

## [Original Research]

## Comparison of Anti-Nociceptive and Anti-Inflammatory/Analgesic Effects of Essential Oils in Experimental Animal Models

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**Abstract:** Previous studies have shown that some essential oils have anti-nociceptive and anti-inflammatory actions; however, the mechanisms underlying how such essential oils exert these actions are not fully understood. In the present study we examined anti-nociceptive and anti-inflammatory properties of essential oils in rats and mice. The anti-nociceptive effects of essential oils on rats were assessed by a Randall Selitto assay. Application of 2% ginger and true lavender essential oils to the hindpaw produced significant anti-nociceptive effects compared to control. Furthermore, stimulatory pressure-induced c-Fos expression in spinal dorsal horn neurons was significantly suppressed by the application of 2% ginger essential oil. As impurities and/or aromatic components may exist in essential oils as a consequence of thermal denaturation, a ginger cell-extract containing non-denatured and purified compounds was tested. In this way, we compared the anti-nociceptive effects of ginger essential oil to those of the ginger cell-extract using a von Frey test after injection of complete Freund's adjuvant into the hindpaw of mice. When applied to the paw, both agents induced significant anti-inflammatory and anti-nociceptive effects compared with control; however there was no significant difference between the two agents in terms of their potency. Interestingly, gas chromatography-mass spectrometry experiments indicated that the principal active ingredients of ginger essential oil were different from those of the ginger cell-extract. These results suggest that the constituents of essential oils produce their anti-inflammatory and anti-nociceptive effects transdermally via different mechanisms.

**Key words:** c-Fos, complete Freund's adjuvant, Ginger cell-extract, ginger essential oil, von Frey test

## INTRODUCTION

Essential oils are natural, volatile, complex compounds characterized by a strong odor. They can be synthesized as secondary metabolites by all plant organs, i.e. buds, flowers, leaves, stems, branches, seeds, fruits, roots, and wood or bark, and are stored in secretory cells, cavities, canals, epidermic cells or glandular trichomes. Essential oils have been largely used on the basis of properties already observed in nature, such as their antibacterial, antifungal and insecticidal activities. To the present time, approximately 3,000 essential oils have been identified, with around 300 of these now commercially available. To this end, essential oils or their constituent compounds are used in perfumes and make-up products, in sanitary products, in dentistry, in agriculture, as food preservatives and additives, and as natural remedies. For example, *d*-limonene, geranyl acetate and *d*-carvone are employed in perfumes, creams, and soaps, as flavor-

ings for food, as fragrances for household cleaning products, and as industrial solvents. Moreover, essential oils are commonly mixed with vegetal oils for use in massage or to be added to bathwater, and are frequently employed in aromatherapy. Furthermore, some essential oils appear to exhibit medicinal properties that have been claimed to cure organ dysfunctions or systemic disorders.<sup>1-3)</sup> For example, it has been reported that essential oils can exert antibacterial activity, increase blood flow, and cause sedation.<sup>4-7)</sup>

An analgesic effect of lavender essential oil has been shown following its oral administration to animals.<sup>8)</sup> In clinical practice, it was reported that lavender oil reduced post-episiotomy pain intensity scores in primiparous women in the days following childbirth compared with a control group. In addition, the period of perineum healing was also shortened significantly.<sup>9)</sup>

Animal studies have shown that individual components of ginger essential oil exert anti-inflammatory effects, while the systemic and transdermal administration of ginger essential oil to animals and humans also induces anti-nociceptive effects.<sup>5)</sup> Further to these observations, the administration of ginger essential oil suppresses

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leukocyte migration under inflammatory conditions *in vivo* and *in vitro*,<sup>6</sup> while Ar-curcumene and  $\alpha$ -pinene, which are oily extracts from the ginger rhizome, reduce the level of lipopolysaccharide-induced IL-8 secretion from bronchial epithelial (BEAS-2B) cells in asthmatic patients.<sup>7</sup> Ar-curcumene also inhibits the expression of cyclo-oxygenase-2 (COX-2), an inducible isoform of prostaglandin H synthase, at the transcriptional level.<sup>10</sup> Furthermore, ginger essential oil contains sesquiterpenoids such as  $\alpha$ -zingiberene,  $\beta$ -sesquiphellandrene and zingiberol, which suppress the release of leukocyte elastase and inhibit the activation of COX-1 and COX-2.<sup>11, 12</sup> Thus, it seems that individual components of ginger essential oil exert both anti-nociceptive and anti-inflammatory effects through different mechanisms; however, the site of action of these compounds remains unclear.

