Mechanism Underlying the Anxiolytic Effect of *Cinnamomum Camphora* Chvar. *Borneol* Essential Oil Revealed by Network Pharmacology

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Research

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Abstract

Background: Anxiety disorder, the most common mental health issue, can cause palpitations, fear, and compulsive behavior, and can severely endanger human health. Most drugs to treat anxiety disorder can cause a variety of side effects, therefore, it is important to seek natural and safe complementary and alternative therapies.

Methods: The open field (OF), elevated plus maze (EPM), and light-dark box (LDB) tests were used to confirm the anxiolytic effect of BEO in mice. Further, we constructed a component-target-signaling pathway network and a protein-protein interaction (PPI) network for the regulation of anxiety by BEO through pharmacological network analyses, and performed Gene Ontology (GO) enrichment analyses of BEO targets, and analyzed the active components and targets of BEO through molecular docking.

Results: In the OF test, BEO significantly prolonged the time spent by the mice in the central area ($p < 0.05$), in a dose dependent manner (r = 0.9992), and also significantly increased the number of central area entries ($p < 0.01$). In the EPM test, BEO significantly increased the time spent in the open arms ($p < 0.01$) and the number of entries into the open arms ($p < 0.01$) in a dose-dependent manner (r = 0.9733, r = 0.9669). In the LDB tests, BEO significantly increased the light area duration ($p < 0.05$) and the transition number ($p < 0.01$) in a dose-dependent manner (r = 0.9166, r = 0.9572), thus confirming its anxiolytic effect. Network pharmacology results showed that 33 active components in BEO acted on 54 targets, mainly through modulation of neuroactive ligand-receptor interactions, G-protein coupled receptor signaling pathways, and RNA polymerase II transcription factor activity. PPI network analysis identified 48 key proteins, including estrogen receptor 1 (ESR1), androgen receptor (AR), and mitogen-activated protein kinase 8 (MAPK8). Molecular docking results showed that the main active components of BEO are borneol, β-caryophyllene, α-cadinol, limonene, and α- selinene, which act on the key targets CNR2, ADRA2B, and ADORA2A.

Conclusion: Our results indicated that BEO has multi-component, multi-target, and multi-pathway characteristics, thus providing a theoretical basis for further research on the mechanism of action of BEO as a potential anxiolytic agent.

1. Background

Anxiety disorder, the most common mental health issue, can cause palpitations, fear, and compulsive behavior, and can severely endanger human health [1]. The main drugs used to treat anxiety disorders are benzodiazepines, which have been used as the first-line treatment for decades. However, these drugs in clinical practice have been found to cause problems such as excessive sedation, headaches, blurred vision, dependence, and withdrawal symptoms [2]. Therefore, safe, and evidence-based complementary or alternative therapies may provide safer and more effective treatments for patients with anxiety.

Recent studies have shown that essential oils can reduce stress, anxiety, and depression, and promote physical and mental health. Essential oils have been used as traditional medicines for centuries, and a

long history of treatment with essential oils has demonstrated their efficacy and safety [3]. Studies have found that oral neroli oil, which is rich in limonene (25%), β-pinene (20%), and linalool (16%), can relieve anxiety [4].

BEO (borneol, 16.4%) is a by-product of natural crystalline borneol (NCB, 98.4% borneol) obtained by steam distillation of *Cinnamomum camphora*. Its main components, including β-caryophyllene (10.7%), limonene (8.2%), α-pinene (7.5%), β-pinene (3.6%), and linalool (0.5%), have been shown to have significant anti-inflammatory effects [5]. In animals, borneol has been reported to have significant anxiolytic effects when injected into the dorsal hippocampus, on the basis of open field (OF), elevated plus maze (EPM), and light-dark box (LDB) tests [6]. Oral β-caryophyllene also has shown significant anxiolytic effects in the above tests [7]. Previous studies have confirmed that intraperitoneal injection of limonene [8] and inhalation of α-pinene [9] have significant anxiolytic effects, on the basis of EPM experiments. Therefore, these components may have potential as anxiolytic agents for administration in humans.

The above findings indicate that all these components are found in BEO, and it anxiolytic effects are worthy of further exploration. To date, the anxiolytic mechanism of action of essential oils has rarely been investigated. Therefore, to determine its mechanism of action, we used classical OF, EPM, and LDB methods to evaluate the anxiolytic efficacy of BEO, the BEO anxiolytic component-target-signaling pathway network, and the protein interaction (PPI) network, as constructed through network pharmacology analysis. We also performed Gene Ontology (GO) enrichment analysis of the targets of BEO and analyzed the binding sites of the active components and their targets through molecular docking to further determine the relationship between the active ingredients and targets, and to provide a theoretical basis for the mechanism of action of these active compounds.

2. Materials And Methods

2.1 Reagents and instruments

BEO was provided by *Chunjingziran* Biotechnology (Shaoxing, Zhejiang Province, China) and was obtained by steam distillation of fresh branches and leaves of *Cinnamomum camphora* chvar. *Borneol*. *Cinnamomum camphora* chvar. *Borneol*. A voucher specimen (768133) was deposited at the South China Institute of Botany, Chinese Academy of Science (Guangzhou, Guangdong, China). The essential oil was dehydrated by the addition of anhydrous Na_2SO_4, collected in a dark brown bottle, and stored at 4°C until use. The components of BEO were previously identified by our research group through gas chromatography-mass spectrometry; the total number of components was 43, and the most abundant component was borneol (16.4%) [5] (Table S1).

2.2 Experimental animals

All experimental animal procedures were approved by the Ethics Committee of the Experimental Animal Center of Jiangnan University [No. 20191015i0151220 (279)], and the care and use of experimental

animals complied with national and international guidelines (2010/63/EU). Adult Institute of Cancer Research (ICR) mice (20 ± 2 g) were housed in an environment with 22 ± 2°C, 55 ± 15% relative humidity and a 12-h light/dark cycle, and were given food and water ad libitum. All animals were acclimated to the experimental environment for at least 5 days before experimentation.

