

## Antidepressant-Like Effect of Aromatherapy with *Magnolia sieboldii* Essential Oils on Depression Mice

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**Abstract:** Globally, depression is the fourth most common disorder. It is difficult to treat and prone to recurrent episodes. To elucidate the antidepressant effect and mechanism of action of *Magnolia sieboldii* aromatherapy, the main components of *M. sieboldii* essential oils were analyzed using GC-MS. A mouse model of depression, established by repeated intraperitoneal injection of reserpine, was used to probe antidepressant efficacy. Behavioral tests were used to evaluate depressive behavior in mice. In vivo animal studies, changes in the number of measured neurons were detected using Nissl staining, and GR protein expression was examined using immunohistochemistry. The relative expression levels of 5-hydroxytryptamine (5-HT<sub>1A</sub>) and brain-derived neurotrophic factor (BDNF) were detected using western blot (WB). The results showed that the essential oils of *M. sieboldii* are mainly composed of  $\beta$ -elemene (22.11%), *trans*- $\beta$ -ocimene (14.87%), germacrene D (7.27%), and nerolidol (4.51%). The expression of GR protein was substantially upregulated in the tissues of mice treated with the essential oils of *M. sieboldii*. WB showed that the components of *M. sieboldii* essential oils modulated the expression of BDNF and 5-HT in serum, which remarkably attenuated depressive behavior in mice. In addition, a medium concentration of *M. sieboldii* substantially ameliorated depressive-like behavior in mice and increased the serum levels of 5-HT<sub>1A</sub> and BDNF. This study suggests that a medium concentration of *M. sieboldii* has a prominent antidepressant effect.

**Keywords:** *Magnolia sieboldii*; essential oils; depression; BDNF; 5-HT. © 2022 ACG Publications. All rights reserved.

### 1. Introduction

Depression is a common, complex, and potentially life-threatening mental disorder that causes severe social and economic burdens worldwide [1]. To date, patients with depression are widespread worldwide, but the exact mechanisms of the development of depression remain unclear [2]. The main problem with the treatment of depression is that clinically used antidepressants are extremely limited [3]. Most antidepressant drugs are expensive, and may cause serious side effects [4]. Major depressive

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disorder is characterized by extremely low cure rates, as well as relatively low remission rates, and is often associated with drug resistance and administration difficulties [5-6]. In addition, antidepressant medications have been linked to sexual dysfunction, as well as gastrointestinal and anxiety issues [7]. Challenges facing new approaches to understanding antidepressant action include focusing on early changes in mood and social processes, as well as the role of neuroplasticity [8].

Great progress has been recently achieved in the study of the pathogenesis of depression. A depression model based on KM mice is relatively remarkable [9]. It is commonly used to evaluate the effects and mechanism of action of neurotransmitters in mice. Models of depression caused by depletion of monoamine levels in the brain using the antihypertensive agent reserpine have been widely used to study the mechanisms of depression [10]. Researchers often study patients with depression and find that depression is not just a brain disease; it involves changes in many systems throughout the body, including changes in the gut microbiome [11-12]. Associating detected abnormal changes with the disease, it is difficult to determine whether these changes are the initial causes of depression, whether they are caused after illness, or both [13]. Among these, the monoamine hypothesis provides a foundation for the study of the pathogenesis or clinical treatment of depression [14]. It was believed that depression is mainly caused by an abnormal decrease in monoamine transmitters between synapses. The modern monoamine theory suggests that the adaptation and plasticity regulation of 5-HT and its monoamine autoreceptors are closely related to the treatment of depression. A series of advances has been recently made in the study of 5-HT and its transporters and receptors. These include the discovery of positive effects of short alleles in the chain region of the 5-HT transporter on mood and cognition and the discovery of pharmacological effects of new 5-HT-related receptor antagonists or agonists [15]. The results showed that the 5-HT receptor ligand drugs exhibited antidepressant effects. Therefore, it is of great significance to seek safe and effective antidepressants that target 5-HT, BDNF, and GR protein levels for the treatment of depression.

Essential oils are natural products with a complex composition, a vital part of non-food for humans and are depurated from various aromatic medicinal herbs [16]. They have received increased research attention because they improve sleep quality and attenuate the syndrome of brain-related disorders [17], and can also have a positive impact on mood, stress, and depression [18]. In the Vedas, 5000 years ago, aromatherapy was reported to regulate the central nervous system [19].

*Magnolia sieboldii* is a small deciduous leaf tree of the genus *Magnolia* (Family: Magnoliaceae), and is widely distributed in the eastern coastal areas of China [20]. Its wood can be made into farm tools, and its flowers can be used to extract aromatic oils. Essential oils from the flowers of *M. sieboldii* inhibited the production of NO and PGE<sub>2</sub> by LPS-stimulated peritoneal macrophages [21]. Nevertheless, few studies have been conducted on the antidepressant effects of essential oils from the flowers of *M. sieboldii* using aromatherapy.

This study was conducted to explore the efficacy of *M. sieboldii* essential oils (MSEOs) on depressive behavioral symptoms in the tail suspension test (TST) and forced swimming test (FST). In addition, we assessed the ingredients with antidepressant properties, including sesquiterpenes (the major component of MSEOs). The levels of monoamine neurotransmitters, as well as the hormones associated with the monoamine system and the hypothalamic-pituitary-adrenal gland (HPA) axis, were assessed in the prefrontal cortex and hippocampus. This study investigated the expression of the brain-derived neurotrophic factor (BDNF), GR protein, and tropomyosin receptor kinase B (TrkB) in the hippocampus. These results can provide a theoretical foundation for the effective use of essential oils, as well as aromatic compounds, in the aromatherapy of depression.

