

A Study on the Mechanism of the Sedative-hypnotic Effect of *Cinnamomum camphora* chvar. *Borneol* Essential Oil Based on Network Pharmacology

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Abstract: In this paper, we investigated the sedative-hypnotic effect of *Cinnamomum camphora* chvar. *Borneol* essential oil (BEO, 16.4% borneol), a by-product of steam distillation of *Cinnamomum camphora* chvar. *Borneol*, from which natural crystalline borneol (NCB, 98.4% borneol) is obtained. Using locomotor activity tests and pentobarbital sodium-induced sleep test, it was found that BEO significantly reduced locomotor activity ($p < 0.05$), shortened sleep latency ($p < 0.0001$), prolonged sleep duration ($p < 0.05$), and had a sedative-hypnotic effect. We constructed the “components-targets-signaling pathways” and “protein–protein interaction” (PPI) network of BEO using network pharmacology. The results show that the 24 active components of BEO acted on 17 targets, mainly through response to alkaloid and catecholamine transport, and neuroactive ligand-receptor interaction. The PPI network identified 12 key proteins, mainly dopamine receptor (DR)D2, opioid receptor mu 1 (OPRM1), and opioid receptor kappa 1 (OPRK1), and we further analyzed the active components and targets of BEO through molecular docking. The results showed that the active components and targets obtained by network pharmacology analyses had good binding activity, which reflected their multi-component, multi-target, multi-pathway action characteristics. This paper provides a theoretical basis for further study of the mechanism of action of BEO in the treatment of insomnia.

Key words: BEO, locomotor activity test, pentobarbital sodium-induced sleep test, sedative-hypnotic effect, network pharmacology, molecular docking

1 Introduction

Insomnia is a common sleep disorder that may be caused by psychological stress, chronic pain, or medications¹⁾. Many patients with insomnia suffer from varying degrees of depression and anxiety, leading to decreased immune function and disorders of the autonomic nervous system, increasing the risk of diabetes, coronary heart disease, and Alzheimer's disease²⁾. The common drugs used to treat insomnia mainly contain benzodiazepines, non-benzodiazepines, selective melatonin receptor agonists, and antidepressants with sedative effects³⁾; these drugs increase proneness to various adverse effects, such as dependence,

tolerance, withdrawal symptoms, and they can have rebound effects with prolonged use⁴⁾. Therefore, natural remedies, such as plants are increasingly being considered to derive safer and more effective treatment, and many researchers are keen to explore new and safe complementary or alternative therapies to treat insomnia.

Natural medicines have been used in China for more than 2,000 years to improve sleep. One of the most typical natural medicines is essential oil¹⁾. Studies in China and other countries have shown the sedative-hypnotic effect of various plant oils, for example, *Rhizoma Ligustici Chuanxiong* Hort. essential oil⁵⁾, and *Myrtus communis* es-

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sential oil⁶⁾. A type of plant essential oil, *Cinnamomum camphora* chvar. *Borneol* essential oil (BEO) (borneol, 16.4%), that is a by-product of the process of steam distillation of *Cinnamomum camphora* chvar. *Borneol* from which natural crystalline borneol (NCB, 98.4% borneol) is obtained, and whose main components also include β -caryophyllene (10.7%), limonene (8.2%), α -pinene (7.5%), and linalool (0.5%), has been shown to have significant anti-inflammatory effects⁷⁾. In addition, oral administration of borneol has been shown to increase the expression of inhibitory neurotransmitters in the rat brain, thereby mediating sedative-hypnotic effects⁸⁾. In addition, Galdino *et al.* demonstrated the significant sedative-hypnotic effect of β -caryophyllene through pentobarbital sodium-induced sleep test in mice⁹⁾. Linck *et al.* confirmed the sedative-hypnotic effect of linalool inhalation by voluntary activity test and pentobarbital sodium-induced sleep test in mice¹⁰⁾. Yang *et al.* confirmed the sedative-hypnotic effect of oral α -pinene by pentobarbital sodium-induced sleep test, and further verified the effect of α -pinene on the sleep-wake cycle by recording electroencephalograms and electromyograms in mice¹¹⁾. The aforementioned studies show that these components have sedative-hypnotic effects by different modes of administration.

