Antimicrobial, Antioxidant, and Anti-Inflammatory Activities of Essential Oils from Five Selected Herbs

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Eucalyptus bridgesiana, Cymbopogon martinii, Thymus vulgaris, Lindernia anagallis, and Pelargonium fragrans are five species of herbs used in Asia. Their essential oils were analyzed by GC-MS, and a total of 36 components were detected. The results of our study indicated that, except for the essential oil of Pelargonium fragrans, all of the essential oils demonstrated obvious antimicrobial activity against a broad range of microorganisms. The C. martinii essential oil, which is rich in geraniol, was the most effective antimicrobial additive. All of the essential oils demonstrated antioxidant activities on 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay, β-carotene/linoleic acid assay, and nitric oxide radical scavenging assay. Furthermore, the T. vulgaris essential oil, which possesses plentiful thymol, exhibited the highest antioxidant activity. For P. acnes-induced secretion of pro-inflammatory cytokines, the essential oils of P. aeruginosa, C. martinii, and T. vulgaris reduced the TNF-α, IL-1β, and IL-8 secretion levels of THP-1 cells.

Key words: Eucalyptus bridgesiana; Cymbopogon martinii; Thymus vulgaris; Lindernia anagallis; Pelargonium fragrans

In recent years, the measurement and utilization of plant essential oils has become increasingly important in scientific research and industrial applications, including pharmaceutical, nutritional, and cosmetic uses, primarily due to the oils’ various potent biological activities, including antimicrobial, antioxidant, and anti-inflammatory activities. The infection process frequently induces inflammation, and free radicals are released from the phagocytes during the inflammatory process. Because various skin disorders, including atopic dermatitis and acne vulgaris, are associated with infection-stimulated inflammation, the presence of antimicrobial, antioxidant, and anti-inflammatory agents might explain the effectiveness of some plant essential oils in the treatment of these syndromes. This fact also indicates that antimicrobial, antioxidant, and anti-inflammatory activities are good targets for natural product development. Moreover, in many regions of the world, aromatic herbs still play major roles in primary health care, particularly in rural areas. Thus, an understanding of the biological activities of herbs can provide many functional components and additives for medical, nutritional, and cosmetic products.

The vast majority of Eucalyptus species belong to the family Myrtaceae and originated in Australia. Several species are widespread throughout the world, including Eucalyptus bridgesiana. Eucalyptus essential oils are found in the leaves, fruits, buds, and bark of the tree, but only a small number of studies have described the essential oil of E. bridgesiana. Cymbopogon martinii produces a geraniol-rich, commercially valuable essential oil. The essential oil of C. martinii has been employed as a folk medicine in many countries, this study is the first to investigate the effectiveness of the essential oil of this common plant. The genus Pelargonium contains a large number of species with scented leaves exhibiting various fragrances, ranging from pleasantly fruity and floral scents to oppressively balsamic aromas. In addition, commercial geranium oil has been extracted from the leaves of several Pelargonium species and cultivars. Pelargonium fragrans might also represent a useful source of commercial geranium oil, but the chemical and biological characteristics of the P. fragrans essential oil have not been fully elucidated. These five herbs are regularly and widely used in many areas, but only a few studies have described the chemical and biological features of these essential oils. Moreover, no studies have yielded information about the essential oils of Lindernia anagallis and P. fragrans. Hence, investigation of these five selected herbs is important.

In this study, we examined the antimicrobial activity of essential oils against several microorganisms, including Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Propionibacterium acnes, Candida albicans, and Pityrosporum ovale. The antioxidant activities of the essential oils were determined by various antioxidant assays, including 2,2-diphenyl-1-
picrylhydrazyl (DPPH) radical scavenging assay, \( \beta \)-carotene/linoleic acid assay, and nitric oxide (NO) radical scavenging assay. Furthermore, the anti-inflammatory activities of the essential oils were determined by 5-lipoxygenase (5-LOX) inhibition assay and by measuring the secretion of pro-inflammatory cytokines from the THP-1 human acute monocytic leukemia cell line. Moreover, we evaluated the major chemical compositions of the essential oils derived from the five selected herbs by gas chromatography-mass spectrometry (GC-MS). These results should help to clarify the applications of these plants for the future.