Aromatherapy has been actively adopted in recent times, and is based on the use of fragrances, such as those in essential oils, to alleviate patient symptoms. For example, for patients with lower back pain, the standard approach of orthopedic surgeons has been to inject analgesic or anaesthetic agents to relieve pain via nerve block approaches. However, pain relief via aromatherapy is non-invasive and involves far less discomfort to the patient. Also, many of the active ingredients in essential oils can be absorbed via the nose, lungs, blood vessels, and skin, thus providing significant treatment modalities in the event that patients cannot be treated orally. The present studies were designed to demonstrate the possible involvement of spinal nerve activity suppression by essential oils to regulate the transmission of pain stimuli in rats via anti-inflammatory and analgesic effects.

In this way, differences in the effects of ginger essential oil and a ginger cell-extract on anti-nociceptive and anti-inflammatory effects were investigated in rats and mice.

## MATERIALS AND METHODS

### 1. Animals

Male Sprague-Dawley rats (5 weeks old at arrival) weighing 125–150 g, and male ICR mice (5 weeks old at arrival) weighing 25–30 g were obtained from Japan SLC (Inc.). Animals were placed into experimental groups and housed in polypropylene cages at a temperature of  $24 \pm 1^\circ\text{C}$  with a 12 h : 12 h dark-light cycle, and provided with free access to standard pellet feed and filtered water. All experiments were carried out between 08:00 am and 17:00 pm in a quiet laboratory room. Animals were habituated to the laboratory environment for 1 week prior to the experiments.

All animal experiments were approved by the Ethical Board for Animal Experiments of Showa University (Approval No. 02136, 03007), Tokyo, Japan.

### 2. Randall Selitto assay using a PAM device

We chose a natural skin care gel product, Mizunoirodori, containing 1,3-butylene glycol, hypromellose, carboxy vinyl polymer, and triethanolamine as the vehicle. Two essential oils produced for domestic use were used: Gin-

ger (*Zingiber officinale*: Charis Seijo Co., Tokyo), and True lavender (*Lavandula officinalis*: Hyperplants, Saiseisya Co., Ltd., Japan). These were diluted individually in approximately 1.0 g of the vehicle to provide a final concentration of 2% essential oil.

A novel tool for measuring mechanical pain threshold, the Pressure Application Measurement (PAM) device (UGO BASILE, Inc. (B)), was used to perform the Randall Selitto assay. After the habituation period, rats were divided into 4 groups ( $n=6$  animals per group). Five consecutive stimulations were applied to the right hindpaw of rats by using the PAM device, and the maximum pressure applied before a withdrawal reflex was elicited was measured. Measurements were repeated five times and the average value calculated. The vehicle or 2% essential oil emulsion was then applied to the skin of the right hindpaw of the control or oil group, respectively, and rubbed in lightly with one hundred circular movements over a 3 min period. The withdrawal reflex was measured 10 min, 1, 2, and 3 h after application, and the analgesic effect analyzed by comparing the difference in pain threshold before and after treatment with essential oil or vehicle.

### 3. Counting of spinal c-Fos neurons

Immunostaining for c-Fos was used as an indicator of the activation by pain stimuli of gene expression in the spinal cord dorsal horn neurons. As such, the analgesic effect of ginger oil application was verified in terms of c-Fos expression.

Ginger essential oil was mixed with the vehicle to give a 2% final oil concentration in the emulsion; this was applied to the skin of the right hindpaw of each rat and rubbed in gently as described above. Rats were then subjected to five stimuli applied at a pressure of 1,500 gf ( $=14.7\text{ N}$ ), following which rats were deeply anesthetised with pentobarbital (55 mg/kg i.p.; Sanofi, Paris, France) and perfused intracardially with 200 ml of 0.1 M phosphate-buffered saline (PBS) followed by 500 ml of 4% paraformaldehyde in 0.1 M PBS. The spinal cord was then removed and post-fixed for 4 h in the same fixative, and cryoprotected overnight in 30% sucrose in PBS. Frontal frozen sections, 40  $\mu\text{m}$  thick, were cut and collected in phosphate buffer to be processed immunohistochemically as free floating sections.

