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Antioxidant Activities and Volatile Constituents of Various Essential Oils

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Thirteen essential oils were examined for their antioxidant activity using three different assay systems. Jasmine, parsley seed, rose, and ylang−ylang oils inhibited hexanal oxidation by over 95% after 40 days at a level of 500 μg/mL in the aldehyde/carboxylic acid assay. Scavenging abilities of the oils for the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical ranged from 39% for angelica seed oil to 90% for jasmine oil at a level of 200 μg/mL. The greatest inhibitory activity toward malonaldehyde (MA) formation from squalene upon UV-irradiation was obtained from parsley seed oil (inhibitory effect, 67%), followed by rose oil (46%), and celery seed oil (23%) at the level of 500 μg/mL. The main compounds of oils showing high antioxidant activity were limonene (composition, 74.6%) in celery seed, benzyl acetate (22.9%) in jasmine, α-pinene (33.7%) in juniper berry, myristicin (44%) in parsley seed, patchouli alcohol (28.8%) in patchouli, citronellol (34.2%) in rose, and germacrene (19.1%) in ylang−ylang.

KEYWORDS: Antioxidant activity; essential oils; jasmine oil; malonaldehyde; parsley seed oil; rose oil; squalene

INTRODUCTION

Plant essential oils have been used for many purposes including flavoring foods and beverages, aroma additives for cosmetics and household products, and masking agents for unpleasant odors. In particular, essential oils have been one of the most important ingredients of cosmetic products such as perfumes and skin care creams. The production of essential oils has been developing steadily in many countries. Over 200 kinds of essential oils are produced annually, with production volumes ranging from 30,000 tons/year for orange oil to less than 100 kg/year for some flower extracts such as jasmine, rose, and lavender (*1*).

Essential oils have been also used as therapeutic agents since ancient times. For example, lavender and chamomile oils are used for insomnia patients. Digestive problems are treated with peppermint oil, rosemary oil, and coriander oil. Muscle aches and pain are treated with German chamomile oil and eucalyptus oil. Chamomile oil, celery oil, juniper oil, and coriander oil are used for their anti-inflammation benefits. Moreover, some of them have been scientifically proven to possess medicinal activities including anti-inflammatory (*2*), antiviral (*3*), antitumor (*4*), anti-hyperglycemic (*5*), and anticarcinogenic (*6*) activities. In addition, discovery of the antioxidant activity has been reported in various essential oils, including rosemary (*7*), lavender (*7*), eucalyptus (*8*), clove (*9*, *10*), oregano (*11*), and Origanum glandulosum (*12*). Consequently, antioxidant activity of essential oils has gained considerable attention among researchers.

Since the benefits derived from essential oils have created renewed demand in their use by the public, explorations of their antioxidant activities for their possible beneficial use in the prevention of oxidative damage is in order. In the present study, the essential oils derived from 13 medicinal plants were examined for their antioxidant activity to find a way to use essential oils to prevent oxidative damages.

MATERIALS AND METHODS

Materials. Hexanal, undecane, α-tocopherol, *N*-methylhydrazine (NMH), sodium dodecyl sulfate (SDS), and 2-methylpyrazine were purchased from Aldrich Chemical Co. (Milwaukee, WI). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was bought from TCI Organic Chemicals (Portland, OR). Squalene was purchased from Sigma Chemical Co. (St. Louis, MO).

Essential oils angelica seed (*Angelica archangelica* L.), celery seed (*Apium graveolens* L.), chamomile (*Anthemis nobilis* L.), ginger (*Zingiber officinale* R.), jasmine (*Jasminum officinale* L.), juniper berry (*Juniperus communis* L.), lavender (*Lavendula officinalis* L.), parsley seed (*Petroselinum sativum* H.), patchouli (*Pogostemon patchouli* B.), peppermint (*Mentha piperita* L.), rose (*Rosa Damascena* M.), sandalwood (*Santalum album* L.), and ylang−ylang (*Cananga odorata* H. & T.) were provided by International Flavors and Fragrances Inc. (New York).

1-Methylpyrazole (1-MP) was synthesized by the previously reported method (*13*). Stock solutions were prepared for SDS (0.20%) in deionized water and the gas chromatographic internal standard, 2-methylpyrazine (1 g/L), in ethyl acetate.