### Materials and Methods

Plants, microorganisms, and cell line. All of the herbs, including Eucalyptus bridgesiana, Cymbopogon martinii, Thymus vulgaris, Lindernia anagallis, Pelargonium fragrans, and Melaleuca alternifo- lia, were purchased from a local market in Nantou, Taiwan. The microorganisms and cell line Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 9027, Staphylococcus aureus ATCC 6538, Propionibacterium acnes ATCC 6919, Candida albicans ATCC 10231, Pityrosporum ovale ATCC 12078, and THP-1 (human acute monocytic leukemia cell line, BCRC 60430) were purchased from the Bioresource Collection and Research Center (BCRC, Hsinchu, Taiwan, ROC), and were employed in antimicrobial and anti-inflammatory activity assays.

Materials. Lipopolysaccharide (LPS; Escherichia coli O127: B8, L3129), bovine serum albumin (BSA), trypan blue, chlorofrom, DPPH, \( \beta \)-carotene, linoleic acid, 5-lipoxygenase, \( \alpha \)-bisabolol, \( \alpha \)-tocopherol, Tween-40, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS), penicillin, streptomycin, trypsin-EDTA, and RPMI-1640 Medium were from Gibco BRL/Invitrogen (Carlsbad, CA). Agar was from Merck (Darmstadt, Germany). Yeast extract, malt extract, peptone, and malt extract broth (MEB) were from HiMedia (Mumbai, India). Glycerol mono-oleate, tryptic soy broth (TSB), reinforced clostridial medium (RCM), dextrose, and Griess reagent were from Fluka (Buchs, Switzerland). Sodium nitroprusside was from Riedel deHaen (Seelze, Germany). Lipopolysaccharide (LPS; Escherichia coli O127: B8, L3129), bovine serum albumin (BSA), trypan blue, chlorofrom, DPPH, \( \beta \)-carotene, linoleic acid, 5-lipoxygenase, \( \alpha \)-bisabolol, \( \alpha \)-tocopherol, Tween-40, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO). Fetal bovine serum (FBS), penicillin, streptomycin, trypsin-EDTA, and RPMI-1640 Medium were from Gibco BRL/Invitrogen (Carlsbad, CA). Agar was from Merck (Darmstadt, Germany). Yeast extract, malt extract, peptone, and malt extract broth (MEB) were from HiMedia (Mumbai, India). Glycerol mono-oleate, tryptic soy broth (TSB), reinforced clostridial medium (RCM), dextrose, and Griess reagent were from Fluka (Buchs, Switzerland). Sodium nitroprusside was from Riedel deHaen (Seelze, Germany). Lipopolysaccharide (LPS; Escherichia coli O127: B8, L3129), bovine serum albumin (BSA), trypan blue, chlorofrom, DPPH, \( \beta \)-carotene, linoleic acid, 5-lipoxygenase, \( \alpha \)-bisabolol, \( \alpha \)-tocopherol, Tween-40, and other chemicals were purchased from Sigma-Aldrich (St. Louis, MO).

Preparation of essential oils. Leaf sections of the herbs were air-dried at room temperature for 2 weeks. The air-dried herbs were steam-distilled with ddH2O for 4 h at 100 °C with a Clevenger-type apparatus. The essential oils collected were filtered with a 0.45-μm filter and maintained at 4 °C until further analysis. The steam-distilled yields of the essential oils are presented in Table 1.

Determination of antimicrobial activity. For bacterial cultivation, E. coli, P. aeruginosa, and S. aureus were incubated at 37 °C in TSB, and P. acnes was incubated anaerobically at 37 °C in RCM. C. albicans and P. ovale were cultured at 30 °C in YMFD medium (0.3% yeast extract, 0.3% malt extract, 0.5% peptone, and 1% dextrose) and Dixon culture medium (MEB with 2% ox-bile, 1% Tween-40, and 0.25% glycerol mono-oleate) respectively. A broth microdilution method was employed to determine the minimum inhibitory concentration (MIC). All tests were performed in corresponding media supplemented with Tween 80 (0.5%). Serial dilutions of the essential oils were prepared in a 96-well microtiter plate over a range 0.02 to 49.00 mg/mL. Overnight broth cultures of the various strains were prepared, and the final concentration in the various wells was adjusted to 2 × 10^5 CFU/mL. Plates were incubated at the corresponding culture temperatures, with incubation times of 24 h for E. coli, P. aeruginosa, S. aureus, and C. albicans, 48 h for P. acnes, and 72 h for P. ovale. MIC was defined as the lowest concentration of an essential oil at which the microorganism does not exhibit visible growth, as indicated by the turbidity of the medium.