## 2. Materials and Methods

### 2.1. Essential Oils and Supplies

The steam distillation method adopted in this study is the most commonly used method to prepare essential oils by using water vapor to remove volatile compounds. Fresh *M. sieboldii* was washed, sliced, air-dried, homogenized, crushed, and placed in a 500-mL distillation bottle through a 20-mesh aperture screen. Distilled water (210 mL) and a certain amount of ionic liquid were added. An electric heating sleeve was used to heat it to boiling. Slight boiling was maintained under atmospheric pressure to

produce a mixture of essential oils and water steam. The obtained oil and water mixture was imported into the oil and water separator. The oil layer was collected to prepare the essential oils of *M. sieboldii*, and the extraction rate of the essential oils was computed. The resulting essential oils were processed using anhydrous sodium sulfate and stored at 4 °C until further use. All sample reagents were of analytical grade and were obtained from Aladdin Biotechnology Company (Shanghai, China). Part of the oil was stored at the Institute of Nature Medicine & Green Chemistry (Guangdong University of Technology, Guangzhou, China) as voucher specimens (No. ZLY-20210620-008). The plant materials were identified by Prof. Nian Liu (Zhongkai University of Agriculture and Engineering, Guangzhou, China), according to the morphological descriptions presented in the China Species Library.

Extraction rate = total mass of essential oils/mass of *M. sieboldii* × 100%

## 2.2. Gas Chromatography-Mass Spectrometry (GC-MS)

The essential oils were analyzed according to the following conditions (Thermo Electron Corporation, USA), using a DB-WAX capillary column (60 m × 0.25 mm × 0.25 μm). Heating procedure: the initial temperature was 70 °C, which was maintained for 2 min, then increased to 120 °C at a rate of 3 °C/min, and finally increased to 230 °C at a rate of 4 °C/min, and the temperature was maintained for 5 min. The flow rate of the carrier gas (H<sub>2</sub>) was 1 mL/min, the inlet temperature was 260 °C, and the sample was injected without a split flow. In addition, the electron impingement ion source, electrons were at 70E energy, the temperature was 280 °C that the transmission line, the ion source was at 230 °C, the quadrupole temperature was 150 °C, the scanning mode was full scan, and the mass scanning scope was 3–600 m/Z.

## 2.3. Experimental Animals

Sixty SPF KM mice (male, 4–5 weeks, 25–30 g, production license number: SCXK20180002) were used in this study. They were subjected to conventional feeding at Guangdong Medical Experimental Animal Center, room temperature 20–26 °C, relative humidity 40–70%, air cleanliness 7, alternate lighting day and night, normal water, and food supply. Acclimatized feeding was performed 1 week before the experiment.

## 2.4. In Vivo Mouse Model

The selected KM mice were divided into six groups (depression model group, fluoxetine-positive drug group, control group, *M. sieboldii* leaf-low concentration group, *M. sieboldii* leaf-medium concentration group, and *M. sieboldii* leaf-high concentration group), according to body weight, with 10 mice in each group. The dosage was based on dose conversion between human and animal body surface area in TCM pharmacological experimental methodology.

The fluoxetine group was injected with fluoxetine hydrochloride 20 mg/kg. The *M. sieboldii* groups (divided into low, medium, and high concentration groups) were sniffed with 625, 1250, and 2500 μL/kg of MSEOs, respectively. The model and control groups were daily sniffed with 0.9% normal saline. All experimental groups, except the control group, received a single intraperitoneal injection of reserpine (6 mg/kg) on day 4 of the experiment. The control group received intraperitoneal injection of normal saline in the same volume on the same day. Behavioral tests were initiated on day 5. The fluoxetine group received an intraperitoneal injection of fluoxetine hydrochloride (20 mg/kg), 30 min before the test.

## 2.5 Behavioral Tests

Depression-related behaviors were assessed using the FST and TST.

For the FST stationary time, the mice were placed in a transparent glass water tank, 25 cm in diameter and 30 cm in height, filled with water at a height of 15 cm. The water temperature ranged from 21 to 25 °C. The total recording time was 360 s. Data were recorded between 120 and 360 s. The percentage of time the mice spent floating, immobile, or with slight limb movement was measured to

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reflect the degree of helplessness. For the TST, the tail of the mice was fixed and kept in an inverted suspension state for 6 min. The amount of time the mice stopped struggling and remained stationary was recorded within the next 4 min. The measurement index was the percentage of time the mice were stationary during suspension to evaluate the degree of helplessness.

Behavioral tests were performed 1 h after the end of daily administration. After the tests, all mice were sacrificed using cervical dislocation. The whole brain was surgically removed for fixation with 4% paraformaldehyde solution or frozen with liquid nitrogen.