Aldehyde/Carboxylic Acid Assay. Essential oils were tested for their abilities to inhibit the oxidation of hexanal to hexanoic acid, according to previously published reports (*14*). Various amounts of the essential oils (0, 10, 20, 50, 100, 200, and 500 μg/mL) were added

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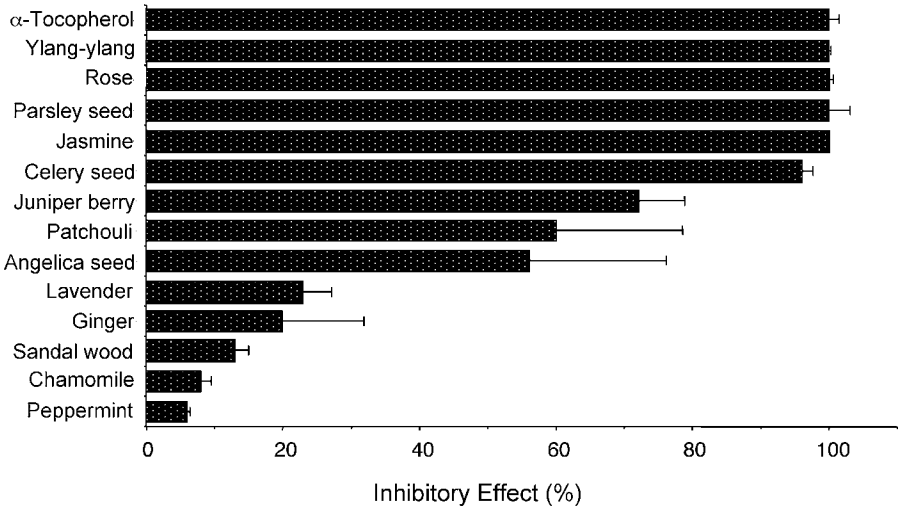

Figure 1. Antioxidant activities of 13 essential oils at the level of 500 μg/mL after 40 days measured via aldehyde/carboxylic acid assay.

to a 2-mL dichloromethane solution containing hexanal (3 mg/mL) and 0.2 mg/mL of undecane as the internal standard. Sample oxidation was initiated by heating the sealed sample vials in a 60 °C water bath for 10 min. Vials were stored at room temperature, in the dark, for the duration of the study. The decrease in hexanal was monitored at 5-day intervals for 40 days. α-Tocopherol was used as the reference antioxidant. The experiments were conducted in triplicate for each concentration.

The hexanal sample was analyzed by an Agilent model 6890 gas chromatograph (GC) equipped with a 30 m × 0.25 mm i.d. (d_f = 1 μm) DB-1 bonded-phase fused silica capillary column (Agilent, Folsom, CA) and a flame-ionization detector (FID). 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 °C to 120 °C at 8 °C/min. Temperature for the injector and detector was 300 °C and 280 °C, respectively.

DPPH Free-Radical-Scavenging Assay. The abilities of the essential oils to scavenge DPPH free radicals were measured by a previously reported method (*15*). The DPPH solution (7.6×10^{-5} M) in dichloromethane was prepared fresh daily prior to UV measurements. Various volumes (10, 20, 50, 100, or 200 μL) of the stock essential oil solutions (1 mg/mL) dissolved in dichloromethane were removed and placed into the vials to give the following concentrations: 10, 20, 50, 100, or 200 μg/mL. The DPPH solution (1 mL) was added to each of these vials. After sample solutions were allowed to stand for 30 min in the dark (25 °C), the absorbance was measured at 517 nm using a Hewlett-Packard 8452A diode array UV spectrophotometer. Blank samples containing the same amount of methanol and DPPH solution were also prepared and measured daily. α-Tocopherol was also monitored for radical-scavenging activity. All experiments were carried out in triplicate.