Determination of antioxidant activity by DPPH radical scavenging assay. For the DPPH scavenging effect, various concentrations of each essential oil and \( \alpha \)-tocopherol were mixed individually into a methanolic solution containing DPPH radicals (0.1 mM), yielding a final volume of 1 mL. The mixture was shaken vigorously and left to stand for 30 min at 25 °C in the dark, and the absorbance was measured at 517 nm. A decrease in absorbance of the DPPH solution indicates DPPH radical scavenging activity, and was calculated by the following formula: % DPPH radical scavenging = ([Absample - Absample]/Ablank) × 100.

Determination of antioxidant activity by \( \beta \)-carotene/linoleic acid assay. In the \( \beta \)-carotene/linoleic acid assay, \( \beta \)-carotene was dissolved in 0.2 mL of chloroform (1 mg/mL) and this was added to a solution of linoleic acid (20 mg) in 200 mg of Tween 40. The chloroform was evaporated under a vacuum at 40 °C for 5 min, and 50 mL of oxygenated, ultra-pure water was added to the emulsion, which was vigorously shaken. Various concentrations of the various essential oils, and \( \alpha \)-tocopherol (0.2 mL) were added individually to 4.8 mL of the emulsion and this was then incubated at 50 °C for 3 h. The absorbance was measured at 470 nm. Readings of all samples were done immediately (t = 0 h) and after 3 h of incubation. The antioxidant activities of the essential oils were evaluated in terms of inhibition of \( \beta \)-carotene bleaching by the following formula: % inhibition of \( \beta \)-carotene bleaching = ([Absample - Absample]/Ablank) × 100.

Determination of antioxidant activity by NO radical scavenging assay. To determine NO radical-scavenging activity, various concentrations of the various essential oils, and \( \alpha \)-tocopherol were mixed individually with 0.1 mL of sodium nitroprusside (100 mM) and phosphate buffer solution (pH 7.4), yielding a final volume of 1 mL. After 2.5 h of incubation at 20 °C, 0.05 mL of the mixture was added to 0.05 mL of Griess reagent over 10 min at 25 °C. The absorbance was determined at 540 nm, and NO radical scavenging activity was calculated by the following formula: % NO radical scavenging = ([Absample - Absample]/Ablank) × 100.

Determination of anti-inflammatory activity by 5-LOX inhibition assay. For 5-lipoxygenase (5-LOX) inhibition activity, linoleic acid was used as substrate for 5-LOX. Various concentrations of 30-μL aliquots of essential oil and \( \alpha \)-bisabolol were mixed individually with 30 μL of linoleic acid and potassium phosphate buffer (0.11 st, pH 6.3) containing 5-LOX (25 U), yielding a final volume of 3 mL. The mixture was incubated at 25 °C for 10 min, and the absorbance was determined at 234 nm. Because linoleic acid is enzymatically converted to a conjugated diene by 5-LOX, which results in a continuous increase in absorbance at 234 nm, inhibition activity was calculated by the following formula: %5-LOX inhibition = ([Absample - Absample]/Ablank) × 100.

