents and for determination of the Kovats indices. The linear velocity of the helium carrier gas was 30 cm/s at a split ratio of 20:1. The oven temperature was programmed from 50 °C (hold for 8 min) to 180 °C (hold for 80 min) at 3 °C/min. The injector and detector temperatures were 300 and 280 °C, respectively.

A Hewlett-Packard model 5890 series II gas chromatograph equipped with a 5791A mass selective detector, at a MS ionization voltage of 70 eV, was used for mass spectral analyses of the volatile constituents. The gas chromatograph conditions were identical to those described above.

RESULTS AND DISCUSSION

Antioxidant Activity of Essential Oils. Antioxidant activities of selected essential oils were examined using four different assays.

Conjugated Diene Assay. The term conjugated diene is defined as a moiety with two double bonds separated by a single bond. The antioxidant effect of test substances can be evaluated by monitoring the conjugated diene formation at the early stage of lipid peroxidation (*6*). The amount of hydroperoxides in the blank samples increased from 13.0 ± 2.6 to 25.4 ± 1.4 mmol/kg after 24 h. The amount of hydroperoxides found in samples with 200 μg/mL essential oil ranged from 10.1 ± 3.4 mmol/kg (thyme oil) to 29.7 ± 6.2 mmol/kg (angelica seed oil) after 24 h. Among the 25 essential oils tested at 200 μg/mL, those that exhibited appreciable antioxidant activity after 24 h are shown in **Figure 1**. The values are given as mean ± SD (n = 3). A standard antioxidant, α-tocopherol, exhibited 66% inhibition of hydroperoxide formation. The greatest antioxidant effect was demonstrated by thyme oil (53%), followed by clove leaf oil (52%) and basil oil (49%). Eucalyptus oil (25%), cinnamon leaf oil (23%), chamomile oil (20%), and anise seed oil (20%) exhibited moderate antioxidant effects. The other essential oils tested, sandalwood oil (16%), bergamot oil (13%), rosemary oil (10%), and bitter orange oil (9%), showed slight antioxidant effects (data not shown). In contrast, some essential oils such as angelica seed oil (−94%), rose oil (−33%), and peppermint oil (−27%) had pro-oxidant activity. Angelica seed oil even exhibited dose-related pro-oxidant activity.

Among the oils examined for antioxidant activity for up to 72 h, a typical result obtained from thyme oil is shown in **Figure 2**. The antioxidant activity of the sample containing 200 μg/mL of thyme oil increased to 53% after 24 h and then decreased after 48 h (38%) and 72 h (27%). The sample containing 100 μg/mL exhibited slight antioxidant activities after 24 h, whereas the sample containing 10 μg/mL showed no appreciable activity except after 48 h.

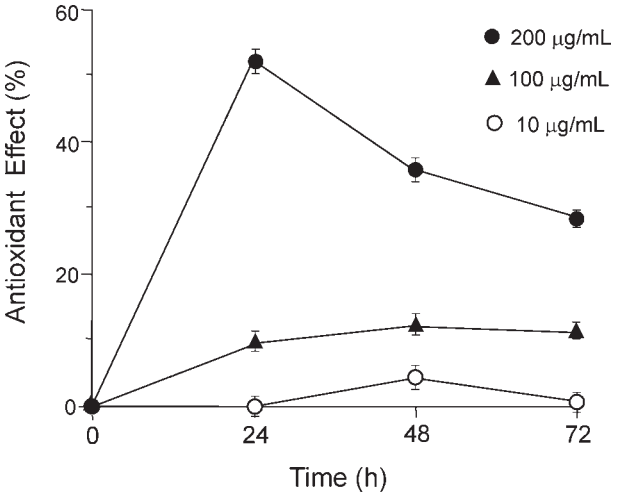

Figure 2. Antioxidant activities of thyme oil samples after 24, 48, and 72 h in the conjugated diene assay.

As mentioned above, the conjugated diene assay monitors hydroperoxides containing dienes, which form in the early stage of lipid peroxidation (*6*). Therefore, when oxidation progresses over time, many oxidants and radicals form and begin to interfere with the assay systems. The assay used in the present study is adequate for monitoring antioxidant activity in the early stage of lipid peroxidation. This may be why a clear dose−response relationship was not observed from the samples allowed to stand for prolonged times in the present study.

