The purpose of the present study was to examine the antidepressant-like effects of an aqueous extract of lavender (LAE) using the forced swimming test (FST), the most widely used animal model of depression. LAE was orally administered to rats three times, i.e., 24, 5, and 1 h prior to the FST. The administration of LAE (3428 mg/kg body weight) showed a significant reduction of the immobility time in the FST, the effect of which was comparable to that of the synthetic antidepressant, imipramine (30 mg/kg). In addition, the same dose of LAE did not change the locomotor activity in the open field test. These results suggest that LAE might have antidepressant-like effects that are independent of motor stimulation. Furthermore, the active ingredients of LAE were suggested to be non-volatile constituents, because linalool, the main aroma constituent of lavender, was completely removed during the preparation of LAE. Possible contribution of rosmarinic acid and that of apigenin glycosides to the antidepressant-like effects of LAE were discussed on the basis of their content in LAE.

Keywords: lavender, depression, antidepressant-like, forced swimming test

Introduction
Depression has become a common psychological illness in recent years. According to an investigation by the World Health Organization International Consortium of Psychiatric Epidemiology (WHO-ICPE), 6.3 – 15.7% of the world’s population has been estimated to get depression once in their life (Andrade et al., 2003). Exposure to chronic stress is known to be associated with a higher incidence of depression, though its mechanism has not been elucidated. Although a wide variety of antidepressant drugs are available to treat depression, most of the synthetic drugs are not without side effects. Therefore, the search for regularly eaten foods with an antidepressant activity seems to be an essential approach to finding an effective antidepressant treatment without side effects.

Lavender (Lavandula angustifolia Mill.) is a famous herb that has a long history in folk medicine and is still therapeutically used today. The essential oil obtained by steam distillation from the fresh flowering tops of this plant is often used in aromatherapy as a relaxant (Lis-Balchin, 1996). Inhalation of the vapor of the lavender essential oil and its main constituent, linalool, has shown sedative effects in both human and animal studies (Buchbauer et al., 1991; Buchbauer et al., 1993; Diego et al., 1998; Kuroda et al., 2005). Other pharmacological effects of this oil, including anticonvulsive (Yamada et al., 1994), anxiolytic (Bradley et al., 2007), antidepressive (Seol et al., 2010), and anticonflict effects (Umezu et al., 2006), have also been reported.

On the other hand, lavender is also used as a tea infusion (i.e., aqueous extracts) to treat restlessness, insomnia, and nervous disorders of the stomach and intestines (Blumenthal et al., 1998). Furthermore, lavender contains aqueous phenolic constituents, such as hydroxycinnamic acids and fla-
vone glycosides (Harborne and Williams, 2002), which have been associated with the antioxidant activities of Lamiaceae plants including lavender (Zheng and Wang, 2001; Dorman et al., 2004; Shan et al., 2005; Torras-Claveria et al., 2007). However, in contrast to the essential oil, few studies have investigated the medicinal properties of the aqueous extracts of lavender.

The purpose of the present study was to examine the antidepressant-like effects of an aqueous extract of lavender (LAE) by using the forced swimming test (FST), the most widely used animal model of depression.

Material and Methods

Animals 6-week-old male Wistar rats (110 – 130 g) were obtained from Japan SLC (Shizuoka, Japan). The rats were group-housed (seven rats/cage; cage size: 420 × 263 × 199 mm height) in a room at a constant temperature (23 ± 1°C) and humidity (50 ± 5%), and a 12-hour light-dark cycle (light: 7:00 – 19:00). Controlled purified diets were prepared according to CE-2 (CLEA Japan, Inc., Shizuoka, Japan). The animals consumed the diets and tap water ad libitum. All animals were handled daily for 7 days to familiarize them with the experimental equipment and to minimize nonspecific stress responses during the experiment. Behavioral tests were performed between 13:00 and 17:00 h. A different set of animals was used for each behavioral test. All experimental procedures were in accordance with the Guidelines of the University of Shizuoka for the Care and Use of the Laboratory Animals, based on those of the American Association for Laboratory Animal Science.

Chemicals Imipramine hydrochloride was purchased from Nacalai Tesque (Kyoto, Japan). (−)-Linalool and rosmarinic acid were purchased from Sigma-Aldrich Japan (Tokyo, Japan). Apigenin was purchased from Wako Pure Chemical Industries (Osaka, Japan). tert-Butylhydroquinone was purchased from Kanto Chemicals (Tokyo, Japan). (-)-Linalool and rosmarinic acid, and apigenin aglycone, respectively, and the UV spectra wavelengths of 210, 310, and 350 nm were used for the quantification of linalool, rosmarinic acid, and apigenin aglycone, respectively, and the UV spectra (wavelengths from 200 to 400 nm) plus retention time were used for their identification.