Serial sections from the lumbar segment were immunostained for c-Fos-like protein according to the avidin-biotin-peroxidase method.<sup>13</sup> The tissue sections were incubated for 30 min at room temperature in a blocking solution of 3% normal goat serum in 0.1 M PBS with 0.3% Triton-X and then incubated overnight at  $4^\circ\text{C}$  in the primary antiserum directed against the c-Fos protein (Oncogene Science). The Fos antibody, a rabbit polyclonal antibody directed against residues 4–17 of the N-terminal region of the peptide, was used at a dilution of 1: 4,000. The incubated sections were washed 3 times in 1% normal goat serum in 0.1 M PBS with 0.3% Triton-X and incubated in biotinylated rabbit anti-sheep IgG for 1 h at room temperature, then washed twice in 1% normal goat

serum in 0.1 M PBS with 0.3% Triton-X and incubated for 1 h in avidin-biotin-peroxidase complex (Vectastain, Vector Laboratories). Finally, the sections were washed three times in 0.1 M PBS and developed in 1-naphthol ammonium carbonate solution (89.5 ml 0.1 M PBS, 10 ml ammonium carbonate (1% in distilled water), 0.5 ml 1-naphthol (N-199-2 Aldrich, 10% in absolute alcohol) and 0.1 ml hydrogen peroxide) for 5 min, and washed three times in PBS to stop the staining reaction. The sections were mounted on gelatin-coated slides and air dried for stain intensification and made alcohol resistant through basic dye enhancement in 0.025% crystal violet (Aldrich) in PBS for 3 min. After two short rinses in PBS to remove excess stain, the sections were differentiated in 70% alcohol and the differentiation time was evaluated under the microscope. After being air dried, the slides were coverslipped. To test the specificity of the primary antibody, controls were performed: preabsorption with the corresponding synthetic peptide or omission of any stage of the protocol abolished the staining.

The c-Fos-like immunoreactivity was studied on L4 to L5 spinal segments. Tissue sections were first examined using dark-field microscopy to determine the segmental level according to Molander et al. (1984),<sup>14</sup> as well as the grey matter landmarks. The sections were then examined under light-field microscopy at  $\times 10$  magnification to localize c-Fos-positive cells. Labelled nuclei were counted using a camera lucida microscope attachment.

For studies of the different experimental parameters (various delays post CFA injection and various CFA doses) for each rat, two calculations were made: (1) the total number of Fos-like immunoreactive neurons in the grey matter for 15 sections through L4-L5 segments (three sections per segment), (2) The number of c-Fos-like immunoreactive neurons per segmental level. For this purpose, four regions were defined: superficial dorsal horn, i.e. laminae I and II; nucleus proprius, i.e. laminae III and IV; neck of the dorsal horn, i.e. laminae V and VI; and the "ventral horn," i.e. laminae VII, VIII and X (ventral).

#### 4. von Frey Test using the adjuvant-induced inflammation model

We used a previously described unilateral adjuvant-induced inflammation model.<sup>15, 16</sup> In this model, the right hindpaws of mice were injected subcutaneously with 20  $\mu$ l of complete Freund's adjuvant (CFA: mycobacterium tuberculosis; Sigma-Aldrich Co., LLC, undiluted) with a 27G needle into the plantar surface. Ginger essential oil (*Zingiber officinale*: Charis Seijo Co., Tokyo) was diluted with Mizunoiroitori to give a 2% final concentration of oil in the emulsion, while Ginger cell-extract (see below; FEC, Ltd., Hyogo) was used in an undiluted manner.