Determination of anti-inflammatory activity by the pro-inflammatory cytokine secretion assay. THP-1 cells were cultured in RPMI-1640 medium with 10% fetal bovine serum at 37 °C with 5% CO2. To examine the anti-inflammatory activity of the essential oils, pro-inflammatory cytokines TNF-α, IL-1β, and IL-8 were employed as indicators of the inflammatory response. The human TNF-α, IL-1β, and IL-8 standards were diluted to concentrations ranging from 8 to 1,000 pg/mL to establish a calibration curve. Anti-human TNF-α antibody, anti-human IL-1β antibody, and anti-human IL-8 antibody were utilized to recognize these pro-inflammatory cytokines with an ELISA reader in accordance with the manufacturer’s protocol (R&D Systems). In the induction of THP-1 cell inflammatory responses, 1 μg/mL of lipopolysaccharide (LPS) and 6 × 10^5 CFU/mL of heat-killed P. acnes were utilized as inducers. The THP-1 cells were cultured in 24-well plates (1 x 10^5 cells/well) with 0.01 μg/mL of essential oils dispersed by 1 μL 25% ethanol over 24 h, and were individually treated with LPS or P. acnes for 48 h. After treatment, the culture medium of the THP-1 cells was collected to evaluate the concentration of pro-inflammatory cytokines.
Determination of the primary components of the essential oils by GC-MS. The essential oils were analyzed with a Hewlett Packard GC (HP6890 Series II) coupled with a mass detector (MSD 5973) equipped with an auto-sampler and a HP-5MS capillary column (inner diameter 30 m × 0.25 mm, film thickness 0.25 μm) (Agilent Technologies, Palo Alto, CA). Helium was employed as the carrier gas. Electron impact ionization of 70 eV was utilized to obtain mass spectra. The oven temperature was maintained isothermally at 40 °C for 4 min, and was programmed to increase to 280 °C at a rate of 4 °C/min. The split ratio was adjusted to 20:1, and the mass range was completed from 40 m/z to 550 m/z. The percentage of components was calculated from total ion chromatograms. Identification of the primary component was completed by comparing mass spectra using the Wiley7n.1 and NIST02.L libraries. The results are presented in Table 4.

Statistical analysis. All analytic measurements were performed in triplicate at least. The results were analyzed by the Student’s t-test, and were expressed as mean ± standard deviation (SD) for each measurement. Differences were recognized as significant when the p values were <0.05.

Results and Discussion

Air-dried leaf sections of five selected herbs were steam-distilled to produce colorless to yellowish essential oils. The steam-distilled yields of the essential oils are presented in Table 1. T. vulgaris and L. anagallis exhibited the lowest yields of essential oil, at 2.83 and 3.89 g/kg respectively. Conversely, P. fragrans, C. martini, and E. bridgesiana produced greater essential oil yields, of 7.34, 6.78, and 14.65 g/kg respectively (Table 1). Differences in essential oil yields are often caused by the characteristics of the plants. In addition, the section of the plant extracted and the extraction procedure influenced the final yield of an essential oil.21

To analyze the antimicrobial activities of the essential oils, the broth microdilution method was employed to determine the minimum inhibitory concentration (MIC) of an essential oil against selected microorganisms, and the M. alternifolia (tea tree) essential oil was used as control. Gram-negative bacteria (E. coli and P. aeruginosa), gram-positive bacteria (S. aureus and P. acnes), and fungi (C. albicans and P. oval) were employed as screening microorganisms. The results are show in Table 2.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Essential oil yield (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eucalyptus bridgesiana</td>
<td>14.65</td>
</tr>
<tr>
<td>Cymbopogon martini</td>
<td>7.34</td>
</tr>
<tr>
<td>Thymus vulgaris</td>
<td>2.83</td>
</tr>
<tr>
<td>Lin dernia anagallis</td>
<td>3.89</td>
</tr>
<tr>
<td>Pelargonium fragrans</td>
<td>6.78</td>
</tr>
<tr>
<td>Melaleuca alternifolia</td>
<td>15.25</td>
</tr>
</tbody>
</table>

Table 2. Minimum Inhibitory Concentrations (μg/mL) for the Antimicrobial Activities of the Essential Oils