### *2.6. Nissl Staining*

Changes in the quantity of nuclei in the hypothalamus, hippocampal formation, and cerebral cortex were detected using Nissl staining. After deep anesthesia, cerebral tissue was extracted by perfusion. Then, it was fixed at 4 °C overnight with gradient glucose precipitation. Coronal hippocampal sections were prepared using a frozen slicing machine, and the thickness of the section was 30 μm. The sections were then placed on tissue culture plates for testing. For Nissl staining: brain sections were rinsed, air dried, and degreased using xylene degreasing. This was followed by gradient rehydration, pipetting 1% tar purple (G1032; Servicebio, Wuhan, China), color fixation using alcohol (75%), alcohol dehydration, xylene degreasing (G10000218; Servicebio), and neutral resin seal sheet.

### *2.7. Immunohistochemical Staining*

Immunohistochemistry was used to detect GR protein expression in the hippocampus. The fixed samples were sectioned in accordance with conventional paraffin sections and the sections were dewaxed in water. Endogenous peroxidase was removed by incubation with hydrogen peroxide for 10 min. The antigen was repaired in 0.02 mol/L of citric acid antigen repair buffer (pH = 6.0) for 10–15 min. BAS (5%) sealing solution was used for 20 min. The sections were incubated with GR antibody (1:1000) at 37 °C for 1 h. Polymerized HRP-labeled antibody (5-HT1A receptor antibody, AF5453, 1:50, Affinity Biosciences, Cincinnati, OH, USA) was added and incubated at 37 °C for 30 min. The samples were gently restained with hematoxylin, dehydrated, made transparent, sealed, and observed under a microscope (Nikon, Japan). The GR protein content in the hippocampus was quantitated using ImageJ™ software (NIH, Bethesda, MD, USA).

### *2.8. Western Blot (WB)*

#### *2.8.1. WB Detection 5-HT1A*

The whole brains of the mice were quickly removed after abdominal anesthesia, and the hippocampus was isolated using an ice bath. Proteins were cleaved using RIPA lysis buffer, and quantitative kits were used to measure protein concentrations (Novagen). The protein concentration in each group was adjusted to 0.5 g/L, and SDS (2×) loading buffer was added. Denaturation was performed at 99 °C for 10 min. Sample (10 μL) was added to each well, Tris-SDS polyacrylamide gel was electro-transferred onto nitrocellulose membrane, and blocked using 5% skimmed milk for 1 h. Then, the membrane was incubated overnight with antibodies for 5-HT1A receptor and PSD-95 at 4 °C. This was followed by washing the membrane using TBST three times, 15 min each. HRP-labeled goat anti-rabbit antibody (1:2000) was added, and the membrane was placed on a shaking table at room temperature for 1 h. The membrane was washed using TBST three times, 15 min each. A gel imaging analysis system was used to detect the western blot bands.

#### *2.8.2. BDNF Protein Measurement*

The hippocampus, hypothalamus, and cerebral cortex were stripped. Total proteins were extracted, and quantified using Bradford method. WB was performed as described above. Membrane was incubated overnight with primary antibody (anti-BDNF rabbit polyclonal antibody; 1:500; Tubulin.

1:1000) at 4 °C or on a shaker at room temperature for 1 h. Then, the membrane was incubated with the secondary antibody (1:5000) on a shaker at room temperature for 1 h, followed by washing with TBST three times. ECL was used for color development and anechoic chamber exposure detection. A gel imaging analyzer was used to scan the color film under visible light and analyze BDNF expression.

### 2.9. Statistical Analysis

Data were presented as the mean  $\pm$  SD. The data were statistically compared using the GraphPad prism software (8.0.2). Turkey's test was used as a post hoc test.  $P < 0.05$  was considered statistically significant.

## 3. Results and Discussion

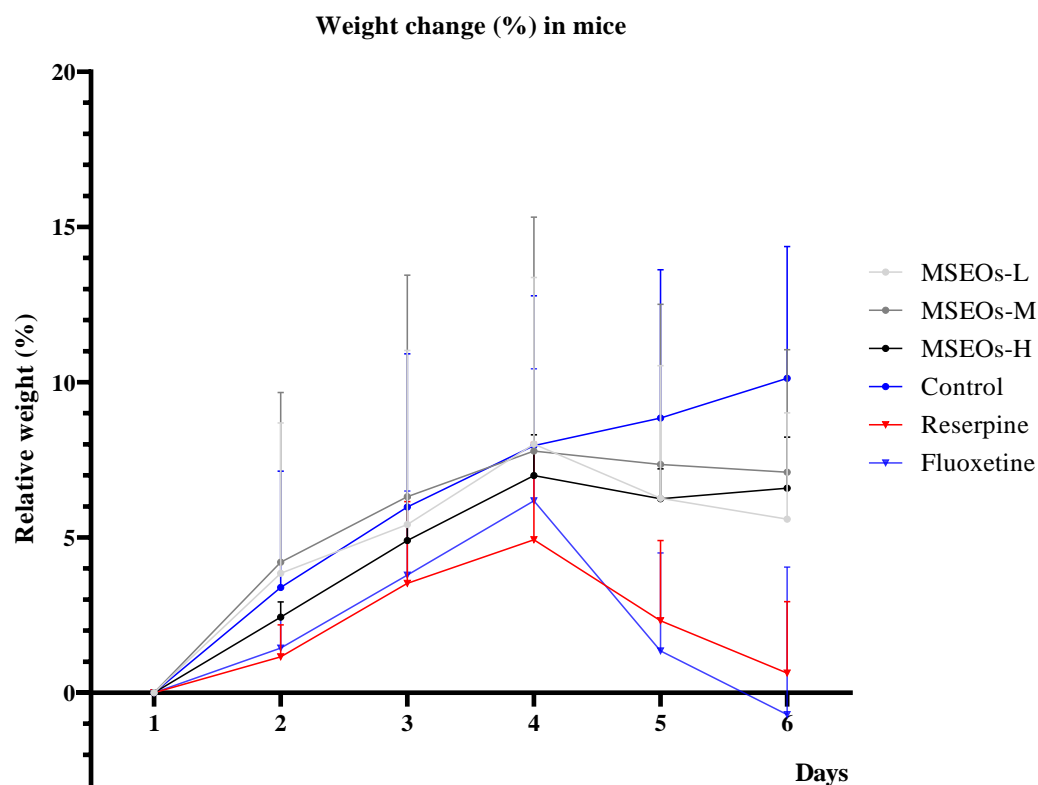
### 3.1. Analysis of Essential Oils Components