Preparation of the cell-extract involved purification of plant-derived water from cells by a low-temperature vacuum extraction method. The extraction device for preparing the cell-extract consisted of a stirring blade and a movable blade for stirring and crushing plants, a unit for reducing the pressure of the vessel containing

the plant matter, and a heating unit for heating the plant matter in the container. Because the heating temperature of the device is relatively low at 55°C or less, aromatic compounds are subjected to minimal modification. Therefore, it is possible to extract volatile aromatic components from the prepared plant mixture without degrading them. For this, the pressure of the device is set to below  $-80$  kPa. In an environment that does not contain externally added water, the reduced pressure, combined with the heating, stirring and crushing of the plant matter, produces steam in a short time. Because aromatic compounds in the plant matter do not react with solvent or water vapor of external origin, these compounds are obtained in their natural state, thus preventing any degradation in their quality due to overheating or reactions with impurities.

Twenty-four hours after the CFA injection, the vehicle or 0.5 g of 2% ginger essential oil or ginger cell-extract was applied to the skin of the right hindpaw of each mouse (control group, oil group) and rubbed in gently with one hundred circular motions over a 3-min period.

The time course of the inflammatory-related response was assessed by measuring three response parameters (paw diameter, von Frey test, and body weight) as described below.

For the von Frey test, mice were placed on a perforated metallic platform (7 $\times$ 7 mm, each opening), on which transparent cylindrical acrylic boxes (12 cm diameter) were placed to separate the mice individually into compartments. The platform was elevated 30 cm from the table surface so that stimulation could be applied from below. Experiments were commenced after mice had been on the platform for approximately 15 min or until exploration activity ceased. Calibrated von Frey monofilaments (ranging from 0.008 to 2.0 g; The Touch Test Sensory Evaluator Set; Linton Instrumentation, Norfolk, UK) were applied perpendicularly to the mid-plantar surface of animals' right hind limb using enough pressure to curve the filament. Withdrawal of the hind limb was considered a valid response when the animal withdrew its limb completely from the platform.<sup>17, 18</sup> Paw elevation caused by normal locomotor activity was ignored. In the absence of a response to the stimulus, the next strongest filament was applied. When a valid response was observed, the filament of the next lowest force was applied to confirm the previously obtained response. The test was repeated three times at intervals of 5 min, and the average value in grams was calculated.

After completion of the von Frey test, the thickness of the foot was measured as an index of edema. Measurements were made with calipers before the experiments, and at various times after the injection of CFA as previously described.<sup>19</sup>

#### 5. Gas chromatography-mass spectrometry (GC-MS) analysis

The separation and identification of compounds in ginger essential oil and ginger cell-extract were performed using a Shimadzu GC-17A gas chromatograph

equipped with a QP-5000 quadrupole mass analyzer (Shimadzu Scientific Instruments, Inc., Columbia, MD). Prior to analysis, 20  $\mu$ l of freshly defrosted ginger essential oil and ginger cell-extract were dissolved in 1,000  $\mu$ l of pentane. One microliter of this dissolved extract was injected manually into the gas chromatograph using a 1:50 inlet split ratio and helium as the carrier gas at a flow rate of 1.4 ml/min. The gas chromatograph contained a nonpolar RTX-5MS column (30 m length, 0.25 mm ID, 0.25  $\mu$ m film thickness; Restek Corporation, Bellefonte, PA, USA). The initial column temperature was 70°C, which was ramped up at 4°C/min to 180°C. Electron ionization detection was in the full-scan, positive ion mode over a mass-to-charge ratio ( $m/z$ ) range of 41 to 300. Compounds were tentatively identified by searching a NIST library and by comparison of arithmetic retention indices to values reported by Adams.<sup>20</sup>

## 6. Statistical analysis

Results are expressed as the mean  $\pm$  standard error of the mean (*S.E.M.*). The statistical significance of differences between groups was analyzed by one-way and two-way analysis of variance (ANOVA) followed by the Bonferroni multiple comparisons test. A value of  $p < 0.05$  was considered to indicate statistical significance.

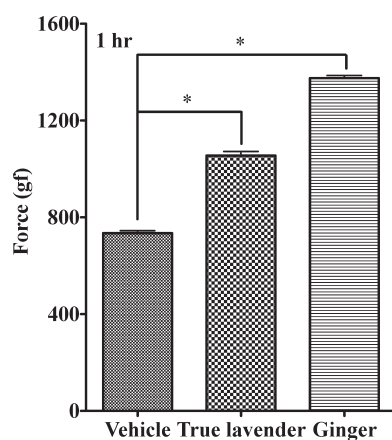
## RESULTS

### 1. Randall Selitto assay using PAM device

Application of ginger essential oil to the hindpaw induced a significant increase in the threshold for 1 h after administration (Fig. 1,  $F_{2, 15} = 571.42$ ), indicating that this essential oil may have anti-nociceptive actions against pressure-induced pain in rats. True lavender essential oil also provided an increased threshold for pain for 2 h, while the analgesic effect of ginger essential oil was maintained for more than 3 h.

