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Effects of Essential Oils from Herbal Plants and Citrus Fruits on DNA Polymerase Inhibitory, Cancer Cell Growth Inhibitory, Antiallergic, and Antioxidant Activities

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ABSTRACT: In this study, the biological activity of 20 essential oils (EOs) from herbal plants and citrus fruits were investigated in terms of mammalian DNA polymerase (pol) inhibitory activity, cancer cell (human colon carcinoma, HCT116) growth inhibitory activity, antiallergic activity, as anti-β-hexosaminidase release activity in rat basophilic leukemia RBL-2H3 cells treated with calcium ionophore A23187, and antioxidant activity by a lipophilic-oxygen radical absorbance capacity method. These EOs showed patterns of inhibition of pol α, a DNA replicative pol, similar to their cancer cell growth inhibitory activity, and their inhibitory activity on pol λ, a DNA repair/recombination pol, by the EOs showed correlation with anti-β-hexosaminidase release activity. Among these EOs, chamomile (*Matricaria chamomilla* L.) was the strongest inhibitor of pols α and λ and showed significant effects on both cancer cell growth and mast cell degranulation. On the basis of these results, chamomile EO can be recommended as a potentially useful, bioactive candidate for therapeutic applications.

KEYWORDS: *essential oil, chamomile, α-bisabolol oxide A, DNA polymerase inhibitory activity, cancer cell growth inhibitory activity, anti-β-hexosaminidase release activity, antioxidant activity, antiallergic activity, anti-inflammatory activity*

INTRODUCTION

Pol (DNA-dependent DNA polymerase, E.C. 2.7.7.7) catalyzes deoxyribonucleotide polymerization in association with a DNA strand, which it "reads" and uses as a template.[1] Pol can add free nucleotides only to the 3′ end of the forming strand, such that new strand elongation occurs in the 5′ → 3′ direction. The newly polymerized molecule is complementary to the template strand and identical to the template's partner strand.[1] Replication, recombination, and repair of DNA in eukaryotes are key systems in which pols have important roles.[2] The human genome encodes ≥15 pols that carry out cellular DNA synthesis.[3−6] Eukaryotic cells contain three replicative pols (α, δ, and ε), mitochondrial pol γ, and ≥11 nonreplicative pols (including β, ζ, η, θ, ι, κ, λ, μ, ν, terminal deoxynucleotidyl transferase (TdT), and REV1).[6] Pols have a highly conserved structure, suggesting their overall catalytic subunits differ very little between species as well as indicating their important, irreplaceable cellular functions, the stability of which provides evolutionary advantages. Pols are not only essential for DNA replication, repair, and recombination, but they are also involved in cell division.

In screening for pol inhibitors from natural products, including food components, nutrients, and chemically synthesized materials for 15 years, more than 100 compounds have been identified as interesting inhibitors.[7,8] In particular, selective pol inhibitors are considered to be a group of potentially useful chemotherapeutic agents, because some inhibitors suppress human cancer cell proliferation and are cytotoxic.[9] Recently, some inhibitors of pol λ, a DNA repair/recombination related pol, have been found to possess anti-inflammatory activity.[7,9,10]

Essential oils (EOs) are natural, volatile, complex compounds that are characterized by a strong odor and formed by aromatic plants as secondary metabolites.[11] EOs have largely been employed for their already observed natural properties, such as their antitumor, anti-inflammatory, antioxidant, and antibacterial activities.[12−15] At present, approximately 3000 EOs are known, 300 of which are commercially important, especially in the pharmaceutical, agronomic, food, sanitary, cosmetic, and perfume industries. EOs or some of their components are used in perfumes and cosmetic products, sanitary products, dentistry, agriculture, as food preservatives and additives, and as natural remedies.[16,17] Moreover, EOs are used in massages, as mixtures with vegetal oil, or in baths, but they are most frequently used in aromatherapy.[11] Some EOs appear to exhibit particular medicinal properties that have been claimed to cure various organ dysfunctions or systemic disorders. Because of new interest in EOs and despite their wide use and our familiarity with them as fragrances, it is important to develop a better

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understanding of their biological mode of action for inventing new applications in human health, agriculture, and their environment.

In this study, the mammalian pol inhibitory activity and other biological activities of 20 kinds of EOs isolated from herbal plants and citrus fruits were examined. As chamomile EO was found to be the strongest pol inhibitor, the major chemical components of this EO were evaluated by gas chromatography−mass spectrometry (GC−MS). These results should contribute in recognizing future, novel applications of EOs from these plants.

