Suppression of allergic and inflammatory responses by essential oils derived from herbal plants and citrus fruits

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Abstract. The aim of the present study was to investigate the biological activity of 20 essential oils (EOs) derived from herbal plants and citrus fruits. The in vitro anti-allergic and anti-inflammatory activities of these oils were investigated, and the EOs which was found to have the strongest activity of the 20 EOs examined, was investigated further to identify its components and bioactive compounds. The in vitro anti-allergic activity was determined by measuring the production of the calcium ionophore, A23187. The in vitro anti-inflammatory activity was determined by measuring the production of tumor necrosis factor-α (TNF-α) in RAW264.7 murine macrophages treated with lipopolysaccharide. Among the EOs examined, lemongrass [Cymbopogon citratus (DC.) Stapf] elicited the strongest anti-allergic and anti-inflammatory effects. A principal component of this EO is citral (3,7-dimethyl-2,6-octadien-1-al) (74.5%), a mixture of the stereoisomers, geranial (34.24%), as determined by gas chromatography-mass spectrometry analysis. The activities of citral and geranial are similar to those of lemongrass EO. These compounds elicited significant in vivo anti-allergic and anti-inflammatory effects, suppressing an immunoglobulin E (IgE)-induced passive cutaneous anaphylactic reaction in mice and a 12-O-tetradecanoylphorbol-13-acetate-induced inflammatory mouse ear edema, respectively. Our data demonstrate that lemongrass EO and its constituents, citral and geranial, may be a therapeutic candidate for allergic and inflammatory diseases.

Introduction

Essential oils (EOs) are natural volatile complex compounds that are characterized by a strong scent and produced by aromatic plants as secondary metabolites (1). EOs have largely been employed for their well-known natural antitumor, anti-inflammatory, antioxidant and anti-bacterial properties (2). Currently, approximately 3,000 EOs have been identified, 300 of which are commercially important, particularly in the pharmaceutical, agronomic, food, sanitary, cosmetic and perfume industries. EOs and some of their components are used in the perfume and cosmetic industry, as well as in the manufacture of sanitary products, dentistry and agriculture; they have also been employed as food preservatives, additives and natural remedies (3,4). EOs are also used in massages as mixtures with vegetable oil, or in baths. Their most frequent use is in aromatherapy (1). Some EOs may have particular medicinal properties that are thought to cure certain organ dysfunctions or systemic disorders. EOs have long been used as the components of fragrances. Over past few years, there is a renewed interest in understanding their biological properties due to their potential novel applications in human health, agriculture and the environment.

The prevalence of allergic diseases, such as allergic rhinitis, atopic dermatitis, asthma and food allergies has increased in the majority of countries (5). Immunologically active mast cells and basophils express the high-affinity receptor for immunoglobulin E (IgE) on their surface and play critical roles in various
biological processes associated with allergic diseases (6). The interaction of multivalent antigens with surface-bound IgE triggers the secretion of the mediators stored in cytoplasmic granules and causes a de novo synthesis of cytokines (7), that in turn activate the migration of neutrophils and macrophages, causing tissue inflammation (8).

Inflammation is an initial host immune reaction mediated by inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) (9). Macrophages play an important role in the host defense against noxious substances (10). Macrophage activation by lipopolysaccharide (LPS), a major component of Gram-negative bacteria outer membranes, leads to the increased production of pro-inflammatory cytokines, thereby mediating the major cytotoxic and pro-apoptotic mechanisms that participate in the innate response in many mammals (11). However, the cytokine overproduction by activated macrophages has been implicated in the pathophysiology of several inflammatory diseases, including rheumatoid arthritis, atherosclerosis, chronic hepatitis, pulmonary fibrosis and inflammatory brain diseases (11). Therefore, LPS-stimulated macrophages serve as a useful model for studying inflammation and the potential mechanisms of action of anti-inflammatory compounds.

In this study, the anti-allergic and anti-inflammatory properties of 20 different types of EOs isolated from herbal plants and citrus fruits were examined. Lemongrass EO exhibited the strongest anti-allergic and anti-inflammatory properties and therefore, the major chemical constituents of this EO were evaluated by gas chromatography (GC)-mass spectrometry (MS). These results are of great significance in recognizing the novel future applications of EOs derived from these plants.