### 2. c-Fos-positive spinal neuron counts

To narrow down the region implicated in the anti-nociceptive effects of ginger essential oil, we measured

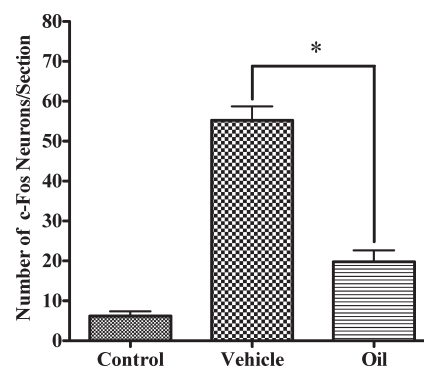


**Fig. 1** Effects of true lavender and ginger essential oils on pressure-induced nociception using the PAM device in rats 1 h after administration. Values are expressed as the mean  $\pm$  SEM. \*  $p < 0.001$  compared with control group.

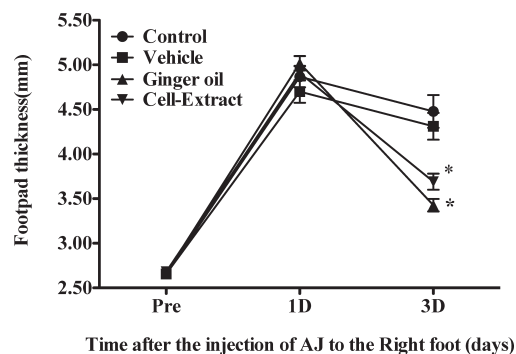
c-Fos-positive neuron counts in the spinal cords of rats treated, or not, with ginger essential oil. Counts in the dorsal horn of the spinal cord were  $6.2 \pm 2.59$  in vehicle-treated rats compared with  $55.2 (\pm 7.92)$  in vehicle-treated rats subjected to hindpaw pressure stimulation (PAM device) (Fig. 2,  $F_{2, 12} = 87.17$ ). These pressure-induced increases of c-Fos-positive cells in the dorsal horn of the spinal cord were almost completely suppressed by pre-treatment of the foot pad with ginger essential oil. Thus, it seems that ginger essential oil inhibits pain transmission at the spinal cord level or in primary sensory neurons in the dorsal root ganglia.

### 3. Adjuvant-induced inflammation model

The injection of CFA into the right hindpaw of mice induced swelling, with a peak in paw edema seen 1 day after CFA injection. Ginger essential oil and the ginger cell-extract potently and significantly suppressed the paw edema induced by CFA at day 3 (Fig. 3,  $F_{3, 108} = 5.78$ ). However, no differences were observed between ginger cell-extract and ginger essential oil with respect to anti-inflammatory effects. Similarly, injection of CFA into the right hindpaw of mice caused a decrease in the reaction threshold as measured by the von Frey test (Fig. 4,



**Fig. 2** Effects of ginger essential oil on PAM stimulation induced increase of number of c-Fos positive neurons in the L4-5 segments of the rat spinal cord. Values are expressed as the mean  $\pm$  SEM. \*  $p < 0.01$  compared with control group.



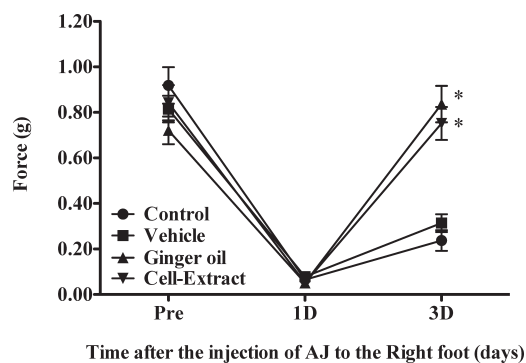
**Fig. 3** Effects of ginger essential oil and ginger cell-extract on CFA-induced swelling at day 4 by evaluating the footpad thickness. Values are expressed as the mean  $\pm$  SEM. \*  $p < 0.001$  compared with vehicle group and control group.