MATERIALS AND METHODS

Materials. The 20 EOs were commercially available and donated by Nagaoka Perfumery Co., Ltd. (Osaka, Japan). The 17 species of hydrodistilled EOs from herbal plants were basil (*Ocimum basilicum* L., produced in Union of the Comoros), caraway (*Carum carvi* L., Europe), carrot seed (*Daucus carota* L., France), celery seed (*Apium graveolens* L., India), chamomile (*Matricaria chamomilla* L., Egypt), citronella (*Cymbopogon winterianus* Jowitt, Indonesia), clary sage (*Salvia sclarea* L., USA), clove (*Syzygium aromaticum* L., Madagascar), cumin (*Cuminum cyminum* L., India), eucalyptus (*Eucalyptus globulus* L., China), lemongrass (*Cymbopogon citratus* (DC.) Stapf, India), marjoram (*Majorana hortensis* Moench., France), nutmeg (*Myristica fragrans* Houtt., Indonesia), sage (*Salvia officinalis* L., Albania), sandalwood (*Santalum album* L., India), spearmint (*Mentha spicata* L., USA), and thyme (*Thymus vulgaris* L., India). Three species of cold-pressed EOs from citrus fruits were lemon (*Citrus limon* L., USA), lime (*Citrus aurantifolia* Swingle, Mexico), and orange (*Citrus sinensis* L., Portugal). Five sesquiterpenes, α-bisabolol oxide A, (*E*)-β-farnesene, chamazulene, α-bisabolol, and β-bisabolene, were purchased from Sigma-Aldrich, Inc. (St. Louis, MO,).

A chemically synthesized DNA template, poly(dA), was purchased from Sigma-Aldrich, Inc. and a customized oligo(dT)$_{18}$ DNA primer was produced by Sigma-Aldrich Japan K.K. (Hokkaido, Japan). Radioactive nucleotide [^{3}H]-labeled 2′-deoxythymidine-5′-triphosphate (dTTP; 43 Ci/mmol) was obtained from Moravek Biochemicals, Inc. (Brea, CA). All other reagents were analytical grade from Nacalai Tesque, Inc. (Kyoto, Japan).

Cells. Three cultured cell lines, human colon carcinoma HCT116, rat basophilic leukemia RBL-2H3, and murine macrophage RAW264.7, were obtained from the American Type Culture Collection (Manassas, VA). HCT116 cells were cultured in McCoy's 5A Medium supplemented with 10% fetal bovine serum (FBS, by vol), 100 units/mL penicillin, and 100 μg/mL streptomycin. RBL-2H3 cells were cultured in Eagle's minimum essential medium supplemented with 4.5 g glucose/L plus 10% FBS, 5 mM L-glutamine, 50 units/mL penicillin, and 50 units/mL streptomycin. RAW264.7 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 4.5 g glucose/L plus 10% FBS, 5 mM L-glutamine, 50 units/mL penicillin, and 50 units/mL streptomycin. All cells were cultured at 37 °C in standard medium in a humidified atmosphere of 5% CO_2/95% air.

Animals. Female 6-week-old ICR mice (25−27 g body wt) were obtained from Japan SLC, Inc. (Hamamatsu, Japan) and maintained on a standard diet (MF; Oriental Yeast Co., Ltd., Osaka, Japan) and provided water ad libitum. All animal studies were approved by the Kobe Gakuin University Animal Committee according to the guidelines for the "Care and Use of Laboratory Animals" of the University.

Measurement of Pol Inhibitory Activity. Pol α was purified from calf thymus by immunoaffinity column chromatography, as described by Tamai et al.[18] Recombinant human His-pol λ was overexpressed and purified according to a method described by Shimazaki et al.[19] The reaction mixtures for these pols have been described previously.[20−22] For pol reactions, poly(dA)/oligo(dT)$_{18}$ (A/T, 2/1) and dTTP were used as the DNA template−primer substrate and nucleotide (dNTP; 2′-deoxynucleotide-5′-triphosphate) substrate, respectively. EOs were dissolved in distilled dimethyl sulfoxide (DMSO) to various concentrations and sonicated for 30 s. Next, 4 μL aliquots of EO solution were mixed with 16 μL of each enzyme (0.05 units) in 50 mM, pH 7.5 Tris-HCl, containing 1 mM dithiothreitol, 50% glycerol (by vol), and 0.1 mM ethylenediaminetetraacetic acid, and held at 0 °C for 10 min. These inhibitor-enzyme mixtures in 8 μL aliquots were next added to 16 μL of enzyme standard reaction mixture and incubated at 37 °C for 60 min. Activity without inhibitor or enzyme was considered 0 or 100% inhibition activity, respectively, and the pol inhibitory activity was determined for each EO concentration. One unit of pol activity was defined as the amount of each enzyme that catalyzed incorporation of 1 nmol dTTP into synthetic DNA template−primers in 60 min, at 37 °C, and under normal reaction conditions.[20,21]

Measurement of Cancer Cell Growth Inhibitory Activity. For the HCT116 cell viability assay, cells were plated at 1×10^4 cells/well in a 96-well microplate and then individual EOs added to wells to a final concentration of 100 μg/mL in 0.5% DMSO. After incubation for 24 h, the cell survival rate was determined using a cell proliferation WST-1 assay.[23] Inhibition activity without inhibitor or HCT116 cells was considered 0 or 100%, respectively, and cancer cell growth inhibitory activity was determined for each EO concentration.

