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The anti-depression effect and mechanism of harmonious rosemary essential oil and its application in microcapsules

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ABSTRACT

Depression imposes a heavy burden on patients and society, and current antidepressants often cause side effects such as dryness in the mouth and constipation. The explorations about aromatic plant essential oils provide a new method for the treatment of depression, but the mechanisms of antidepressant effect of these oils are not deeply enough. Here, the rosemary essential oil analyzed by GC-O and GC-MS techniques, whose main aromatic compounds are determined as 1,8-cineole (OAV = 1869.27, AI = 9.4), linalool (OAV = 1668.90, AI = 5.4), and ethyl decanoate (OAV = 1169.09, AI = 6.9). The network pharmacology was employed to investigate the possible pathway of action for antidepressant effects of the rosemary essential oil, and Gface software that can capture facial expressions was used to harmony the aroma to make the oil more pleasant. Then, the harmonious essential oil was proved to have antidepressant effects. For addressing issues of strong volatility and oxidation susceptibility of essential oil, dendrimer-like γ-cyclodextrin was prepared and encapsulate the essential oil. The obtained microcapsule prolonged fragrance duration and presented good stability, which are applied in aromatherapy and daily care products. This study lays a theoretical and methodological foundation for essential oil efficacy research and provides new strategies for designing and producing functional products related to essential oils.

1. Introduction

Depression is a common mood disorder in clinical practice, characterized by persistent low mood, loss of interest, reduced physical activity and so on [1]. In the United States, depression that significantly impacts patients' lives causes an economic loss of $43.7 billion annually, imposing a heavy burden on families and society [2]. Currently, clinical treatment for depression mainly relies on a variety of antidepressants, however, these drugs often exacerbate these feelings of bodily discomfort and come with side effects such as dryness in the mouth and constipation [3]. Therefore, developing safer novel antidepressants is currently a hot topic.

As a common mental disorder, the mechanism of depression is highly complex [4]. The widely recognized theory is the monoamine hypothesis, which suggests that a decrease in neurotransmitters such as dopamine and serotonin contributes to the onset of depression [5,6]. Indeed, some patients with severe depression do not respond well to long-term use of monoamine-based antidepressants, indicating a degree of treatment resistance [7]. Therefore, relying solely on the monoamine hypothesis for developing antidepressants is insufficient. Additionally, research suggests that elevated pro-inflammatory cytokines in the blood, such as TNF-α and IL-1β, can trigger inflammation and lead to the onset of depression [8,9]. Serotonin can regulate the release of inflammatory cells and the expression levels of cytokines through binding with specific receptors to influence the intensity and duration of neuroinflammation, then affect the activity of inflammatory cells and depressed emotion [10].

The aromatic plant essential oils have been reported that they have certain effects in combating depression and neurological regulation [11–14]. Shen et al. used rat olfactory experiments to demonstrate that inhaling lavender essential oil can influence the autonomic nervous system, leading to increased body weight and enhanced appetite in rats [15]. Tidesley et al. indicates that sage essential oil has beneficial effects on human emotions and cognitive abilities [16]. Mora et al. studied the antidepressant effects of the 60 % ethanol extract of sage, finding that it significantly reduced immobility time in the rat forced swim test, comparable to the effects of intraperitoneal injections of 10 mg kg^{-1} fluoxetine or 12.5 mg kg^{-1} imipramine [17]. The antidepressant effects of

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rose essential oil and geranium essential oil have also been proved [18]. Rosemary, a perennial evergreen subshrub belonging to the Lamiaceae family, has been extensively cultivated in southwestern China. It has also been successfully introduced in Shanghai, therefore, developing the added value of rosemary is particularly important at present. Although there are some reports about the antidepressant effects of rosemary essential oil, the mechanisms and applications in antidepressant efficacy of rosemary essential oil is lacking [19]. Additionally, other aroma substances are needed to blend and improve its unpleasant aroma [20]. In addition, the essential oil itself is easily susceptible to factors such as light and heat, and spoilt by oxidation, resulting in its poor stability and effectiveness.

Encapsulating essential oil within microcapsules can protect the oil from most of the adverse external factors to extend its shelf life. Cyclodextrins with the special structure that is hydrophilic externally and hydrophobic internally are considered ideal materials to form microcapsules. In addition, γ-cyclodextrin (γ-CD) has a cavity diameter larger and better solubility than α-cyclodextrin and β-cyclodextrin. Therefore, γ-CD can be a potential candidate as the wall material of microcapsules to encapsulate the rosemary essential oil [21]. And dendritic macromolecule of γ-CD has strong affinity with fabrics or skin.

In this study, the components of rosemary essential oil were identified, while the fragrance of the oil was blended to enhance its antidepressant effect. Moreover, the Antidepressant mechanism of rosemary essential oil was explored using the network pharmacology and animal experiments [22]. Furthermore, γ-CD was dendrimerized to encapsulate the blended oil to create microcapsules for the use in aromatherapy and personal care products. This research lays a theoretical and methodological foundation for the study of exploring the efficacy and mechanism of essential oils, and provides a new strategy for designing functional products related to essential oils.

2. Materials and methods

2.1. Material

Rosemary essential oil was purchased from Anhui Burnt Sweet Biotechnology Co., Ltd. γ-CD, hexadecanol, anhydrous ethanol, acetyl chloride, tween 80, sodium laureth sulfate (AES), sodium dodecyl sulfate (K12), sodium chloride, boric acid, ethylenediaminetetraacetic acid (EDTA), phosphate buffered saline (PBS), cocamidopropyl betaine (CAB-35), propylene glycol, coconut oil diethanolamide (CDEA), kathon, citric acid were purchased from Shanghai Yuanye Biotechnology Co., Ltd. 1,8-cineole (Food Grade) and d-limonene (Food Grade) was bought from Shanghai Meixin Chemical Technology Co., Ltd. The standard compounds were purchased from Meryer (Shanghai) Chemical Technology Co., Ltd., including methyl nonanoate, α-pinene, camphene, β-pinene, sabinene, myrcene, α-phellandrene, α-terpinene, d-limonene, 1,8-cineole, γ-terpinene, 3-octanone, p-cymene, terpinolene, toluene, 1-octen-3-yl-acetate, 3-octanol, nonanal, ethyl octanoate, α-thujone, 1-octen-3-ol, styrene, camphor, bornyl acetate, β-elemene, linalool, terpinen-4-ol, β-caryophyllene, aromadendrene, ethyl decanoate, 1,3-cycloheptadiene, 3-carene, α-caryophyllene, α-terpineol, verbenone, δ-cadinene, p-isopropyl benzaldehyde, perilla aldehyde, caryophyllene oxide, trans-nerolidol, octanoic acid, perhydrofarnesyl acetone, eugenol, carvacrol, decanoic acid and fluoxetine hydrochloride. Silk was purchased from Guangxi Hengye Silk Group Co., Ltd. C57 rats, 5-HT ELISA Kit, DA ELISA Kit, TNF-α ELISA Kit, IL-1β ELISA Kit were purchased from ELK (Wuhan) Biotechnology Co., Ltd. Soy wax, beeswax, and candle wicks were bought from Jiaxing Hudong Daily Chemical Additives Co., Ltd.

2.2. GC-O analysis

Agilent 7890 gas chromatography (GC) system equipped with a flame ionization detector (FID) and an olfactory detector Gerstel ODP-2 was used to analyze the aromatic compounds of rosemary essential oil. The used chromatographic column was HP-INNOVAX capillary column (60 m × 0.25 mm, 0.25 μm). Sample injection conditions were consistent with GC-MS, with an injection volume of 1 μL. To prevent condensation of volatile aromatic components, the temperature at the olfactory detector was maintained at 250 °C throughout. To maintain olfactory sensitivity, humidified air was used to uniformly purify the olfactory detector port.

The aroma intensity (AI), retention time (RT), and aroma characteristics of aromatic compounds of rosemary essential oil were evaluated by a scoring system. The AI scores range from 0 to 10. Here, 0 indicates no perceptible aroma, 5 indicates a clear perception of aroma, and 10 indicates a strong perception of aroma. Each experiment was repeated three times per person, and the average value was calculated. This group consists of 15 graduate students from Shanghai Institute of Technology. All members have completed a professional sensory training program lasting three months to prevent olfactory fatigue. During the training, three different concentrations of aromatic compounds related to rosemary essential oil were provided to ensure that the team members were thoroughly familiar with the corresponding AI values.

2.3. GC-MS analysis

The Agilent 7890 gas chromatography (GC) system coupled with a 5973C mass spectrometer (MS) was used to analyze the aroma compounds of rosemary essential oil. A HP-INNOWAX capillary column (60 m × 0.25 mm, 0.25 μm) was employed with helium as the carrier gas at a flow rate of 1 mL/min. Mass spectrometry was performed in electron ionization mode with a source temperature of 230 °C and electron energy of 70 eV. The inlet temperature was set at 250 °C. The initial column temperature was 50 °C, ramped at 5 °C/min to 140 °C and held for 5 min, then ramped at 3 °C/min to 230 °C and held for 10 min. For sample preparation, 0.107 g of rosemary essential oil was diluted with 4.892 g of anhydrous ethanol and spiked with 50 μL of internal standard (21.06 g/L methyl nonanoate). A 1 μL aliquot was injected in splitless mode. Qualitative analysis was conducted by comparing the obtained mass spectra with those in the NIST11 and Wiley7n libraries. RI values (Retention Indices) of aroma compounds were calculated using RT values (Retention Times) of normal alkanes (C9~C23). RI values were evaluated by formula (1):

$$RI = n \times 100 + 100 \times \frac{RT - T_n}{T_{n+1} - T_n} \quad (1)$$

Here, n represents the number of carbon atoms. T_n and T_{n+1} denote the retention times of two adjacent normal alkanes.

Subsequently, the identification of aroma compounds was further refined based on the significant ion fragments produced in the mass spectra. Quantitative analysis of volatile aroma compounds was conducted by the standard curve method [23,24]. The x-axis represented the ratio of the concentration of aroma compound standards to that of the internal standard, while the y-axis represented the ratio of peak areas between the aroma compound standards and the internal standard. Each compound was diluted to five concentration gradients (stock solution: 5 %, 10 %, 20 %, 40 %, 80 %) with anhydrous ethanol to fit the calibration curve (Table S1, Supporting Information). All experiments were repeated three times, and the average values were taken.

2.4. Identification of the main aromatic compounds in rosemary essential oil

This study employed a combination of AI (Aroma Intensity) and OAV (Odor Activity Value) to determine the main aromatic compounds of rosemary essential oil. The AI value represents the aroma intensity of aroma compounds, assessing their contribution to the overall aroma; a higher AI value indicates a greater contribution to the overall aroma profile. OAV indicates the Odor Activity Value, where an OAV greater

than 1 indicates that the volatile compound can be perceived by the senses. A higher OAV suggests a greater contribution of that compound to the overall aroma profile of rosemary. OAV value was calculated by formula (2):

$$\mathrm{OAV} = \frac{\text{Concentration}}{\text{Threshold}} \quad (2)$$

The threshold used in this study is the odor threshold of aroma compounds in water [25], which was obtained from threshold books consultation and experimental determination (Table S1, Supporting Information).