Aldehyde/Carboxylic Acid Assay. This assay is convenient for evaluating the effects of antioxidants against slow oxidation phenomena occurring over prolonged periods of time, as shown in the shelf life of foods (*6*). In the present study, the inhibitory effects toward oxidation of hexanal to hexanoic acid were demonstrated by various concentrations (10, 20, 50, 100, 200, and 500 μg/mL) of the essential oils and α-tocopherol after 40 days of storage. **Figure 3** shows the antioxidant effects exhibited by the essential oils tested with this assay. The oils were tested at five concentrations, but the typical results for two concentrations (20 and 200 μg/mL) are shown. This figure clearly shows the oils that exhibited strong antioxidant activity (anise star, basil, cinnamon leaf, clove leaf, and thyme) and the ones that did not show appreciable activity (bergamot, bitter orange, eucalyptus, lemon, rosemary, and sage). After 40 days, the most potent oils, cinnamon leaf and thyme, showed complete inhibition of hexanal oxidation at a concentration of 10 μg/mL. This was comparable to the standard α-tocopherol at the same concentration. Anise star, basil, and clove leaf oils showed 100, 94, and 94% inhibition at 20 μg/mL, respectively. Moderate inhibitory activity (67%) was observed for 100 μg/mL of bergamot oil, which increased to 100% at higher concentrations (as shown in **Figure 3**). Both lemon and bitter orange oils demonstrated slight inhibitory activities at the lower concentrations, but >80% inhibition at 500 μg/mL. No appreciable levels of inhibition were observed for aloe vera or rosemary essential oils. The activities demonstrated by thyme and basil essential oils in this study were similar to those shown by Lee and Shibamoto (*12*) for volatile extracts. In contrast to this study, they demonstrated that rosemary leaf volatile extract possessed some degree of antioxidant activity,

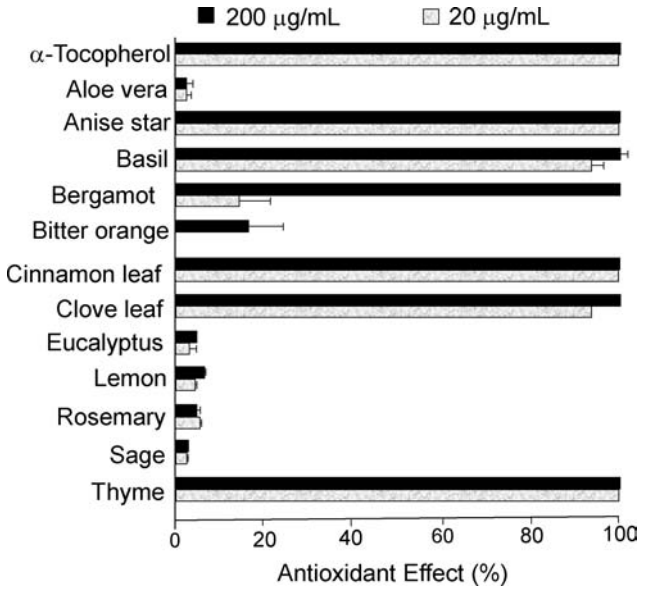

Figure 3. Antioxidant effects exhibited by essential oils at concentrations of 20 and 200 μg/mL in the aldehyde/carboxylic acid assay.

whereas cinnamon bark antioxidant activities were minor. The difference in antioxidant activities exhibited by these two oils in both studies may be attributed to the part of the plant that the oil/volatile extract was isolated from. For instance, the volatile constituents found in leaves of plants have been suggested to play a role in preventing photo-oxidative damage to the plant. As such, the volatile extracts taken from the leaves of a plant may demonstrate stronger antioxidant activities than volatile extracts isolated from the bark of the same plant.

DPPH Free Radical Scavenging Assay. Recently, the DPPH assay has become quite popular in natural antioxidant studies because this method is simple and highly sensitive. This assay is based on the theory that a hydrogen donor is an antioxidant and measures the ability of a potential antioxidant compound to reduce the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH$^{\bullet}$) (*6*). Of the 12 essential oils tested with this assay, those that exhibited potent dose-related activity are shown in **Figure 4**. The values are given as the mean of three replicated results. The standard deviations were not included because they were all $< 5\%$. At 10 μg/mL, clove leaf oil was almost as effective as the standard α-tocopherol (68 vs 72%). At higher concentrations, its effect was comparable to that of α-tocopherol. Clove leaf, basil, and cinnamon leaf oils exhibited $> 80\%$ effect at a concentration of 200 μg/mL. On the other hand, the antioxidant activity demonstrated by thyme (60% at 200 μg/mL) was not as potent as α-tocopherol at 10 μg/mL. Aloe vera, anise star, and bergamot oils exhibited only slight activities at 200 μg/mL. Bitter orange, eucalyptus, lemon, rosemary, and sage essential oils did not show appreciable antioxidant activities.