The mice were randomly divided into five groups (ten per group: five males and five females), which included a control group and a positive control group (2 mg/kg diazepam in saline solvent, administered intraperitoneally) and three BEO groups with different BEO doses (150, 300, or 600 mg/kg in corn oil solvent, administered orally) [10]. The administration continued for 7 days; at 30 minutes after the last administration, OF, EPM, and LDB tests were performed sequentially.

2.3 Behavioral procedures

2.3.1 Open field tests

OF test is among the most used behavioral tests for the evaluation of anxiety in rodents. The test is based on the animals' tendency to move around the walls of the box rather that explore the central area. Our experiments were performed according to a protocol modified from a previous study [10]. The OF test uses an OF box and a video capture system. In this study, the OF test consisted of an arena of 45 cm length × 45 cm width × 45 cm depth. At the beginning of the experiment, the mice were quickly placed in the center of the OF, the video collection and timer were started simultaneously, and activity was recorded for 5 minutes. After completion, we used 75% alcohol to clean and wipe the OF to eliminate the influence of animal odor on subsequent experiments. Without changing the total moving distance, the increase in the number of times in which the animal entered the central area and the percentage of time spent in the central area with respect to the total time were recorded and used as anxiolytic effect indices. We used Etho Vision XT 11 (Noldus Information Technology, Leesburg, VA) for data analysis.

2.3.2 Elevated plus maze test

The mice EPM test was based on a method slightly modified from that described previously [8]. The equipment consisted of EPM hardware and a video capture system. The EPM hardware consisted of two opposite open arms (30 cm × 6 cm × 15 cm), two opposite closed arms (30 cm × 6 cm × 15 cm), and a central platform (6 cm × 6 cm) connected by four arms. The distance between the bottom of the maze and the ground was 50 cm. At the beginning of the experiment, the mice were quickly placed onto the central platform with their heads facing the open arm, then allowed to explore freely. Simultaneously, the video acquisition and timer were started, and the number of times in which the mouse entered the open arm, the staying time, the number of times the mouse entered the closed arm, and the staying time within 5 minutes were recorded. After the video collection, we again wiped the maze with 75% alcohol to eliminate the influence of animal odor on subsequent experiments. Anxiolytic drugs decrease the natural aversion of mice to the open arms and promote exploratory behavior. Therefore, the percentage of entries to the open arms with respect to the total number of arm entries, and the percentage of exercise time in

the open arms with respect to the total time were used as indicators to evaluate the anxiety levels in the mice. We used Etho Vision XT 11 for data analysis.

2.3.3 Light-dark box test

The LDB (Cat. No. 40553, UGO, USA) test was established on the basis of rodents' aversion to bright new environments. When mice are less anxious, the time and distance taken to enter the bright box increases. However, when mice are more anxious, they tend to move into the dark box. At the beginning of the test, the mice were placed into the light box, the board in the middle of the LDB was removed, and the animals were allowed to explore freely for 5 minutes, during which their behavioral indices were recorded. The percentage of time that the mice spent in the light box with respect to the total test time, and the number of entries into the light box were used as indicators [10]. After each test, the LDB was wiped with 75% alcohol to eliminate the influence of animal odor on subsequent experimental animals.

2.4 Grip-strength measurement

A grip strength meter (YLS-13A, Yiyan Technology Development Co., Ltd., Shandong, China) was used to assess forelimb grip strength. Mice were lifted by their tails so that their forepaws could grip the strength meter wire, then gently pulled back with their tails parallel to the surface of the table until they lost their grip on the wire. The maximum force was recorded in gram-force (gf). Three tests were performed on each mouse, and the average score was used for statistical analysis [11].

2.5 Network pharmacology analysis

2.5.1 Compound-target-pathway network

We have identified BEO components in our previous study [5] (Table S1). **S**implified molecular input line entry systems (SMILE) and strings of the components were obtained by searching the Traditional Chinese Medicine Integrated Database (TCMID) (database http://www.megabionet.org/tcmid/) and imported into the Swiss Target Prediction database (http://www.swisstargetprediction.ch/) to identify potential targets for the BEO components. The Swiss Target Prediction database predicts targets of active molecules on the basis of chemical structure and ligand similarity, as well as cross validation and arrangement analyses [12]. The predicted targets for all BEO components were obtained from limited search species in humans. Next, the DisGeNET database (http://www.disgenet.org/web/DisGeNET/menu/home) was used to screen potential targets for anxiety treatment, and the targets for the BEO components and anxiety treatment were intersected to obtain the targets that could potentially be used as anxiolytics.

On the basis of the above prediction results, the Clue GO module in *Cytoscape* 3.2.1 software was used to annotate GO enrichment analysis of the targets of the BEO active components. The Kyoto Encyclopedia of Genes and Genomes (KEGG) Mapper tool from the KEGG database (http://www.kegg.jp/) was used to identify enriched pathways of the targets. *Cytoscape* 3.2.1 was used to construct a component-target-signaling pathway network in which nodes representing active components of BEO, potential targets, and

associated signaling pathways were connected by edges. The mechanisms of action of BEO in treating anxiety were determined by analysis of the constructed network.

2.5.2 Gene Ontology enrichment analysis

Clue GO analysis allows similar descriptors to be used for the functions of target products across databases. It was used to classify and analyze the targets from biological processes, molecular functions, and cellular components ($p \leq 0.05$) to predict the anxiolytic mechanism of action BEO [13].

2.5.3 Construction of protein interaction networks

The String database (https://string-db.org/Version 10.5) was used to analyze known and predicted PPI databases[14]. We imported the targets of the BEO active ingredients into the String database, limited the research species to humans, obtained protein interaction relationships, and saved them in TSV format. We imported node1, node2, and combined score information in the file into *Cytoscape* software to construct the PPI networks and obtain the network analysis results. To produce the PPI, we further set the node size; color, which ranged from dark red to yellow to light green, reflecting large to small values; and edge thickness, reflecting the size of the combined score.