Malonaldehyde/Gas Chromatography (MA/GC) Assay. The antioxidant activities of essential oils were also evaluated by analyzing malonaldehyde (MA) formed in oxidized squalene upon UV-irradiation after derivatizing to 1-MP with NMH (*13*). Stock solutions of squalene (137 mg) dissolved in various concentrations of each essential oil (50, 100, or 500 μg/mL) were prepared in 25 mL hexane. A stock solution for control samples (without essential oil) was made by dissolving 137 mg of squalene into 25 mL of hexane. From each of these stock solutions, 5-mL aliquots of each were taken and placed into quartz test tubes. These test tubes were then placed under a gentle stream of nitrogen and were slowly turned so as 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 then placed in a Rayonet RPR-100 chamber reactor equipped with eight lamps (λ = 300, 0.68 ± 0.2 mW/cm^2) and were irradiated at 31 °C for 12 h.

Following irradiation, 5 mL of the 0.20% SDS solution and 100 μL of NMH were added to each test tube. Test tubes were then covered and stirred for 1 h (at 25 °C). After stirring, samples were extracted by solid-phase extraction (SPE) with C-18 cartridges (Varian, Inc., Lake Forest, CA). Prior to use, cartridges were conditioned with ethyl acetate (5 mL), methanol (5 mL), and then deionized water (5 mL, twice). After conditioning, samples were loaded onto each cartridge and were eluted. Cartridges were then rinsed twice with 2.5 mL of deionized water and were dried for 1 h. MA, as its derivative, 1-MP, was then eluted out with ethyl acetate (5 mL). This extract was then filtered through anhydrous sodium sulfate into concentration tubes. After removing the solvent with a purified nitrogen stream until a volume of 0.1 mL was obtained, the internal standard, 2-methylpyrazine (150 μL of the stock solution), was added to each extract. Finally, the sides of the concentration tubes were washed with ethyl acetate until a final volume of 0.5 mL was obtained. Extracts were then analyzed by GC.

An Agilent model 6890 gas chromatograph equipped with an NPD and a 30 m × 0.25 mm i.d. × 0.25 μm film thickness bonded-phase DB-1 fused silica capillary column (J&W Scientific, Folsom, CA) was used for the quantitative analysis of the MA derivative, 1-MP. The linear velocity of the helium carrier gas was 18 cm/s at a split ratio of 20:1. The oven temperature was programmed from 100 °C to 180 °C at 3 °C/min. The injector and detector temperatures were 250 °C and 300 °C, respectively.

Identification of Major Compounds in Selected Essential Oils. The GC Kovats retention index *I* (*16*) and the mass spectrometry (MS) fragmentation pattern of each component were compared to those of the authentic compound to identify the major volatile chemicals in the essential oils that exhibited appreciable antioxidant activities. Kovats Indices of major constituents in the essential oils were obtained by an Agilent model 6890 gas chromatograph equipped with an FID and a 60 m × 0.25 mm i.d. (d_f = 0.25 μm) bonded-phase DB-Wax fused silica capillary column (J&W Scientific, Folsom, CA). The linear velocity of the helium carrier gas was 26 cm/s at a split ratio of 20:1. The oven temperature was held at 60 °C for 8 min and then was programmed to 180 °C at 3 °C/min and was held for 80 min. The injector and detector temperatures were 300 °C and 280 °C, respectively.

An Agilent model 5890 Series II gas chromatograph equipped with a 60 m × 0.25 mm i.d. (d_f = 0.25 μm) bonded-phase DB-Wax fused silica capillary column (J&W Scientific, Folsom, CA) and interfaced to an Agilent model 5791A mass spectrometer was used for mass spectral identification at an MS ionization voltage of 70 eV. The GC conditions were the same as described above.

RESULTS AND DISCUSSION

Aldehyde/Carboxylic Acid Assay. Numerous antioxidant assays based on the different mechanisms have been used for testing antioxidant activities of chemicals or mixtures of chemicals. The aldehyde/carboxylic acid assay is useful for determining long-term antioxidant activity of chemicals. **Figure 1** shows the antioxidant activities of 13 essential oils at the level of 500 μg/mL after 40 days. α-Tocopherol was also tested for

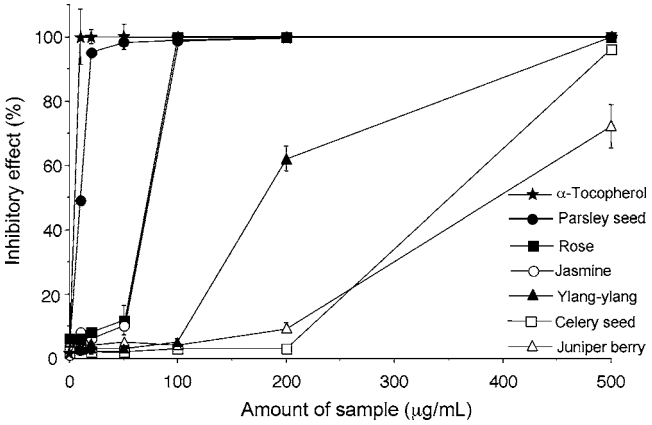

Figure 2. Dose-related activities of the essential oils inhibited hexanal oxidation by more than 70% at the level of 500 μg/mL after 40 days (refer to **Figure 1**).