Measurement of Anti-β-hexosaminidase Release Activity. It has been reported that the release of β-hexosaminidase correlates well with histamine release, a major component of mast cell granules,[24] so that degranulation of mast cell was determined using a β-hexosaminidase release assay as described previously,[25] with slight modifications. Briefly, RBL-2H3 cells at 8×10^4 cells/well in 24-well plates were washed with Tyrode's buffer (in mM, 137 NaCl, 5.6 glucose, 11.9 $NaHCO_3$, 2.7 KCl, and 0.32 NaH_2PO_4) containing 1 mM $CaCl_2$ and 0.5 mM $MgCl_2$, and then each EO added individually to a final concentration of 100 μg/mL in 0.5% DMSO. The cells were then stimulated with 5 μM A23187 and incubated for 30 min. The cell supernatant and total cell lysate dissolved in 2% Triton X-100 were collected and mixed with substrate solution (2 mM *p*-nitrophenyl-*N*-acetyl-β-D-glucosaminide in 0.1 M sodium citrate buffer, pH 4.5). The mixture was incubated for 90 min at 37 °C and then the reaction terminated by adding stopping buffer composed of 0.2 M glycine buffer at pH 11.0. The 405 nm absorbance was measured with a microplate reader (Vmax-K; Molecular Devices, LLC, Sunnyvale, CA). The EO-mediated anti-β-hexosaminidase release activity was expressed as the percentage inhibition, calculated using the following formula: % activity = [(β-hexosaminidase release without EO − β-hexosaminidase release with EO)/β-hexosaminidase release without EO] × 100.

Measurement of Antioxidant Activity. The modified lipophilic-oxygen radical absorbance capacity (L-ORAC) method was used to study the EOs' antioxidant capacities.[26,27] The EOs and Trolox standards were dissolved in 7% randomly methylated β-cyclodextrin (RMCD) solvent (50/50 acetone/water, v/v), and other reagents were prepared in 75 mM, pH 7.4 phosphate buffer. In the final assay mixture, fluorescein (FL, 6.3×10^{-8} M) was used as a target for free radical attack and 2,2′-azobis(2-amidino-propane) dihydrochloride (AAPH, 1.28×10^{-2} M) as a peroxyl radical generator. Seven percent RMCD was used as the blank and Trolox (12.5, 25, 50, and 100 μM) was used as the positive control standards. A fluorescence microplate reader (SH-9000 Lab; Hitachi High-Technologies Corp., Tokyo, Japan) was used to measure the FL fluorescence for 90 min after AAPH addition and all measurements expressed relative to the initial reading. Final results were calculated using the FL decay-curve area differences between the blank and a sample and all data expressed as moles of Trolox equivalent per gram (mol of TE/g).

GC−MS Analysis. GC−MS was performed with an Agilent 6890 gas chromatograph coupled with an Agilent 5972A MSD mass spectrometer (Agilent Technologies Japan, Ltd., Tokyo, Japan). The chromatograph was fitted with an InertCap WAX (polyethylene glycol) fused silica capillary column (60 m × 0.25 mm i.d., film thickness 0.25 μm; GL Sciences, Inc., Tokyo, Japan), with purified helium (≥99.99995%) carrier gas at 149 kPa inlet pressure, in split/splitless mode, and at 1.2 mL/min. The oven temperature was programmed for 5 min at 70 °C and then 3 °C/min to 240 °C, with