$F_{3,108}=6.03$ ). CFA did not affect the body-weight of mice. These results suggest that a constituent compound in ginger produces a robust analgesic action against inflammatory-related nociception.

#### 4. GC-MS analysis

Chromatograms for ginger essential oil and ginger cell-extract are presented in Figs. 5 and 6, while Table 1 presents data for the relative volatile compound contents of ginger essential oil and ginger cell-extract, expressed as percentages of the area under the chromatograms. For the GC/MS analysis of ginger essential oil, 15 compounds were identified in the oil obtained in a 0.4% (v/w) yield. Among them,  $\alpha$ -zingiberene (29.7% of the total area),  $\alpha$ -curcumene (12.2%), and  $\beta$ -sesquiphellandrene (11.3%) were the major constituents. On the other hand, GC/MS analysis of the ginger cell-extract identified 13 compounds in the oil obtained in a 0.4% (v/w) yield.



**Fig. 4** Effect of ginger essential oil or ginger cell-extract on CFA-induced allodynia as measured by the von Frey test in mice. The vertical axis represents the paw withdrawal threshold using von Frey filaments measured in grams. The horizontal axis represents the day of testing at baseline (PRE) and on day 1 (1D), and day 3 (3D). Values are expressed as the mean  $\pm$  SEM. \*  $p < 0.001$  compared with vehicle group and control group.

These included  $\alpha$ -pinene (21.6%), 1,8-cineole (19.1%),  $\alpha$ -zingiberene (6.8%), and borneol (6.7%) as the major components.

While it seems that the ginger cell-extract and ginger essential oil have similar potencies for producing anti-inflammatory effects, it also appears that the mechanisms underlying these effects are different.

## DISCUSSION

In agreement with previous studies,<sup>21)</sup> at different times after the intra-plantar injection of CFA, c-Fos-positive neuron counts increased in the ipsilateral spinal cord and were localized predominantly in the superficial (I-II) and deep (V-VI) laminae of the dorsal horn of the L4-L5 segments. This laminar pattern coincides with the spinal areas containing neurons activated by noxious stimuli.<sup>22)</sup>

According to Nackley AG et al., a peripheral cannabinoid mechanism suppresses dorsal horn c-Fos protein expression and pain behavior in a rat model of inflammation.<sup>23)</sup> Our results demonstrating the inhibition of c-Fos expression by ginger plant cell-extract and ginger essential oil are thus indicative of a peripheral analgesic action of compounds in these agents.

Aromatherapy is the most widely used complementary therapy used in palliative care, providing relaxation, stress relief and emotional/psychological support; hence its often being referred to as “holistic aromatherapy.” It is generally provided in the form of aroma massage. The understanding of the term “clinical aromatherapy” is evolving as an advanced level of aromatherapy practice where essential oils are also used for symptom management. In the UK, the integration of clinical aromatherapy as part of specialist palliative care has provided some of the best available evidence to substantiate claims of essential oils acting as anti-microbial, anti-inflammatory, analgesic and anti-spasmodic, anxiolytic and sedative agents.<sup>24)</sup> The number of facilities using aromatherapy is

**Table 1** Chemical composition of ginger essential oil and ginger cell-extract

	Ginger essential oil		Ginger cell-extract	
	Area (%)	RT (min)	Area (%)	RT (min)
$\alpha$ -Pinene	2.2	5.7	21.6	6.4
Camphene	6.9	6.5	1.2	6.8
<i>d</i> -Limonene	0.7	11.0	0.0	—
$\beta$ -Phellandrene	3.4	11.3	1.2	11.5
1,8-Cineole	2.7	11.5	19.1	11.7
Terpinolene	0.24	15.6	3.8	16.3
Linalool	0.08	16.9	1.8	17.3
Borneol	1.5	21.7	6.7	21.8
$\alpha$ -Terpineol	0.6	23.1	2.6	23.4
$\beta$ -Elemene	1.7	30.1	2.2	26.5
$\beta$ -Bisabolene	4.9	32.9	0.0	—
$\alpha$ -Zingiberene	29.7	33.4	6.8	33.3
$\alpha$ -Farnesene	3.4	33.6	0.6	33.7
$\beta$ -Sesquiphellandrene	11.3	34.1	4.2	34.1
Total	81.4	75.3		

RT: retention time (min).