2.5.4 Component-target molecular docking of the potential active components

To further verify the reliability of the target prediction results, we performed molecular docking validation on the screened active ingredients and their associated targets. First, the chemical structures of the main active ingredients were optimized with Chem 3D 15.0, and then the ligand rotatable bonds were determined with AutoDock 4.2.6, after which the 3D structures of the key targets were obtained from the Protein Data Bank (PDB) database (http://www.rcsb.org/pdb). Then the targets were de-watered and de-liganded with PyMOL 2.3.4 software; hydrogenated and subjected to calculation of charges in AutoDock 4.2.6 software; docked with AutoDock Vina 1.1.2 software; and visualized with PyMOL 2.3.4 software. The match between active components and target proteins was assessed according to the docking score value. A binding energy less than − 4.25 kcal/mol is generally considered to suggest some binding activity of the ligand to the receptor; less than − 5.0 kcal/mol implies good binding activity, and less than − 7.25 kcal implies strong binding activity [13, 15].

2.6 Data analysis

Prism 6 (GraphPad, San Diego, CA, USA) and OriginLab-9.0s (OriginLab, Northampton, MA, USA) were used for data analysis, and plots and results are expressed as mean ± standard deviation. The data were analyzed with one-way analysis of variance with Dunnett's multiple comparisons test; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; and ****$p < 0.0001$ were considered statistically significant.

3. Results

3.1 Open field test

In the OF test (Fig. 1), there was no significant difference in the total movement distance between groups, thus indicating that the tested substance had no significant effect on the total activity of the mice (Fig. 1A, $p > 0.05$). Compared with the control group, the groups with BEO treatment (300 or 600 mg/kg) showed significantly longer time in the central area (Fig. 1B, $p < 0.05$), in a dose-dependent manner ($r = 0.9992$). BEO (600 mg/kg) also significantly increased the number of center area entries (Fig. 1C, $p < 0.01$), thus indicating its anxiolytic effect, which was comparable to that of the positive control (diazepam). The above difference was not due to the difference in the total distance moved by the mice.

3.2 Elevated plus maze test

In the EPM test (Fig. 2), the BEO group showed significant differences with respect to the control group when the dose reached 600 mg/kg, and the effect was equivalent to that seen with the positive control ($p < 0.01$). The time spent in open arms (Fig. 2A) and the number of entries onto open arms (Fig. 2B) showed a dose-dependent increase ($r = 0.9733$ and 0.9669), thus indicating the good anxiolytic effects of BEO.

3.3. Light-dark box test

In the LDB test (Fig. 3), BEO treatment (300 or 600 mg/kg), as compared with the control group, significantly increased the light area duration (Fig. 3A, $p < 0.05$) and the transition number (Fig. 3B, $p < 0.01$), similarly to the effects of the positive control. In addition, the light area duration and transition number were dependent on the dose ($r = 0.9166$ and 0.9572).

3.4 Coordinated movement ability

To determine whether BEO influences movement coordination, we subjected the mice to grip meter testing (Fig. 3C). BEO, compared with the control, had no significant effect on grip strength ($p > 0.05$), thus indicating that BEO did not affect movement or coordination ability in these mice, in agreement with previous observations after intraperitoneal injection of borneol [16].

3.5 Component-target-pathway BEO networks involved in the regulation of anxiety

3.5.1 Components and targets

Using BEO components [5] (Table S1) obtained from previous research from our group, and examining anxiety-related targets through database searches, we screened 33 components associated with anxiety and identified 54 corresponding targets (Fig. 4A). The target genes cannabinoid receptor 2 (CNR2), androgen receptor (AR), estrogen receptor 1 (ESR1), acetylcholinesterase (ACHE), solute carrier family 6 member 4 (SLC6A4), and cytochrome P450 family 2 subfamily C member 19 (CYP2C19) were associated with 17, 16, 14, 14, 12, and 11 BEO components, respectively, and were the main targets involved in the anxiolytic effect of BEO (Fig. 4A).

3.5.2 Targets and signaling pathways

On the basis of results from KEGG signaling pathway analysis (Fig. 4B), signaling pathways for the 54 targets were found to involve mainly neuroactive ligand-receptor interactions (16 targets), metabolic pathways (13 targets), calcium signaling pathways (9 targets), cancer pathways (9 targets), neurodegeneration pathways (7 targets), Alzheimer's disease pathways (6 targets), and cAMP signaling pathways (6 targets).

3.5.3 Gene Ontology enrichment analysis

To further understand the interacting genes, we performed GO enrichment analysis, which revealed the following findings. (1) The enrichment analysis of biological processes (Fig. 5A) showed that the ten terms with the largest number of targets among 97 biological processes mainly included regulation of ion homeostasis and organic hydroxy compound transport (11 targets), G-protein coupled receptor signaling pathways, steroid hormone mediated signaling pathways and response to alkaloids (10 targets), and response to ammonium ions (7 targets). (2) The enrichment analysis of molecular functions revealed terms (Fig. 5A) including RNA polymerase II transcription factor activity (five targets), steroid hormone receptor binding (four targets), neurotransmitter, sodium symporter activity, and oxidoreductase activity (three targets). (3) The enrichment analysis of cellular components (Fig. 5A) confirmed that the targets were mainly located in caveolae.

3.5.4 Protein interaction network

The PPI network was constructed with the String database and *Cytoscape* software (Fig. 5B). Among 54 target proteins, six targets [ACHE, BCHE, thymidylate synthetase (TYMS), carboxylesterase 2 (CES2), macrophage migration inhibitory factor (MIF) and prolyl endopeptidase (PREP)] did not interact with other targets. According to database calculations, AR and E1A binding protein p300 (EP300), peroxisome proliferator activated receptor gamma (PPARY) and EP300, EP300 and ESR1 had the strongest interaction, with a combined score of 0.999. According to the target interactive network diagram (Fig. 5B), AR and ESR1 were located in the center of the targets, with the highest degree value, followed by mitogen-activated protein kinase 8 (MAPK8), PPARG, nuclear receptor subfamily 3 group C member 1 (NR3C1), EP300, prostaglandin-endoperoxide synthase 2 (PTGS2), 5-hydroxytryptamine receptor 2A (HTR2A), dopamine receptor D2 (DRD2), and metabotropic glutamate receptor 5 (MGR5).