Previous work on the radical-scavenging activities of citrus essential oils using DPPH assay indicated that bergamot oils exhibited moderate antioxidant activities (*16*). Even though the bergamot oil tested for antioxidant effect using DPPH assay did not show significant activity in the present study, this may be due to differences in the oil sources.

MA/GC Assay. In this assay, malonaldehyde is derivatized with *N*-methylhydrazine to form 1-methylpyrazole (*13*). As a consequence of enhanced stability and nitrogen incorporation into the MA molecule, analysis was performed using a gas chromatograph equipped with a nitrogen−phosphorus detector. In the present study, the antioxidant activities of essential oils were tested by their ability to inhibit MA formation from

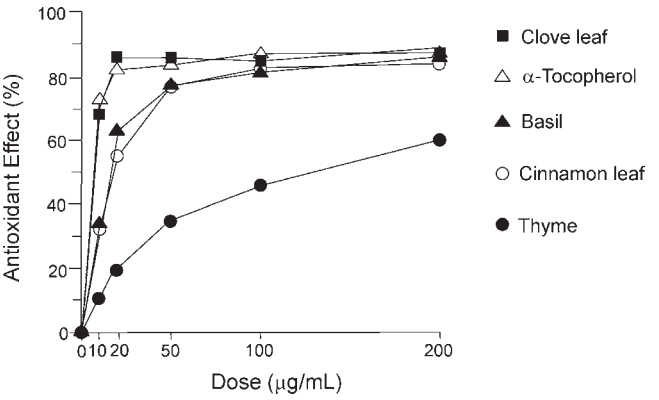

Figure 4. Potent dose-related antioxidant activities exhibited by clove leaf, basil, cinnamon leaf, and thyme essential oils in the DPPH free radical scavenging assay.

squalene upon ultraviolet irradiation ($\lambda = 300$ nm). Anise star, basil, bergamot, bitter orange, cinnamon leaf, clove leaf, eucalyptus, lemon, sage, and thyme oils were selected for testing because they demonstrated moderate to strong antioxidant activities in the other antioxidant assays. **Figure 5** shows the antioxidant effects of essential oils, which exhibited appreciable effects. The values are the mean of three replications. The standard deviations are not shown because they were all $< 0.3\%$. The potency of thyme oil was also demonstrated. Thyme oil inhibited MA formation by $83.2 \pm 0.1\%$ at the concentration of 50 μg/mL. At higher concentrations, the potency of thyme oil decreased. It is difficult to explain this observed phenomenon; however, it may be that the combined effects of many kinds of volatile constituents present in thyme oil vary at different concentrations. Clove leaf oil also exhibited strong antioxidant activities at 500 μg/mL ($79.6 \pm 0.2\%$), comparable to that of the reference compound, α-tocopherol, at the same concentration. At 500 μg/mL, bitter orange oil showed a relatively high effect ($53.5 \pm 0.4\%$). At all concentrations tested, anise star, basil, and cinnamon leaf oil showed moderate antioxidant activities. Bergamot, eucalyptus, lemon, and sage oils showed pro-oxidant activities at some of the concentrations tested in the present study. In this assay, α-tocopherol, basil, and bitter orange exhibited clear dose−response activities.

A previous study using the MA/GC assay indicated that a clove bud aroma extract exhibited antioxidant activities toward horse blood plasma oxidized with Fenton's reagent by 25% at 40 μg/mL and by 35% at 100 μg/mL (*17*). These effects are comparable to those exhibited by clove leaf oil in the present study with the MA/GC assay. At the highest concentration (400 μg/mL) tested, clove bud volatile extract inhibited MA formation by 48%, whereas clove leaf oil showed 80% effect at 500 μg/mL in this assay. Eucalyptus aroma extract also showed antioxidant activities at all concentrations (20−400 μg/mL) tested against MA formation in the previous study (*18*), unlike the pro-oxidant effects shown by eucalyptus oil in the present study. This difference in observed antioxidant activity may be attributed to the reaction mechanisms associated with the two model systems. Unlike the Fenton-induced oxidation in the previous study (*18*), which used Fenton's reagent to initiate and accelerate oxidation, the oxidation of squalene in the present study occurs by both autoxidative and photo-oxidative means via an "ene" type reaction (*19*). Therefore, between the two oxidation systems, the mechanisms by which a volatile extract and the constituents present demonstrate antioxidant action may be different. It is possible that whereas some of the oils exhibited antioxidant actions in the conjugated diene assay, the aldehyde/carboxylic