Materials and methods

Materials. The 20 commercially available EOs were donated by Nagaoka Perfumery Co., Ltd. (Osaka, Japan). The 17 types of hydrodistilled EOs used in the study were from herbal plants, including basil (Ocimum basilicum L., produced in the 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 (Thymbus vulgaris L., India). The cold-pressed EOs were derived from 3 species of citrus fruits: lemon (Citrus limon L., USA), lime (C. aurantifolia Swingle, Mexico) and orange (C. sinensis L., Portugal). Five terpenoids, citral (3,7-dimethyl-2,6-octadien-1-ol), geraniol, geranyl acetate, linalool and camphene were purchased from Sigma-Aldrich, Inc. (St. Louis, MO, USA). Tranilast and glycyrrhetinic acid, anti-allergic and anti-inflammatory controls, respectively, were also obtained from Sigma-Aldrich, Inc. Geraniol was prepared by the oxidation of geraniol with manganese dioxide, as previously described (12). For western blot analyses, anti-nuclear factor-κB (NF-κB) p65 and anti-inhibitor of NF-κB (IκB)-α antibodies were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA). Horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (IgG) antibody was obtained from Thermo Scientific (Kanagawa, Japan). All other reagents were of analytical grade and were obtained from Nacalai Tesque, Inc. (Kyoto, Japan).

Cells. RBL-2H3 rat basophilic leukemia and RAW264.7 murine macrophage cell lines were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA). The RBL-2H3 cells were cultured in Eagle's minimum essential medium supplemented with 4.5 g glucose/l plus 10% fetal bovine serum (FBS), 5 mM L-glutamine, 50 U/ml penicillin and 50 U/ml streptomycin. The 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 U/ml penicillin and 50 U/ml streptomycin. All cells were cultured at 37°C in their respective media in a humidified atmosphere of 5% CO2/95% air.

Measurement of β-hexosaminidase release. Mast cell degranulation-mediated histamine release is accompanied by the release of β-hexosaminidase (13). Therefore, we performed a β-hexosaminidase release assay, as previously described (14) with slight modifications to determine the degranulation of mast cells as a measure of the anti-allergenic activity of the examined compounds. Briefly, the RBL-2H3 cells at 8x10⁴ cells/well in 24-well plates were washed with Tyrode's buffer (137 mM NaCl, 5.6 mM glucose, 11.9 mM NaHCO₃, 2.7 mM KCl and 0.32 mM NaH₂PO₄) containing 1 mM CaCl₂ and 0.5 mM MgCl₂, and the EOs (in 0.5% DMSO) were then added to the culture wells at a final concentration of 100 µg/ml. 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-β-D-glucosaminide in 0.1 M sodium citrate buffer, pH 4.5). The mixture was incubated for 90 min at 37°C and the reaction was then terminated by the addition of a stopping buffer containing a 0.2 M glycine buffer at pH 11.0. Absorbance at 405 nm was measured using a microplate reader (Vmax-K; Molecular Devices, LLC, Sunnyvale, CA, USA). The EO-mediated inhibition of β-hexosaminidase release and activity was expressed as the percentage inhibition and calculated using the following formula: % activity = [(β-hexosaminidase release in the absence of EO - β-hexosaminidase release in the presence of EO)/β-hexosaminidase release in the absence of EO] x 100.

Measurement of the EO-mediated inhibition of TNF-α. The RAW264.7 cells were placed in a 12-well plate at 5x10⁴ cells/well and incubated for 24 h. The cells were then pre-treated with each EO at a final concentration of 100 µg/ml in 0.5% DMSO for 30 min prior to the addition of LPS (100 ng/ml). Following LPS stimulation for 24 h, the cell culture medium was collected to quantify the secreted TNF-α using a commercially available enzyme-linked immunosorbent assay (ELISA) development system (Bay Bioscience Co., Ltd., Kobe, Japan), in accordance with the manufacturer's instructions. The EO-mediated inhibition of TNF-α production was expressed as the percentage inhibition, calculated using the following formula: % activity = [TNF-α production in the absence of EO - TNF-α production in the absence of EO] x 100.
production in the presence of EO)/TNF-α production in the absence of EO) x100.