3.5.5 Active component-target protein molecular docking analysis

The top five components derived from BEO were borneol (16.4%), β-caryophyllene (10.7%), camphor (10.6%), limonene (8.2%), and α-pinene (7.5%) (Table S1). The components with the top five "degree" values according to network pharmacology were borneol (26), linalool (16), methyl eugenol (16), α-cadinol (14), and α-selinene (11) (Table 1). For these 54 targets associated with anxiety, molecular docking was performed. The system with the lowest docking energy for each component and the targets

were shown in Table 1. The docking energy of all components was lower than − 5 kcal/mol, thus indicating that the components have good binding activity to the target proteins. The docking energy of more than 50% of the components with the targets was less than − 7.25 kcal/mol, thus showing strong binding activity [13]. The docking results (Fig S1) indicated that the above components and targets are the active components and targets of the anxiolytic effect of BEO.

Table 1
Molecular docking score

Molecular name	Content (%) *	Target	Score (Kcal/mol)
Borneol	16.4	CNR2	-7.6
β-caryophyllene	10.7	CNR2	-9.5
Camphor	10.6	EP300	-7.4
Limonene	8.2	ADRA2B	-7.2
α-Pinene	7.5	EP300	-7.7
α-selinene	0.7	ADORA2A	-9.0
Linalool	0.5	MIF	-6.5
Methyl eugenol	0.3	LRRK2	-7.1
α-Cadinol	0.1	CNR2	-9.0
*Preliminary work acquisition			

As shown in Fig. 6A, β-caryophyllene, α-cadinol, and borneol had the lowest binding energy with CNR2, showing strong binding activity (Table 1). For the CNR2 protein, the small molecule binding pocket is composed of typical hydrophobic amino acids such as F94, P184, F106, F183, I110, V113, F87, and F91, which form a hydrophobic binding pocket, whereas the S90, a polar amino acid, can form hydrogen bonds with small molecules. β-caryophyllene, which is composed of carbon atoms, has a large volume, can interact with the hydrophobic binding cavity on the CNR2 protein, and has the lowest energy (Fig. 6A). Compared with β-caryophyllene, α-cadinol is smaller, and thus the hydrophobic interaction formed between the surrounding amino acids is relatively weak. However, the hydroxyl -OH on the α-cadinol molecule can form a hydrogen bond with the S90 amino acid; therefore, it still has strong binding activity (Fig. 6A). Compared with β-caryophyllene and α-cadinol, borneol molecules have relatively fewer carbon atoms; consequently, when it binds the CNR2 protein, it cannot completely occupy the binding cavity, and its binding activity remains relatively low (Fig. 6A). Study have shown that β-caryophyllene acts on CNR2 and exerts anxiolytic effects [7], in agreement with the results from this study, showing that CNR2 is an important target for the anxiolytic effect of BEO. Among them, limonene and ADRA2B (Fig. 6B), α-selinene, and ADORA2A also have high binding activity (Fig. 6C).

4. Discussion

The results of OF test indicated that BEO significantly prolonged the time spent in the central area by approximately 0.52-fold (Fig. 1). Studies have shown that β-caryophyllene gavage in mice at a dose of 200 mg/kg also significantly prolongs the time spent in the central area by approximately 0.34-fold [10]. However, the actual concentration of β-caryophyllene in this study was only 64 mg/kg (Table S1), thus indicating that the active components in BEO are derived from β-caryophyllene. For example, intra-dorsal hippocampal injection of pure borneol [6] has anxiolytic effects, thereby indicating that borneol is also an active anxiolytic compound found in BEO.

The results of EPM test indicated that BEO prolonged the time spent in the open arms by approximately 1.2-fold and increased the number of entries on open arms by approximately 1.5-fold (Fig. 2) that in the control group. The β-caryophyllene used in the OF test also significantly prolonged the time spent on the open arms by approximately 0.6-fold when the mice were intragastrically administered a dose of 200 mg/kg. The number of entries into open arms significantly increased by approximately 0.4-fold [10], in agreement with previous results showing that the active components of BEO are derived not only from β-caryophyllene but also from other components. According to preliminary experiments (Table S1), the components of BEO, in addition to β-caryophyllene (10.6%), borneol (16.4%), and limonene (8.2%), have been tested with the EPM and demonstrated to possess anxiolytic effects [6, 8]. The above results indicate that β-caryophyllene, limonene, and borneol are the active components involved in the anxiolytic effect of BEO.

The results of LDB test indicated that BEO treatment (600 mg/kg), compared with the control group, significantly prolonged the light area duration by approximately 0.45-fold and increased the transition number by approximately 0.67-fold (Fig. 3). The β-caryophyllene described above also prolonged the light area duration by approximately 0.47-fold at a dose of 200 mg/kg and increased the transition number by 0.4-fold [10]. The actual concentration of β-caryophyllene found in BEO in this study was only 64 mg/kg (Table S1), thereby indicating that other components in BEO and β-caryophyllene have synergistic effects.

Further network pharmacological analysis results shown that CNR2 is associated with anxiety and depression [7], and AR has been confirmed to be involved in brain function and the regulation of anxiety [17]. ESR1 has also been confirmed to be associated with anxiety and to be a cause of frequent anxiety in women [18]. Furthermore, low ACHE activity has been associated with an increased risk of depression and anxiety in adolescents [19], and SLC6A4 is also associated with anxiety and depression [20]. Selective serotonin reuptake inhibitors are the main treatments for major depression and anxiety, and CYP2C19 polymorphisms are known to affect the metabolism of these drugs, thereby affecting their efficacy and safety [21].

In addition, studies have shown that borneol acts on the transient receptor potential cation channel vanilloid subfamily member 1 (TRPV1) and exerts analgesic effects [22]. Moreover, α-pinene, β-pinene, camphor, and limonene inhibit ACHE activity, and limonene also inhibits BCHE activity [19], whereas β-caryophyllene acts on CNR2, thereby producing its anxiolytic effect [7]. These results show that these components and targets are important for the regulation of anxiety with BEO.