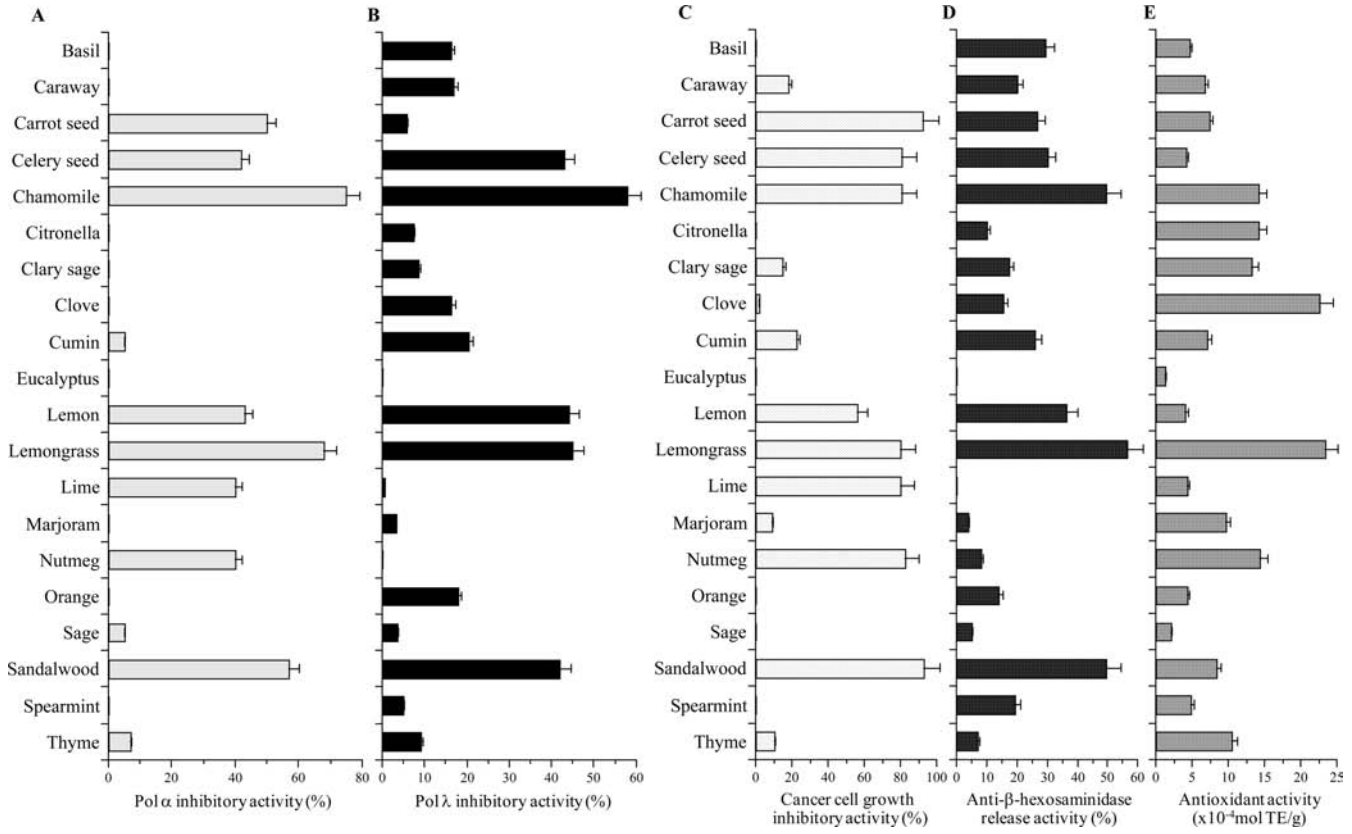

Figure 1. Biological activity of EOs isolated from 20 plant species. (A) Calf pol α inhibitory activity; (B) human pol λ inhibitory activity; (C) cancer cell (HCT116) growth inhibitory activity by WST-1 assay; (D) anti-β-hexosaminidase release activity in RBL-2H3 cells treated with A23187; and (E) antioxidant activity by L-ORAC assay; EOs, 100 μg/mL; A, B, and E, data, mean ± SD (n = 3); and C and D, data, mean ± SD (n = 5).

both the injector and transfer line held at 240 °C. The mass spectrometer operated at 70 eV electron impact, with an ion source temperature of 150 °C, and data collected in full scan mode over a mass scan range was m/z 27−400. Each 0.6 μL sample was injected in a split ratio of 1:60. Qualitative analysis was performed by similarity searches of private and commercial mass spectral databases (Wiley 275 and the National Institute of Standards and Technology 02).

Measurement of Anti-Tumor Necrosis Factor-α (TNF-α) Expression Activity. RAW264.7 cells were placed in a 12-well plate at 5×10^4 cells/well and incubated for 24 h. The cells were then pretreated with a test compound, at a final concentration of 100 μg/mL in 0.5% DMSO, for 30 min before the addition of 100 ng/mL of lipopolysaccharide (LPS), a major component of Gram-negative bacteria outer membranes. After LPS stimulation for 24 h, the cell culture medium was collected to measure the culture medium concentration of secreted TNF-α, quantified using a commercially available enzyme-linked immunosorbent assay (ELISA) development system (Bay Bioscience Co., Ltd., Kobe, Japan) in accordance with the manufacturer's protocol.

Measurement of Antianaphylactic Activity. The passive cutaneous anaphylaxis (PCA) reaction was measured according to a previous report.[25] Mice were sensitized by intradermal injection of 0.1 μg of antidinitrophenyl (DNP) immunoglobulin E (IgE) in the ear and 4 h later intravenously challenged with 0.2 mL (1 mg/mL) of DNP-labeled human serum albumin containing 2% Evans blue dye. In a series of experiments, a test compound (100 mg/kg) or saline was administered orally 2 h before the antigen challenge, with saline used in the control group. The mice were subsequently sacrificed and the ears removed and weighed 30 min after the challenge. After dissolution of the ears in 200 μL of 1 N KOH, they were incubated overnight at 37 °C for measurement of the amount of Evans blue dye present in the exudates. For this, the dissolved tissue solution was added to 400 μL of a mixture of acetone and 0.6 N phosphoric acid (5/13, v/v) and the 620 nm optical density was measured. The amount of dye in the exudates was calculated from an Evans blue standard curve and the results expressed as a percentage of the mean exudate dye amount from the treated test mice compared with controls.