Based on the above findings, the sedative-hypnotic function of BEO, which is rich in the above components, is worth investigating. Secondly, previous studies on the sedative-hypnotic properties of essential oils have not investigated their mechanism of action. Therefore, in this study, we investigated the sedative-hypnotic effect of BEO by using mice locomotor activity test and pentobarbital sodium-induced sleep test and used network pharmacology to construct the “components-targets-signaling pathways” network and “protein-protein interaction” (PPI) network of BEO in regulating insomnia, which provide useful insights into the mechanisms underlying the action of these active ingredients of BEO.

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*, a voucher specimen (768133), was deposited at South China Institute of Botany, Chinese Academy of Science (Guangzhou, Guangdong). The essential oil was dehydrated by adding anhydrous Na_2SO_4 , and collected in a brown bottle, then stored at 4°C until future use. The BEO components were previously identified by our research group using gas chromatography mass spectrometry; BEO contains 42 components, the most abundant being borneol (16.4%)⁷⁾ (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[280]), and the care and use of experimental animals were conducted in accordance with national and international guidelines (2010/63/EU). Adult ICR (Institute of Cancer Research) mice (20 ± 2 g) were raised in an environment of $22 \pm 2^\circ\text{C}$, $55 \pm 15\%$ relative humidity, and a 12-h light/dark cycle; the animals were allowed to eat and drink freely. The mice were adapted to the experimental environment for at least 5 days before the experiments.

The mice were randomly divided into five groups (10 in each group, half male and half female), included a control group and a positive control group (2 mg/kg diazepam, saline as a solvent, intraperitoneally) and three BEO groups with different BEO doses administered (150, 300, and 600 mg/kg, corn oil as a solvent, intragastrically)⁹⁾. The administration was continued for 14 days, and 30 min after the last administration, the locomotor activity test and pentobarbital sodium-induced sleep test were carried out in sequence. In the pentobarbital sodium-induced sleep test, pentobarbital sodium was injected at the dosage of 50 mg/kg⁶⁾.

2.3 Locomotor activity test in mice

The test system was composed of two parts: a voluntary activity test chamber ($40 \times 40 \times 40$ cm) and a video acquisition system. The test was performed according to the method used previously, with slight modifications¹²⁾. The material used in the voluntary activity test chamber was a medical organic board. Each group of mice was tested for voluntary activity 30 min after the last dose. The mice were acclimated to the environment for 3 min, and the behavioral parameters were tracked for 10 min to ascertain the differences between the groups. The distance travelled (cm), maximum speed (cm/s), average speed (cm/s), resting time (s) were used as the test indicators. Data analysis was performed using the software Etho Vision XT 11 (Noldus Information Technology, Leesburg, VA, USA). After the data collection, the chamber was cleaned and wiped, feces and urine were removed, and the odor left behind by the mice was eliminated before the next mouse was tested to avoid the influence of odor and residue on the next mouse.

2.4 Pentobarbital sodium-induced sleep test

After the completion of the voluntary activity test, each group of mice was injected intraperitoneally with a threshold dose of 50 mg/kg (the minimum required dose to induce sleep in 100% of the animals measured in the pre-experiment) of pentobarbital sodium (in saline), and the time between the injection of the drug and the disappearance of

the righting reflex was the sleep latency, while the time between the disappearance of the righting reflex and its reappearance was the sleep time; the sleep latency and sleep time of the mice were recorded⁵⁾.

2.5 Measurement of grip strength of mice

A grip strength meter (YLS-13A, Yiyan Technology Development Co., Ltd., Shandong, China) was used to assess forelimb grip strength. Mice were lifted up by their tails, so their forepaws could grip the wire of the strength meter, and 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 grams-force (gf). Three tests were performed on each mouse, and the average score was used for statistical analysis¹³⁾.

2.6 Network pharmacology analysis

2.6.1 "Components-targets-signaling pathways" network

We have identified BEO components in our previous study⁷⁾ (Table S1). Simplified molecular input line entry systems (SMILES) 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 of BEO components. The Swiss Target Prediction database predicts the targets of active molecules based on the chemical structure of the molecules and ligand similarities, as well as by cross validation and arrangement analyses¹⁴⁾. The predicted targets of 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 of sedative-hypnotics, and the targets of BEO components, and sedative-hypnotics were intersected to obtain the targets and components that could be sedative-hypnotic in BEO.