GC-MS. GC-MS was performed using 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 x 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 as follows: 5 min at 70°C and then 3°C increments/min to 240°C, with both the injector and transfer line held at 240°C. The mass spectrometer operated at a 70 eV electron impact, with an ion source temperature of 150°C, and the data collected in full scan mode over a mass scan range revealed an m/z of 27-400. Each 0.6 µl sample was injected in a split ratio of 1:60. A 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).

Cell treatment and preparation of nuclear and whole cellular lysates and western blot analysis. The RAW264.7 cells were pre-treated with 10 µg/ml of a test compound for 30 min followed by treatment with 100 ng/ml LPS. After 30 min, the nuclear proteins and whole cell lysates were collected, using the methods described in the study by Nishiumi et al (15). The protein concentrations of the nuclear proteins and whole cell lysates were measured using the BCA Protein Assay kit (Pierce, Rockford, IL, USA), according to the manufacturer’s instructions. Briefly, 25 µl of each sample or 2 mg/ml BSA solution (as a standard) were added to a 96-well microplate well, and then 200 µl of BCA Working Reagent were added to each well. The microplate was incubated at 37°C for 30 min, and the protein concentration was determined by measuring the absorbance at 575 nm. The nuclear proteins and whole cell lysates were subjected to western blot analysis to evaluate the nuclear translocation of NF-κB and the protein expression of IκB-α.

The nuclear proteins (30-50 µg of protein/superscript p65)/superscript p65) and the protein expression of IκB-α were quantified using an image analysis software (NIH Image). The protein expression ratio of the test compound-treated samples compared with that of the control group (i.e., the cells incubated in the absence of the test compound).

Measurement of anti-inflammatory activity. The mouse inflammatory test was performed according to the method described in the study by Gschwendt et al (16). Briefly, 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 section of a mouse ear and, 30 min later, an acetone solution containing a test compound at 500 µg/20 µl of a DNP-labeled human serum albumin solution was infiltrated into the ears of mice compared with that of the controls.

Results

EO-induced inhibition of β-hexosaminidase release in mast cells. First, the inhibitory effects of 20 species of EOs on mast cell degranulation were investigated. β-hexosaminidase
release is widely used as an indicator for evaluating the extent of degranulation by mast cells and the corresponding release of histamine and other chemical mediators, which is an important process in initiating the immediate type of hypersensitive reaction. The effects of the EOs (at 100 µg/ml) on the release of a chemical mediator (β-hexosaminidase) was investigated in the RBL-2H3 cells treated with the calcium ionophore, A23187. The EOs did not influence the growth of RBL-2H3 cells and did not inhibit β-hexosaminidase enzyme activity (data not shown). The degree of degranulation was calculated by assessing the β-hexosaminidase activity in the supernatant and cell lysate. Among the 20 EOs examined, treatment with EOs from chamomile, lemongrass and sandalwood produced >40% inhibition of mast cell degranulation, as assessed by β-hexosaminidase release. Moreover, of the EOs examined, lemongrass EO showed the strongest inhibitory activity (Fig. 1A). On the other hand, EOs from eucalyptus and lime did not influence β-hexosaminidase release. A23187 is known to induce a calcium flux, which may further induce protein kinase C (PKC) activation, a process essential for mast cell degranulation. It is hence plausible that the β-hexosaminidase inhibition induced by treatment with EOs may affect PKC activity. The mechanisms through which EOs inhibit mast cell degranulation require further investigation.

EO-induced inhibition of TNF-α production in macrophages. We then investigated whether plant EOs, at 100 µg/ml, inhibit the production of TNF-α induced by LPS stimulation in cultured cells. The inflammatory cytokine, TNF-α, activates the NF-κB signaling pathway by binding to the TNF-α receptor, thereby initiating an inflammatory response implicated in various inflammatory diseases (17). In cultured RAW264.7 murine macrophage cells, none of the compounds examined showed cytotoxicity at 100 µg/ml (data not shown); the EOs...
had no effect on the proliferation of peritoneal macrophages. The RAW264.7 cells produced 693 pg/ml of TNF-α following stimulation with LPS. The rate of TNF-α production in the controls was set at 0%. Among the EOs examined, those derived from lemongrass showed the strongest effect on TNF-α production (>50% inhibition) and those from chamomile, lemon and sandalwood displayed the second strongest effects (30-50% inhibition) (Fig. 1B). EOs derived from eucalyptus, lime and nutmeg failed to affect TNF-α production (0% inhibition).