Measurement of Anti-Inflammatory Activity. The mouse inflammatory test was performed according to Gschwendt's method.[28] In brief, an acetone solution containing a test compound at 500 μg/20 μL or a vehicle control of 20 μL of acetone was applied to the inner part of a mouse ear and, 30 min later, an acetone solution of 12-*O*-tetradecanoylphorbol-13-acetate (TPA, a chemical edema inducer) at 0.5 μg/20 μL was applied to the same part of the ear; acetone, followed by a TPA application, served as a control. After 7 h, a 6 mm diameter disk was obtained from the ear and weighed. Anti-inflammatory activity was determined as the percentage of prevented increase in ear disk weight compared to the controls, using: percentage activity = [(TPA only) − (tested compound plus TPA)]/[(TPA only) − (vehicle)] × 100.

RESULTS

Effect of EOs on the Activities of Mammalian Pols α and λ. First, the inhibitory activities of 20 species of EOs toward mammalian pols was investigated, using pol α and pol λ as a representative DNA replicative pol and DNA repair/recombination-related pol, respectively.[4,5] The inhibitory activity of calf pol α and human pol λ against 100 μg/mL of each EO is shown in Figure 1A and B, respectively. Among the 20 EOs tested, chamomile EO showed the strongest inhibition of both pols α and λ, with inhibitory activities of 75.0 and 57.8%, respectively. The inhibitory effects of these EOs on pol α were ranked as follows: EOs from chamomile > lemongrass > sandalwood > carrot seed > lemon, with no inhibition observed by EOs from basil, caraway, citronella, clary sage, clove, eucalyptus, marjoram, orange, and spearmint. The inhibition

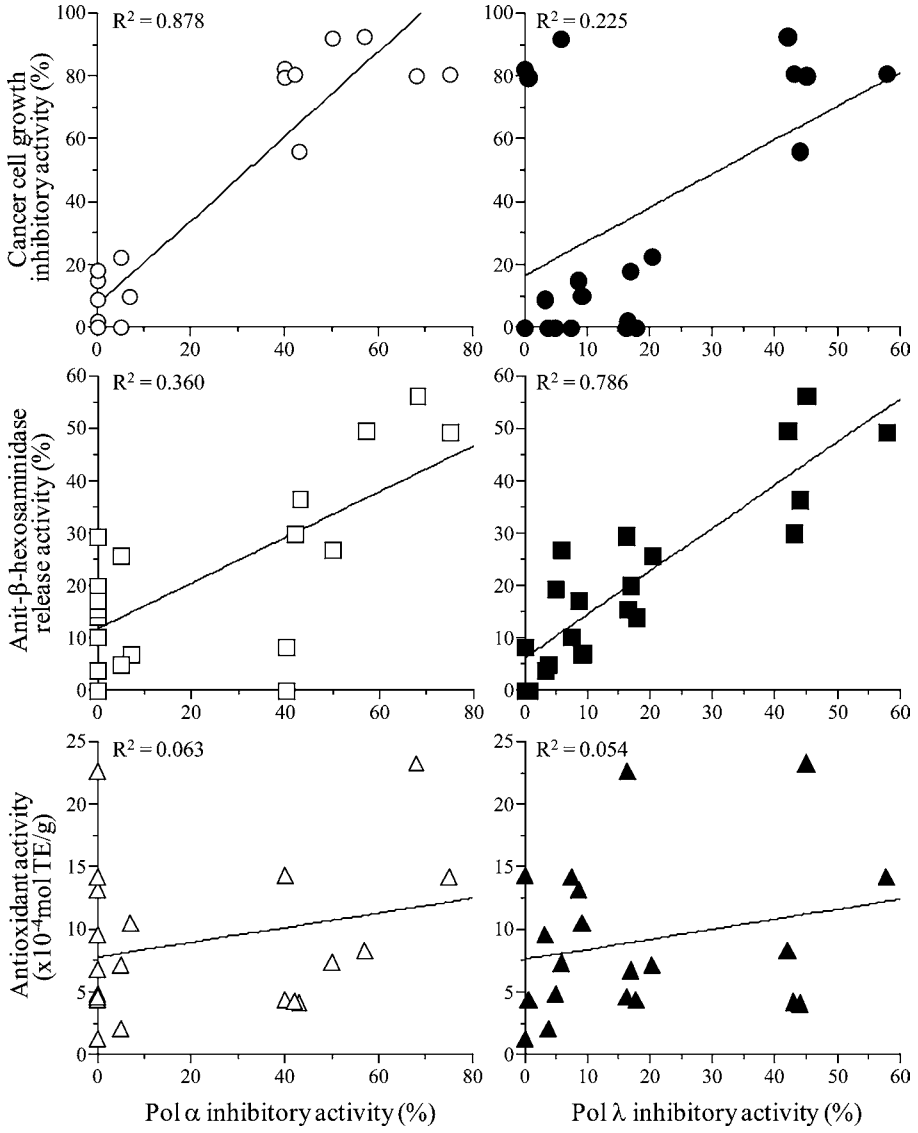

Figure 2. The relationship between mammalian pols α and λ inhibitory activities versus cancer cell growth inhibition, anti-β-hexosaminidase release, and antioxidant activities by 100 μg/mL EOs isolated from 20 plants species. Left and right panels, inhibitory activity of calf pol α (white) and human pol λ (black), respectively; upper, middle, and lower panels, cancer cell growth inhibitory activity (circles), anti-β-hexosaminidase release activity (squares), and antioxidant activity (triangles), respectively; data based on Figure 1; and correlation coefficient values shown in each panel.