Based on analysis of the KEGG signaling pathway (Fig. 4B), among them, the neuroactive ligand-receptor interaction signaling pathway controls and regulates many important biological functions such as emotion, memory, and endocrine function [23]. Anxiety and metabolic disorders are closely related, and both have common pathological manifestations, such as chronic inflammation [24]; moreover, long-term stress and anxiety can induce tumors and promote the development of cancer [25]. Both depression and anxiety are early manifestations of neurodegenerative diseases and Alzheimer's disease [26]. In addition, the serotonergic synapse pathway is associated with the occurrence of anxiety [23]. The cAMP signaling pathway also has anxiolytic effects, through regulating intracellular cAMP levels [27]. Therefore, BEO regulates anxiety through multiple signaling pathways, thus underscoring its multi-component, multi-target, and multi-pathway function. Furthermore, our current research is essentially consistent with previous results and thus should have value for theoretical guidance. Based on the analysis of GO enrichment analysis (Fig. 5A), among these, G-protein coupled receptor signaling pathways are involved in the development of anxiety disorders and may be a potential therapeutic target [28]. The steroid hormone mediated signaling pathway has also been confirmed to be associated with anxiety [29]. Interestingly, the sedative and hypnotic mechanism of action of a compound found in Anshen essential oil is similar to those of components in BEO and are also associated with calcium ion transport into the cytosol, response to ammonium ions, and RNA polymerase II transcription factor activity [13]. The above studies are essentially consistent with the findings of this study and indicate that BEO regulates anxiety through modulating ion homeostasis, transcriptional regulation, and signal transduction. Among the result of PPI network, AR and ESR1 are associated with a variety of human behaviors, including anxiety [30]. PPARG has been confirmed to be associated with pathological anxiety [31]. glucocorticoid receptor (NR3C1) [32] and HTR2A [33] are also associated with anxiety disorders. An estrogen-dependent interaction between ESR1 and EP300[34] and an interaction between NR3C1 and EP300 in transcription activation[35] have been reported. ADORA2A and D2D2 have been shown to have a synergistic effect on anxiety disorders [36]. The above results are consistent with those of this study. Subsequently, we further focused on the potential therapeutic targets of anxiety disorders and used molecular docking analysis of the active components and potential targets obtained from the screening.

Based on analysis of the molecular docking, among these, ADRA2B [37] and ADORA2A [36] have been reported to be closely associated with anxiety. In addition, camphor, α-pinene, linalool, and methyl eugenol have high affinity for EP300, MIF, and leucine rich repeat kinase 2 (LRRK2) targets. Furthermore, EP300 [38], MIF [39], and LRRK2 [40] have been confirmed to be associated with anxiety (Fig. S1), thus indicating that the above components and targets are significantly related to anxiety and are worthy of further investigation. This study determined and confirmed that the main active components in BEO, including borneol, β-caryophyllene, α-cadinol, limonene, and α-selinene, act on key targets, such as CNR2, ADRA2B, and ADORA2A. This study therefore provides new avenues for further research on the pharmacodynamic basis of BEO for the treatment of anxiety. Moreover, it should promote future basic research to determine novel targeted drugs for the treatment of anxiety disorders.

5. Conclusion

In this study, the OF, EPM, and LDB tests were used to confirm the anxiolytic effect of BEO in mice. Component-target-pathway network and PPI network pharmacology analysis of the anxiolytic process of BEO revealed 33 active components regulating 54 targets through neuroactive ligand-receptor interaction, G-protein coupled receptor signaling pathways, RNA polymerase II transcription factor activity, and other pathways working together in anxiety disorders. Our molecular docking results showed that the main active components of BEO are borneol, β-caryophyllene, α-cadinol, limonene, and α-selinene, which act on the key targets CNR2, ADRA2B, and ADORA2A. These relationships reveal the multi-component, multi-target nature of BEO. This study provides a theoretical basis for the mechanism of action of BEO as a potential treatment for anxiety and provides a novel means to explore auxiliary and alternative therapies for anxiety.

Abbreviations

Cinnamomum camphora chvar. *borneol* essential oil (BEO); natural crystalline borneol (NCB); protein-protein interaction (PPI); open field (OF); elevated plus maze (EPM); light-dark box (LDB); Gene Ontology (GO); estrogen receptor 1 (ESR1); androgen receptor (AR); mitogen-activated protein kinase 8 (MAPK8); cannabinoid receptor 2 (CNR2); androgen receptor (AR); estrogen receptor 1 (ESR1); acetylcholinesterase (ACHE); solute carrier family 6 member 4 (SLC6A4); cytochrome P450 family 2 subfamily C member 19 (CYP2C19); transient receptor potential cation channel vanilloid subfamily member 1 (TRPV1); thymidylate synthetase (TYMS); carboxylesterase 2 (CES2); macrophage migration inhibitory factor (MIF); prolyl endopeptidase (PREP); E1A binding protein p300 (EP300); peroxisome proliferator activated receptor gamma (PPARY); prostaglandin-endoperoxide synthase 2 (PTGS2); 5-hydroxytryptamine receptor 2A (HTR2A); dopamine receptor D2 (DRD2); metabotropic glutamate receptor 5 (MGR5); leucine rich repeat kinase 2 (LRRK2); adenosine A2a receptor (ADORA2A).

Declarations

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Authors' contributions

Shanshan Xiao: conceptualization, methodology, software, investigation, data curation, and writing the original draft; Hang Yu: resources, writing the review and editing, and supervision; Yunfei Xie: validation, formal analysis, visualization, and supervision; Yahui Guo: resources and project administration; Jiajia Fan: resources and funding acquisition; Weirong Yao: conceptualization, validation, formal analysis, visualization, writing the review and editing, supervision, and data curation.

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Availability of data and materials

The datasets used during the current study are available from the corresponding author upon reasonable request.

Ethical Approval and Consent to participate

All experimental animal procedures were approved by the Ethics Committee of the Experimental Animal Center of Jiangnan University (Wuxi, Jiangsu Province, JN. No. 20191015i0151220 (279)]

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests.