2.5. Network pharmacology

2.5.1. Collection and screening of targets for aromatic compounds

The compounds identified from rosemary essential oil were screened based on their gastrointestinal absorption (GI absorption) and blood-brain barrier permeability (BBB) values calculated using Swiss ADME. Compounds with a GI absorption value categorized as "high" and a BBB value categorized as "yes" were considered active compounds of rosemary essential oil. The SMILES files of these ADME-screened active ingredients were then imported into Swiss Target Prediction and SuperPred databases to obtain their targets. Targets with a probability greater than 0 were filtered, resulting in a database of targets associated with the aromatic compounds of rosemary essential oil.

2.5.2. Obtaining disease targets and intersecting targets

Search for depression-related targets using "depression" as a search term in GeneCards, OMIM, and TTD databases. Integrate disease targets from all three databases to create a depression-related target database. Venny 2.1.0 was used to input both the target database of aroma compounds from rosemary essential oil and the depression-related target database, then, their intersection was obtained to form a Venn diagram.

2.5.3. Construction of protein-protein interaction (PPI) networks

The intersection of targets to the STRING database was uploaded, and "*Homo sapiens*" as the organism type was selected. Then, the minimum interaction score threshold was set to 0.9, and disconnected nodes in the network were hided. After that, the data file in TSV format was downloaded and imported into Cytoscape 3.10.1 for analysis. Finally, a protein-protein interaction (PPI) network graph sorted by "degree" values was construct.

2.5.4. GO functional enrichment and KEGG pathway enrichment analysis

The intersection of targets was uploaded to Metascape, the species was set as "*Homo sapiens*", and Gene Ontology (GO) functional and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis were performed. Finally, the data results were exported and visualized.

2.5.5. Construction of essential oil-compound-target-disease-pathway network

The top 20 KEGG pathways most relevant to depression based on enrichment analysis was selected. A network in Cytoscape 3.10.1 was built to illustrate the relationships among essential oil compounds, targets related to the disease, and pathways involved. "Degree" refers to the number of edges connecting a node, indicating its influence and importance within the network. A higher degree suggests more connections with other nodes, indicating a significant role in disease treatment. Based on the "Degree" values and the contribution of aromatic compounds to rosemary essential oil, the primary active ingredients responsible for exerting antidepressant effects were speculatd. When the degree of aromatic compounds exceeds 100, it can be inferred that these components may significantly contribute to the antidepressant effects of rosemary essential oil. However, the overall aromatic contribution of these compounds to rosemary essential oil should be further scrutinized. Therefore, it is necessary to identify compounds with both OAV >1 and AI >5 among those with degree >100. Regarding the main aromatic compounds of rosemary essential oil, their degree values should be checked; if they exceed the median, they should also be included for further validation. Additionally, the top twenty pathways highly correlated with depression can be selected and representative key antidepressant targets can be identified within these pathways according to related literature.

2.5.6. Molecular docking validation

Deepsite was utilized for predicting the binding sites of aromatic compounds with the aforementioned targets, followed AutoDock was used to explore the affinity and binding modes of aromatic compounds with these targets. The docking process was automated and set to be performed 50 times using AutoDock. Generally, docking results were considered acceptable if the binding energy was less than -1.2 kcal/mol or less than -5 kJ/mol; lower binding energies indicated tighter binding [26]. Therefore, the conformation with the lowest binding energy can be selected from the docking results. It was also important to consider whether the aromatic compounds interact with key antidepressant amino acid residues in the target sites. Finally, Pymol and Ligplot were used to analyze and visualize the docking results: Pymol 2.2.0 was employed to create three-dimensional structural diagrams of the binding sites, while LigPlot software was used to generate two-dimensional interaction plots between the compound and the target.

2.6. Coordinating fragrances

After the molecular docking validation, the aromatic compounds were evaluated for human olfactory perception ratings. The olfaction assessors were the aforementioned sensory evaluation group. Pleasure ratings ranged from 0 to 10, where, 0 represented unacceptable smell, 5 represented acceptable smell, and 10 represented very enjoyable smell. Three parallel experiments were carried out, and the average score is taken. Gface software (Facial expression analysis system 1.15) was used to capture facial expressions to digitize olfactory perceptions, combining facial expressions with olfactory ratings, the pleasant aromatic compounds were identifeied. These compounds were mixed with rosemary essential oil to create harmonious blends, referred to as the harmonious essential oil in subsequent papers. Lastly, Gface and human olfactory perception ratings were combined to evaluate the pleasure of rosemary essential oil and the harmonious essential oil for the purpose of assessing the olfactory comfort of the harmonious essential oil.

2.7. Animal experiments

2.7.1. Experimental procedure overview

All of the animal operations were carried out in accordance with the guidelines for experimental animal care and under the supervision of Laboratory Animal Welfare & Ethics Committee with the license number of 2024-08-23A. C57 rats were selected as experimental subjects to construct a rat model of depression through a chronic mild stress procedure ($n = 5$). The main implementation methods including intermittent illumination, deprivation of water and food, stroboscopic irradiation, exposure to white noise, cage tilting, dampened bedding, and housing in pairs. Different stressors were applied in the morning and evening over seven days, with weights recorded before and after modeling. Subsequently, an olfactory experiment was conducted where rats were divided into appropriate drug administration groups. Each rat was exposed to 1 mL of the test solution dripped onto filter paper and placed in a relatively enclosed space (Dimensions: 28 cm $\times$ 21 cm $\times$ 17.5 cm) for seven days, with daily replacement of the filter paper. There were five groups. The blank group referred to the rat that was not exposed to the olfactory experiment. Group fluoxetine hydrochloride (Antidepressant group) received a daily gavage of 0.2 mL of fluoxetine hydrochloride suspension (30 mg/kg) for 7 days. The other rats were

exposed to the scent of rosemary essential oil (Rosemary essential oil group), harmonious essential oil (Harmonious fragrance group), and aromatic compounds from rosemary essential oil selected for both antidepressant effects and pleasant aroma after molecular docking and fragrance adjustment (Monomer fragrance raw material group). Their weight changes were compared to infer the antidepressant effects of each treatment. Furthermore, to validate the antidepressant efficacy of the drugs, depression tests were conducted using the tail suspension test and the rat forced swim test. Finally, following the instructions of the enzyme-linked immunosorbent assay (ELISA) kit, concentrations of neurotransmitters serotonin (5-HT) and dopamine (DA) in the rat brain, as well as concentrations of TNF-α and IL-1β in serum, were measured to compare the antidepressant effects of each group to select the core material for subsequent microcapsules.

2.7.2. Experiment on depression assessment in rats

For the tail suspension test, after drug administration, a segment of the rat's tail approximately 2 cm from the tip was secured to a custom-made tail suspension apparatus. This positioning resulted in the rat being suspended in an inverted position, with its head approximately 5 cm above the surface. Each animal was separated by boards on both sides to prevent visual contact and mutual interference. The rats were observed for cumulative immobility time during the last 5 min of a 6-min observation period. Immobility was defined as the cessation of struggling with the body hanging motionless in a vertical position.

For the rat forced swimming test, rats were individually placed into water at a temperature of (25 ± 1)°C with a depth of 10 cm, where they were forced to swim for 6 min. The first 2 min served as an adaptation period, followed by recording the cumulative immobility time during the subsequent 4 min. Immobility was defined as when the rat ceased struggling in the water, adopting a floating posture with only minor movements to keep its head above water.

2.8. Statistical analysis

All quantitative data are expressed as the mean ± standard deviation of at least five independent experiments, with each animal experiment using 25 rats divided into 5 rats per group (n = 5). GraphPad Prism 10.4.0 was used to perform the analysis of variance (ANOVA), followed by Tukey's honest significant difference test for comparisons between multiple groups. Statistical significance was considered for p values < 0.05.

2.9. Microcapsule

2.9.1. Modification and characterization of γ-CD

5 g of γ-CD and 25 g of acetyl chloride were weighed and dissolved in 50 mL deionized water. The temperature of the water bath was adjusted to 40 °C and stirred for 1 h. Then 6 g of hexadecanol was added and the temperature of the mixture was adjusted to 60 °C. After heating and stirring for 2 h, 3 g of anhydrous ethanol was added and the reaction was lasted for 1 h. After that, the reaction product was neutralized with saturated sodium bicarbonate solution. Finally, the mixture was filtered and dry to obtain the modified γ-CD.

The NICOLET IZ10 Fourier Transform Infrared Spectrometer (FTIR) from Thermo Fisher Scientific was used to obtain the infrared spectrograms of γ-CD, modified γ-CD, and hexadecanol. The wavelength range was 4000∼400 cm^{-1}.

2.9.2. Preparation of essential oil microcapsules

Modified γ-CD was taken as the wall material and dissolved in deionized water. The harmonious essential oil and the emulsifier Tween 80 were added, and the mixture stirred to obtained the final product of essential oil microcapsules.

2.9.3. Single-factor experiment

In this study, two single-factor experiments were conducted to explore the optimal conditions for preparing essential oil microcapsules. The factors were core-to-wall ratio and emulsifier dosage. All experiments were repeated tree times.

For the core-to-wall ratio experiment, ratios of 1:10, 1:5, 2:7, 2:5, and 1:1 were tested. Tween 80 was chosen as the emulsifier at a ratio of 1:2 with the essential oil. The stirring temperature was 60 °C, the speed was 1000 rpm, and the stirring time was 3 h. The effects of different core-to-wall ratios on the particle size and dispersity of the essential oil microcapsules were examined using the Nano ZS90 particle size analyzer (British Malvern Instrument Company).

Upon the optimal core-to-wall ratio, the dosage of Tween 80 was 0.1 g, 0.2 g, 0.3 g, 0.4 g, and 0.5 g, respectively. The stirring temperature was 60 °C, the speed was 1000 rpm, and the stirring time was 3 h. The effects of different emulsifier dosages on the particle size and dispersity of the essential oil microcapsules were investigated under these conditions.

2.9.4. Characterization of essential oil microcapsules

A Gemini SEM 300 scanning electron microscope (Germany ZEISS Instrument Company) was used to capture images of samples. A STAR thermogravimetric analyzer (Swiss Mettler Instrument Company) was used to assess the thermal stability of essential oil microcapsules, essential oil, and γ-CD modified samples. 4 mg of powdered sample was placed in the thermogravimetric analyzer, heated from 30 °C to 800 °C at a rate of 10 °C/min under a nitrogen atmosphere to obtain TG and DTG curves to analyze the microcapsules' thermal stability towards essential oils. An HERACLES II electronic nose (France Alpha MOS) was used to measure aroma profiles of essential oils encapsulated for 1 day and 30 days at 25 °C to evaluate the sustained release effect of essential oil microcapsules.