Preparation of LAE One hundred grams of dried lavender (Lavandula angustifolia Mill.) flowers were extracted with 2 L of distilled water at 90°C for 1 h. The extract separated from the residue was evaporated in vacuo and lyophilized to give 25 g of LAE. The LAE was stored in a desiccator at 4°C until needed.

Treatments LAE and imipramine hydrochloride were dissolved in distilled water just before use. All solutions were orally administered at a volume of 1.0 mL/100 g body weight of the rat. The control group only received distilled water.

FST procedure The FST was performed according to the method of Porsolt et al., with some modifications (Porsolt et al., 1977, Kageyama et al., 2010). Briefly, 35 male rats were divided into five groups (seven rats per group): one group served as the control, another group received imipramine (30 mg/kg body weight), and the other three groups received LAE at three different doses (857, 1714, and 3428 mg/kg). The rats were placed in an acrylic cylinder (45 cm × 19.2 cm i.d.) filled with water at 25 ± 1°C to a depth of 17 cm for 15 min without any treatment (pre-test session). Twenty-four hours later, the rats were exposed to the same conditions for 5 min (test session). Between the pre-test and test sessions, the test solutions were orally administered three times, the first just after the pre-test session, then 5 h before the test session, and finally 1 h before the test session. Time spent by the rats in active movement and immobility was recorded during the 5-min test session by a video camera. The tapes were evaluated by observers not informed of the treatment that each animal had received. After each test, the tank was refilled with fresh water.

Open field test (OFT) procedure The locomotor activity in a novel environment was recorded employing the OFT. Fourteen male rats were divided into two groups (seven rats per group): one group served as the control and the other group received LAE at a dose of 3428 mg/kg. The test solutions were orally administered three times, i.e., 24, 5, and 1 h before the test. The arena was a circular black box (70 cm diameter and 40 cm height). The test was begun by placing the animals at the center of the arena. The test used a CCD camera (2 frames/s) and a computer video tracking system Image OF (O’harara & Co., Ltd., Tokyo, Japan), modified software based on the public domain NIH Image program (i). Locomotion (cm) and percent center (%) were analyzed for 5 min. Defecation was estimated by counting the number of fecal boluses deposited in the arena.

High performance liquid chromatography (HPLC) An Agilent 1200 Series HPLC system equipped with a diode array detector (Agilent Technologies, Palo Alto, CA) and a Capcell Pak C18 MG column (250 mm × 4.6 mm i.d.; particle size, 5 µm; Shiseido, Tokyo, Japan) were used. The operating conditions were as follows: column oven temperature, 40°C; mobile phase, a linear gradient from 100% solvent A (water, pH 2.5 adjusted with phosphoric acid/acetonic acid, and apigenin aglycone, respectively, and the UV spectra (wavelengths from 200 to 400 nm) plus retention time were used for their identification.
Determination of total apigenin glycosides in LAE  To determine the total concentration of apigenin glycosides in LAE, hydrolysis was performed by a method of Hertog et al. (1992) with some modifications. Two milliliters of 62.5% aqueous methanol containing 25 mg of LAE and 4 mg of TBHQ were mixed with 0.5 mL of 10 M HCl. After refluxing at 90°C for 4 h, the solution was cooled to room temperature and extracted by adding ethyl acetate (5 mL) and water (3 mL). The ethyl acetate layer was washed twice with water (5 mL), dried over sodium sulfate, and subjected to HPLC analysis. The total concentration of apigenin glycosides in LAE was calculated by the difference between the concentration of apigenin aglycone before and after hydrolysis.

Statistical analysis  Data are expressed as mean ± standard error of the mean (SEM) and were analyzed using Microsoft Excel 2007 (Microsoft Japan, Tokyo, Japan) with the add-in software Statistical Analysis System (OMS Publishing, Saitama, Japan). For the analysis of the immobility time in the FST, a fixed-sequence testing procedure (Dmitrienko et al., 2005) was adopted to control the family-wise type I error rate (FWER) for the two steps of statistical analysis, which were performed in the following pre-specified order: first, the difference between the vehicle control and imipramine (a positive control) was examined by the Student’s t-test at a significance level of 0.05 (two-tailed) in order to assess the validity of the experiment; then, if the result of imipramine was significant, the effect of three different doses of LAE compared to the vehicle control was examined by Williams’ test (Williams, 1972) at a significance level of 0.025 (one-tailed) on the assumption that a dose-dependent effect exists. On the other hand, if the result of imipramine was not significant, the analysis was stopped so that the overall FWER did not exceed a level of 0.05 (two-tailed). In the case of data from the OFT, the difference between the vehicle control and LAE at a dose of 3428 mg/kg was examined by the Student’s t-test at a significance level of 0.05 (two-tailed). Prior to the Student’s t-test and Williams’ test, homogeneity of variance was checked using F-test and Bartlett’s test, respectively, and a significance level of 0.05 was used for each test.