Association between the EO-induced inhibition of β-hexosaminidase release by mast cells and TNF-α production by macrophages. The possible association between the observed inhibition of β-hexosaminidase in mast cells and TNF-α inhibition in macrophages was confirmed by comparing the effects of the 20 EOs on these biological activities. The inhibition of β-hexosaminidase release showed a high correlation with TNF-α inhibition, with a significant correlation coefficient (R²=0.783). These results led us to speculate that the anti-allergic activity related to the inhibition of β-hexosaminidase release and the anti-inflammatory effects induced by the suppression of TNF-α may be EO-related events within a shared biological pathway.

Among the 20 EOs examined, lemongrass EO produced the strongest inhibitory activities in the cultured cells (Fig. 1); thereafter we focused on lemongrass EO.

Identification of lemongrass EO components. The association between the biological activity of lemongrass EO and EO composition was clarified by an analysis of the primary EO components by GC-MS. In total, 138 compounds were detected in the EO, of which 40 components were identified, and the 15 major compounds are listed in Table I. The 2 main compounds representing >30% of the total content were geranial and neral (40.16 and 34.24%, respectively). A mixture of these stereoisomeric monoterpenoid aldehydes is referred to as citral. Of the identified components, 5 compounds, citral [1], geraniol [3], geranyl acetate [4], linalool [5] and camphene [6], were commercially available. Geranial [2] was chemically synthesized. Preparations of compounds 1-6 contain terpenoids of low molecular weight, and their corresponding chemical structures are shown in Fig. 2.

Effects of major lemongrass EO components on β-hexosaminidase release and TNF-α production in cultured cells. Based on the above results, the inhibitory effects of 100 µg/ml of lemongrass EO and its major components, compounds 1-6, on β-hexosaminidase release by mast cells and TNF-α production by macrophages were investigated. In the RBL-2H3 and RAW264.7 cells, these compounds did not show cytotoxicity at 100 µg/ml (data not shown).

Citral [1] (a mixture of neral and geraniol) and geranial [2], which is a trans-isomer of citral, markedly inhibited the release of β-hexosaminidase induced by the calcium ionophore, A23187, and this inhibitory effect was similar to that elicited by lemongrass EO (Fig. 3A). On the other hand, 4 other components, geraniol [3], geranyl acetate [4], linalool [5] and camphene [6], exerted weak or moderate effects, and the inhibitory effect on the release of β-hexosaminidase elicited by compounds 3-6 was 2-fold weaker than that elicited by compounds 1-2 or lemongrass EO.

The effects of compounds 1-6 on TNF-α production were similar to those on β-hexosaminidase release, and compounds 1-2 and lemongrass EO exerted stronger inhibitory effects than compounds 3-6 (Fig. 3B). These results

### Table I. Chemical composition of lemongrass essential oil (EO).

<table>
<thead>
<tr>
<th>Components</th>
<th>Composition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geranial [1] [2]</td>
<td>40.16</td>
</tr>
<tr>
<td>Neral [1]</td>
<td>34.24</td>
</tr>
<tr>
<td>Geraniol [3]</td>
<td>5.11</td>
</tr>
<tr>
<td>Geranyl acetate [4]</td>
<td>2.89</td>
</tr>
<tr>
<td>Linalool [5]</td>
<td>1.45</td>
</tr>
<tr>
<td>Methylheptenone</td>
<td>1.44</td>
</tr>
<tr>
<td>4-Nonanone</td>
<td>1.38</td>
</tr>
<tr>
<td>β-Caryophyllene</td>
<td>1.09</td>
</tr>
<tr>
<td>γ-Cadinene</td>
<td>0.96</td>
</tr>
<tr>
<td>Limonene</td>
<td>0.33</td>
</tr>
<tr>
<td>α-Pinene</td>
<td>0.28</td>
</tr>
<tr>
<td>Tricyclene</td>
<td>0.18</td>
</tr>
<tr>
<td>Acetone</td>
<td>0.13</td>
</tr>
<tr>
<td>4-Heptanone</td>
<td>0.06</td>
</tr>
<tr>
<td>Others</td>
<td>8.91</td>
</tr>
</tbody>
</table>
suggest that citral [1] and/or geranial [2] are the bioactive components conferring the biological activities to lemongrass EO. Therefore, we sought to explore compounds 1-2 further.