2.10. The application of essential oil microcapsules

2.10.1. Preparation of essential oil microcapsule aromatherapy candles

The soy wax and beeswax were mixed with a 1:1 ratio in a double boiler setup. The mixture was stirred to accelerate melting until the wax reaches around 60 °C, then it was poured into a glass container. The essential oil microcapsules was added into the wax and stir thoroughly with a glass rod until evenly mixed. A concentration of 5 % of microcapsules can provide a noticeable fragrance. Once the wax mixture was cooled to a certain extent, a candle wick should be insert into the center. Finally, decorate with dried flowers or other embellishments according to personal preference, such as fruit peels or small twigs.

2.10.2. Preparation of essential oil microcapsule shower gel

10 mL of sodium laureth sulfate (AES) and 5 mL of sodium dodecyl sulfate (K12) surfactants were dissolved in 80 mL of deionized water at 60 °C. Then, 1.5 g of sequentially sodium chloride, 0.5 g of borax, and 0.2 g of EDTA were added and dissolved. 3 mL of cocamidopropyl betaine (CAB-35) and 5 mL of propylene glycol were added and the mixture was stirring for 30 min until evenly mixed. Then, the temperature of the mixture was cooled down to 50 °C, 0.5 g of pearlizing agents and 1 mL of essential oil microcapsules were added. Finally, 1 mL of coconut fatty acid diethanolamide (CDEA) and preservatives like 0.2 mL of Kathon were incorporate into the mixture. After adjusting the pH using citric acid to achieve a pH range of 4.5–6.5, the product was placed to naturally defoam for over 24 h at room temperature to obtained the essential oil microcapsule shower gel.

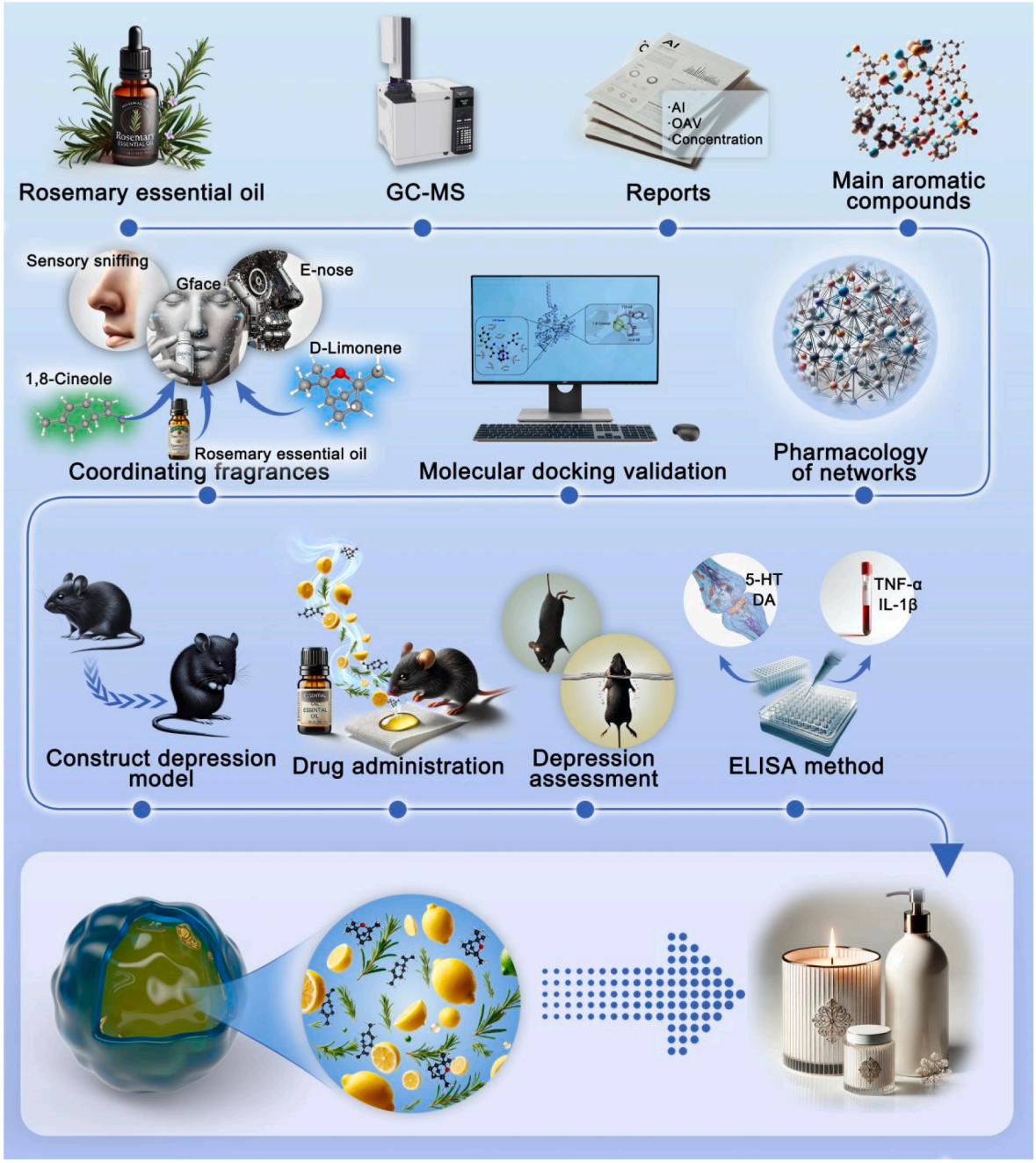
Silk fabric with similar protein composition to human skin was used to simulate the human skin surface. Silk fabric was cut into 5 cm × 5 cm squares and completely moistened with distilled water at 37 °C. Surface water droplets are then gently removed with a tissue to simulate a moist skin environment. Equivalent concentrations of essential oil microcapsule shower gel and plain essential oil shower gel were applied to the

prepared simulator surface. After 5 min, the simulator was rinsed with warm water and any remaining surface water droplets are removed with a tissue. The aromas were smelled and compared until the scent was no longer detectable to observe the duration time of fragrance to assesses the retention time of essential oil microcapsules on the surface of a simulated human skin model.

3. Results and discussions

3.1. The preparation and antidepressant mechanism of harmonious rosemary essential oil and its applications

In this study, rosemary essential oil was blended to be a candidate of novel anti-depressive material. At first, GC-O and GC-MS were used to identify the aroma compounds of rosemary essential oil to determine its main aromatic compounds. Subsequently, a combined analysis of network pharmacology and the main aromatic compounds was conducted to infer the most significant anti-depressive compounds in rosemary essential oil. Pleasant aromatic compounds were selected by Gface and enose combined with human sensory evaluation to formulate an anti-depressive essential oil with high pleasantness. Then, animal experiments were employed to analyze the anti-depressive mechanisms of the blended rosemary essential oil. Furthermore, γ-CD was modified to be the wall material of microcapsule, in which the essential oil was encapsulated, resulting in microcapsules with prolonged fragrance retention and good stability. These microcapsules are suitable for use in aromatherapy materials and personal care products (Scheme 1).



Scheme 1. The preparation and antidepressant mechanism of harmonious rosemary essential oil and its applications.

3.2. Aroma compounds of rosemary essential oil

To accurately characterize rosemary essential oil, GC-O analysis was conducted, and volatile aroma compounds were evaluated based on direct intensity method. In total, 44 substances were detected, including 23 terpenes, 5 alcohols, 5 esters, 3 aldehydes, 2 phenols, 2 acids, 2 ketones, and 2 other substances (toluene and styrene) (Fig. 1A and B). Among these, 11 aroma compounds had AI values exceeding 5, with 8 of them belonging to the terpene class, indicating their significant contribution to the aroma profile of rosemary essential oil. Notably, the top three compounds by AI value were 1,8-cineole (AI: 9.4), camphor (AI: 8.9), and β-caryophyllene (AI: 7.2), each characterized by a cooling, minty, and spicy camphoraceous aroma, which were prominent features of the rosemary essential oil used in this study. Additionally, the esters ethyl decanoate (AI: 6.9), ethyl octanoate (AI: 4.3), bornyl acetate (AI: 3.7), 1-octen-3-yl-acetate (AI: 1.2), and trans-nerolidol (AI: 0.9) imparted green, fruity, wine-like, and floral notes to the rosemary essential oil. Phenolic, alcoholic, aldehydic, ketonic, and acidic compounds also contributed to the herbaceous, spicy, woody, and acidic aroma characteristics of the oil (Fig. S1A, Supporting Information), while toluene (AI: 0.3) and styrene (AI: 0.2) made negligible contributions to the overall aroma profile of rosemary essential oil.

To accurately quantify the aromatic compounds in rosemary essential oil, the concentrations of these compounds were determined using GC-MS with a standard curve method (Table S1, Supporting Information). The rosemary essential oil was then reconstituted based on the measured concentrations to verify the accuracy of the analysis (Fig. 1C; Fig. S1, Supporting Information). It is evident that the top ten aromatic compounds are β-caryophyllene, camphor, verbenone, camphene, α-thujone, terpinen-4-ol, p-cymene, α-pinene, 1,8-cineole, and β-pinene. This indicates that the concentration of terpene compounds in rosemary essential oil is significantly higher than that of other types, with camphor being more concentrated than verbenone and 1,8-cineole, suggesting that this rosemary essential oil is camphor-rich rosemary oil. Fig. 1D clearly shows the odor activity values (OAV) of the aromatic compounds in rosemary essential oil. Compounds with OAVs greater than 1000 are 1,8-cineole (OAV = 1869.27), linalool (OAV = 1668.90), and ethyl decanoate (OAV = 1169.09). This indicates that these three