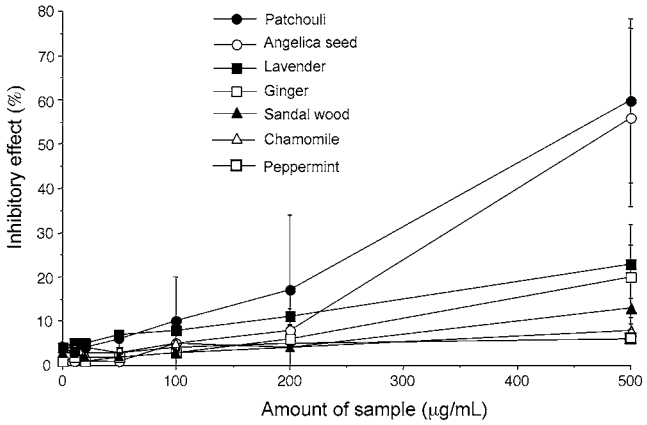

Figure 3. Dose-related activities of the essential oils inhibited hexanal oxidation by less than 60% at the level of 500 μg/mL (refer to **Figure 1**).

validation of the assay. Ylang−ylang, rose, parsley seed, and jasmine oils inhibited hexanal oxidation by nearly 100% over 40 days. Celery seed oil inhibited hexanal oxidation by 96%. Juniper berry, patchouli, and angelica seed oils exhibited moderate activities (56−72%).

Figure 2 shows the dose-related activities of the essential oils that inhibited hexanal oxidation by more than 70% at the concentration of 500 μg/mL after 40 days. Parsley seed demonstrated 95% inhibitory activity against hexanal oxidation at 20 μg/mL. This was comparable to that of the standard, α-tocopherol at 10 μg/mL (>99%). Inhibitory effect of rose oil increased significantly from the concentrations of 50 μg/mL (12%) to 100 μg/mL (100%). Ylang−ylang oil began to show activity above the concentration of 200 μg/mL (62%). **Figure 3** shows the dose-related activities of the essential oils that inhibited hexanal oxidation by less than 60% at the concentration of 500 μg/mL. Only patchouli and angelica seed oils exhibited appreciable dose-related activities in this group. No significant activities were observed for chamomile, ginger, lavender, peppermint, and sandalwood essential oils in the present study.

DPPH Free-Radical-Scavenging Assay. In this assay, the ability of an antioxidant compound to donate hydrogens and thus reduce stable DPPH• to nonradicals was spectrophotometrically measured at 517 nm. The abilities of the antioxidant compound to scavenge DPPH• after a certain time corresponds inversely to the remaining DPPH• present. **Figure 4** shows the antioxidant activity of the 13 essential oils at the concentration

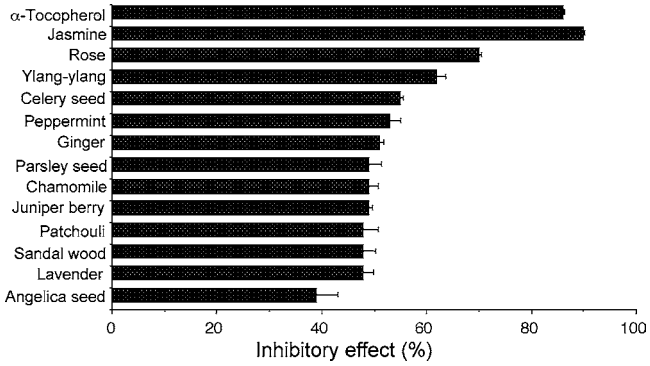

Figure 4. Antioxidant activities of 13 essential oils at the level of 200 μg/mL measured via DPPH• scavenging assay.