Sesquiterpenes are natural terpenes that contain 15 carbon atoms in their molecules. Sesquiterpenoids are widely distributed and abundant in Family Magnoliaceae. They often exist in essential oils in the form of alcohols, ketones, lactones, etc., and are the main components of the high boiling point part of the essential oil. Owing to its strong aroma and biological activity, it is an important raw material in the pharmaceutical, food, and cosmetic industries. The table 1 shows that the essential oils of *M. sieboldii* are rich in terpenoids, especially sesquiterpenoids, with a content of 48.19%. A total of 36 compound base peaks were detected in the essential oil samples.  $\beta$ -elemene (22.11%), *trans*- $\beta$ -ocimene (14.87%), germacrene D (7.27%), nerolidol (4.51%), and *cis*-caryophyllene (3.63%) were the most abundant and the chemical structures of these main compounds are shown in Figure S1. These results indicate that these compounds may be the main therapeutic components of MSEOs in the management of depression. With the development of clinical research on aromatherapy, as a rare surviving plant of the Archean Quaternary ice age, *M. sieboldii* has become a popular ornamental tree species in gardens, and the medicinal functions of its flowers and leaves have gradually attracted attention [22]. The leaves of *M. sieboldii* are rich in magnolol and terpenoids. As one of the anti-inflammatory and antioxidant plants, it has diuretic and detumescent effects. Moreover, it is used to treat carbuncles and cough along with lung deficiency and eliminate phlegm. It is also frequently used in combination with acupuncture and moxibustion to treat hypertension and obesity in traditional Chinese medicine [23]. However, these studies did not shed light on the antidepressant effect of *M. sieboldii*.

### 3.2 Body Weight

A poor appetite is one of the main symptoms of depression. Because chronic lack of appetite, poor digestion, and malabsorption lead to a lack of nutrition and weight loss, depression is further aggravated. The collected data were drawn into a broken line chart of the rate of change in adult weight. The present study showed that the weight of the mice sniffed with MSEOs rapidly recovered after weight loss in the model group. The medium dose of MSEOs showed higher resistance to weight loss compared to that of the model group. This suggests that essential oils may have a therapeutic effect on the loss of appetite and indigestion caused by depression by acting on receptors in the mice nasal cavity, which are recognized by olfactory cells, and stimulate the olfactory area of the brain.

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**Figure 1.** Rate of weight change in mice

**Table 1.** Analysis of essential oils composition of *M. sieboldii* by gas chromatography-mass spectrometry

No	Compounds <sup>i</sup>	RI <sup>ii</sup>	Exp.RI	Ref.	Relative content (%)
1	$\beta$ -Pinene	861	990	[24]	0.13
2	Terpilene	877	1012	[25]	0.87
3	Trans- $\beta$ -Ocimene	895	1039	[26]	14.87
4	$\gamma$ -Terpinene	928	1055	[25]	1.72
5	Terpinolene	978	1308	[27]	0.61
6	fenchon	998	1325	[25]	0.17
7	Linalool	1011	1102	[28]	0.8
8	Alloocimene	1046	1118	[24]	2.91
9	Camphor	1012	1121	[24]	0.12
10	4-Carvomenthenol	1042	1170	[29]	2.99
11	Citronellol	1126	1240	[30]	0.28
12	2-Carene	1246	1001	[31]	0.25
13	Citronellyl acetate	1265	1336	[32]	0.5
14	$\alpha$ -Copaene	1278	1358	[25]	0.3
15	$\beta$ -Elemen	1293	1394	[26]	22.11
16	cis-Caryophyllene	1316	1408	[33]	3.63

Table 1 continued..

No	Compounds <sup>i</sup>	RI <sup>ii</sup>	Exp.RI	Ref.	Relative content (%)
17	$\alpha$ -Guaiene	1335	1587	[25]	0.76
18	Humulene	1347	1447	[26]	1.84
19	$\beta$ -Farnesene	1350	1455	[24]	1.43
20	(+)-Valencene	1354	1494	[31]	0.35
21	Germacrene D	1367	1442	[26]	7.27
22	Curcumene	1373	1483	[34]	0.76
23	$\alpha$ -Bergamotene	1381	1390	[25]	3.49
24	$\alpha$ -muurolene	1385	1472	[26]	0.69
25	$\beta$ -Bisabolene	1390	1478	[26]	1.15
26	$\gamma$ -Cadinene	1395	1514	[28]	0.45
27	$\delta$ -Cadinene	1401	1484	[26]	2.84
28	(-)- $\alpha$ -Selinene	1431	1464	[26]	0.58
29	Nerolidol	1436	1551	[34]	4.51
30	$\alpha$ -Bulnesene	1483	1501	[25]	1.3
31	$\tau$ -Cadinol	1490	1635	[26]	0.55
32	$\alpha$ -Cadinol	1492	1657	[29]	0.83
33	$\alpha$ -Muurolol	1495	1629	[31]	0.38
34	$\tau$ -Muurolol	1501	1617	[25]	2.07
35	(+) spatulenol	1517	1571	[34]	0.72
36	Safranal	1603	1189	[29]	2.71
	Total identified/%				86.94
	Total monoterpenes				21.36
	Total oxygen monoterpenes				0.29
	Total sesquiterpenes				48.19
	Total oxygenated sesquiterpenes				0.72
	Others				16.38