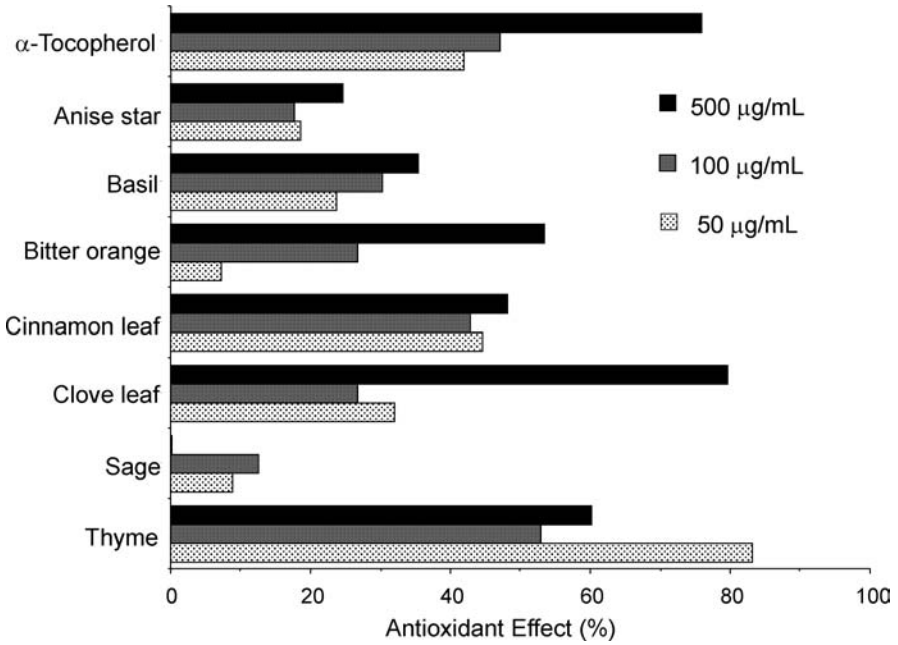

Figure 5. Antioxidant effects exhibited by the essential oils in the MA/GC assay.

acid assay, or the DPPH free radical scavenging assay, their prooxidant effects were observed under ultraviolet irradiation in the MA/GC assay as a consequence of the oil undergoing photochemical changes due to the transformation of constituents and the formation of new photochemical products that enhance oxidation.

Isolation and Identification of Volatile Constituents in Selected Essential oils. The volatile constituents identified in the 10 essential oils used in the MA/GC assay are summarized in **Table 1**. The volatile constituents from celery seed, jasmine, juniper berry, parsley seed, rose, and ylang-ylang essential oils were previously identified (*11*). On the basis of comparisons to mass spectral fragmentation patterns of authentic chemicals, 54 constituents were identified from the oils from anise star, basil, bergamot, bitter orange, cinnamon leaf, clove leaf, eucalyptus, lemon, sage, and thyme. The major constituents for anise star essential oil were anethole (86%), *p*-allylanisole (4%), and *p*-anisaldehyde (2%). The major compounds found in sage essential oil were α-thujone (27%), camphor (25%), and 1,8-cineole (8%). In eucalyptus essential oil, the main constituent, 1,8-cineole, comprised almost 90% of the essential oil. Lee and Shibamoto (*18*) found that 500 μg/mL of 1,8-cineole inhibited hexanal oxidation by 96% after 30 days in the aldehyde/carboxylic acid assay. The presence of 1,8-cineole may have contributed to the strong antioxidant activity demonstrated by 100 μg/mL of eucalyptus oil in the aldehyde/carboxylic acid assay. The three compounds that were present in most of the essential oils were α-pinene, limonene, and *p*-cymene. Among nine essential oils, the presence of α-pinene ranged from 0.09 to 3.50%. Limonene was particularly abundant in bergamot, lemon, and bitter orange oils (approximately 38, 68, and 93%, respectively). In thyme essential oil, *p*-cymene comprised almost 45% of the total oil. The other major constituents present in thyme essential oil were thymol (23%) and camphene (5%). Thymol showed strong antioxidant actions in the MA/GC and aldehyde/carboxylic acid assays (*18*). Eugenol, the major component identified from clove leaf oil (77%) and in cinnamon leaf and basil oils (13%), had shown antioxidant activities in various assays (*18*). Basil oil was also characterized by the presence of linalool (53%) and isoanethole (19%). For both cinnamon leaf and clove leaf oils, *β-trans*-caryophyllene was a major constituent present (53 and 17%, respectively) (*20*).