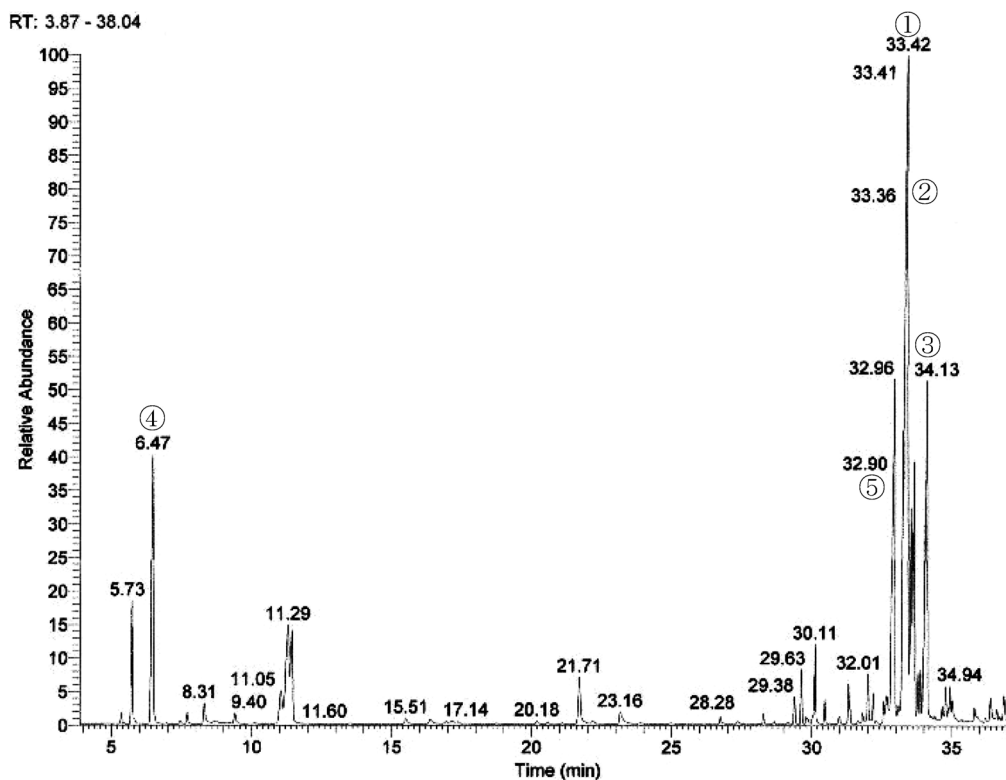


Fig. 5 Gas chromatogram of ginger essential oil. Peaks : ①  $\alpha$ -zingiberene, ②  $\alpha$ -curcumene, ③  $\beta$ -sesquiphellandrene, ④ camphene, ⑤  $\beta$ -bisabolene.