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Figures

Fig.1 Shanshan Xiao et.al

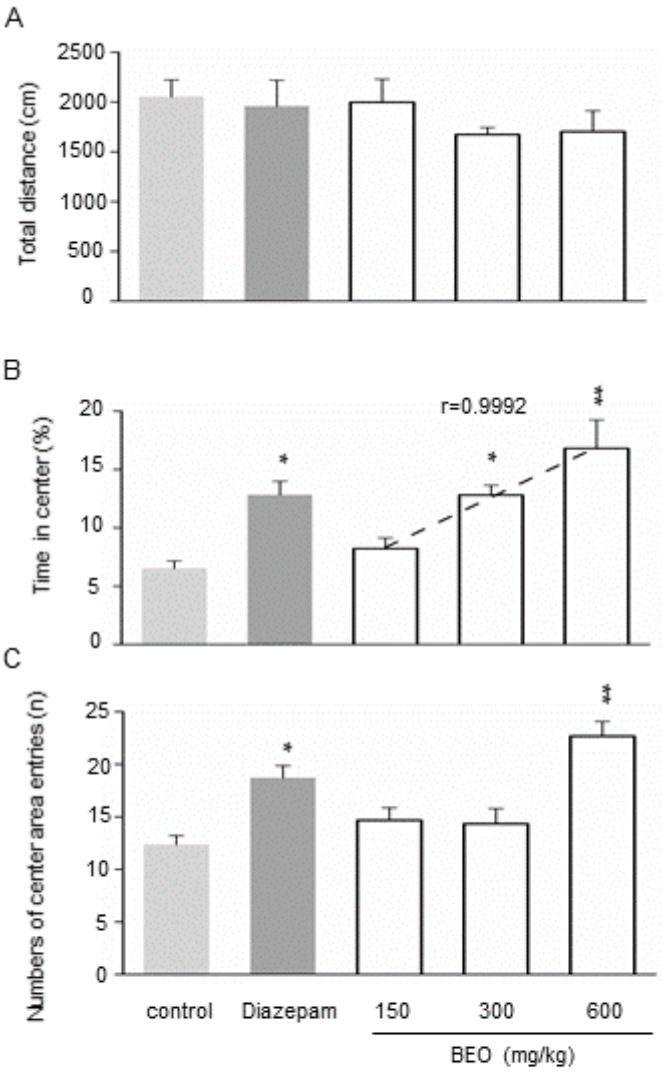

Figure 1

Effect of Cinnamomum camphora chvar. Borneol essential oil (BEO) on mice: total distance (A), time at the center (B), and number of center area entries (C) Data are expressed as the mean ± SEM (n = 10), **p < 0.01; ***p < 0.001; ****p < 0.0001 compared with the control group.

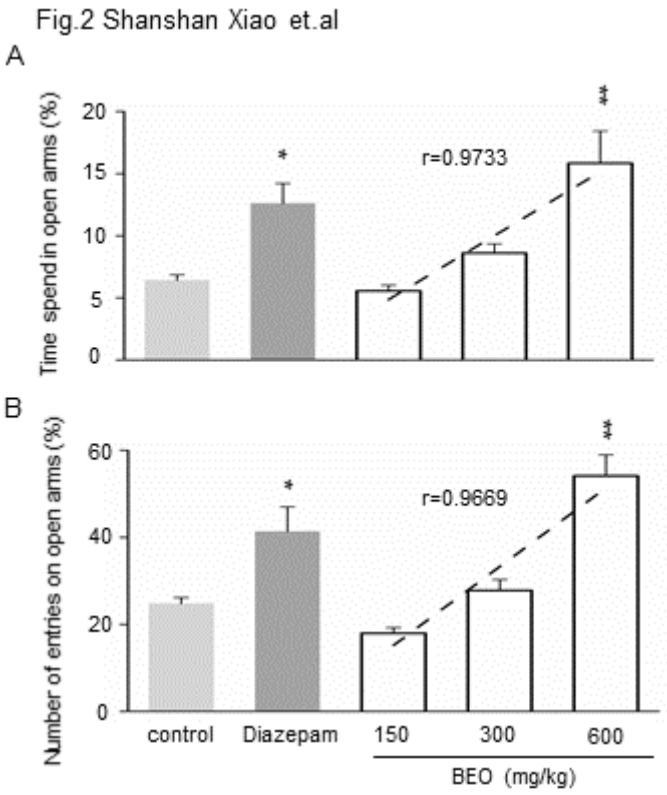

Figure 2

Effect of BEO on mice: time spent in open arms (%) (A) and number of entries to open arms (%) (B). Data are expressed as the mean ± SEM (n = 10), **p < 0.01; ***p < 0.001; ****p < 0.0001 compared with the control group.