Results from this study suggest that the antioxidant potency exhibited by essential oils is dependent on the mechanisms of action by which the constituents exert their antioxidant effects and the testing conditions of the assays. Under the influence of ultraviolet radiation, the antioxidant effectiveness of some essential oils toward inhibiting lipid oxidation decreased or became negative.

Lipoxygenase Inhibitory Activity of Essential Oils. The results of the 15-lipoxygenase inhibitor screening assay on essential oils are shown in **Figures 6**−**8**. **Figure 6** shows the essential oils that exhibited strong lipoxygenase inhibitory activities. Values are the average of two experiments. A standard lipoxygenase inhibitory chemical, NDGA, inhibited 15-lipoxygenase activity by 56% at 0.5 μg/mL and showed reverse dose−response activity. The same phenomenon was observed in the case of ylang-ylang oil. Aloe vera oil exhibited the greatest lipoxygenase inhibitory effect (96%) at a concentration of 5 μg/mL, followed by thyme oil (86%) at a concentration of 0.5 μg/mL and bergamot oil (85%) at a concentration of 0.5 μg/mL. Aloe vera oil showed clear dose−response activity. The topical application of aloe sap on wounds and inflammations has been a domestic practice for a long time. Reports also indicate that essence from aloe contains anti-inflammatory compounds (*7*). **Figure 7** shows the essential oils that exhibited moderate (30−50%) to slight (10−30%) lipoxygenase inhibitory activities. Jasmine oil inhibited 15-lipoxygenase activity by 47% at a concentration of 0.5 μg/mL. The lipoxygenase inhibitory activities of angelica seed oil and rosemary oil at the concentration of 5 μg/mL were 44 and 42%, respectively. Clove leaf oil, which exhibited strong antioxidant activity in the conjugated diene assay, showed moderate lipoxygenase inhibitory activity (33% at 5 μg/mL).

The anti-inflammatory activities of many of these plants have also been demonstrated in other inflammatory assays (*7*−*10*). In addition, various researchers have also found that the volatile components present in these oils possess anti-inflammatory activities. For instance, eugenol, the major volatile component of clove essential oil, was shown to inhibit 5-lipoxygenase

Table 1. Volatile Compounds Identified in Essential Oils Used in the MA/GC Assay and Their GC Peak Areas

		concentration (GC peak area %)[a]									
compound	I^b	anise star	basil	bergamot	bitter orange	cinnamon leaf	clove leaf	eucalyptus	lemon	sage	thyme
α-tricyclene	1011										0.87
α-pinene	1039	0.45	0.09	1.62	0.37	0.16		3.50	2.29	2.82	0.45
camphene	1082									6.23	5.17
p-menthene	1116										0.25
β-pinene	1124		0.22	8.07					15.57	1.22	
sabinene	1130		0.08		0.13					0.43	
δ-3-carene	1132	0.12									
β-myrcene	1153		0.17		1.74					0.15	0.15
α-phellandrene	1177	0.10						0.35			
limonene	1208	0.80	0.14	38.46	92.81			0.44	68.25	1.79	0.23
β-phellandrene	1216	0.06				0.15					
1,8-cineole	1218	0.17	2.22	0.24				89.86		8.01	
γ-terpinene	1253			5.53				0.27	5.99		
p-cymene	1280	0.17		0.49		0.23		3.67	1.13	1.25	44.84
α-terpinolene	1280			0.33					0.13		0.12
α-thujone	1444									26.98	
p-cymenene	1449							0.12			
β-thujone	1456									3.37	
α-cubene	1457					0.37	0.24			0.13	
cis-limonene oxide	1468								0.14		
trans-limonene oxide	1481								0.09		
α-copaene	1519					0.51	0.61				
camphor	1537		0.24							24.72	
linalool	1545	1.54	53.36		0.17				0.5	0.39	1.23
linalyl acetate	1549			23.49	0.10						
α-bergamotene	1572		1.20						0.18		
β-*cis*-caryophyllene	1600					4.99					0.43
β-*trans*-caryophyllene	1626			0.49		53.22	17.16			1.70	
terpinen-4-ol	1628	0.14								0.07	0.09
β-citral	1661			0.16					0.67		
isoanethole	1670		19.57								
p-allylanisole	1683	3.98									
α-caryophyllene	1690					6.07	2.05			3.27	
γ-muurolene	1690									0.30	
α-terpineol	1708	0.14		0.29							4.31
α-citral	1721			0.30					1.06		
isoborneol	1721									4.53	
β-bisabolene	1730										0.14
δ-cadinene	1751					0.56	0.34			3.80	0.09
geranyl acetate	1761			0.59							0.07
anethole	1820	86.00									
benzyl alcohol	1822										0.49
(−)-calamenene	1842					0.28	0.19				
safrole	1876					0.25					
p-cymen-8-ol	1877									0.13	
caryophyllene oxide	1997					1.23	0.46			0.63	
p-anisaldehyde	2030	1.93									
(*E*)-cinnamaldehyde	2030					0.11					
cinnamyl acetate	2103					0.09					
thymol	2135										22.83
eugenol	2156		13.32			13.88	76.51				
carvacrol	2159									0.18	0.04
eugenyl acetate	2243					0.39					
anisyl methyl ketone	2257	0.43									