Based on the above prediction results, the Clue Gene Ontology (GO) module of the *Cytoscape* 3.2.1 software was used to annotate GO enrichment analysis of the targets of active components of BEO. The Kyoto Encyclopedia of Genes and Genomes (KEGG) Mapper tool of the KEGG database (<http://www.kegg.jp/>) was used to find enriched pathways of targets. *Cytoscape* 3.2.1 was used to construct an "active components-targets-signaling pathways" network in which nodes representing BEO active components, potential targets, and associated signal pathways were connected by edges. The underlying mechanisms of sedative-hypnotic effects of BEO were determined by analyzing the constructed network.

2.6.2 Annotated analysis of target biological functions

The biological processes of BEO active ingredient targets were enriched by *Cytoscape*'s Clue GO plug-in to further search for major functional annotations that are signifi-

cantly enriched in their active ingredient action targets¹⁵⁾.

2.6.3 Construction of a protein-protein interaction (PPI) network

The String database ([https://string-db.org/Version 10.5](https://string-db.org/Version%2010.5)) was used to analyze known and predicted protein-protein interactions¹⁶⁾. The BEO active ingredient action targets were imported into the String database, limiting the study species to human, and protein interactions were obtained and saved in TSV format. The node1, node2, and combined score information in this file were imported into *Cytoscape* 3.2.1 software to plot the protein interaction network and obtain the results of network analysis. We further set the node size and color; the color was set from dark red to light yellow; the node size reflected the 'degree value' from large to small, and the thickness of the edge reflected the 'combine score' size, and finally obtained the protein interaction network.

2.6.4 Active component-target molecular docking

To further verify the reliability of the target prediction results, molecular docking validation was performed on the screened active ingredients and their associated targets. Firstly, the chemical structures of the main active ingredients were optimized by Chem 3D 15.0, and then the ligand rotatable bonds were determined by 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>); this was de-watered and de-liganded by PyMOL 2.3.4 software, hydrogenated and charge calculated by AutoDock 4.2.6 software, and finally docked by AutoDock Vina 1.1.2 software, and visualized by PyMOL 2.3.4 software. The match between the active ingredient and the target protein was judged according to the docking score value. It is generally considered that a binding energy less than -4.25 kcal/mol suggests 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/mol implies strong binding activity^{15, 17)}.

2.7 Data analysis

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

3 Results

3.1 Locomotor activity test

BEO (300, 600 mg/kg) significantly reduced the total distance by the mice in 10 min compared with the control