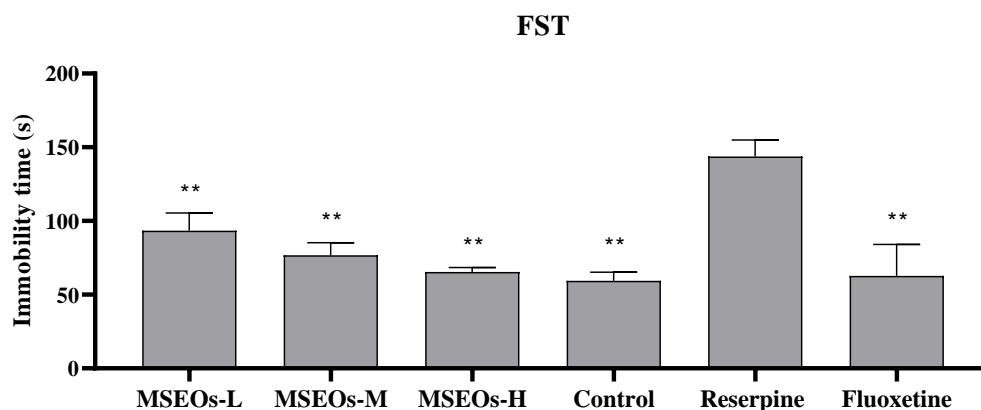
<sup>i</sup> Methyl silicon capillary column (30 m  $\times$  0.25 mm, 0.25 micron film thickness) for elution sequence of listed compounds.

<sup>ii</sup> Retention Index (RI) of N-alkanes (C<sub>6</sub>-C<sub>40</sub>) on the Same Methyl Silicon Capillary Column.

### 3.3. FST

Compared to that of the model group, the FST showed reduced immobility time of MSEOs-sniffed mice. Mice sniffed with the highest dose of MSEOs exhibited the shortest immobility time; thus, the best antidepressant activity. Therefore, it can be speculated that *M. sieboldii* can improve brain activity by stimulating the nerve center of the mouse brain (Figure 2).

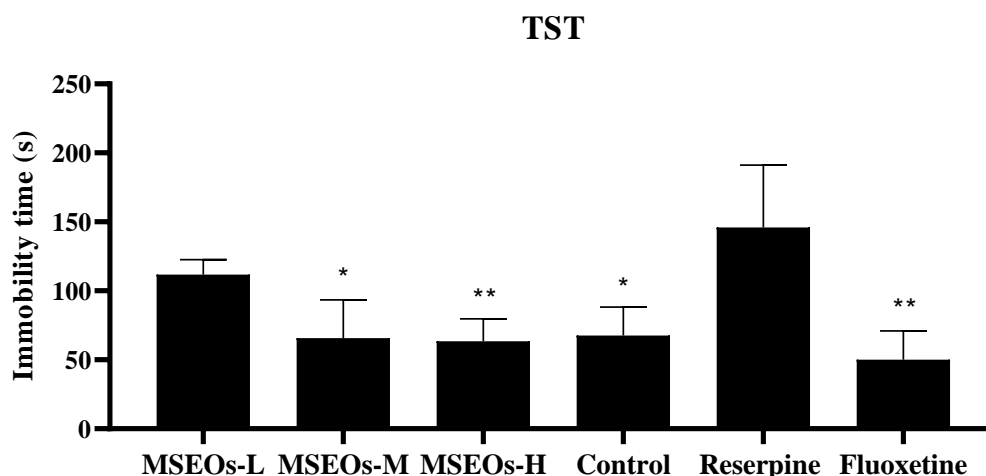
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**Figure 2.** Mouse antidepressant -FST immobility time

### 3.4. TST

Mice sniffed the three concentrations of MSEOs exhibited reduced immobility time in the TST compared to that of the model group. Mice sniffed with the highest concentration of MSEOs displayed the shortest immobility time, compared to that of other groups, which indicated that *M. sieboldii* had a remarkable antidepressant effect. In addition, it can be speculated that the essential oils of *M. sieboldii* can promote the secretion of dopamine and 5-HT (Figure 3).