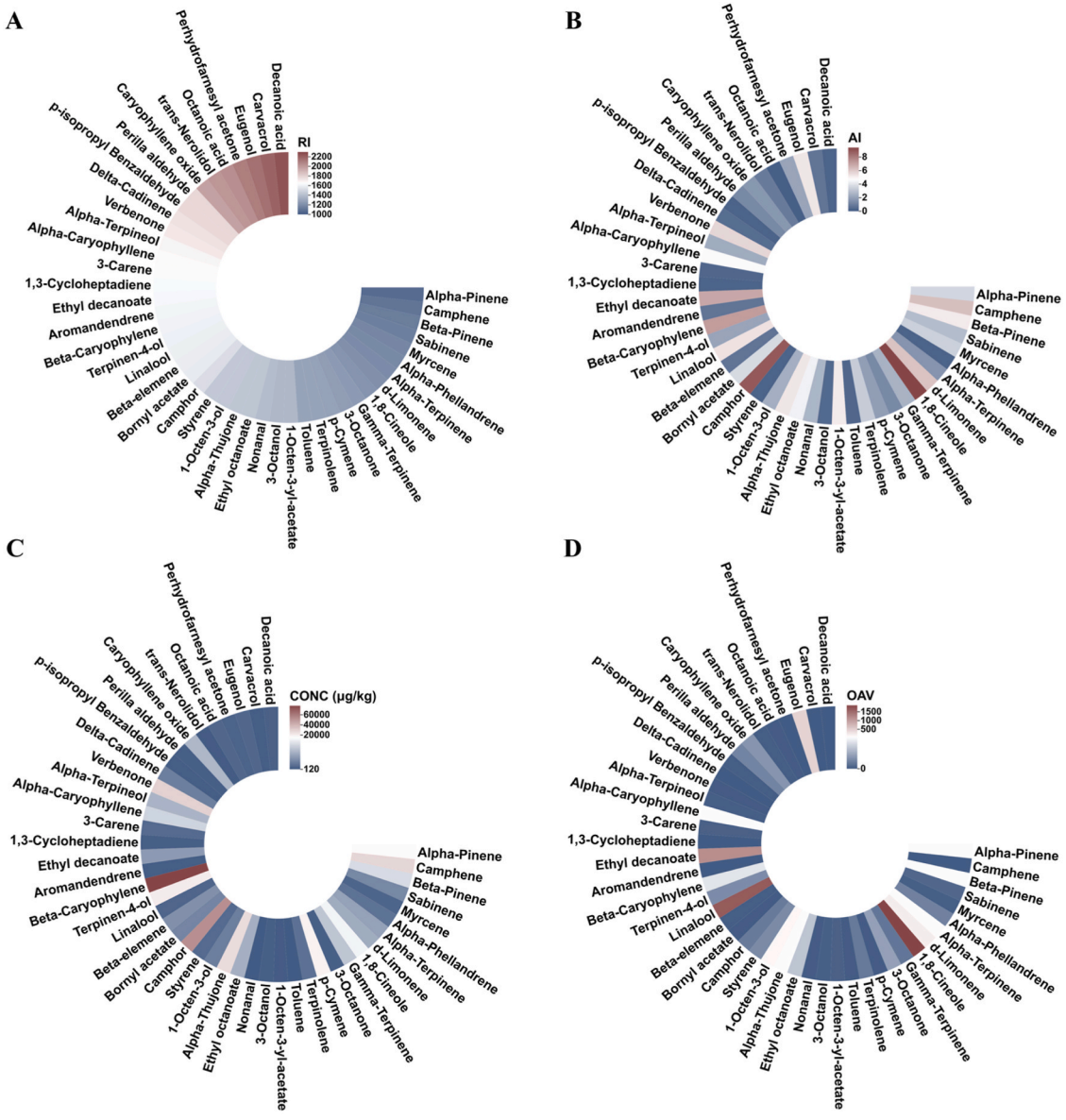


Fig. 1. Odorants identified in rosemary essential oil. A) RI values of aroma compounds. B) AI values of aroma compounds. C) Concentration of aroma compounds. D) OAV values of aroma compounds.

compounds make a significant contribution to the overall aromatic profile of rosemary essential oil. Combining this with the entire Fig. 1, the high AI value, concentration, and OAV of 1,8-cineole suggest that it is the most important component in the aroma profile of rosemary essential oil. Linalool and ethyl decanoate also have AI values greater than 5, indicating that their presence is clearly perceptible. Therefore, the primary aromatic constituents of the rosemary essential oil in this study are 1,8-cineole, linalool, and ethyl decanoate. Of course, any aromatic compound with an OAV greater than 1 contributes to the aroma fingerprint of rosemary essential oil. For example, while β-caryophyllene has a high concentration, its higher threshold results in a lower OAV value. This means it contributes to the overall aroma profile of rosemary essential oil, but its contribution is not significant.

3.3. Pharmacology of networks

3.3.1. Rosemary essential oil aroma compounds target database

After ADME screening of the identified aromatic compounds in rosemary essential oil, 22 bioavailable active aromatic compounds were obtained. Upon importing these compounds into the Swiss Target Prediction and SuperPred databases, 678 targets with a probability greater than 0 were identified (Table 1).

3.3.2. Potential depression targets of rosemary essential oil

By searching for "depression" in GeneCards, OMIM, and TTD databases, a total of 3701 depression-related targets were identified. Using Venny 2.1.0 to intersect the rosemary essential oil aroma compounds target database with the depression-related target database, 366 overlapping targets were obtained. These 366 targets represent the potential depression targets of rosemary essential oil.

3.3.3. PPI network and the screening of core targets

The overlapping targets were uploaded to the STRING database to obtain interaction relationships, which were then imported into Cytoscape 3.10.1 for analysis. The protein-protein interaction (PPI) network was constructed and sorted by "Degree" values (Fig. 2B). Larger Nodes areas indicate more connections, suggesting that these proteins interact with more targets and have a greater impact on the antidepressant effects of rosemary essential oil. SRC, PRKACA, STAT3, PIK3R1, MAPK1, and MAPK3 among the 264 targets may be core targets for the antidepressant action of rosemary essential oil.

Table 1
Active aroma compounds found in rosemary essential oil.

NO.	Aroma compounds	GI absorption	BBB permeant
GC1	1,8-Cineole	High	Yes
GC2	3-Octanone	High	Yes
GC3	1-Octen-3-yl-acetate	High	Yes
GC4	3-Octanol	High	Yes
GC5	Nonanal	High	Yes
GC6	Ethyl octanoate	High	Yes
GC7	α-Thujone	High	Yes
GC8	Camphor	High	Yes
GC9	Bornyl acetate	High	Yes
GC10	Linalool	High	Yes
GC11	Terpinen-4-ol	High	Yes
GC12	Ethyl decanoate	High	Yes
GC13	α-Terpineol	High	Yes
GC14	Verbenone	High	Yes
GC15	p-isopropyl Benzaldehyde	High	Yes
GC16	Perilla aldehyde	High	Yes
GC17	Caryophyllene oxide	High	Yes
GC18	trans-Nerolidol	High	Yes
GC19	Octanoic acid	High	Yes
GC20	Eugenol	High	Yes
GC21	Carvacrol	High	Yes
GC22	Decanoic acid	High	Yes

3.3.4. Gene function and pathway analysis

GO functional enrichment analysis was performed on 264 core targets, resulting in a total of 3055 GO terms: 2515 for Biological Process (BP), 182 for Cellular Component (CC), and 358 for Molecular Function (MF). The top ten terms ranked by "Count" values for each category were visualized (Fig. 2C). BP mainly involves cellular response to nitrogen compound, response to hormone, behavior, regulation of system process, synaptic signaling, circulatory system process, response to salt, response to inorganic substance, positive regulation of MAPK cascade, regulation of secretion by cell, etc. CC is mostly about dendrite, receptor complex, perinuclear region of cytoplasm, membrane raft, side of membrane, monoatomic ion channel complex, cell projection membrane, glutamatergic synapse, transcription regulator complex, focal adhesion, etc. MF basically includes protein kinase activity, monoatomic ion transmembrane transporter activity, oxidoreductase activity, transcription factor binding, kinase binding, protein domain specific binding, protein homodimerization activity, neurotransmitter receptor activity, amide binding, nuclear receptor activity, etc.

KEGG pathway analysis identified a total of 221 pathways, with the top 20 pathways ranked by "Count" values visualized (Fig. 2D). The signaling pathways closely related to rosemary essential oil include neuroactive ligand-receptor interaction, pathways in cancer, calcium signaling pathway, chemical carcinogenesis - receptor activation, lipid and atherosclerosis, cAMP signaling pathway, morphine addiction, serotonergic synapse, pathways of neurodegeneration - multiple diseases, inflammatory mediator regulation of TRP channels, neutrophil extracellular trap formation, efferocytosis, Th17 cell differentiation, bladder cancer, dopaminergic synapse, cGMP-PKG signaling pathway, JAK-STAT signaling pathway, transcriptional misregulation in cancer, adrenergic signaling in cardiomyocytes, adherens junction.

3.3.5. Network of essential oil-aroma compounds-targets-depression-pathways

The top 20 KEGG enrichment pathways with closely relate to depression were selected, and the connections among the essential oil aroma compounds disease targets, and the relevant pathways were established. The data were imported into Cytoscape 3.10.1 software to construct an interaction network of essential oil-aroma compounds-targets-depression-pathways (Fig. S3, Supporting Information). Based on the "Degree" values and the contribution of aroma compounds to rosemary essential oil, the main active compounds contributing to the antidepressant effect are inferred to be 1-octen-3-yl-acetate (OAV = 2.09, AI = 5.2, Degree = 112), ethyl decanoate (OAV = 1169.09, AI = 6.9, Degree = 112), 1,8-cineole (OAV = 1869.27, AI = 9.4, Degree = 98), and linalool (OAV = 1668.90, AI = 5.4, Degree = 86).

Therefore, rosemary essential oil, particularly its active compound 1,8-cineole, may modulate neurotransmitter activity by interacting with various brain receptors, including those for serotonin (5-HT) and dopamine (DA), thereby regulating mood and alleviating anxiety and depression. It may also influence intracellular calcium levels, impacting neurotransmission and neural plasticity, and potentially improving depressive symptoms through modulation of calcium channels and signaling pathways. Additionally, rosemary oil may enhance cAMP signaling, which regulates neurotransmitter release and neuronal adaptation, thus promoting the activity of both 5-HT and DA systems. Its effects on serotonin synthesis, release, and receptor activity may further improve mood regulation. Moreover, rosemary oil may enhance dopamine function and brain reward pathways, counteracting depressive symptoms. Its anti-inflammatory properties, may reduce neuroinflammation by lowering pro-inflammatory cytokines such as TNF-α and IL-1β, potentially mitigating depression-related neurodamage. It can be observed that the pathways highly related to depression among the top 20 pathways are strongly connected with the monoamine hypothesis and inflammatory response. Therefore, the representative key antidepressant targets among the top 20 pathways are selected as MAOA, SLC6A4, HRH4, and DRD2.