activities against pol λ produced a ranking of: EOs from chamomile > lemongrass > lemon > celery seed > sandalwood, with no effects by EOs from eucalyptus and nutmeg. These results indicated that the inhibitory effect on pol α showed a different pattern from that on pol λ. When DNA, activated via calf thymus DNA digestion by bovine deoxyribonuclease I, and dNTP were used as the DNA template−primer substrate and nucleotide substrate, respectively, instead of synthesized DNA [poly(dA)/oligo(dT)$_{18}$ (A/T = 2/1)] and dTTP, respectively, the inhibitory effects of these EOs did not change (data not shown). These results suggested that the pol inhibitory activity using native DNA exhibited the same tendency as that using synthesized DNA.

Effects of EOs on Cultured Human Cancer Cell Growth. Pols, especially DNA replicative pols, have recently emerged as important cellular targets for chemical intervention in the development of anticancer agents. As EOs could therefore be useful in chemotherapy, the cytotoxic effect of these EOs at 100 μg/mL against HCT116 human colon carcinoma cultured cell line was investigated. It was found that EOs from carrot seed, celery seed, chamomile, lemongrass, lime, nutmeg, and sandalwood suppressed HCT116 cell growth at >80% (Figure 1C). In contrast, 6 EOs, from basil, citronella, eucalyptus, orange, sage, and spearmint, had no effect on the growth of this cancer cell type.

Effects of EOs on the Inhibition of Mast Cell Degranulation. The value for released β-hexosaminidase has frequently been used as an indicator for evaluating the extent of degranulation by mast cells. The release of histamine and other chemical mediators from mast cells is an important process in initiating the immediate type of anaphylactic reaction. Thus, the effect of EOs on a chemical mediator, β-hexosaminidase, release was investigated in rat basophilic leukemia RBL-2H3 cells treated with calcium ionophore A23187. The results confirmed that these EOs did not influence the cell growth of RBL-2H3 and also did not inhibit β-hexosaminidase enzyme activity (data not shown). The degree of degranulation was calculated from the β-hexosaminidase activity in the supernatant and cell lysate.

EOs from chamomile, lemongrass, and sandalwood at 100 μg/mL exhibited >40% inhibition of mast cell degranulation related to anti-β-hexosaminidase release, while EOs from eucalyptus and lime did not influence release activity (Figure 1D). As A23187 induces calcium ion flux and protein kinase C (PKC) activities, the latter being essential for mast cell degranulation,[29] the EOs having anti-β-hexosaminidase release activity may have affected PKC activities. Further studies are necessary to explain the inhibitory mechanism of these EOs for mast cell degranulation.

Effects of EOs on Antioxidant Activity. Free radicals leading to oxidation of biomolecules have been implicated in several diseases.[30] Here, the antioxidant activity of these EOs was evaluated using a L-ORAC assay, with Trolox as the positive standard, each EO at 100 μg/mL, and assessed as mol of TE/g (Figure 1E). Among the EOs tested, those from clove and lemongrass showed the strongest antioxidant activities (>0.02 mol TE/g) and those from chamomile, citronella, clary sage, nutmeg, and thyme the second strongest activities (0.01−0.02 mol TE/g). No EOs entirely lacked antioxidant activity (0.00 mol TE/g).

Relationship of Mammalian Pol Inhibitory Activity and Other Biological Activities by EOs. The possible relationship between the observed mammalian pols α and λ inhibitions and cancer cell growth inhibitory activity, anti-β-hexosaminidase release activity or antioxidant activity was confirmed by comparing the effects of the 20 EOs on these biological activities (Figure 2). Pol α inhibitory activities showed a higher correlation with cancer cell growth inhibitory activity than pol λ inhibitory activities, with the highest correlation coefficient (0.878) of all combinations of biological activities. In contrast, pol λ inhibition showed a higher correlation (0.786) with β-hexosaminidase release inhibition than did pol α inhibition. Conversely, neither pol inhibition was related to antioxidation, with correlation coefficients between these activities and antioxidation at <0.1. These results led to the speculation that human cancer cell growth and mast cell degranulation related to β-hexosaminidase release activities might have been caused by mammalian pols α and λ activities, respectively.

In particular here, chamomile EO showed the strongest inhibition of pols α and λ in the 20 EOs tested. This EO also exhibited significant effects on both cancer cell growth inhibition and anti-β-hexosaminidase release. Therefore, further experimentation focused on chamomile EO.