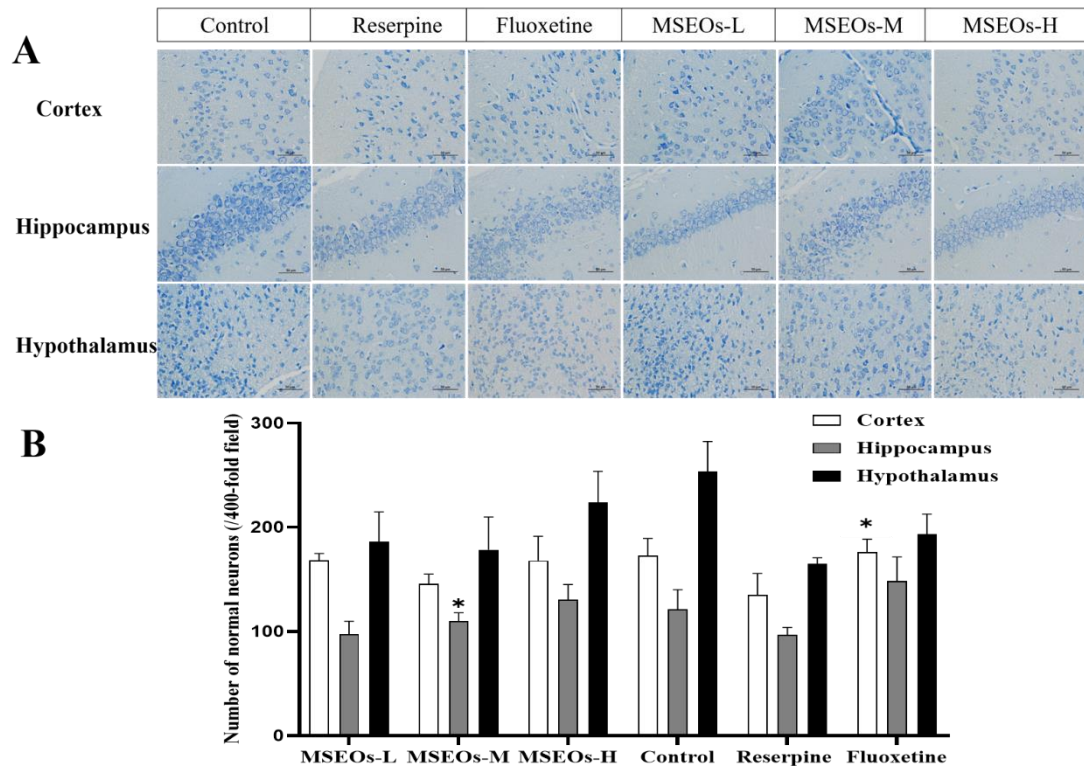


**Figure 3.** Antidepressant -TST immobility time

### 3.5 Nissl Staining

The number of neurons in MSEOs-treated mice treated was higher than that in the model group. In addition, the number of neurons in the hippocampus of the mice sniffed with the medium dose of MSEOs was substantially higher than that of other experimental groups. This suggests that the active components in *M. sieboldii* may play an antidepressant role, by protecting the neurons through the blood-brain barrier (Figure 4).





**Figure 4.** Nissl staining mouse brain tissue

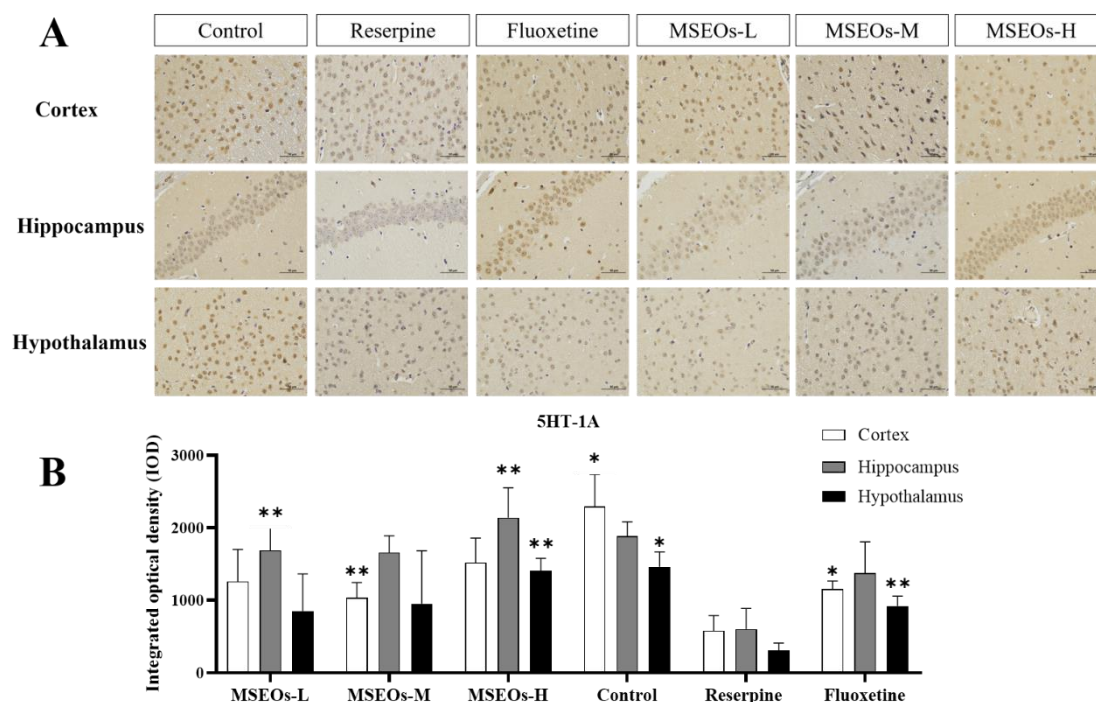
(A) Nissl staining of cerebral cortex, hippocampus and hypothalamus in drug-treated brain tissue. (B) Histogram of the number of neurons in each tissue in Nissl staining. Through Anova and multi-range tests, there was an average differential signal with different super script values ( $P < 0.05$ )