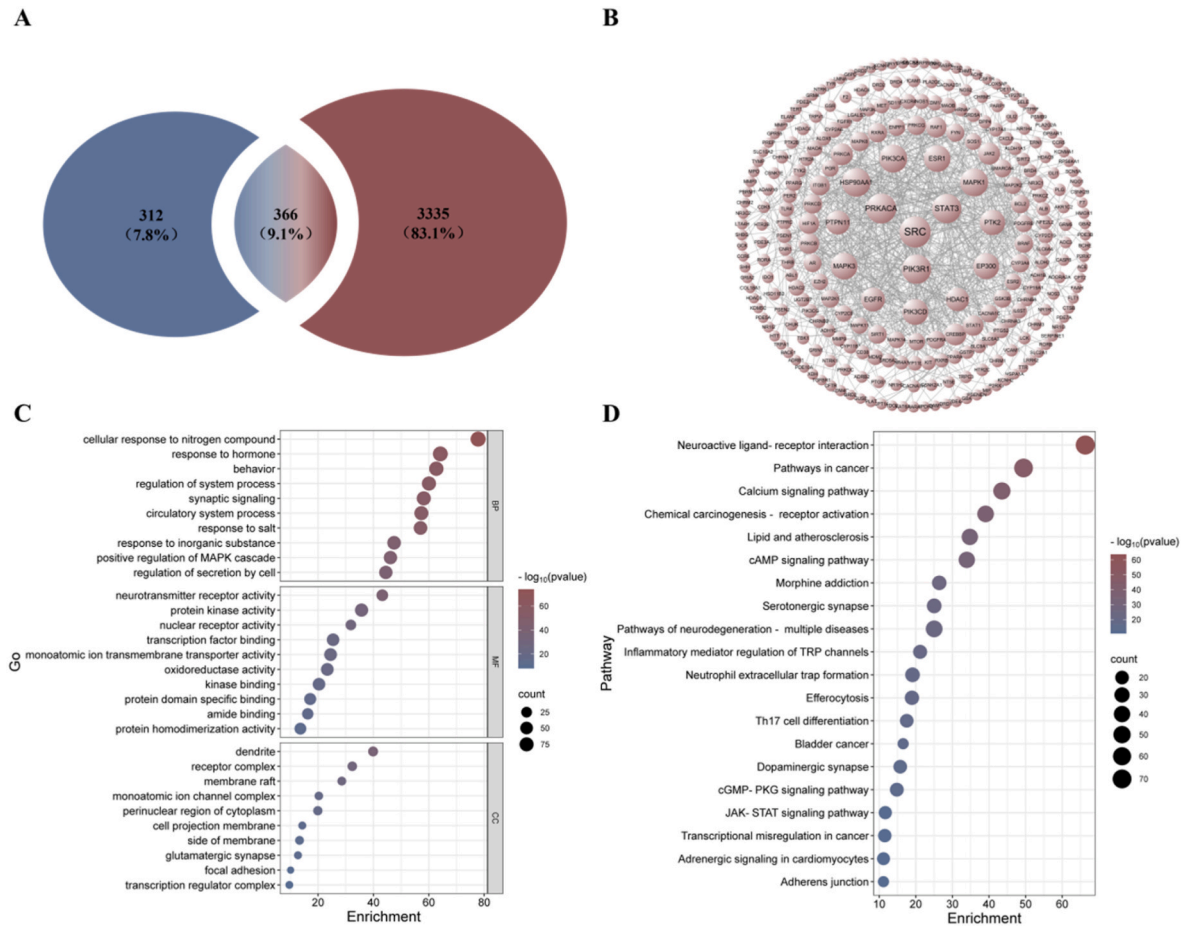


Fig. 2. A) The Venn diagram of overlapping targets. B) PPI network diagram. C) GO functional enrichment analysis. D) KEGG pathway analysis.

3.3.6. Molecular docking validation

Deepsite was used to predict the binding sites of targets and ligands, as shown in Fig. 3A panels (a), (b), and (c), MAO has three binding sites at [3.4, −0.1, −7.8], [−2.5, −0.1, −1.7], and [29.4, 47.8, −29.7], with key amino acid residues Phe208, Asn181, and Tyr407 [27]. As shown in Fig. 3A panels (d) and (e), SLC6A4 has two binding sites at [−0.5, −1.8, 1.0] and [−4.5, −9.8, 25.0], with key amino acid residues Ile172, Ser438, and Tyr95 [28]. As depicted in Fig. 3A panels (f) and (g), HRH4 has two binding sites at [−8.3, −10.1, 14.6] and [15.6, 5.8, −11.3], with key amino acid residues Tyr95, Phe344, and Asp94 [29]. As illustrated in Fig. 3A panels (h), (i), and (j), DRD2 has three binding sites at [−19.3, −2.1, 9.4], [8.6, 3.8, −10.5], and [32.5, −0.1, −24.5], with key amino acid residues Ile184, Ser197, Cys118, and Phe390 [30].

Using the AutoDock molecular docking software, we performed semi-flexible docking of 1-octen-3-yl-acetate, ethyl decanoate, 1,8-cineole, and linalool with MAOA, SLC6A4, HRH4, and DRD2. The docking results are shown in Fig. 3 and Table 2. When docking 1-octen-3-yl-acetate, 1,8-cineole, and linalool with MAOA, hydrogen bonding interactions were observed with amino acid residues Tyr69 and Ala68. Ethyl decanoate, when docked with MAOA, formed hydrogen bonds with the amino acid residue Arg51. All four aroma compounds interacted hydrophobically with the key amino acid residue Tyr407. For docking with SLC6A4, 1-octen-3-yl-acetate formed hydrogen bonds with the amino acid residue Ser438, while ethyl decanoate formed hydrogen bonds with Arg104. 1,8-cineole did not form hydrogen bonds with SLC6A4, and linalool formed hydrogen bonds with Thr439. Additionally, 1-octen-3-yl-acetate, 1,8-cineole, and linalool interacted hydrophobically with key amino acid residues Ile172, Ser438, and Tyr95, whereas ethyl decanoate interacted hydrophobically with Ile172. When docking with HRH4, 1-octen-3-yl-acetate formed hydrogen bonds with Tyr340 and Lys336. Ethyl decanoate interacted with Tyr95, and 1,8-cineole did not form hydrogen bonds with HRH4. Linalool formed hydrogen bonds with Lys336 and Glu165. All four aroma compounds interacted hydrophobically with key amino acid residues Tyr95 and Phe344. For DRD2, 1-octen-3-yl-acetate formed hydrogen bonds with His393, ethyl decanoate with Tyr416, and linalool with Ser194. 1,8-cineole did not form hydrogen bonds with DRD2. All four aroma compounds interacted hydrophobically with key amino acid residues Cys118 and Phe390.

More importantly, the binding energies of these four aromatic compounds with the four key antidepressant targets are all less than −1.2 kcal/mol, indicating successful docking and suggesting that these compounds may have antidepressant effects. Comparing the binding energies, it can be observed that 1,8-cineole has lower binding energies with the four key antidepressant targets compared to the other three aromatic compounds. Therefore, it can be inferred that 1,8-cineole binds more tightly with the antidepressant targets in rosemary essential oil and may exert a significant antidepressant effect. Furthermore, the successful docking of 1,8-Cineole with HRH4 suggests a potential interaction between this compound and the histamine receptor, which may play a crucial role in modulating neuroinflammation. Therefore, 1,8-Cineole has shown the potential to modulate the immune response and decrease the production of inflammatory mediators, highlighting its dual function in both neurotransmitter regulation and inflammation reduction.

3.4. Harmonious rosemary essential oil

The results of sensory evaluation of the human olfactory response using Gface are shown in Fig. 4. The likelihood of a "smirk" was almost

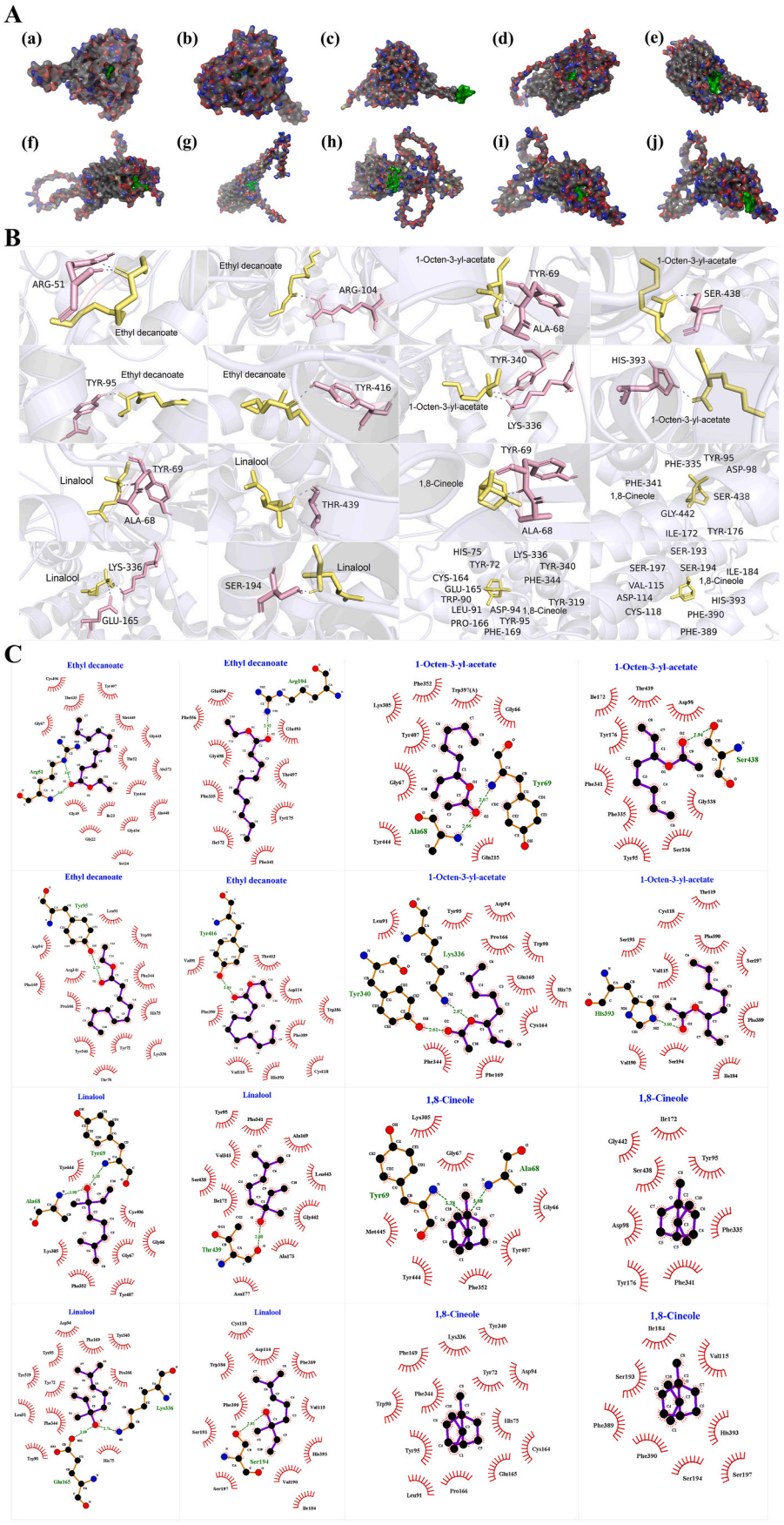


Fig. 3. Molecular docking results presentation. A) Binding sites prediction. B) 3D interaction plots of aroma compounds with antidepressant target. C) 2D interaction plots of aroma compounds with antidepressant target.

Table 2
Binding information of key antidepressant targets and actual docking binding energy.