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>E. coli</th>
<th>P. aeruginosa</th>
<th>S. aureus</th>
<th>P. acnes</th>
<th>C. albicans</th>
<th>P. oval</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. bridgesiana</td>
<td>6.00 ± 0.00</td>
<td>&gt;20.00</td>
<td>3.87 ± 0.00</td>
<td>3.12 ± 0.00</td>
<td>17.50 ± 0.00</td>
<td>2.04 ± 0.07</td>
</tr>
<tr>
<td>C. martini</td>
<td>1.54 ± 0.07</td>
<td>&gt;20.00</td>
<td>1.04 ± 0.07</td>
<td>0.91 ± 0.07</td>
<td>2.57 ± 0.00</td>
<td>1.20 ± 0.07</td>
</tr>
<tr>
<td>T. vulgaris</td>
<td>1.87 ± 0.21</td>
<td>&gt;20.00</td>
<td>1.54 ± 0.07</td>
<td>5.87 ± 0.00</td>
<td>1.62 ± 0.00</td>
<td>1.83 ± 0.14</td>
</tr>
<tr>
<td>L. anagallis</td>
<td>2.50 ± 0.16</td>
<td>&gt;20.00</td>
<td>1.70 ± 0.07</td>
<td>5.12 ± 0.00</td>
<td>2.75 ± 0.00</td>
<td>2.04 ± 0.07</td>
</tr>
<tr>
<td>P. fragrans</td>
<td>&gt;20.00</td>
<td>&gt;20.00</td>
<td>&gt;20.00</td>
<td>&gt;20.00</td>
<td>&gt;20.00</td>
<td>&gt;20.00</td>
</tr>
<tr>
<td>M. alternifolia*</td>
<td>1.58 ± 0.07</td>
<td>&gt;20.00</td>
<td>1.00 ± 0.00</td>
<td>1.12 ± 0.00</td>
<td>1.37 ± 0.00</td>
<td>0.74 ± 0.01</td>
</tr>
</tbody>
</table>

* M. alternifolia was the control. Values are presented as mean ± SD (n = 3).
been due to the different principles of these antioxidant assays. That is to say the antioxidant activities are not comparable when different assay systems are used.\textsuperscript{26,27} These results also indicate that the antioxidant properties of these essential oils are distinct. In the NO radical scavenging assay, the essential oil of \textit{T. vulgaris} exhibited the lowest IC\textsubscript{50} value. Although the IC\textsubscript{50} value of the \textit{T. vulgaris} essential oil was greater than that of \textit{α}-tocopherol (the positive control), the \textit{T. vulgaris} essential oil might prove an effective antioxidant in many applications. The antioxidant activities of the \textit{T. vulgaris} essential oil were similar to those determined in previous studies\textsuperscript{28,29}, but the antioxidant activities of the \textit{L. anagallis} and \textit{P. fragrans} essential oils are described for the first time here.

To determine anti-inflammatory activities, 5-lipoxygenase (5-LOX) inhibition assay and pro-inflammatory cytokine secretion assay were employed as screening experiments. As for 5-LOX inhibition activity, the results indicate that all of the essential oils repress the enzymatic activity of 5-LOX. The IC\textsubscript{50} values ranged from 0.005 to 1.50 µg/mL (Table 3). Compared with the positive controls (tea tree oil and \textit{α}-bisabolol), the \textit{T. vulgaris} essential oil exhibited stronger anti-inflammatory activity. Moreover, in the pro-inflammatory cytokine secretion assay, THP-1 cells were utilized to test the inflammatory response by measuring the amounts of pro-inflammatory cytokines TNF-α, IL-1β, and IL-8. First, to verify the effects of these essential oils in THP-1 cells and to determine proper working concentration, the essential oils were added directly to THP-1 cells and the secretion of pro-inflammatory cytokines and cell viability were analyzed. For this reason, to avoid the possibility that a high concentration essential oil might influence THP-1 cells, we chose a relative low concentration (0.01 μg/mL) in subsequent experiments. The levels of pro-inflammatory cytokines in the THP-1 cells treated with 0.01 μg/mL of each of the essential oils did not exhibit noticeable differences (data not shown). Furthermore, we examined the cell viability of essential oil treated THP-1 cells by a standard tetrazolium (MTT) method, and the results indicated that no essential oil at a concentration of 0.01 µg/mL showed cytotoxicity for THP-1 cells (data not show). These results indicate that none of the essential oils directly affects the secretion of pro-inflammatory cytokines or the survival of THP-1 cells.