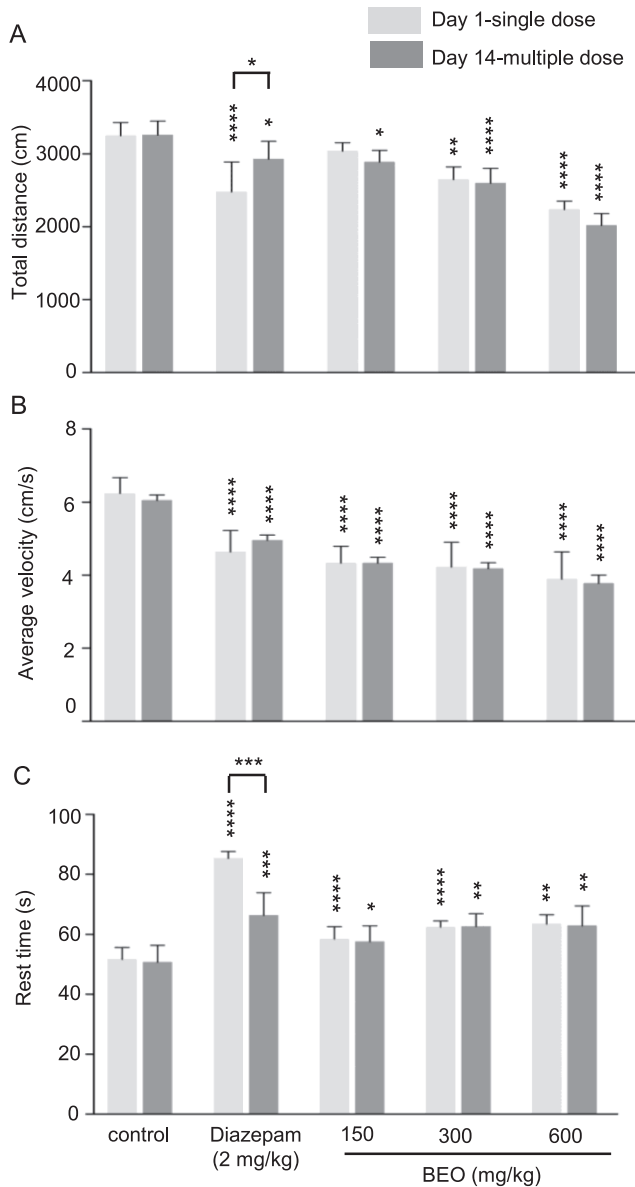


Fig. 1 Effect of *Cinnamomum camphora* chvar. Borneol essential oil (BEO) on total distance (A), average velocity (B), and rest time (C) of mice. Data are expressed as the mean $\pm$ SEM ($n = 10$), ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ compared to the control.

group (Fig. 1A, $p < 0.01$); BEO (150, 300, 600 mg/kg) significantly reduced the average speed of movement of the mice (Fig. 1B, $p < 0.0001$) and increased their resting time (Fig. 1C, $p < 0.05$). The results show that BEO (300, 600 mg/kg) significantly reduced the locomotor activity of mice, had a significant sedative-hypnotic effect ($p < 0.05$), and its effect was comparable to that of the positive control (diazepam). Notably, after 14 days of continuous diazepam administration, there was a significant increase in the total distance (Fig. 1A, $p < 0.05$) and a significant decrease in resting