Key target	Key amino acid residues	Aroma compounds	Binding energy (kcal/mol)
MAOA	Phe208, Asn181, Tyr407	1,8-Cineole	−6.66
		Linalool	−5.45
		Ethyl decanoate	−5.7
		1-Octen-3-yl-acetate	−5.2
SLC6A4	Ile172, Ser438, Tyr95	1,8-Cineole	−5.36
		Linalool	−5.21
		Ethyl decanoate	−4.24
		1-Octen-3-yl-acetate	−4.02
HRH4	Tyr95, Phe344, Asp94	1,8-Cineole	−5.93
		Linalool	−5.06
		Ethyl decanoate	−4.94
		1-Octen-3-yl-acetate	−5.21
DRD2	Ile184, Ser197, Cys118, Phe390	1,8-Cineole	−4.75
		Linalool	−4.61
		Ethyl decanoate	−3.9
		1-Octen-3-yl-acetate	−4.14

zero, indicating that the facial expressions of the 15 participants during the sniffing were natural and not feigned smiles, thus ensuring high data reliability. Among the four aromatic compounds validated through molecular docking, 1,8-cineole, which has a camphor and menthol-like aroma, was notably pleasant and comfortable with almost zero discomfort, suggesting that the scent of 1,8-cineole is agreeable. The other three aromatic compounds caused varying degrees of discomfort in the participants, so it is recommended to increase the concentration of 1,8-cineole in coordinating fragrance. Furthermore, the sensory evaluation scores from the participants are consistent with the Gface results, indicating the reliability of the findings (Fig. 4A and B).

When smelling the rosemary essential oil, participants found its aroma to be very pungent and herbal. To moderately mask and coordinate this herbal scent, we chose to add d-limonene, which has a citrus fruit fragrance, to balance the herbal aroma. Literature has also proven that d-limonene has good antidepressant effects [31–33]. Subsequently, gradient experiments were conducted to determine the final ratio. The ratios designed for rosemary essential oil, 1,8-cineole, and d-limonene were 1:1:1, 1:2:2, and 1:3:3. When the ratio was 1:1:1, the diffusion of the rosemary essential oil was insufficient, resulting in a dull aroma, and the herbal scent's pungency was not yet masked or coordinated by the citrus fruit scent. Increasing the ratios of 1,8-cineole and d-limonene, it was found that at a 1:3:3 ratio, d-limonene effectively coordinated the pungency of the herbal scent in the rosemary essential oil, and 1,8-cineole improved the diffusion of the rosemary essential oil. The final ratio for the harmonious fragrance essential oil was set to 1:3:3 for rosemary essential oil, 1,8-cineole, and d-limonene. As shown in Fig. 4B, the comfort level of the harmonious fragrance group (HF) was higher than that of the essential oil group (EO). Fig. 4D and E provide a complete display of the electronic nose aroma fingerprint spectra for rosemary essential oil and harmonious fragrance essential oil.

3.5. Antidepressant effect

After applying different stimulation methods in the morning and evening for seven consecutive days (Fig. 5A), the weight of all rats decreased by approximately 1 g–2 g before and after modeling, indicating that the rats were successfully induced into a depressive state (Fig. 5B).

Among the compounds selected from molecular docking and coordinating fragrance, 1,8-cineole in rosemary essential oil has both antidepressant effects and a pleasant aroma. Therefore, rats were divided into five groups, including Blank group, Rosemary essential oil group, Monomer fragrance raw material group, Harmonious fragrance group and Antidepressant group. The Blank group received no olfactory or gavage treatment and served as the control group. Other groups were exposed to the test substances via olfaction daily for 7 days.

After treatment, the weight of the rats in Blank group decreased to below 20 g, while the weights of rats in other groups increased compared to after modeling, with the highest increase in Antidepressant group (WT = 24.02 g), followed by Harmonious fragrance group (WT = 22.67 g). This suggests that rosemary essential oil, 1,8-cineole, and harmonious fragrance essential oil all have certain antidepressant effects, with the harmonious fragrance essential oil showing the best effect, second only to the antidepressant medication (Fig. 5C).

3.5.1. Assessment of antidepressant effect

In the tail suspension test (Fig. 5D), the Blank group exhibited the longest immobility time, recording 129.6 s (Immobility >100 s). This suggests that the rats in this group displayed significant anhedonia and a lack of reward mechanisms, with markedly increased despair and depression-like symptoms. The immobility time progressively decreased from the Blank group to the Antidepressant group, indicating varying degrees of depression relief with different treatments. The Rosemary essential oil group had an immobility time of 111.0 s (Immobility >100 s), showing that while these rats were still depressed, they experienced some relief compared to the Blank group. However, the immobility time in this group was notably longer than that of the 1,8-cineole group (88.8 s), the Harmonious Fragrance group (58.4 s), and the Antidepressant group (35.8 s).

In the forced swimming test (Fig. 5E), the Blank group again had the longest immobility time, with 141.2 s (Immobility >100 s), confirming the significant depression-like symptoms observed in this group. Similar to the tail suspension test, the immobility time decreased progressively in the groups receiving different treatments, indicating varying levels of antidepressant effects. The Rosemary essential oil group had an immobility time of 118.7 s (Immobility >100 s), showing a mild reduction in depressive symptoms compared to the Blank group. However, this time was still significantly longer than the Harmonious fragrance (57.5 s) and Antidepressant groups (34.3 s), suggesting that rosemary essential oil had a limited antidepressant effect. Additionally, the immobility time in the Rosemary essential oil group was 1.3 times longer than the 1,8-Cineole group and twice as long as the Harmonious fragrance group, indicating that 1,8-cineole has a more potent antidepressant effect compared to rosemary essential oil. This suggests that increasing the concentration of 1,8-cineole in fragrance formulations could further enhance the antidepressant properties of the essential oils.

These results indicate varying degrees of depression relief with different treatments, with the Rosemary essential oil group showing moderate improvement relative to the Blank group but not as strong as the other treatments tested. Specifically, while the Rosemary essential oil group demonstrated a statistically significant reduction in immobility time in both the tail suspension and forced swimming tests, the effect was less pronounced compared to the 1,8-cineole and Harmonious Fragrance groups. This suggests that while rosemary essential oil can provide some relief from depression-like symptoms, its antidepressant potential may be limited, possibly due to the complexity of its constituent compounds and their interaction with the brain's neurochemical pathways. Notably, the relatively stronger effects observed in the 1,8-cineole and Harmonious Fragrance groups suggest that 1,8-cineole in the rosemary essential oil may play a key role in relieving depression.

As shown in Fig. 5F, the concentration of 5-HT in the brain of rats from the Blank group was very low, at 85.93 ng/mg. In contrast, the concentrations of 5-HT were significantly higher in the other treated groups. The concentration levels followed this order: Antidepressant group (CONC = 315.74 ng/mg) > Harmonious fragrance group (CONC = 277.05 ng/mg) > Monomer fragrance raw material group (1,8-Cineole) (CONC = 234.32 ng/mg) > Rosemary essential oil group

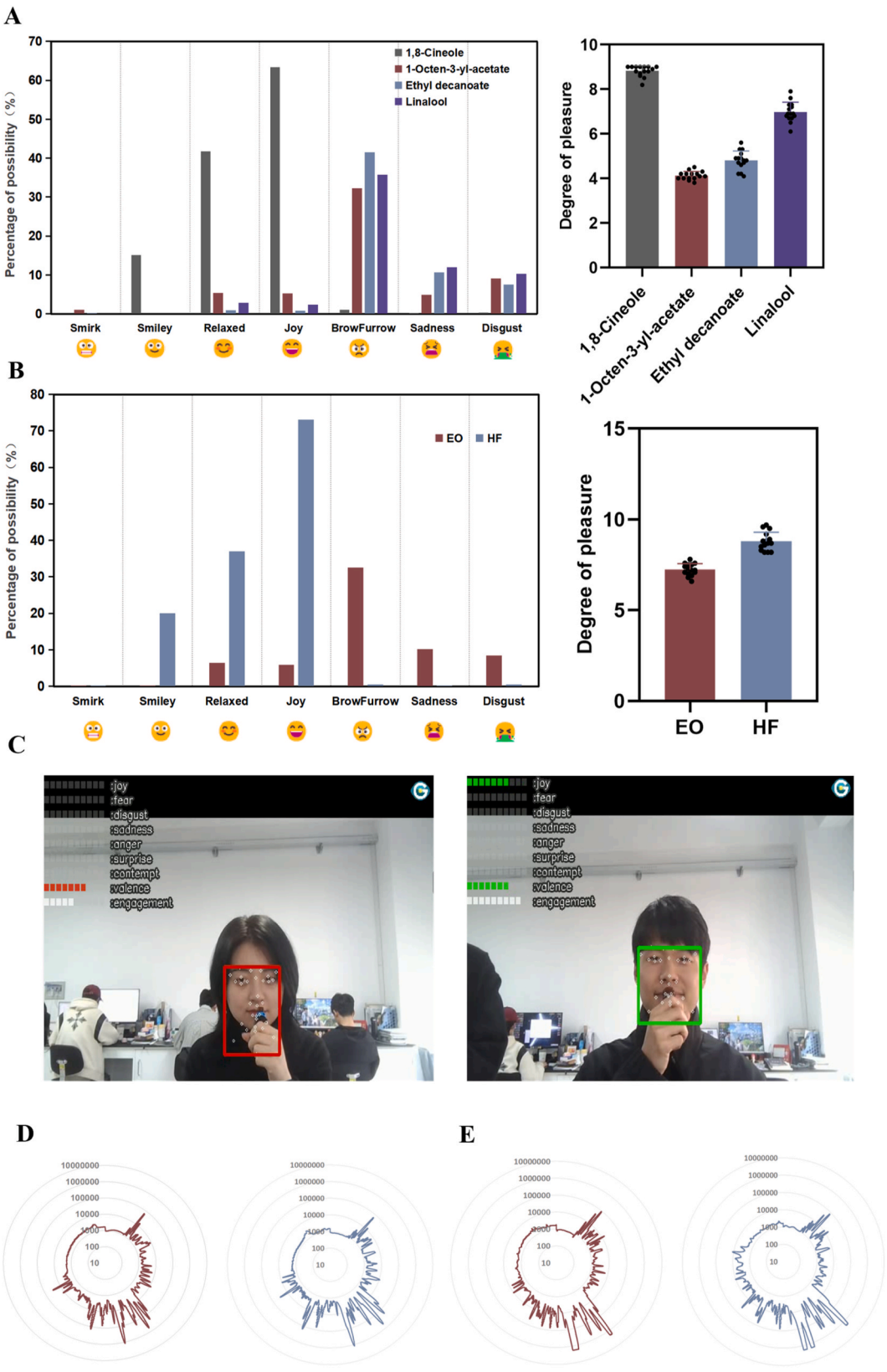


Fig. 4. Fragrance coordination and sensory processes. A) Gface and olfactory sensory data of 1,8-cineole, 1-octen-3-yl-acetate, ethyldecanoate and linalool. B) Gface and olfactory sensory data of rosemary essential oil (EO) and harmonious fragrance essential oil (HF). C) Gface test photo. D) Electronic nose graph of rosemary essential oil. The red trace corresponds to the measurement on the DB-5 polar column, and the blue trace corresponds to the measurement on the DB-1701. E) Electronic nose graph of harmonious fragrance essential oil. The red trace corresponds to the measurement on the DB-5 polar column, and the blue trace corresponds to the measurement on the DB-1701.