Results  Immobility time in the FST  LAE (857, 1714, and 3428 mg/kg) and imipramine (30 mg/kg) were orally administered to the rats 24, 5, and 1 h before the test. No deaths or evident toxic effects, such as vomiting, diarrhea or abnormal behavior, were observed in any of the groups. Figure 1 shows the effects of imipramine and LAE on the immobility time in the FST. The mean immobility time and SEM in rats treated with imipramine was 154 ± 16 s, which was significantly lower (t = 3.13, df = 12, p = 0.009) than that of the vehicle control rats (236 ± 21 s), indicating that the test had sufficient sensitivity to detect antidepressant-like action of the positive control drug at a dose previously reported to be effective in the FST in rats (Butterweck et al., 2001). On the other hand, the immobility time in rats treated with LAE at the low, medium, and high doses was 242 ± 18, 234 ± 12, and 178 ± 23 s, respectively, indicating a dose-dependent reduction of immobility time. Williams’ test revealed a significant effect of LAE at a dose of 3428 mg/kg as compared to the vehicle control group (Williams’ test statistic of 2.17, four groups, df
cess was determined to be 24.2 mg/g on a dry weight basis, whereas linalool was not detected after the evaporation-lyophilization process (Fig. 2).

Concentration of rosmarinic acid, apigenin, and apigenin glycosides in LAE

The concentration of rosmarinic acid, apigenin, and apigenin glycosides in LAE was determined as shown in Table 2. The results showed that apigenin presents mostly as glycosides in LAE.

Discussion

The FST (Porsolt et al., 1977) is the most widely used animal model for screening potential antidepressant activity. = 24, p < 0.025 [one-tailed]). The percent of reduction in the immobility time in rats treated with LAE (3428 mg/kg) was 25%, which was comparable to some extent to that of the positive control imipramine (35%).

Locomotor activity in the OFT LAE (3428 mg/kg) was orally administered to the rats 24, 5, and 1 h before the test. Table 1 shows the effects of LAE on the locomotion (cm), time spent in the center of the arena (%), and number of defecations in the OFT, among which the last two parameters are used as indicators of anxiolytic effects (Prut and Belzung, 2003). The administration of LAE did not significantly affect any of these parameters in the OFT but reduced the locomotion (p = 0.13) by 14% (95% confidence interval: −5 to +32%). This might suggest a mild sedative action of LAE. A number of antidepressants including imipramine have also been reported to decrease the open field activity in rats at doses that exert antidepressant action in the FST (Porsolt et al., 1978).

Concentration of linalool in LAE before and after the evaporation-lyophilization process The concentration of linalool in LAE before the evaporation-lyophilization process was determined to be 24.2 mg/g on a dry weight basis, whereas linalool was not detected after the evaporation-lyophilization process (Fig. 2).

Concentration of rosmarinic acid, apigenin, and apigenin glycosides in LAE The concentration of rosmarinic acid, apigenin, and apigenin glycosides in LAE was determined as shown in Table 2. The results showed that apigenin presents mostly as glycosides in LAE.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (μmol/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rosmarinic acid</td>
<td>37.1</td>
</tr>
<tr>
<td>Apigenin glycosides</td>
<td>5.5</td>
</tr>
<tr>
<td>Apigenin</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

Table 1. Effects of LAE on the OFT in rats.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>LAE (3428 mg/kg)</th>
<th>t (df = 12)</th>
<th>p (two-tailed)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Locomotion cm</td>
<td>1909 ± 67</td>
<td>1647 ± 147</td>
<td>1.63</td>
<td>0.13</td>
</tr>
<tr>
<td>Center %</td>
<td>27 ± 2</td>
<td>24 ± 2</td>
<td>1.15</td>
<td>0.27</td>
</tr>
<tr>
<td>Defecation Number</td>
<td>0.8 ± 0.6</td>
<td>0.5 ± 0.5</td>
<td>0.24</td>
<td>0.82</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM (n = 7). * Student’s t-statistic. # Degrees of freedom.