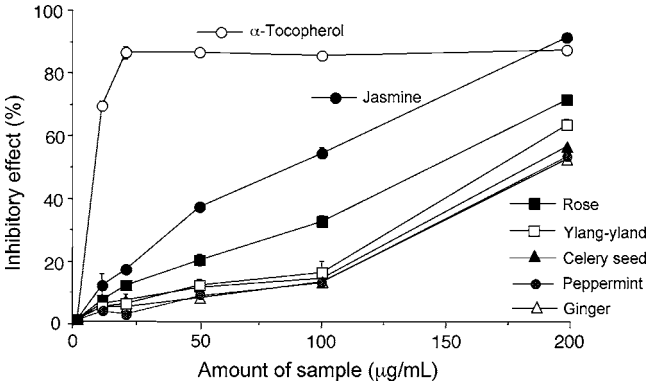

Figure 5. Dose-related activities of essential oils exhibited by over 50% effect measured via DPPH• scavenging assay.

of 200 μg/mL as measured by the DPPH• scavenging assay. The greatest effect was obtained by jasmine oil (90%), which was more effective than α-tocopherol (86%), followed by rose oil (70%) and ylang−ylang oil (62%). Most oils showed moderate activity ranging from 39% (angelica seed oil) to 55% (celery seed oil).

Figure 5 shows that the dose-related activities of the essential oils exhibited over 50% effect. Compared to the standard, α-tocopherol, which demonstrated 68% DPPH• scavenging activity at 10 μg/mL, the activities of the essential oils at this concentration were considerably low (in the range of approximately 2−11%). However, all essential oils tested showed dose-response activities.

MA/GC Assay. MA is known to form from lipids, such as fatty acids and their esters (*17*), at the final stage of lipid peroxidation as a secondary oxidation product (*18*). Moreover, the toxicity of MA has received considerable attention because of its adduct formation with DNA and proteins (*19*). Therefore, it is significantly important to find ways to prevent the formation of MA from lipids upon oxidation, even though the complete formation mechanisms of MA are not well understood. The main objective focused on the effect of essential oils toward inhibition of toxic MA formation from the oxidized skin surface lipid squalene upon UV irradiation. Squalene was chosen because skin surface lipids are susceptible to oxidative damage caused by reactive oxygen species (ROS) in the environment and ultraviolet (UV) radiation and subsequently form toxic secondary lipid peroxidation products such as MA (*20*, *21*). Moreover, skin care products generally contain 0.1−0.3% of the fragrance ingredient made from essential oils (*22*).

In this experiment, the antioxidant activities of jasmine, parsley seed, rose, celery seed, ylang−ylang, juniper berry, and patchouli oils were chosen on the basis of their moderate to

Table 1. Major Components of Essential Oils, Which Exhibited Appreciable Antioxidant Activities in the MA/GC Assay

		GC peak area %						
compound	RI[a]	celery seed	jasmine	juniper berry	parsley seed	patchouli	rose	ylang-ylang
ethanol	937	b					1.0	
α-pinene	1021			33.7	15.5			
camphene	1063			1.9				
β-pinene	1092			1.4	11.7			
sabinene	1107			10.8				
β-myrcene	1153			12.4				
α-terpinene	1161			1.3				
limonene	1178	74.6		4.5				
β-phellandrene	1183				2.9			
γ-terpinene	1224			2.4				
p-cymene	1264			1.4				
α-terpinolene	1280			1.4				
pentyl benzene	1421							
p-methyl anisole	1441							1.6
β-patchoulene	1485					4.1		
α-guaiene	1525					18.2		
linalool	1540		6.41				1.9	
linalyl acetate	1552							8.3
β-elemene	1595			2.6				
β-caryophyllene	1599			1.8			1.2	11.1
terpinen-4-ol	1612			1.7				
γ-elemene	1641			2.6				
methyl benzoate	1641					14.3		
β-humulene	1674							
α-humulene	1690			1.8				3.6
heptadecane	1700						2.7	
germacrene	1705			2.0				19.1
geranial	1707				1.1			
β-selinene	1734	11.0						
γ-guaiene	1761					20.5		
benzyl acetate	1765		22.9					
(*Z*,*Z*) or (*Z*,*E*)-farnesene	1770							12.4
γ-cadinene	1771			2.0				
citronellol1	1773						34.2	
(*E*,*E*)-farnecene	1778							12.6
nerol	1799						7.9	1.5
geraniol	1851						16.3	
benzyl alcohol	1909		6.5					
cis-jasmone	1984		2.9					
methyl eugenol	2022						8.1	
(*E*)-nerolidol	2033						1.0	
p-cresol	2105		1.4					
cis-3-hexenyl benzoate	2155		1.1					
patchouli alcohol	2156					28.8		
eugenol	2180		3.0				2.1	
cinnamyl acetate	2197							4.8
methyl palmitate	2245		1.2					
α-cadinol	2259							1.0
1-allyl-2,3,4,5-tetramethoxybenzene	2265				4.4			
(*E*,*E*)-farnesyl acetate	2255							1.7
myristicin	2323				44.0			
isophytol	2327		7.5					
(*E*,*E*)-farnesol	2377						0.8	1.0
apiole	2431				12.1			
cis-phytol	2570		15.0					