### 3.6. Immunohistochemical Staining

#### 3.6.1. Effect of MSEOs on the Expression of 5-HT1A

Altered activity in the 5-HT system of the brain has been linked to the symptoms of several behavioral disorders symptoms, including depression [35]. According to the modern monoamine hypothesis, the adaptive and plastic regulation of 5-HT1A is closely related to the treatment of depression [15]. Figure 5 shows that, compared with that of the model group, the distribution of 5-HT1A in different parts of the brain tissues of mice in the three groups was increased, and the expression of 5-HT1A in at least one part of each concentration of MSEOs groups was remarkable. Mice sniffed with the highest dose of MSEOs displayed the highest upregulation of brain 5-HT1A expression. Therefore, it can be speculated that the antidepressant effect of *M. sieboldii* can be achieved by its effective components upregulating the expression of 5-HT1A in the brain tissue. In addition, honokiol, as an important regulatory substance, regulates the expression of 5-HT in the brain by inhibiting choline abnormalities and neuronal apoptotic targets, and ultimately increasing the expression of BDNF to achieve antidepressive effects. Improving monoamine levels has recently achieved good results in the study of central nervous system disorders [14].

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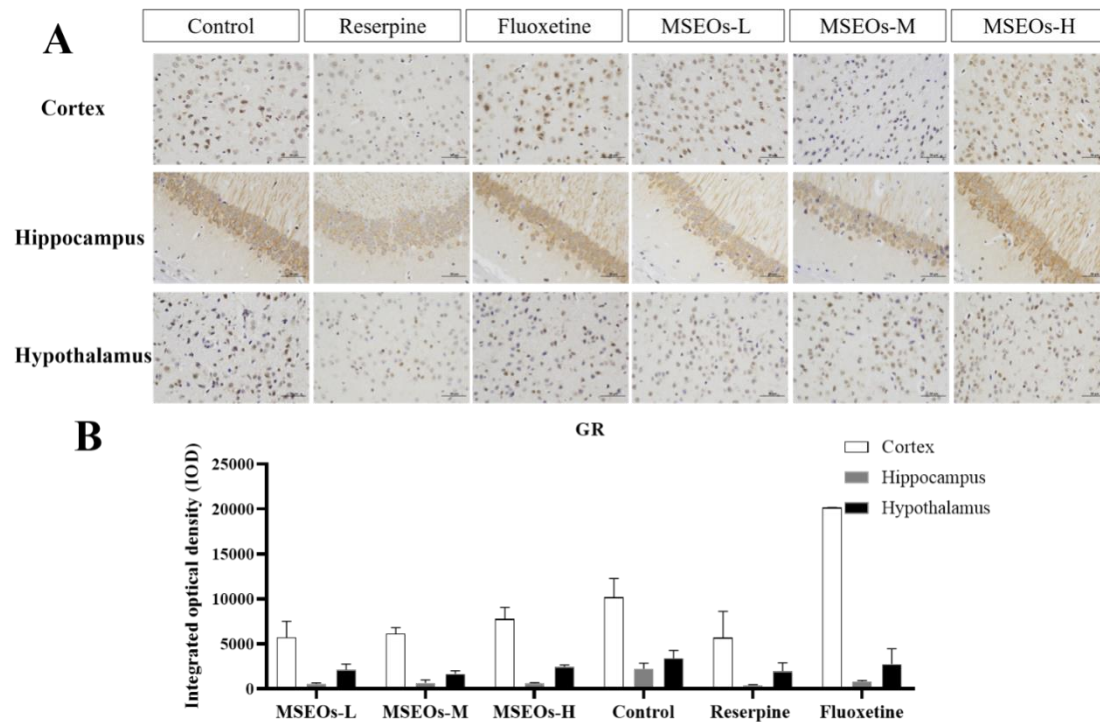


**Figure 5.** Distribution of factor 5HT-1A in mouse brain tissue

(A) Immunohistochemical study of cerebral cortex, hippocampus and hypothalamus in drug-treated brain tissue (9200). (B) Bar chart of 5HT-1A expression in mouse brain tissue. Through anova and multi-range tests, there was an average differential signal with different super script values ( $P < 0.05$ )