### Table 3. IC\textsubscript{50} (µg/mL) Values for the Antioxidant and Anti-Inflammatory Activities of the Essential Oils

<table>
<thead>
<tr>
<th>Essential oils</th>
<th>DPPH radical scavenging assay</th>
<th>β-Carotene/Linoleic acid assay</th>
<th>NO radical scavenging assay</th>
<th>Anti-inflammatory activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{E. bridgesiana}</td>
<td>50.60 ± 0.25</td>
<td>27.54 ± 0.35</td>
<td>24.40 ± 0.52</td>
<td>0.74 ± 0.02</td>
</tr>
<tr>
<td>\textit{C. martini}</td>
<td>51.42 ± 1.25</td>
<td>0.99 ± 0.03</td>
<td>56.93 ± 0.44</td>
<td>1.50 ± 0.04</td>
</tr>
<tr>
<td>\textit{T. vulgaris}</td>
<td>0.10 ± 0.00</td>
<td>0.14 ± 0.02</td>
<td>18.44 ± 0.32</td>
<td>0.005 ± 0.00</td>
</tr>
<tr>
<td>\textit{L. anagallis}</td>
<td>16.46 ± 0.95</td>
<td>11.82 ± 0.75</td>
<td>31.81 ± 0.44</td>
<td>0.62 ± 0.04</td>
</tr>
<tr>
<td>\textit{P. fragrans}</td>
<td>5.68 ± 0.32</td>
<td>0.99 ± 0.02</td>
<td>46.69 ± 0.22</td>
<td>0.54 ± 0.03</td>
</tr>
<tr>
<td>\textit{M. alternifolia}</td>
<td>29.70 ± 0.65</td>
<td>7.80 ± 0.67</td>
<td>34.09 ± 0.39</td>
<td>0.06 ± 0.00</td>
</tr>
<tr>
<td>\textit{α}-tocopherol\textsuperscript{a}</td>
<td>0.041 ± 0.00</td>
<td>—</td>
<td>7.37 ± 0.80</td>
<td>—</td>
</tr>
<tr>
<td>\textit{α}-bisabolol\textsuperscript{a}</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.049 ± 0.00</td>
</tr>
</tbody>
</table>

\textsuperscript{a}M. alternifolia, \textit{α}-tocopherol, and \textit{α}-bisabolol were the controls. Values are presented as mean ± SD (n = 3).
consists of 82.6% 1,8-cineole.\textsuperscript{10} The variations in the percentages of essential oil component might be due to extraction of essential oil from different plant resources and by different extraction procedures. In the composition of the \textit{C. martinii} essential oil, geraniol (52.49\%) and geranyl acetate (12.01\%) were the major compounds (Table 4). This result was similar to that of an earlier study.\textsuperscript{32} In our study, the \textit{C. martinii} essential oil also showed effective antimicrobial activity against most microorganisms (Table 2), possibly because geraniol, the primary component of the \textit{C. martinii} essential oil, causes leakage of K\textsuperscript{+} and Mg\textsuperscript{2+} ions from microorganism cells through changes in the compositions of the cell membranes.\textsuperscript{24} Moreover, geraniol also shows antioxidant and anti-inflammatory activities in the \textit{C. martinii} essential oil.\textsuperscript{33} In the \textit{T. vulgaris} essential oil, the primary compounds were \textit{p}-cymene (14.63\%) and thymol (21.46\%). This correlates with the antioxidant activities of the \textit{T. vulgaris} essential oil, because essential oils with elevated proportions of thymol and carvacrol exhibit upregulated antioxidant activities.\textsuperscript{28} Also, previous research has indicated that carvacrol in thyme oils suppresses the LPS-induced expression of cyclooxygenase-2 (COX-2) mRNA and protein, which can cause repression of inflammation.\textsuperscript{34} Thus, the \textit{T. vulgaris} essential oil (which consists of 2.03%
carvacrol) exhibited a significant anti-inflammatory activity in this study. This is the first study to analyze the chemical compositions of the essential oils of \textit{L. anagallis} and \textit{P. fragrans} (Table 4). In the \textit{L. anagallis} essential oil, the major components were \(\alpha\)-menthanone (30.75\%), menthol (10.30\%), and pulegone (18.41\%). Menthol and pulegone have previously shown antimicrobial and antioxidant activities as components of essential oils.\cite{35,36} Hence, the \textit{L. anagallis} essential oil also possesses the potential to be an effective antimicrobial and antioxidant agent. In the \textit{P. fragrans} essential oil, the major components were (\(\sim\))-spathulenol (13.91\%), fenchone (7.37\%), methyl eugenol ether (7.25\%), and several monoterpens, such as (\(\sim\))-pinene (3.09\%) and limonene (3.78\%). Monoterpens are typical essential-oil constituents, which normally do not exhibit strong antimicrobial activity at low concentrations.\cite{37} However, many monoterpens show excellent antioxidant activities in essential oils. Accordingly, the \textit{P. fragrans} essential oil was not an effective antimicrobial agent. The constituents yield the chemical and biological activities of essential oil. Hence, evaluation of activities of individual compounds can help in identifying the relationships among constituents and activities. Accordingly, we aim to pursue this further study in the future.