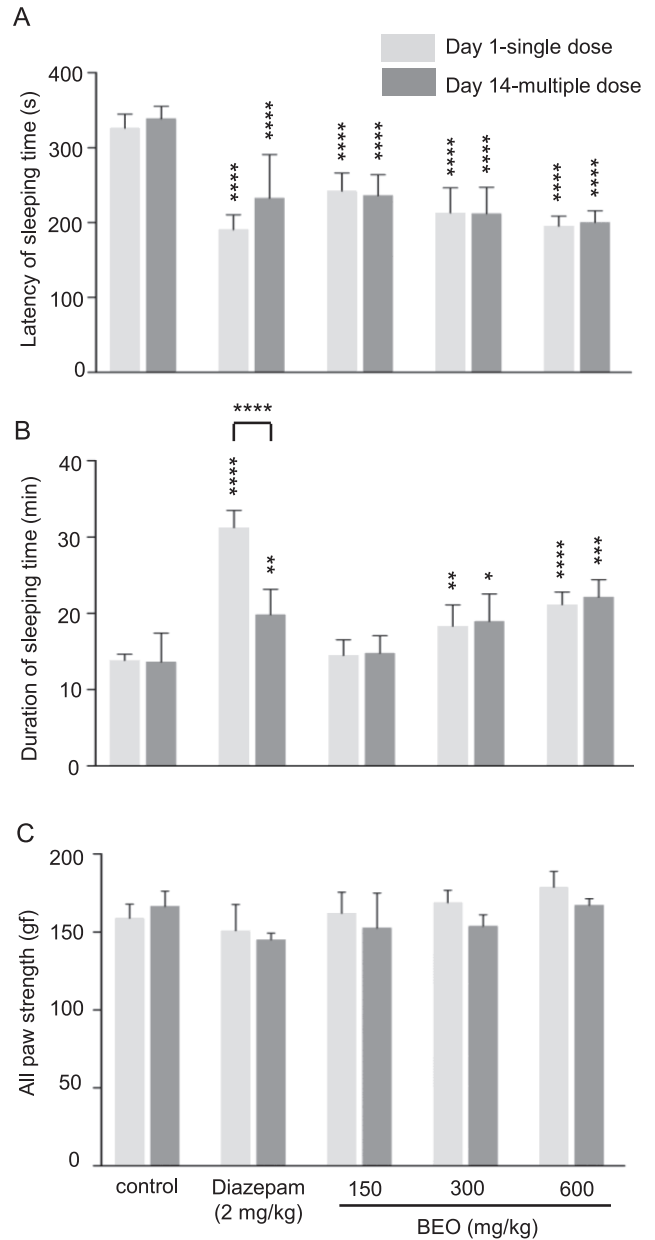


Fig. 2 Effect of BEO on latency of sleeping time (A), duration of sleeping time (B), and all paw strength (C) of mice.

Data are expressed as the mean $\pm$ SEM ($n = 10$), ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ compared to the control.

time (Fig. 1C, $p < 0.001$) compared to 1 day of administration, showing significant desensitization, which is also consistent with previous studies¹². There was no significant desensitizing effect of BEO with continuous use in this study; during the study it was found that low-dose BEO (150 mg/kg) had a significant sedative effect after 14 days of continuous use, which was comparable to the effect of the medium- and high-dose groups, thereby suggesting

that BEO has great potential in the treatment of insomnia.

3.2 Pentobarbital sodium-induced sleep test

As shown in Fig. 2, BEO (150, 300, 600 mg/kg) significantly reduced the sleep latency of pentobarbital sodium-induced mice compared with the blank group ($p < 0.0001$); BEO (300, 600 mg/kg) significantly prolonged the sleep duration of pentobarbital sodium-induced mice ($p < 0.05$), which was comparable to the effect of diazepam. These results show that BEO (300, 600 mg/kg) had a significant sedative-hypnotic effect. The sleep duration of mice that were administered diazepam for 14 consecutive days was

significantly reduced ($p < 0.0001$) compared to that of mice who were administered diazepam for 1 day, which is consistent with the findings obtained from the test of locomotor activity in mice and with previous studies¹⁾. This indicates that BEO has a lower tolerance compared to diazepam and has greater potential for long-term application.

3.3 Motor coordination in mice

To verify whether there was a sedative-hypnotic effect of BEO on motor coordination function in mice, the grip strength of mice was tested by a grip strength meter and