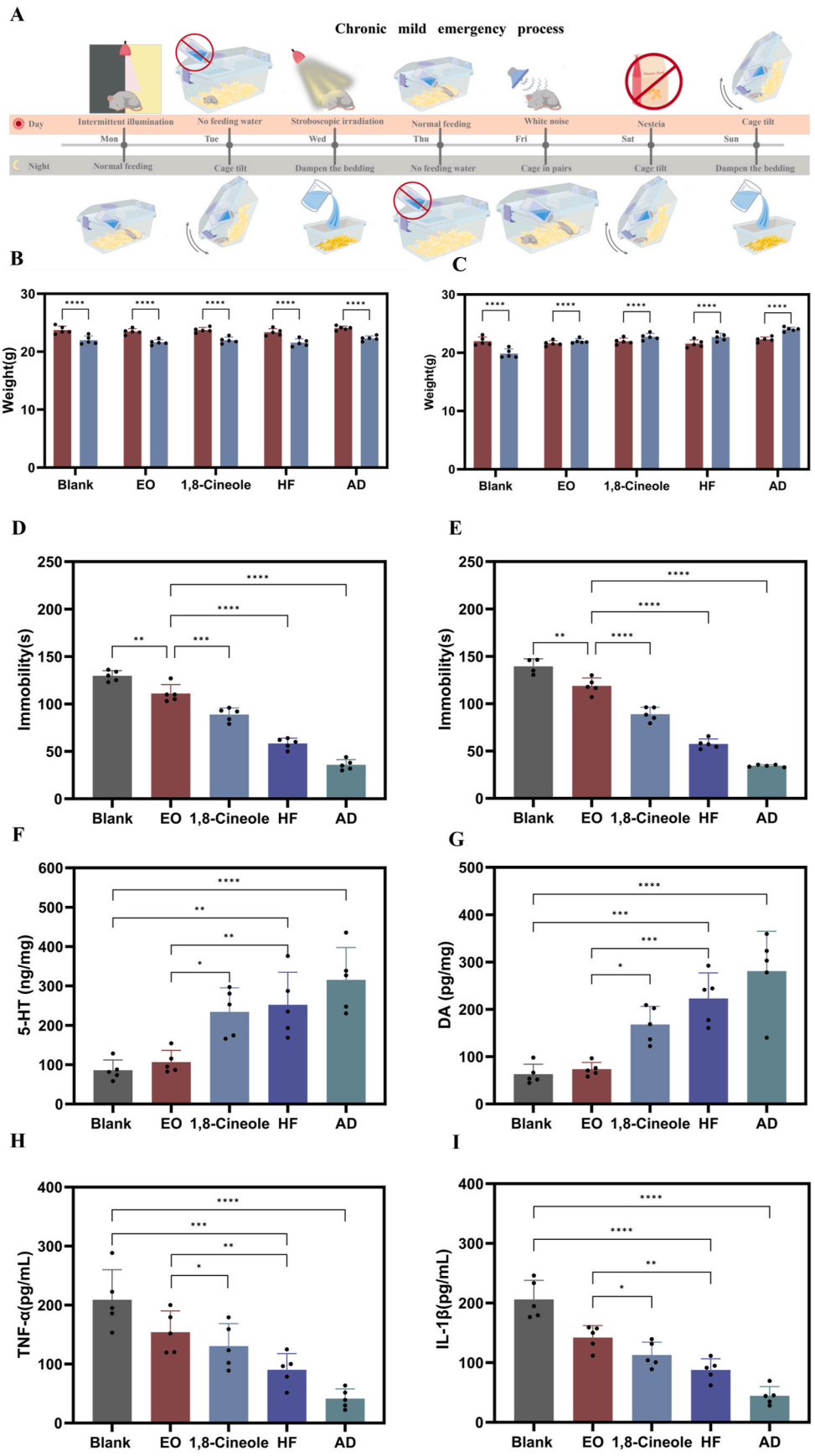


Fig. 5. A) Rat depression model. B) The weights of rats before modeling and immediately after modeling. C) The weights of rats immediately after modeling and after 7 days of medication. D) The immobility time in tail suspension test of rats. E) The immobility time in forced swimming test of rats. F) The concentrations of 5-HT. G) The concentrations of DA. H) The concentrations of TNF-α. I) The concentrations of IL-1β. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$, ****: $p < 0.0001$.

(CONC = 106.76 ng/mg) > Blank group (CONC = 85.93 ng/mg). Notably, the 5-HT concentration in the Blank group was about one-third of that in the Harmonious fragrance group and about one-quarter of that in the Antidepressant group. The 5-HT concentration of Rosemary essential oil group were two-fifths of those in 1,8-Cineole group and one-third of those in Harmonious fragrance group.

Similarly, as shown in Fig. 5G, the concentration of DA in the Blank group was also very low, with a value of 62.97 pg/mg. The concentrations of DA in the treated groups were significantly higher. The concentration levels of DA followed the same order as 5-HT: Antidepressant group (CONC = 280.86 pg/mg) > Harmonious fragrance group (CONC = 223.17 pg/mg) > Monomer fragrance raw material group (1,8-Cineole) (CONC = 167.85 pg/mg) > Rosemary essential oil group (CONC = 73.49 pg/mg) > Blank group (CONC = 62.97 pg/mg). Like 5-HT, the DA concentration in the Blank group was about one-third of that in the Harmonious fragrance group and about one-quarter of that in the Antidepressant group. The DA concentrations of Rosemary essential oil group were two-fifths of those in 1,8-Cineole group and one-third of those in Harmonious fragrance group.

In contrast, the Blank group showed a high concentration of TNF-α in their serum, with a value of 209.14 pg/mL, which exceeded 200 pg/mL. The concentration of TNF-α in the different treatment groups followed this order: Blank group (CONC = 209.14 pg/mL) > Rosemary essential oil group (CONC = 154.23 pg/mL) > Monomer fragrance raw material group (1,8-Cineole) (CONC = 130.60 pg/mL) > Harmonious fragrance group (CONC = 90.33 pg/mL) > Antidepressant group (CONC = 41.30 pg/mL). The TNF-α concentration in the Blank group was approximately two times higher than in the Harmonious fragrance group and five times higher than in the Antidepressant group. The TNF-α concentration of Rosemary essential oil group were 1.2 times those in 1,8-Cineole group and 1.7 times those in Harmonious fragrance group (Fig. 5H).

Similarly, the Blank group had a high concentration of IL-1β in their serum, with a value of 206.05 pg/mL, which also exceeded 200 pg/mL. The concentrations of IL-1β in the different groups were ranked as follows: Blank group (CONC = 206.05 pg/mL) > Rosemary essential oil group (CONC = 142.07 pg/mL) > Monomer fragrance raw material group (1,8-Cineole) (CONC = 112.91 pg/mL) > Harmonious fragrance group (CONC = 88.02 pg/mL) > Antidepressant group (CONC = 44.42 pg/mL). The IL-1β concentration in the Blank group was 2.3 times higher than in the Harmonious fragrance group and 4.6 times higher than in the Antidepressant group (Fig. 5I).

Notably, the 1,8-Cineole group, a key component of rosemary essential oil, demonstrated a significantly higher 5-HT concentration compared to the Rosemary essential oil group (106.76 ng/mg), indicating that 1,8-Cineole plays a critical role in enhancing serotonin levels. This aligns with the observed superior antidepressant effects of 1,8-Cineole compared to whole rosemary essential oil. And the Harmonious fragrance essential oil showed a marked increase in DA levels, surpassing rosemary essential oil, though not reaching the levels seen in the antidepressant group. The higher concentration of both 5-HT and DA in the 1,8-Cineole and Harmonious fragrance groups compared to the Rosemary essential oil group further supports the notion that 1,8-Cineole is a potent antidepressant compound, even though it is not as effective as conventional antidepressants. Also, 1,8-Cineole have shown the potential to modulate the immune response and decrease the production of inflammatory mediators. This reduction in inflammation could enhance the activity of serotonin and dopamine, two neurotransmitters that play a pivotal role in mood regulation. Moreover, both compounds have demonstrated antioxidant properties, which further support their potential antidepressant-like effects by protecting the brain from oxidative damage that could exacerbate mood disorders.

In conclusion, the anti-inflammatory action of 1,8-Cineole and Harmonious fragrance oils not only suggests a mechanism through which they may alleviate inflammation-driven depressive symptoms, but it also highlights the importance of targeting inflammatory pathways in the development of new treatments for depression. By combining anti-inflammatory and neuroprotective effects, these oils could offer a promising adjunctive therapy for managing depression, particularly in individuals with underlying inflammation-related mood disturbances.

3.6. Microcapsule of harmonious rosemary essential oil

3.6.1. Modification characterization of γ-CD

The γ-CD was modified into the dendritic macromolecule that has strong affinity with fabrics or skin. Fourier-transform infrared spectroscopy (FTIR) was then used to analyze the infrared spectra of hexadecanol, γ-CD, and modified γ-CD (Fig. 6A). Comparing the FTIR spectra of γ-CD, modified γ-CD, and hexadecanol, the appearance of absorption peaks at 1066, 1227, 1446, and 1767 cm^{-1} in the spectrum of modified γ-CD indicates the presence of ester groups, confirming the successful modification.

3.6.2. Preparation of microcapsules

The core-to-shell ratio and the amount of added emulsifier were main influencing factors to the particle size of microcapsules. According to Fig. 6B, the general trend of the microcapsule particle size curve for essential oils showed that the particle size first decreased and then gradually increased as the core-to-wall ratio increased. The smallest particle size and the lowest dispersion were achieved when the core-to-wall ratio was 1:5, with an average particle size of 1897 nm and a PDI of 0.352. Therefore, the core-to-wall ratio of 1:5 was chosen as the optimal single-factor condition for preparing essential oil microcapsules. Fig. 6C indicated that the general trend of the microcapsule particle size curve for essential oils was that the particle size gradually decreased with increasing emulsifier amount, while the dispersion first increased, then decreased, and finally increased again before decreasing. When the emulsifier amount was 0.5 g, the microcapsule particle size was smallest, with an average of 1964 nm and the lowest PDI of 0.179, indicating the best dispersion. Considering these factors, the core-to-wall ratio of 1:5 and emulsifier amount of 0.5 g were selected as the optimal single-factor conditions for preparing essential oil microcapsules.