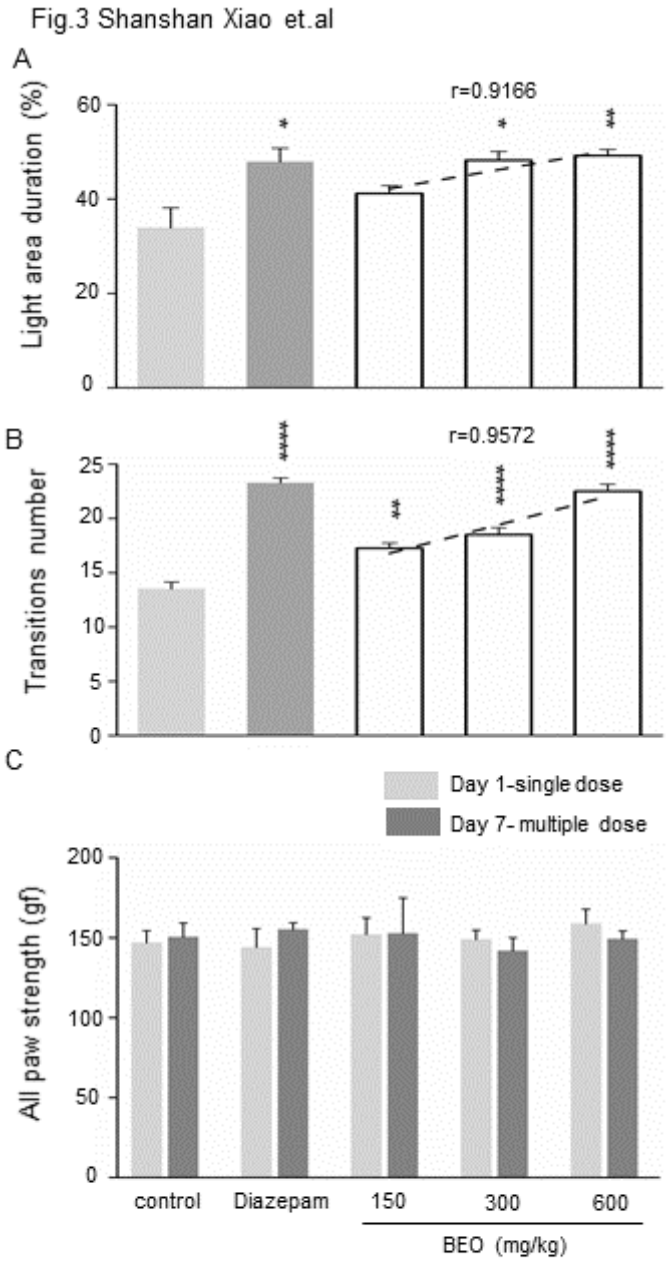

Figure 3

Effect of BEO on mice: light area duration (%) (A), transition number (B), and grip strength test (C). Data are expressed as the mean ± SEM (n = 10), **p < 0.01; ***p < 0.001; ****p < 0.0001 compared with the control group.

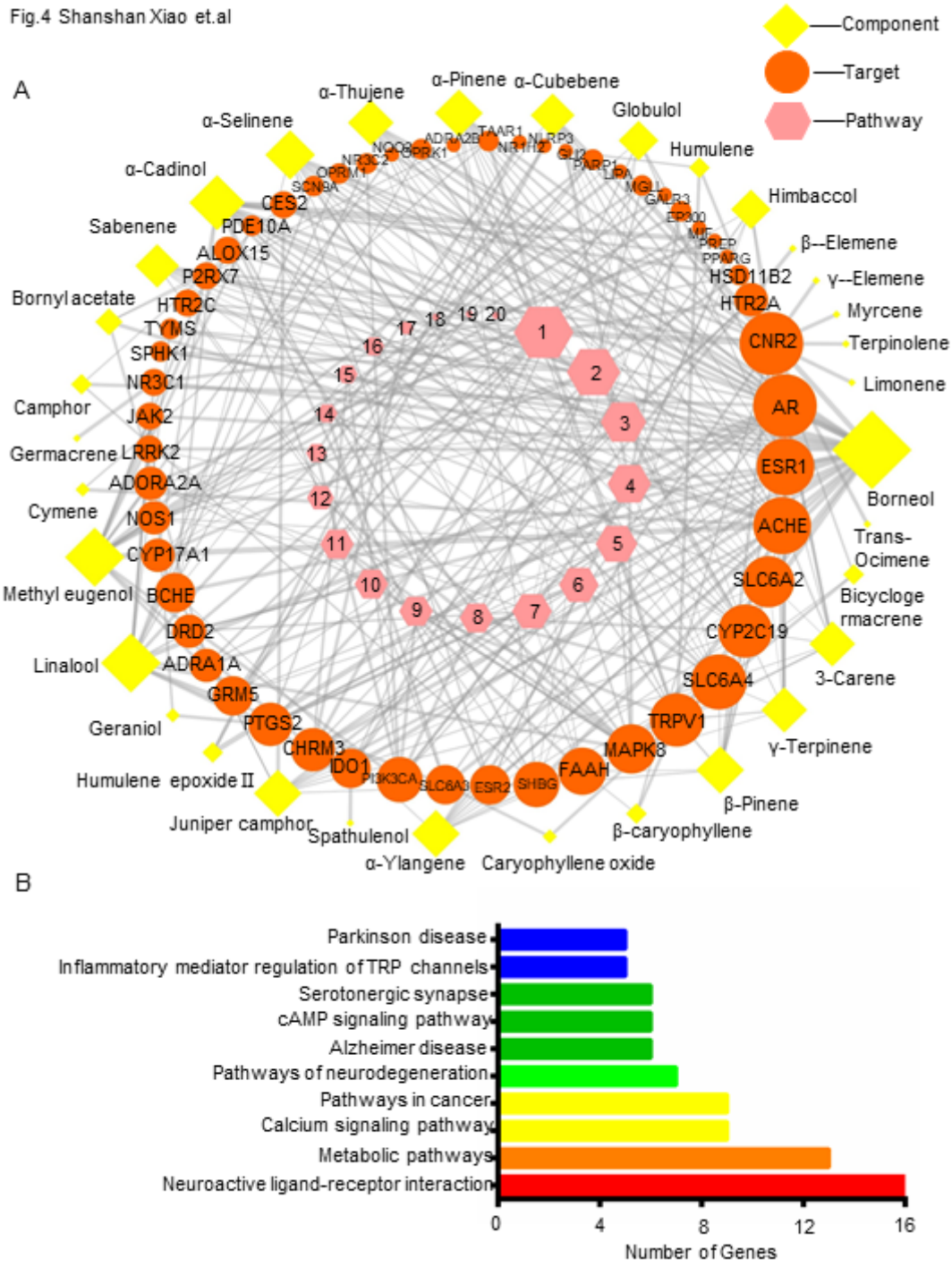

Figure 4

Anxiety pharmacology component-target-pathway network regulated by BEO. Rhomboidal yellow nodes represent components, round orange nodes represent targets, and hexagonal pink nodes represent pathways. 1. Neuroactive ligand-receptor interaction; 2. metabolic pathways; 3. calcium signaling pathway; 4. pathway in cancer; 5. pathways of neurodegeneration; 6. Alzheimer's disease; 7. cAMP signaling pathway; 8. serotonergic synapse; 9. inflammatory mediator regulation of TRP channels; 10. Parkinson's disease; 11. retrograde endocannabinoid signaling; 12. necroptosis; 13. insulin resistance; 14.

synaptic vesicle cycle; 15. cholinergic synapse; 16. aldosterone-regulated sodium reabsorption; 17. taste transduction; 18. cholesterol metabolism; 19. drug metabolism; 20. renin-angiotensin system.