[a] GC peak area % of <0.04 is not shown. [b] Kovats index on a DB-WAX bonded phase fused silica capillary column.

activity (*18*). Linalool and linalyl acetate, major constituents present in basil, bergamot, and bitter orange essential oils, respectively, also were found to possess anti-inflammatory activities (*19*) as demonstrated in carrageenan-induced edema in rats.

On the other hand, some oils exhibited lipoxygenase-inducing activity (as shown in **Figure 8**). Ginger and bitter orange oils showed slight lipoxygenase inhibitory activities at 5 μg/mL, but they showed lipoxygenase-inducing activities at lower concentrations (0.5 and 1 μg/mL). In contrast, chamomile oil inhibited 15-lipoxygenase at 0.5 μg/mL but showed strong lipoxygenase-inducing activity at 5 μg/mL (−123%). The lipoxygenase-inducing activity of these essential oils may be due to the possible presence of polyacetylenes, which cause skin-sensitizing effects (*21*).

In mammals, lipoxygenases catalyze reactions on arachidonic acid that generate metabolites important to the mediation of inflammatory responses. Because of their involvement in lipid oxidation and inflammation, lipoxygenases have been implicated

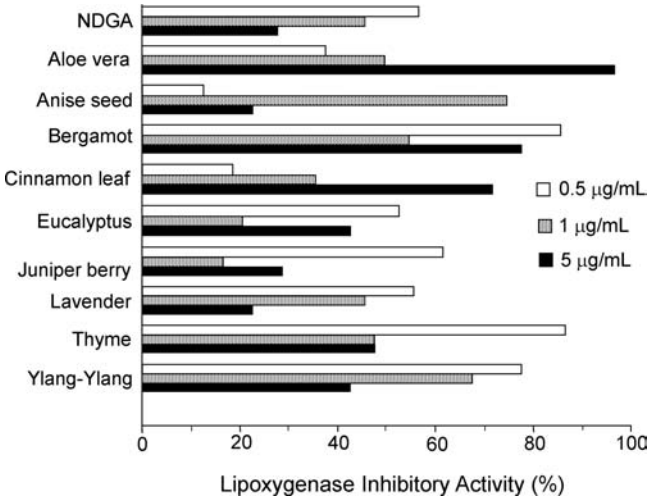

Figure 6. Results for essential oils showing strong effects in the lipoxygenase inhibitory assay.

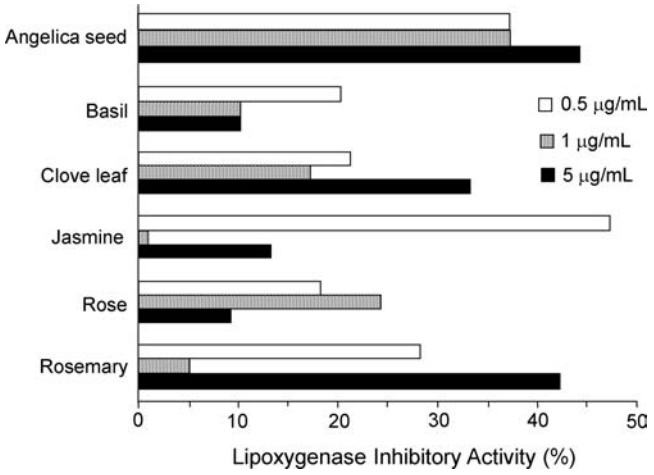

Figure 7. Results for essential oils showing moderate effects in the lipoxygenase inhibitory assay.

in the development of inflammatory vascular diseases such as atherosclerosis and diabetes (*22*−*24*).