Fig. 2. HPLC chromatograms of LAE before (A) and after (B) the evaporation-lyophilization process. A wavelength of 210 nm was used for recording the chromatograms.
The immobility displayed by rodents when subjected to an unavoidable stress such as forced swimming was assumed to be a state of despair, in which the animal has learned that escape is impossible (Porsolt et al., 1977). Additionally, the immobility time in the FST has been shown to be reduced by treatment with known antidepressant drugs (Porsolt et al., 1977; Porsolt et al., 1978). Moreover, a strong correlation has been reported between the clinical efficacy of the antidepressant drugs and their potency in the FST (Willner, 1984). Nevertheless, psychostimulants, such as d-amphetamine and caffeine, have been reported to decrease the immobility time in the FST apparently due to motor stimulation rather than to persistent attempts to escape (Porsolt et al., 1977; Porsolt et al., 1978). These false-positive results can be excluded by an OFT, in which clinically effective antidepressant drugs reduced the locomotor activity, but psychostimulants markedly increased it (Porsolt et al., 1978). In the present study, the oral administration of LAE to rats at a dose of 3428 mg/kg showed a significant reduction of the immobility time during the FST, the effect of which was comparable to that of the synthetic antidepressant imipramine at a dose of 30 mg/kg (Fig. 1). In addition, the same dose of LAE did not affect the locomotor activity during the OFT (Table 1), indicating that the antidepressant-like effect of LAE in the FST cannot be attributed to motor stimulation.

In this study, an antidepressant-like effect of LAE in rats was demonstrated after acute treatment (three administrations in 24 h) at a relatively high dose of 3428 mg/kg. On the other hand, clinical efficacy of antidepressants such as imipramine in humans becomes evident after chronic treatment for 2–3 weeks, even though most of them are acutely effective in the FST at doses 10- to 100-fold greater than those used in clinical practice on a milligram per kilogram per day basis (Borsini and Meli, 1988; Detke et al., 1997). In addition, it has been demonstrated that low doses of the antidepressants desipramine and fluoxetine that were inactive in the FST after acute treatment became active after chronic treatment (Detke et al., 1997). These findings suggest that LAE at doses less than 3425 mg/kg might be effective in the FST after chronic treatment and this time course might agree more with possible use of LAE in humans. Thus, chronic treatment remains to be studied in order to fully understand the efficacy of LAE in the FST.

Specific constituents responsible for the antidepressant-like effects of LAE are still unclear. It has been reported that vapor inhalation of the lavender essential oil did not affect or even increase the immobility time in the FST presumably due to its sedative effect (Komiya et al., 2006; Lim et al., 2005). In contrast, intraperitoneal administration of the lavender essential oil was reported to reduce the immobility time in the FST (Seol et al., 2010). These controversial results might be due to differences in the mode of administration. On the other hand, the oral administration of lavender tincture (an aqueous ethanolic extract) was reported to enhance the therapeutic effect of imipramine in depressed patients (Akhondzadeh et al., 2003), whereas the active constituents responsible for this adjuvant effect have not been clarified. In the present study, linalool, the main essential oil constituent of lavender, was completely removed by the evaporation-lyophilization process (Fig. 2). Therefore, the antidepressant-like effects of LAE can be attributed to its non-volatile constituents. Lavender has been reported to contain non-volatile phenolic compounds such as hydroxycinnamic acids and flavone glycosides (Harborne and Williams, 2002), which can be extracted by hot water and were possibly present in the LAE used in this study. Among the phenolic compounds previously found in lavender, apigenin, which is mostly present as a glycoside in lavender (Harborne and Williams, 2002), and rosmarinic acid have been reported to have an antidepressant-like activity in the FST (Takeda et al., 2002a; Takeda et al., 2002b; Nakazawa et al., 2003; Yi et al., 2008).

In order to estimate the contribution of rosmarinic acid and apigenin glycosides to the antidepressant-like effects of LAE, the concentration of these compounds in LAE was determined (Table 2). From these data, the amount of rosmarinic acid in the effective dose of LAE was calculated to be 127 μmol/kg (37.1 μmol/g × 3.428 g/kg body weight). This value is greater than 99.1 μmol/kg, a reported oral dose acutely effective in the FST in mice (Takeda et al., 2002b). Therefore, rosmarinic acid seems to be one of the active compounds for the antidepressant-like effects of LAE. In the same way, the total amount of apigenin glycosides in the effective dose of LAE was calculated to be 18.9 μmol/kg. This value is less than 37.0 μmol/kg, a reported oral dose of apigenin aglycone effective in the FST in mice, not acutely but after 14 days treatment (Yi et al., 2008). In addition, bioavailability of apigenin glycosides in rats might be lower than that of apigenin aglycone, because intestinal permeability of apigenin 7-O-glucoside, an apigenin glycoside found in lavender (Harborne and Williams, 2002), was reported to be much lower than that of apigenin aglycone in both the caco-2 cell culture model and perfused rat intestinal model (Liu and Hu, 2002). Therefore, roles of apigenin glycosides in the efficacy of LAE after acute treatment seem to be less important. Compounds other than rosmarinic acid and apigenin glycosides remain to be investigated for their possible roles in the antidepressant-like effects of LAE.

In conclusion, LAE showed an antidepressant-like effect in the FST after acute treatment and rosmarinic acid was suggested to be one of the active ingredients in LAE. Fur-
ther studies are needed to clarify the efficacy of LAE after chronic treatment and contribution of other compounds to the antidepressant-like effects of LAE.

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