3.6.3. Characterization of essential oil microcapsules

There are two weight loss stages of modified γ-CD (Fig. 6D). The first occurred at 166.5 °C with a relatively slow rate, while the second thermal weight loss occurred at 397.8 °C with a rapid rate, continuing until complete loss at 435.0 °C. This study used thermogravimetric analysis to investigate the stability of the modified γ-CD encapsulating the essential oil. The quality of the essential oil decreased with increasing temperature due to its susceptibility to volatilization. Initially, the rate of quality reduction was slow, but as the temperature rose, the rate increased. At 117.8 °C, the essential oil quality dropped to 50 %, and it completely volatilized by 244.3 °C (Fig. 6E). In contrast, the microcapsules of essential oil began to lose weight at 201.4 °C, suggesting that the wall material began to rupture. Between 201.4 °C and 253.5 °C, the rate of weight loss increased, then slowed after 253.5 °C, and accelerated again at 307.0 °C, with complete loss by 343.5 °C (Fig. 6F). Comparing Fig. 6D, E, and F, it was concluded that the modified γ-CD significantly improved the temperature range of essential oil loss and reduced the rate of quality loss, enhancing the stability of both the essential oil and its microcapsules.

The morphology of the essential oil microcapsules was observed and shown in Fig. 6G. The essential oil microcapsules generally appeared in square and irregular shapes, with some capsules displaying clumped and aggregated forms of varying sizes. The presence of aggregation may be attributed to the adhesive properties of the emulsifier causing adhesion. Overall, the essential oil was encapsulated by the modified γ-CD wall material.

Additionally, the HERACLES II electronic nose (France Alpha MOS) was used to analyze samples of harmonious fragrance essential oil (HF), freshly prepared essential oil microcapsules (FP), and long-stored

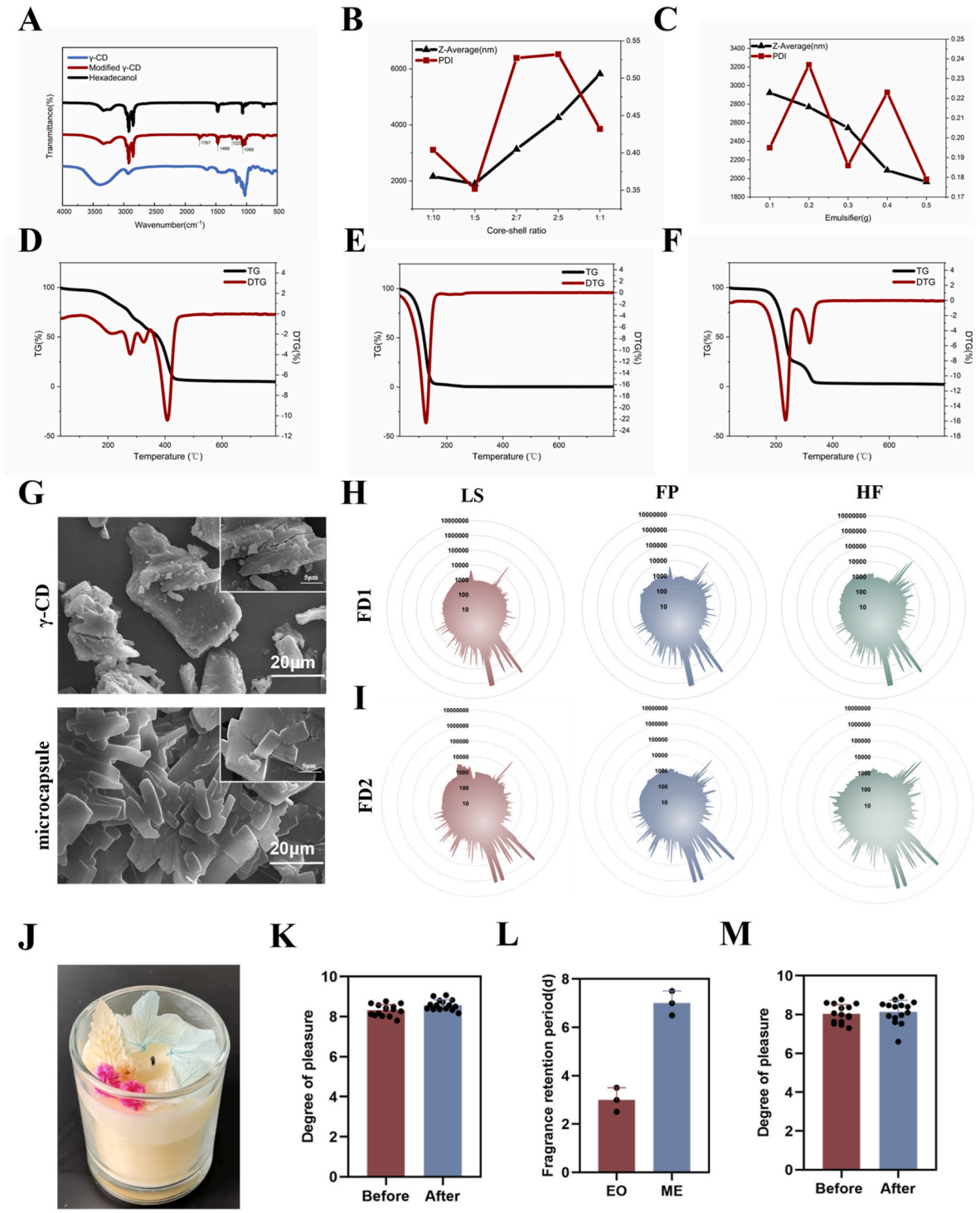


Fig. 6. Preparation and applications of microcapsules. A) Fourier-transform infrared spectra of cetyl alcohol, γ-CD, and modified γ-CD. B) The impact of different core-to-shell ratios on the particle size and dispersion of essential oil microcapsules. C) The impact of varying emulsifier dosages on the particle size and dispersion of essential oil microcapsules. D) TGA of modified γ-CD. E) TGA of harmonious fragrance essential oil. F) TGA of essential oil microcapsules. G) SEM image of γ-CD and microcapsule. H) E-nose measurement on the DB-5 polar column. I) E-nose measurement on the DB-1701 polar column. J) The aromatherapy candle photo. K) The degree of pleasure before and after lighting the candle. L) The release time of fragrance for the essential oil microcapsule shower gel and the essential oil shower gel. M) The degree of pleasure before and after using the shower gel.

essential oil microcapsules (LS). By comparing the aroma profiles detected with two different polar columns (DB-5 and DB-1701), it was found that harmonious fragrance essential oil exhibited several high-content and complex peaks. According to Fig. 6I, there were some differences in aroma between the essential oil before and after encapsulation. This was likely due to changes in the aroma release ratio of the microcapsules compared to the exposed essential oil, with the wall material slowing the release rate, or due to the impact of the wall material and emulsifiers used in the microcapsule preparation process on the aroma structure. However, the characteristic peaks of compounds with anti-depressant effects remained, with negligible impact. The electronic nose radar plots of long-stored microcapsules and freshly

prepared microcapsules largely overlapped, with only minor reductions in aroma observed in a few areas for the 30-day stored microcapsules compared to the freshly prepared ones. This indicated that the essential oil microcapsules in this study had high-quality aroma and good stability, with the ability to release aroma slowly.

3.7. Applications of harmonious rosemary essential oil

The essential oil microcapsule aromatherapy candles prepared in this study had a very faint fragrance when unlit but provided a long-lasting scent with a slow release. When they were lit, a pleasant fruity aroma with hints of rosemary was emitted, accompanied by a mint-like freshness. The candle can effectively alleviate fatigue and providing antidepressant effects. The shower gel containing essential oil microcapsules showed no sign of layering, and had a moderate viscosity. According to Fig. 6L, the fragrance retention time of the essential oil microcapsule shower gel on human skin was 2.3 times that of the essential oil shower gel, indicating that it achieved the goal of slow fragrance release. This could effectively extend the anti-depressant effects of the essential oil in personal care products. As is shown in Fig. 6K and M, both the candles and the shower gel provided a high level of satisfaction to the users before and after use, with scores generally around 8.

4. Conclusion

This study explored rosemary essential oil as a novel antidepressant material by analyzing its antidepressant mechanisms through GC-MS technique, network pharmacology and animal experiments, and preparing essential oil microcapsule materials with antidepressant effects. Here, the rosemary essential oil analyzed by GC-O and GC-MS techniques, whose main aromatic compounds are determined as 1,8-cineole (OAV = 1869.27, AI = 9.4), linalool (OAV = 1668.90, AI = 5.4), and ethyl decanoate (OAV = 1169.09, AI = 6.9). Molecular docking technology was used to validate this hypothesis, and through Gface and sensory combination methods, a more effective harmonious fragrance essential oil (rosemary essential oil: 1,8-cineole: d-limonene = 1:3:3) was formulated. In the rat tail suspension test and forced swim test, the rosemary essential oil group showed a reduction in depressive state compared to the blank group, with an immobility time twice that of the harmonious fragrance group and three times that of the antidepressant group, indicating that the harmonious fragrance oil was superior to rosemary essential oil in both antidepressant efficacy and aroma quality. After the verification of the above experiment, we found that rosemary essential oil exerts antidepressant effects through multiple mechanisms, which involve neurotransmitter systems (such as 5-HT and DA) and anti-inflammatory actions (e.g., inhibition of TNF-α and IL-1β). Moreover, γ-CD was modified to be dendritic macromolecules used as wall materials to prepare essential oil microcapsules. The optimal single-factor conditions for preparing the essential oil microcapsules were determined to be a core-to-wall ratio of 1:5 and an emulsifier amount of 0.5 g, based on minimizing particle size and achieving the best dispersion. The microencapsulation enhanced the stability the harmonious essential oil. The electronic nose radar plots of freshly prepared essential oil microcapsules (stored at 25 °C for 1 day) and long-stored essential oil microcapsules (stored at 25 °C for 30 days) were nearly identical. This indicated that the microcapsules produced in this study had high-quality aroma and released fragrance slowly. The microcapsule emulsion (500 mg/L) retained aroma on human skin 2.3 times longer than the essential oil alone (500 mg/L).

In summary, the essential oil microcapsules developed in this study demonstrated superior antidepressant efficacy, aroma quality, scent retention time, and stability compared to rosemary essential oil itself. Their application in scented candles could release pleasant aromas during burning, and their use in shower gels would extend fragrance duration, providing a theoretical basis and practical application for essential oil use.

CRediT authorship contribution statement

Yunwei Niu: Visualization, Resources, Project administration, Funding acquisition, Conceptualization. **Liyang Xu:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Mengdong Qiao:** Validation, Supervision, Resources, Conceptualization. **Yamei Wang:** Writing – review & editing, Supervision, Resources, Project administration, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no competing financial interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mtbio.2025.101546.

Data availability

Data will be made available on request.

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