Inhibitory Effects of Identified Chamomile EO Components on Mammalian Pol α and λ Activities. The relationship between chamomile EO biological activities and EO composition was clarified by analysis of the primary EO components by GC−MS. As a result, 222 compounds were detected and 83 components identified (Table 1). The major compounds representing >5% of the mixture were α-bisabolol oxide A, (*E*)-β-farnesene, α-bisabolol oxide B, and bisabolone oxide (37.9, 20.8, 6.47, and 5.78%, respectively). Of the identified components, 5 compounds, α-bisabolol oxide A (**1**), (*E*)-β-farnesene (**2**), chamazulene (**3**), α-bisabolol (**4**), and β-bisabolene (**5**), were commercially purchasable as reagents and possessed sesquiterpene structures of low molecular weight (Figure 3).

On the basis of the above results, the inhibitory effects of five compounds (**1**−**5**) on calf pol α and human pol λ were investigated by determining the 50% inhibitory concentration (IC_{50}) values of these compounds against these mammalian pols (Table 2). Only compound **4** inhibited DNA replicative pol α activity, while the other four compounds showed no effects. Conversely, the inhibitory effects of these compounds on DNA repair/recombination related pol λ allowed ranking of their effects as: **1** > **4** > **3**, with the remaining compounds showing no effect. These results suggested that multiple component effects might largely produce the concurrent inhibitory activities on pols α and λ by chamomile EO, such as by **4** and **1**, respectively.

Table 1. Chemical Composition from Chamomile EO

components	composition (%)
α-Bisabolol oxide A (**1**)	37.9
(*E*)-β-Farnesene (**2**)	20.8
α-Bisabolol oxide B	6.47
Bisabolone oxide	5.78
Chamazulene (**3**)	2.70
α-Bisabolol (**4**)	1.93
Germacrene D	1.58
Palmitic acid	1.55
Bicyclogermacrene	1.09
Decanoic acid	1.01
Pentacosane	1.00
α-Farnesene	0.98
Spathulenol	0.81
epi-α-Cadinol	0.75
β-Bisabolene (**5**)	0.13
Others	15.5

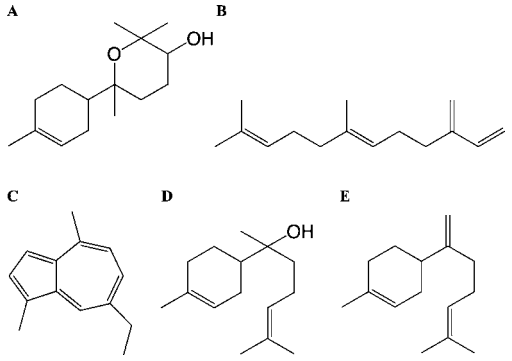

Figure 3. Structure of chamomile EO major components. (A) α-bisabolol oxide A (**1**); (B) (*E*)-β-farnesene (**2**); (C) chamazulene (**3**); (D) α-bisabolol (**4**); and (E) β-bisabolene (**5**).

Table 2. IC_{50} Values of Major Chamomile EO Components against Mammalian Pols α and λ Activities[a]

components	IC_{50} values (μM)	
	pol α	pol λ
α-Bisabolol oxide A (**1**)	>200	34 ± 2.0
(*E*)-β-Farnesene (**2**)	>200	>200
Chamazulene (**3**)	>200	84 ± 5.0
α-Bisabolol (**4**)	81 ± 4.8	77 ± 4.6
β-Bisabolene (**5**)	>200	>200

[a]Chamomile components with each pol (0.05 units); 1 unit of pol activity, amount of enzyme catalyzing incorporation of 1 nmol of dNTP into DNA template−primers in 60 min at 37 °C under enzyme's normal reaction conditions; enzyme activities in absence of inhibitor and pol taken as 0 and 100% inhibition, respectively; and data, mean ± SD (n = 3).

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Effects of α-Bisabolol Oxide A (1) on Biological Activities. As α-bisabolol oxide A (**1**), chamomile EO's major component, showed the strongest pol λ inhibition of the sesquiterpenes tested, its biological activities were further examined (Table 3). This compound did not influence cell growth of HCT116 human colon carcinoma cells, suggesting that it exerted no inhibition of pol α, a DNA replicative pol. While the L-ORAC assay did not detect antioxidant activity by **1**, this compound inhibited mast cell degranulation related to the release of β-hexosaminidase in RBL-2H3 rat basophilic leukemia cells treated with calcium ionophore A23187, suppressed the protein expression of TNF-α, a pleiotropic inflammatory cytokine, in RAW264.7 murine macrophage cells treated with LPS, inhibited the IgE-induced PCA reaction in mouse in vivo, and suppressed the TPA-induced inflammatory edema of mouse ear in vivo. These antiallergic/anti-inflammatory activities by **1** might have been caused by pol λ inhibition, and these **1**-caused biologically active effects were produced by nearly the same concentrations as by EO from chamomile (Figure 1 and Tables 2 and 3), suggesting that the antiallergy and anti-inflammatory effects of chamomile EO should be concluded to be largely generated by the major component, compound **1**.