[a] Kovats index on DBWax column. [b] Values are less than 1.0% or not detected.

strong activities demonstrated in the previous two assays for further testing against MA formation from UV irradiated squalene. In the absence of essential oils, the control samples generated 1.09 ± 0.01 nmol MA/mg squalene.

Figure 6 shows the results of the MA/GC assay on selected oils. The decreasing order of the overall antioxidant activity of the essential oils tested was parsley seed (at concentration of 500 μg/mL) > rose (100 μg/mL) > ylang−ylang (100 μg/mL) > celery seed (500 μg/mL) > jasmine (500 μg/mL) > patchouli (100 μg/mL) > juniper berry (500 μg/mL). Of all oils tested at the concentration of 500 μg/mL, parsley seed oil exhibited the greatest activity (67%). Also, only parsley seed oil showed appreciable dose-related activity. The inhibitory activity of rose oil (54%) surpassed that of α-tocopherol (47%) at the concentration of 100 μg/mL but was lower (46%) than that of α-tocopherol at 500 μg/mL. Celery seed oil (23%) and ylang−ylang oil (26%) showed slight inhibitory effects at the concentrations of 500 μg/mL and 100 μg/mL, respectively. Slight pro-oxidant activity was observed in celery seed oil (−8%) at the concentration of 50 μg/mL. Also, some contradictory results were obtained from jasmine and patchouli oils. Jasmine oil exhibited moderate pro-oxidant activity (−29%) at the concentration of 50 μg/mL, and it showed slight antioxidant activity (21%) at the concentration of 500 μg/mL. Patchouli oil exhibited

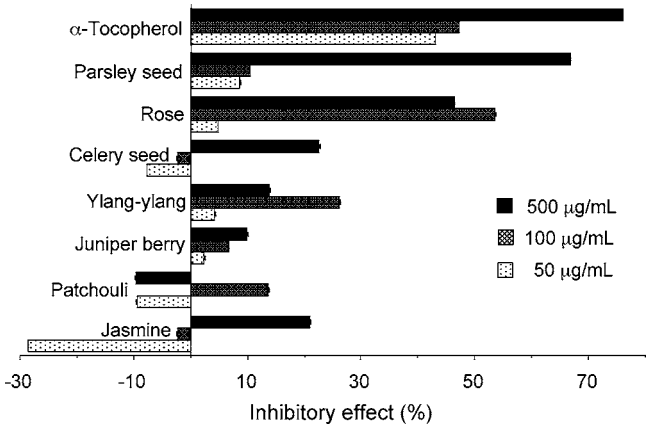

Figure 6. Antioxidant activity of selected essential oils measured via MA/GC assay.

pro-oxidant activity at the concentrations of 50 μg/mL (−10%) and 500 μg/mL (−9%) but exhibited antioxidant activity at the concentration of 100 μg/mL (14%). These contradictory results, as well as a lack of dose dependence, may be due to the presence of numerous chemicals in each essential oil. Therefore, it is necessary to discuss these effects associated with the major constituents of each essential oil.