The inhibitory activity of citral [1] and geranial [2] against LPS-induced inflammatory responses in cultured macrophages. NF-κB activation is known to be the rate-controlling factor during an inflammatory response. Therefore, the inhibitory effects of lemongrass EO and its major components, citral [1] and geranial [2], on the LPS-induced nuclear translocation of NF-κB were examined in RAW264.7 cells (Fig. 4A). Western blot analysis revealed that 10 µg/ml of citral [1] and geranial [2] inhibited the LPS-induced NF-κB nuclear translocation, with 40 and 52% inhibition, respectively. Lemongrass EO, at 10 µg/ml, also inhibited the nuclear translocation of NF-κB, and this inhibitory activity was similar to that of compounds 1-2.

Stimulation with LPS results in the activation of Toll-like receptor 4 and that of downstream IκB kinases (IKKs), which in turn phosphorylate IκB, resulting in IκB degradation and the translocation of NF-κB into the nucleus (18). IκB-α inhibits NF-κB by masking the nuclear localization signals (NLS) of NF-κB proteins and maintaining them in a sequestered and inactive state in the cytoplasm (19). In addition, IκB-α blocks the ability of the NF-κB transcription factor to bind to DNA, which is required for the proper functioning of NF-κB (20). Therefore, the effects of lemongrass EO and compounds 1-2 on the LPS-induced decrease in the protein expression of IκB-α...
were examined in the RAW264.7 cells (Fig. 4B). IκB-α protein expression was reduced by 59% following LPS stimulation (Fig. 4B). The amount of IκB-α protein in the cells treated with compounds 1-2 (10 µg/ml each) was almost the same as that in the LPS-treated cells (the IκB-α protein level was approximately 35% compared to that in the control untreated cells), suggesting that compounds 1-2 did not influence the protein expression of IκB-α. These results demonstrate that lemongrass EO and its major components suppress the NF-κB nuclear translocation through an IκB-α-independent mechanism. The effects of these compounds at other points in the NF-κB signaling pathway required further investigation.

In vivo anti-allergic and anti-inflammatory activities of citral [1] and geranial [2]. The IgE-mediated PCA reaction in vivo is a method used for studying the mechanisms of the immediate hypersensitivity reaction. As shown in Fig. 5A, 100 mg/kg of tranilast, a commonly used anti-allergic drug that targets mast cell degranulation and inhibits the PCA reaction, was used as a positive control. Lemongrass EO and its major components, citral [1] and geranial [2], at 100 mg/kg also inhibited the PCA reaction in the mice by 60.7, 57.7 and 77.4%, respectively. The inhibitory effects of lemongrass EO and compounds 1-2 were >2-fold stronger than those of tranilast, suggesting a great potential for use as anti-allergic compounds.

We examined the anti-inflammatory activity of lemongrass EO, citral [1] and geranial [2] by examining their effects on TPA-induced inflammatory edema of the mouse ear in vivo. The application of TPA (0.5 µg) to the mouse ears induced edema, resulting in a 241% increase in the weight of the ear disk 7 h after application. Pre-treatment with 500 µg/ear of these compounds significantly suppressed inflammation, and the strength of the inhibitory effect was ranked as follows: geranial [2] > lemongrass EO > citral [1] (Fig. 5B). Therefore, the in vivo anti-inflammatory effects of these compounds displayed the same order as their in vitro anti-allergic effects (Fig. 5A). These results suggest that the anti-allergic effects of the components of lemongrass EO positively correlate with the anti-inflammatory activity observed. Glycyrrhetinic acid (500 µg/ear), a known anti-inflammatory agent, had a 40.5% anti-inflammatory effect in this assay system, and these compounds were found to be approximately 1.5-fold more effective than this agent.

The biological effects elicited by compounds 1-2 were similar to those produced by almost the same concentrations of lemongrass EO (Figs. 3-5), suggesting that the anti-allergy and anti-inflammatory effects of lemongrass EO are largely due to its 2 major components, citral [1] and/or geranial [2].