The present study evaluated essential oils for antioxidant and lipoxygenase inhibitory activities. Some essential oils, such as clove leaf oil and thyme oil, demonstrated appreciable inhibitory activities in both antioxidant and lipoxygenase inhibitory assays. Further investigation into the dual antioxidant and lipoxygenase inhibitory activities of these essential oils would be worthwhile if they could be shown to be effective in managing diseases characterized by both oxidative damage and inflammation. Other oils, such as aloe vera, demonstrated a strong potential for lipoxygenase inhibitory properties but lacked antioxidant activity or vice versa (as demonstrated by sandalwood oil).

The results obtained in the present study demonstrate the wide range of antioxidant/lipoxygenase inhibitory activities of essential oils. This suggests that various other factors, such as the many constituents in the essential oils, may need to be investigated to account for the differences in these activities. The volatile oil constituents and their respective amounts in the essential oils may affect the biological activities (including antioxidant and anti-inflammatory) of the overall essential oil. Therefore, identification and characterization of the active components in these essential oils is needed to assess the efficacy, as well as safety, of essential oils.

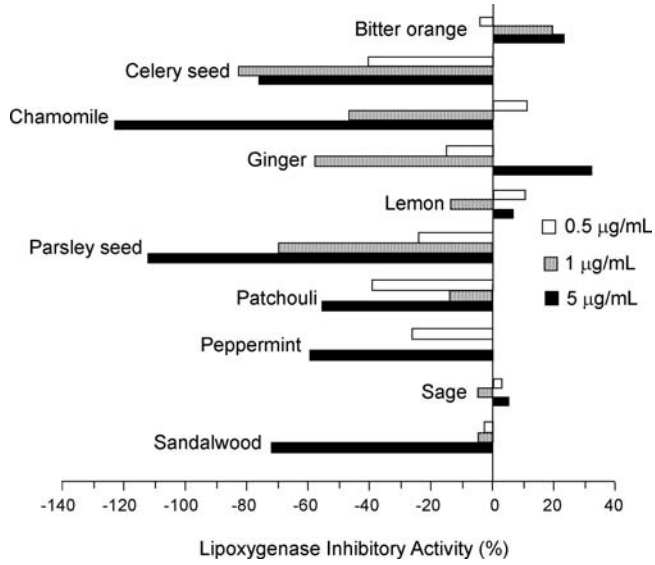

Figure 8. Results for essential oils exhibiting lipoxygenase inducing effects in the lipoxygenase inhibitory assay.

ABBREVIATIONS USED

DPPH, 1,1-diphenyl-2-picrylhydrazyl free radical; MA/GC, malonaldehyde/gas chromatography assay; LISA, lipoxygenase inhibitor screening assay; NDGA, nordihydroguaretic acid; 15-LOX, 15-lipoxygenase soybean enzyme.

LITERATURE CITED

(1) Billot, M.; Wells, F. V. In *Perfumery Technology, Art: Science: Industry*; Wiley: New York, 1975.

(2) Tisserand, R. B. In *The Art of Aromatherapy*; Healing Arts Press: Rochester, VT, 1997.

(3) Koo, B.-S.; Park, K.-S.; Ha, J.-H.; Park, J. H.; Jim, J.-C.; Lee, D.-U. Inhibitory effects of the fragrance inhalation of essential oil from *Acorus gramineus* on central nervous system. *Biol. Pharm. Bul.* **2003**, *26*, 978–982.

(4) Schnaubelt, K. Essential oil therapy according to traditional Chinese medical concepts. *Int. J. Aromather.* **2005**, *15*, 98–105.

(5) Tanaka, A.; Horiuchi, M.; Umano, K.; Shibamoto, T. Antioxidant and anti-inflammatory activities of water distillate and its dichloromethane extract from licorice root (*Glycyrrhiza uralensis*) and chemical composition of dichloromethane extract. *J. Sci. Food Agric.* **2008**, *88*, 1158–1165.

(6) Moon, J.-K.; Shibamoto, T. Antioxidant assays for plant and food components. *J. Agric. Food Chem.* **2009**, *57*, 1655–1666.