Major Constituents of Selected Essential Oils and Their Antioxidant Activity. **Table 1** shows the analytical results of the essential oils tested by the MA/GC assay. Parsley seed oil, which exhibited the greatest antioxidant activity, was comprised mainly of myristicin (composition, 44%), α-pinene (15.5%), β-pinene (11.7%), and apiole (1-allyl-2,5-dimethoxy-3,4-methylene dioxybenzene, 12.1%%). These were also the major constituents, composing over 80% of the oil, in a variety of parsley seed oils reported previously (*23*). The antioxidant activity of parsley oil, of which composition was similar to that of the parsley seed oil used in the present study, has been reported (*24*). In this report, myristicin possessed moderate antioxidant activity, and apiole might be the major contributor to the antioxidant activity of parsley oil. The other major components of parsley seed oil, α-pinene and β-pinene, may not contribute significantly to inhibiting MA formation because juniper berry oil, which was composed of over 35% α-pinene (33.7%) and β-pinene (1.4%), did not exhibit appreciable inhibitory effect toward MA formation. There are many reports on the composition (*25*) and biological activities of juniper berry essential oils. For example, Sardinian Juniperus essential oil, which is composed of 67.6% α-pinene and myrcene, have been shown to possess antimicrobial properties (*26*).

In addition to juniper berry oil, celery seed oil was rich in monoterpene hydrocarbons (74.6% of *d*-limonene), among the essential oils tested for MA inhibitory effect. On the other hand, rose and ylang−ylang oils were rich in sesquiterpene hydrocarbons and monoterpenoids. The rose oil contained citronellol in the greatest amount (34.2%), followed by geraniol (16.3%), methyl eugenol (8.1%), and nerol (7.9%). These concentrations are consistent with the chemical composition of a rose oil previously reported (*27*). Citronellol inhibited MA formation by 27.5%, nerol inhibited MA formation by 42.7%, and geraniol inhibited MA formation by 34.9% at the concentration of 1000 ppm when they were tested by the thiobarbituric acid (TBA) assay (*28*). Therefore, these oxygenated monoterpenes must contribute significantly to the MA inhibitory activity of rose oil. The MA/GC assay should be comparable to the TBA assay because both methods measure MA formed by lipid peroxidation (*29*). Ylang−ylang oil contained germacrene in the greatest amount (19.1%), followed by (*E*,*E*)-farnecene (12.6%), (*Z*,*Z*) or (*Z*,*E*)-farnecene (12.4%), and β-caryophyllene (11.1%). These are also consistent with the chemical composition reported previously in ylang−ylang oil (*30*).

The main compounds identified in jasmine oil were benzyl acetate (22.9%), *cis*-phytol (15%), isophytol (7.5%), benzyl alcohol (6.5%), and linalool (6.4%). There are no reports on the antioxidant activities of benzyl acetate and phytols. Benzyl alcohol inhibited hexanal oxidation by 60% over 40 days (*31*). However, linalool reportedly possessed pro-oxidant activity (−32.9% inhibition at the concentration of 1000 ppm) in the TBA assay (*28*). A unique component of patchouli oil is patchouli alcohol (octahydro-4, 8a, 9,9-tetramethyl-1,6-methanonaphthalene), which composed 28.8% of the oil, but its antioxidant activity is unknown. The other main components of patchouli oil were γ-guaiene (20.5%) and α-guaiene (18.2%), of which antioxidant activities are also unknown. There are only a few reports on the antioxidant activity of pure component of essential oils. However, there are many reports on biological activities as well as the antioxidant activity of essential oils. For example, antioxidant activity was obtained from Australian tea tree oil (*32*) and antifungal activity from the essential oil of the leaves of Sesuvium portulacastrum (*33*).

In the present study, varying degrees of antioxidant actions were observed in essential oils on the basis of their radical-scavenging activities and inhibition of the lipid oxidation cycle. For some oils, anti- or pro-oxidant behaviors were demonstrated, depending on the conditions of the assay system and the concentrations at which the oils were tested. Further assessments of these oils and their constituents must be performed in various assay systems with different dose concentrations before an accurate evaluation of their biological activity can be given. For oils such as parsley seed and rose, the results obtained from this study suggest consistent antioxidant actions in the three lipophilic assay systems, at times comparable to or greater than that of α-tocopherol. For these oils, the medicinal benefit derived from their use may include prevention of oxidative skin damage and subsequent disease progression.

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