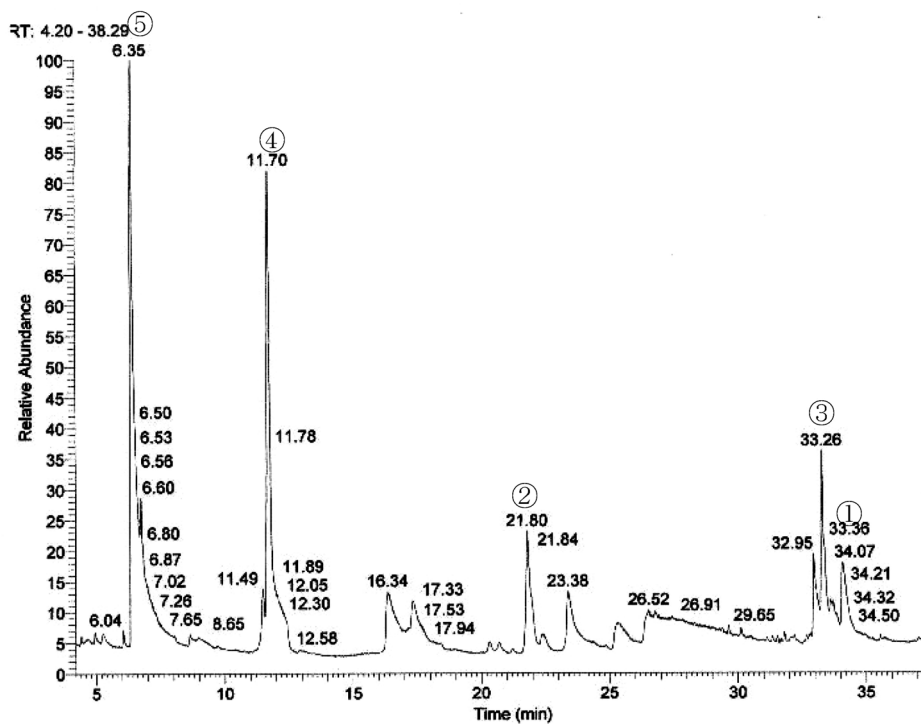


Fig. 6 Gas chromatogram of ginger cell-extract. Peaks : ①  $\alpha$ -pinene, ② 1,8-cineole, ③  $\alpha$ -zingiberene, ④ borneol, ⑤  $\beta$ -sesquiphellandrene.

growing constantly, particularly in relation to hospitals.

Zingiberene, gingerol, and shogaol are well-known components of ginger that have anti-inflammatory effects. It is known that oils with these as constituents inhibit the inflammatory mediators COX-2 and prostaglandin.<sup>25)</sup> However, it has not been clarified as yet which components exert anti-inflammatory effects.

In the present study, ginger cell-extract and ginger essential oil produced anti-nociceptive effects accompanied by anti-inflammatory effects. The gas chromatography analysis to verify the results concerning the anti-inflammatory effects of these two agents indicated that the order of major constituents providing these effects in the ginger cell-extract was totally different from that of ginger essential oil. The present study showed that these two agents had similar potencies for providing anti-inflammatory effects. We also found here that ginger and true lavender essential oils had anti-nociceptive effects in rats. Interestingly, true lavender essential oil also exerts robust anti-anxiety effects in mice (Mori et al., unpublished observation), meaning that this oil could serve as an effective adjunct in palliative care medicine.

It is well known that anti-cancer agents reduce appetite as one of their side effects.<sup>26)</sup> According to the report of Shoji et al., orally administered yokukansan (Kampo medicine), an anxiolytic agent, suppressed c-Fos expression in the spinal cords of rats subjected to restraint stress,<sup>27)</sup> thus revealing that c-Fos expression is not only involved in nociception. Given the results of the present experiments, the causal relationship between the anti-anxiety effects of true lavender essential oil and the suppression of c-Fos expression requires further research.

Gilligan reported that a blend of *Pimpinella anisum* (aniseed), *Foeniculum vulgare* var. *dulce* (sweet fennel), *Anthemis nobilis* (Roman chamomile) and *Mentha piperita* (peppermint) reduces symptoms in patients suffering from nausea in a hospice and palliative care program.<sup>28)</sup> Moreover, aroma massage was shown in one study to be more effective than conventional drugs to treat opioid-induced constipation in patients with advanced cancer.<sup>29)</sup>

Gethin et al., in a survey in which 1,444 representatives of healthcare organisations across 36 countries provided responses, reported that a blend of true lavender, lemon grass, and lemon essential oils in the form of a room spray was effective, according to patients and caregivers, for reducing the smell of necrotic tissue.<sup>30)</sup> Essential oils such as true lavender may also alleviate anxiety in oncology patients. Recently, we found that systemic injection of lavender oil robustly suppressed anxiety-like behavior in mice placed in an elevated plus maze.<sup>31)</sup> This finding indicates that constituents in essential oils may be able to regulate central nervous system activity, thus contributing to the alleviation of anxiety.

In summary, the properties of essential oils discussed here may be highly valuable for use in palliative care environments. The results presented here indicate that

several essential oils have anti-nociceptive and anti-inflammatory effects. While further research is required, our findings add to the body of knowledge underlying the importance of essential oils as an adjunct to standard medications in the treatment of patient symptoms.

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