(7) Vazquez, B.; Avila, G.; Segura, D.; Escalante, B. Anti-inflammatory activity of extracts from aloe vera gel. *J. Ethnopharmacol.* **1996**, *55*, 69–75.

(8) Trouillas, P.; Calliste, C.-A.; Allais, D.-P.; Simon, A.; Marfak, A.; Delage, C.; Duroux, J.-L. Antioxidant, anti-inflammatory and antiproliferative properties of sixteen water plant extracts used in the Limousin countryside as herbal teas. *Food Chem.* **2003**, *80*, 399–407.

(9) Grzanna, R.; Lindmark, L.; Frondoza, C. G. Ginger—an herbal medicinal product with broad anti-inflammatory actions. *J. Medic. Food.* **2005**, *8*, 125–132.

(10) Chao, L. K.; Hua, K.-F.; Hsu, H.-Y.; Cheng, S.-S.; Liu, J.-Y.; Chang, S.-T. Study on the anti-inflammatory activity of essential oil from leaves of *Cinnamomum osmophloeum*. *J. Agric. Food Chem.* **2005**, *53*, 7274–7278.

(11) Wei, A.; Shibamoto, T. Antioxidant activities and volatile constituents of various essential oils. *J. Agric. Food Chem.* **2007**, *55*, 1737–1742.

(12) Lee, K. G.; Shibamoto, T. Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. *J. Agric. Food Chem.* **2002**, *50*, 4947–4952.

(13) Umano, K.; Dennis, K. J.; Shibamoto, T. Analysis of free malonaldehyde in photoirradiated corn oil and beef fat via a pyrazole derivative. *Lipids* **1988**, *23*, 811–814.
(14) Heinonen, I. M.; Lehtonen, P. J.; Hopia, A. I. Antioxidant activity of berry and fruit wines and liquors. *J. Agric. Food Chem.* **1998**, *46*, 25–31.
(15) Macku, C.; Shibamoto, T. Volatile antioxidants produced from heated corn oil/glycine model system. *J. Agric. Food Chem.* **1991**, *39*, 1990–1993.
(16) Choi, H.-S.; Song, H. S.; Ukeda, H.; Sawamura, M. Radical-scavenging activities of citrus essential oils and their components: detection using 1,1-diphenyl-2-picrylhydrazyl. *J. Agric. Food Chem.* **2000**, *48*, 4156–4161.
(17) Lee, K.-G.; Shibamoto, T. Antioxidant property of aroma extract isolated from clove bud [*Syzygiun aromaticum* (L.) Merr. et Perry]. *Food Chem.* **2001**, *74*, 443–448.
(18) Lee, K.-G.; Shibamoto, T. Antioxidant activities of volatile components isolated from *Eucalyptus* species. *J. Sci. Food Agric.* **2001**, *81*, 1573–1579.
(19) Yeo, H. C. H.; Shibamoto, T. Formation of formaldehyde and malonaldehyde by photooxidation of squalene. *Lipids* **1992**, *27*, 50–53.
(20) Lee, S.-J.; Umano, K.; Shibamoto, T.; Lee, K.-G. Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thumus vulgaris* L.) and their antioxidant properties. *Food Chem.* **2005**, *91*, 131–137.
(21) Christensen, L. P.; Brandt, K. Bioactive polyacetylenes in food plants of the Apiaceae family: occurrence, bioactivity and analysis. *J. Pharm. Biomed. Anal.* **2006**, *41*, 683–693.
(22) Huo, Y.; Zhao, L.; Hyman, M. C.; Shashkin, P.; Harry, B. L.; Burcin, T.; Forlow, B.; Stark, M. A.; Smith, D. F.; Clarke, S.; Srinivasan, S.; Hedrick, C. C.; Praticò, D.; Witxtum, J. L.; Nadler, J. L.; Funk, C. D.; Ley, K. Critical role of macrophage 12/15-lipoxygenase for atherosclerosis in apolipoprotein E-deficient mice. *Circulation* **2004**, *110*, 2024–2031.
(23) Cathcart, M. K.; Folcik, V. A. Lipoxygenases and atherosclerosis: protection versus pathogenesis. *Free Radical Biol. Med.* **2000**, *28*, 1726–1734.
(24) Natarajan, R.; Nadler, J. L. Lipid inflammatory mediators in diabetic vascular disease. *Arterioscler. Thromb. Vasc. Biol.* **2004**, *24*, 1542–1548.

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