In summary, except for the \textit{P. fragrans} essential oil, all of the essential oils examined here clearly exhibited antimicrobial activity against a broad range of microorganisms, and the \textit{C. martini} essential oil was the most effective. All of the essential oils showed antioxidant activities, and the \textit{T. vulgaris} essential oil exhibited the highest activity. Moreover, the \textit{L. anagallis} essential oil showed potential as a useful antimicrobial and antioxidant agent. All of the essential oils exhibited anti-inflammatory activities, and the \textit{T. vulgaris} essential oil showed the greatest activity in general. These results should help to clarify functional applications of these folk herbs and their essential oils for the future.

\textbf{References}


\begin{table}[h]
\centering
\caption{Primary Component Percentages (%) of the Essential Oils}
\begin{tabular}{|c|c|c|c|c|c|}
\hline
RT\textsuperscript{a} (min) & E. bridgesiana & C. martini & T. vulgaris & L. anagallis & P. fragrans \\
\hline
9.29 & \(\alpha\)-thuiene & — & — & — & 2.55 \\
9.42 & \(\alpha\)-pinene & 1.58 & — & — & 3.09 \\
11.14 & 4-thuiene & — & — & — & 3.60 \\
12.31 & 3-octanol & — & — & 2.83 & — \\
13.20 & \(\nu\)-cymene & — & — & 14.63 & — \\
13.35 & limonene & — & — & — & 3.78 \\
13.78 & 1.8-cineole & 39.13 & — & — & — \\
14.51 & \(\gamma\)-terpinene & — & — & 1.59 & — \\
15.77 & fenchone & — & — & — & 7.37 \\
16.22 & linalool & — & 1.15 & — & 4.02 \\
17.08 & camphor & — & — & 3.11 & — \\
18.50 & \(\alpha\)-menthanone & — & — & 30.75 & — \\
18.58 & menthone & — & — & — & 2.25 \\
18.76 & linderol & — & — & 5.41 & — \\
18.81 & menthol & — & — & 10.30 & — \\
19.79 & \(\alpha\)-terpinol & 8.52 & — & — & — \\
21.13 & thymol methyl ether & — & — & 4.35 & — \\
21.47 & carvacrol methyl ether & — & — & 5.35 & — \\
21.53 & pulegone & — & — & 18.41 & — \\
22.39 & geraniol & 52.49 & — & — & — \\
23.64 & thymol & — & — & 21.46 & — \\
23.90 & carvacrol & — & — & 2.03 & — \\
25.08 & \(\alpha\)-terpinyl acetate & 6.44 & — & — & — \\
26.28 & geranyl acetate & — & 12.01 & — & — \\
26.98 & methyl eugenol ether & — & — & — & 7.25 \\
27.10 & \(\beta\)-caryophyllene & — & 0.58 & 1.99 & — \\
27.74 & aromadendrene & 2.39 & — & — & — \\
29.97 & \(\beta\)-bisabolene & — & — & 4.39 & — \\
32.11 & caryophyllene oxide & — & — & — & 2.65 \\
32.23 & (\(\sim\))-spathulenol & — & — & — & 13.91 \\
32.26 & (\(\sim\))-globulol & 5.10 & — & — & — \\
32.43 & viridiflorol & 1.83 & — & — & — \\
36.01 & nerolidol & — & 2.38 & — & — \\
36.79 & geranyl hexanoate & — & 2.96 & — & — \\
38.99 & farnesyl acetate & — & 0.55 & — & — \\
41.67 & neryl propanoate & — & 2.48 & — & — \\
\hline
\textsuperscript{a}RT, retention time; —, not detected.
\end{tabular}
\end{table}