### 3.6.2 Effect of MSEOs on The Expression of GR protein

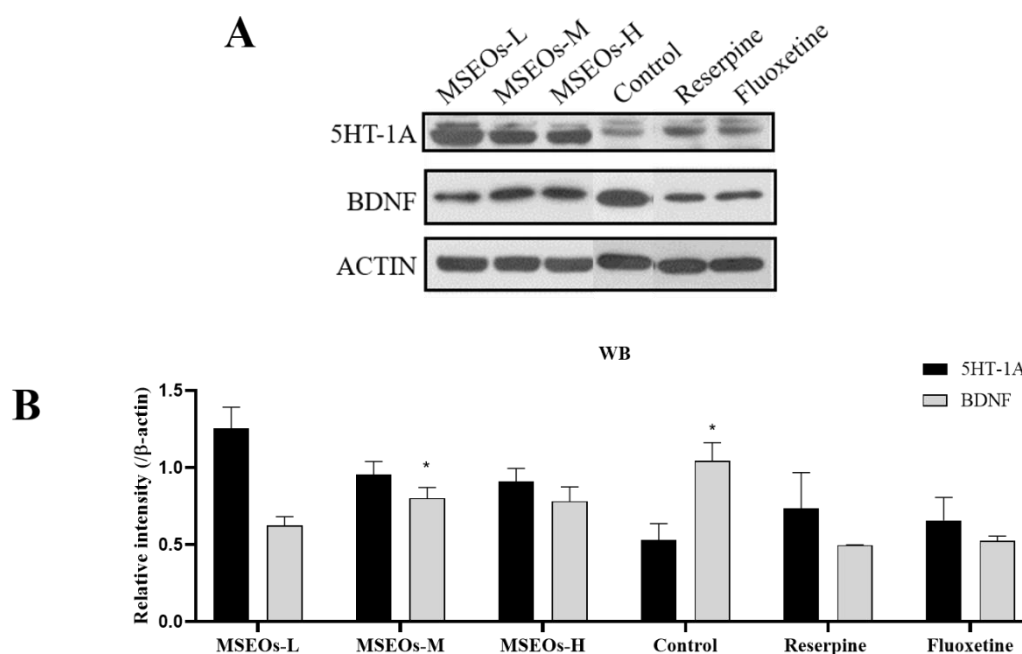
Altered activity in the brain's serotonin (5HT) system has been linked to a variety of behavioral disorder symptoms, covering Angst. The effects of the 5HT-1A receptor have been suggested for use as the regulator of mood balance [35]. According to the modern monoamine hypothesis, the adaptive and plasticity regulation of 5-HT1A is closely related to the treatment of depression [15]. It can be seen from the Figure 6 that compared with the reserpine model group, the distribution of 5HT-1A in different parts of the brain tissues of mice in the three groups is increased, and the expression of 5HT-1A in at least one part of each concentration of *M. sieboldii* essential oils group showed great significance. Among them, MSEOs-H group had the best up-regulation effect on the expression of 5HT-1A in mouse brain tissue. Therefore, it can be speculated that the anti-depression effect of *M. sieboldii* can be achieved by its effective component up-regulating the expression of 5HT-1A in brain tissue.



**Figure 6.** Immunohistochemistry of GR protein expression in mouse brain tissue after sample treatment (A) Immunohistochemical study of cerebral cortex, hippocampus and hypothalamus in drug-treated brain Tissue. (B) Bar chart of GR protein expression in mouse brain tissue.

### 3.7. Effect of MSEOs on The Expression of BDNF and TrkB

BDNF, the most abundant neurotrophic factor, has receptors that are closely distributed throughout the nervous system. It repairs stress-induced nerve injury by binding to its receptor, TrkB [36]. In addition, antidepressants are associated with the recovery of BDNF levels [37]. Mice sniffed with the three concentrations of MSEOs exhibited upregulated expression of BDNF compared to that of the model group (Figure 7). The expression of BDNF in the model group was higher than that in the control group. Besides, the expression of BDNF in the essential oil groups was overtop the administration group. Thus, our process may not have been very successful, or the data for analyzing the results are not perfect.

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**Figure 7.** Relative expression levels of 5HT-1A and BDNF protein in mice

(A) Western blotting bands of BDNF and 5HT-A proteins in mouse brain tissue. (B) Bar chart of 5HT-A and expression of BDNF protein in the brain tissue of mice. Through Anova and multi-range tests, there was an average differential signal with different super script values ( $P < 0.05$ )

The initial goal of the project was to determine the antidepressant effect of the aromatherapy of *M. sieboldii* and the association between monoamine levels and brain disease. This study confirmed the existence of active compounds in *M. sieboldii*, such as magnolol, nerolol, and  $\beta$ -elemene, which may be closely related to changes in 5-HT, BDNF, and GR protein levels [38]. In conclusion, we investigated the main components and antidepressant effects of the essential oils from *M. sieboldii* leaves. Our data are based on a depression model, in which mice treated with different drugs are observed for mood and the expression of depression-related molecules in the tissues. Moreover, all three concentrations of MSEOs upregulated apparent mood (body weight) and the expression of related proteins in the brain tissue of the mice. The compounds in MSEOs may activate the P38/MAPK pathway in the brain tissue through the blood–brain barrier, increase the expression of GR protein, BDNF, and 5-HT1A in the mouse brain tissue, and prevent neuronal apoptosis. In addition, MSEOs promote the balance of monoamine levels between synapses, promote the secretion of 5-HT, and activation of the intracellular secondary messenger cascade. Further research on the antidepressant mechanisms of MSEOs suggests that, except for behavioral and molecular studies of the brain tissue, the practical application of metabolomics should be included. Pathological changes in depression can affect multiple organs. Our study has preliminarily revealed the effect of MSEOs on the brain and the influence of MSEOs on monoamine levels and speculated its possible mechanism. Future studies should investigate the mechanisms by which different natural plant extracts increase monoamine levels, and, based on these studies, clarify the use and dosage of aromatherapy, to meet the ever-changing demand for natural functional health care products and establish a theoretical basis for the development and research of aromatherapy products.

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## Supporting Information

Supporting information accompanies this paper on <http://www.acgpubs.org/journal/records-of-natural-products>

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