Discussion

Over the years, EOs have attracted much attention in scientific research and are increasingly used in pharmaceutical, nutritional and cosmetic industrial applications (21,22), primarily due to their potent anti-microbial (23), antioxidant (24), anticancer (25) and anti-inflammatory activities (26) of EOs. The infection process induces inflammation, during which inflammatory mediators, such as cytokines are released from phagocytes (27). As various skin disorders, including atopic dermatitis (28) and acne vulgaris (29), are associated with infection-induced inflammation, the presence of anti-inflammatory agents may explain the effectiveness of certain plant-derived EOs in the treatment of these syndromes. In several regions worldwide, aromatic herbs play major roles in primary health care, particularly in rural areas (30). Thus, an understanding of herbal biological activities may further the effectiveness and the use of many of their functional components, particularly as beneficial additives in medical, nutritional and cosmetic products.

In the present study, 20 EOs were selected as known herbal plant- and citrus fruit-derived EOs. Among the plant-derived EOs worldwide, the compounds selected represent the most well known and most commonly used materials in perfume preparations. These EOs were isolated by hydrodistillation or cold-pressing and were confirmed to be free of any contamination, such as heavy metals and pesticides.
β-hexosaminidase release from RBL-2H3 cells treated with the calcium ionophore, A23187 (Fig. 1A) and the production of TNF-α, a pleiotropic inflammatory cytokine, by RAW264.7 murine macrophages treated with LPS (Fig. 1B). We observed that individual constituents of lemongrass EO, including citral [1], which is a mixture of stereoisomers, geranial (trans-isomer) [2] and neral (cis-isomer), comprising 74.40% of lemongrass EO (Table I), inhibited NF-κB nuclear translocation by LPS, and suppressed TNF-α production in macrophage cell culture experiments in vitro (Figs. 3-5). These compounds also exhibited potent anti-inflammatory activity in vivo. Lemongrass EO and its major components, such as citral [1] and geranial [2] exhibited anti-inflammatory activity in a TPA-induced mouse model of ear edema, although the underlying molecular mechanisms remain unelucidated (Fig. 5B). Since the activation of NF-κB has been observed in a model of TPA-induced ear edema (31), we hypothesized that the anti-inflammatory effects may be, at least in part, dependent on the inhibition of NF-κB activation. At present, citral [1] and geranial [2] are regarded as promising anti-inflammatory agents. Our study indicates that these compounds are useful as NF-κB inhibitors and may be potent chemopreventive agents against inflammation. Among the lemongrass EO components, citral [1] and geranial [2] inhibited the release of β-hexosaminidase and the production of TNF-α, while geraniol [3] and geranyl acetate [4] did not have any effects (Fig. 3), suggesting that the ketone group of citral may be important for these observed inhibitory activities.

Lemongrass is native to India and tropical Asia. It is widely used as an herb in Asian cuisine. It has a subtle citrus flavor and can be dried and powdered, or used fresh. Lemongrass is commonly used in teas, soups and curries. It is also suitable for poultry, fish, beef and seafood. It is often used as a tea in African countries, such as Togo and the Democratic Republic of Congo, as well as in Latin American countries, such as Mexico. Among the components of lemongrass, geranial [2] has a strong lemon odor. The lemon odor of neral is less intense, but sweeter. Citral [1], on the other hand, is an aromatic compound used in perfumery for its citrus effect. Citral [1] is also used as a flavor and for fortifying lemon oil. This plant containing citral [1] is used in folk medicine as an anti-spasmodic, hypotensive, anti-convulsant, analgesic, anti-inflammatory, anti-spasmodic, hypotensive, anti-convulsant, analgesic, anti-inflammatory, anti-diarreal and antioxidant agent, as well as in the treatment of nervous and gastrointestinal disorders and fevers (32). In this study, lemongrass EO was found to have anti-inflammatory activity; however, the mechanisms behind these different bioactivities were not examined in detail. The association between the above-mentioned biological activities and the blocking of the nuclear translocation of NF-κB and TNF-α production requires further investigation.

Modern aromatherapy for allergic and inflammatory diseases has been developed primarily based on clinical trials of EOs by several pioneers; however, scientific research on the physiological role of these EOs against allergic and inflammatory responses is still at a primitive stage. The elucidation of the pharmacological functions of lemongrass EO in vivo may provide a rationale for the clinical application of this EO as an anti-allergic and anti-inflammatory substance. In conclusion, our data provide strong scientific evidence and highlight the benefits of lemongrass EO and its constituents, citral [1] and geranial [2], for use in medical and/or cosmetic applications.

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