Table 3. Biological Activities of α-Bisabolol Oxide A[a]

biological activity	result	decision
(1) Cancer cell (HCT116) growth inhibitory activity	0%	None
(WST-1 assay)	at 100 μM	
(2) Antioxidant activity	0 mol TE/g	None
(L-ORAC method)	at 100 μM	
(3) Anti-β-hexosaminidase release activity	52.2 ± 5.3%	Existence
in cultured RBL-2H3 cells treated with A23187	at 100 μM	
(4) Anti-TNF-α expression activity	58.0 ± 5.5%	Existence
in cultured RAW264.7 cells treated with LPS	at 100 μM	
(5) Antianaphylactic activity	50.5 ± 7.4%	Existence
using IgE-induced mouse (PCA reaction)	at 400 mg/kg	
(6) Anti-inflammatory activity	64.6 ± 8.4%	Existence
using TPA-induced mouse ear	at 500 μg/ear	

[a]Details in Materials and Methods; 1, 3, 4, 5, and 6, data, mean ± SD (n = 5); and 2, data, mean ± SD (n = 3).

DISCUSSION

In recent years, the measurement and utilization of EOs has become increasingly important in scientific research and industrial applications, including pharmaceutical, nutritional, and cosmetic uses,[31,32] primarily because of EOs' various potent biological activities, including antimicrobial,[33] antioxidant,[34] anticancer,[35] and anti-inflammatory activities.[36] The infection process frequently induces inflammation, in which inflammatory mediators, such as cytokines, are released from phagocytes during the inflammatory process.[37] Because various skin disorders, including atopic dermatitis[38] and acne vulgaris,[39] are associated with infection-stimulated inflammation, the presence of anti-inflammatory agents might explain the effectiveness of some plant EOs in the treatment of these syndromes. In many regions of the world, aromatic herbs still play major roles in primary health care, particularly in rural areas.[40] Thus, an understanding of herbal biological activities can allow the understanding and use of the many functional components, particularly as beneficial additives for medical, nutritional, and cosmetic products.

In this study, 20 EOs were selected from among the herbal plants and citrus fruits, representing the most famous and most used materials for perfumes among the world's plant EOs. These EOs were isolated by hydrodistillation or cold-pressing and were confirmed free of any contamination, such as heavy metals and pesticides by instrumental analyses, such as GC/MS (data not shown). Among the 20 EOs, chamomile EO exhibited the strongest inhibitory activity on mammalian pols α and λ (Figure 1). From subsequent experiments, α-bisabolol oxide A (**1**), comprising 37.9% of chamomile EO (Table 1), appeared to have penetrated macrophage cells, reached the nucleus, inhibited pol λ activity, and specifically caused pol λ inhibition, resulting in antiallergic/anti-inflammatory activity (Table 3). Compound **1** and α-bisabolol (**4**) possessed the first and second strongest pol λ inhibition among the chamomile EO components tested (Table 2). As these compounds have a hydroxyl group in the bisabolol backbone (Figure 3), this group might be important for the observed inhibitory activity.

Chamomile is a herbal plant that has been used for centuries in many human cultures to treat various inflammatory conditions, such as eczema, ulcers, gout, neuralgia, and rheumatic pains.[41,42] Dried flowers of *M. chamomilla* L. are used in tea preparations, which are consumed at a rate of more than a million cups per day.[43] Chamomile has been approved by the German Commission E for oral consumption in the management of various inflammatory gastrointestinal tract diseases and for topical applications in the treatment of various skin and inflammatory disorders of certain mucosal surfaces, such as the oral cavity and anogenital areas.[44] Recent studies have demonstrated its antioxidant, hypocholesterolemic, antiparasitic, antiaging, and anticancer properties, supporting its longstanding traditional use for treating various human ailments.[45−47] The relationship between the above-mentioned biological activities and mammalian pol inhibition will be stringently investigated in future studies.

In conclusion, chamomile EO containing α-bisabolol oxide A (**1**) could be recommended on a scientific basis for medical and/or cosmetic uses. In the present results, a positive relationship was found between the inhibition of mast cell degranulation, related to anti-β-hexosaminidase release, and pol λ inhibitory activities that might be useful as a new and convenient in vitro assay in screening for novel antiallergic and/or anti-inflammatory compounds.

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Notes

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■ ABBREVIATIONS USED

EO, essential oil; Pol, DNA polymerase (E.C.2.7.7.7); TdT, deoxynucleotidyl transferase; GC-MS, gas chromatography−mass spectrometry; dTTP, 2′-deoxythymidine-5′-triphosphate; FBS, fetal bovine serum; dNTP, 2′-deoxynucleotide-5′-triphosphate; DMSO, dimethyl sulfoxide; L-ORAC, lipophilic-oxygen radical absorbance capacity; RMCD, randomly methylated β-cyclodextrin; FL, fluorescein; AAPH, 2,2′-azobis-(2-amidino-propane) dihydrochloride; TE, Trolox equivalent; TNF-α, Necrosis Factor-α; LPS, lipopolysaccharide; ELISA, enzyme-linked immunosorbent assay; PCA, passive cutaneous anaphylaxis; DNP, dinitrophenyl; IgE, immunoglobulin E, TPA, 12-*O*-tetradecanoylphorbol-13-acetate; PKC, protein kinase C

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