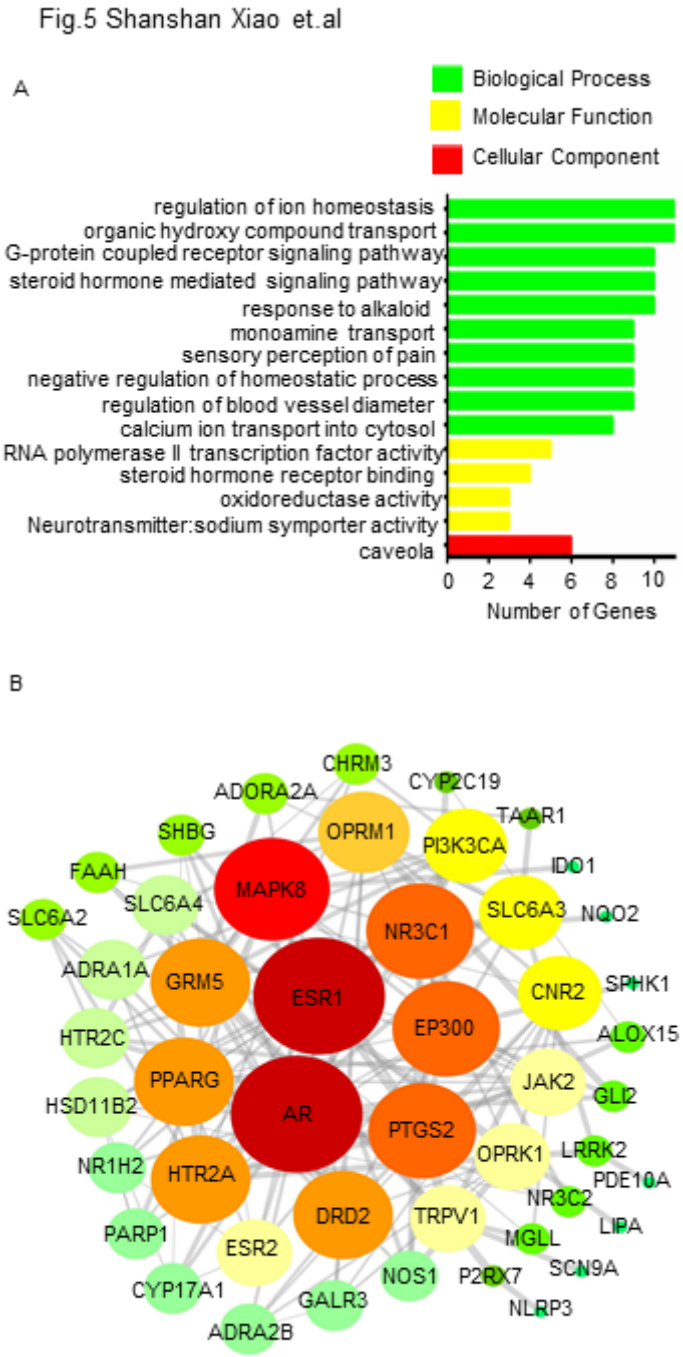

Figure 5

Gene Ontology (GO) enrichment analysis (A) and protein-protein interaction (PPI) network (B) of the 54 genes.

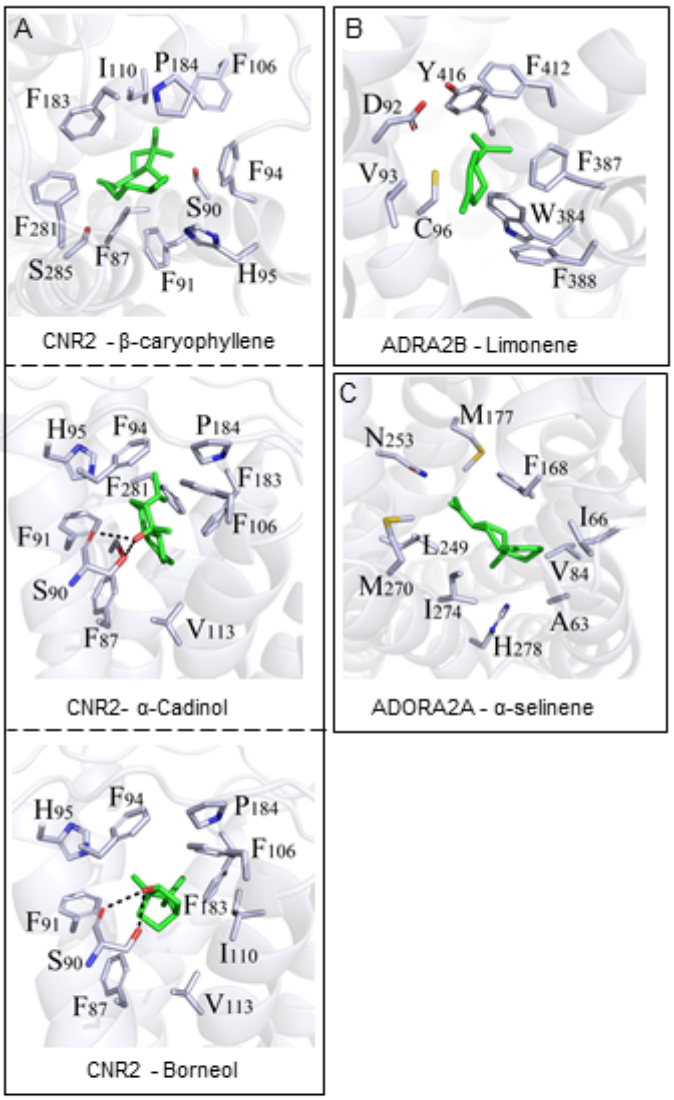

Figure 6

Molecular docking diagram for β-caryophyllene, α-cadinol, borneol (A), limonene (B), and α-selinene (C).

Supplementary Files

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