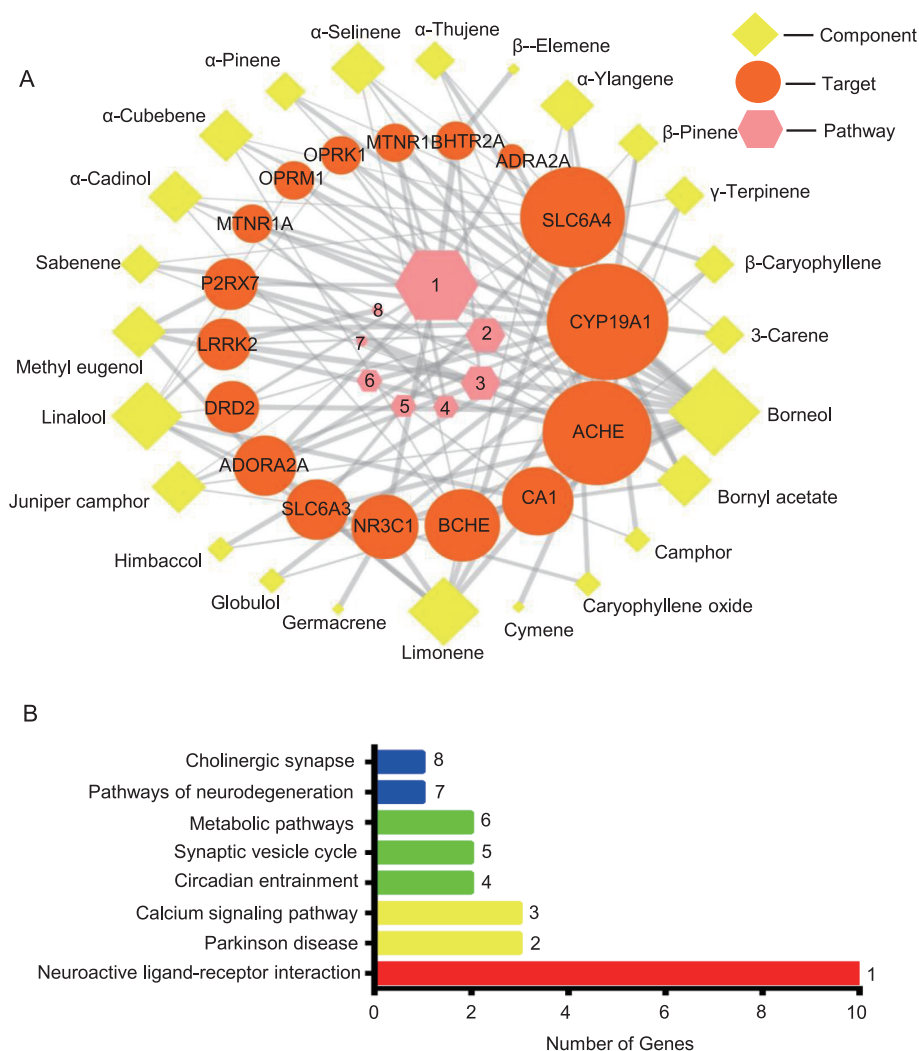


Fig. 3 Sedative-hypnotic pharmacology network of the “components-targets-signaling pathways” regulated by BEO.

(A) The yellow rhomboidal nodes represent components, the orange round nodes represent targets, and the pink hexagonal nodes represent pathways. The size of the nodes represents the degree of influence of components, or the degree of effect on the targets and pathways. The larger the node, the greater the degree. The thickness of the connecting grey lines represents the combined score; the thicker the line, the greater the combined score. (B) Enriched the signaling pathway of targets. 1. Neuroactive ligand-receptor interaction; 2. parkinson's disease; 3. calcium signaling pathway; 4. circadian entrainment; 5. synaptic vesicle cycle; 6. metabolic pathways; 7. pathways of neurodegeneration; 8. cholinergic synapse. The pathway number in (B) corresponds to the pathway number in (A).

the results are shown in Fig. 2C. Compared with the control group, there was no significant difference between the grip strength of mice administered BEO for 1 and 14 days ($p > 0.05$), indicating that BEO did not affect the motor coordination of mice; this result is consistent with that of previous studies on intraperitoneal injection of borneol¹⁸.

3.4 Network pharmacology analysis

3.4.1 BEO components and targets

As shown in Table S1, the BEO components were obtained from the previous study by our group⁷. A total of 24 components of BEO modulating insomnia and 17 corresponding action targets were obtained through database search (Fig. 3A). Among the 24 active components of BEO, borneol had the highest number of connections to the targets (12), followed by limonene (7) and linalool (7), which can be considered as the main active components of BEO in the regulation of insomnia. Among the target genes, cytochrome P450 family 19 subfamily A member 1 (CYP19A1), acetylcholinesterase (ACHE), and solute carrier family 6 member 4 (SLC6A4) are associated with 18, 15, and 14 BEO components, respectively, and can be considered as the main active targets of BEO in the regulation of insomnia.

3.4.2 Targets and signaling pathways

Based on the analysis of the KEGG signaling pathway (Fig. 3B), the 17 targets were mainly involved in the signaling pathways of neuroactive ligand-receptor interaction (involving 10 targets), Parkinson's disease (involving 3 targets), calcium signaling pathway (involving 3 targets), and circadian entrainment (involving 2 targets), thereby indicating that the active component targets of BEO are distributed in different pathways and act in a coordinated manner through each pathway.

3.4.3 Gene Ontology enrichment analysis

Figure 4A shows the Gene Ontology enrichment analysis of the targets; the results show that the 17 targets of the BEO components are mainly involved in response to alkaloid and catecholamine transport (involving 7 targets), ammonium transport, response to ammonium ion, and regulation of amine transport (involving 6 targets), etc.

3.4.4 Protein-protein interaction network analysis

The protein-protein interaction network was constructed by using STRING database and Cytoscape software (Fig. 4B). Five of the 17 target proteins (carbonic anhydrase 1 [CA1], BCHE, purinergic receptor P2X7 (P2RX7), nuclear receptor subfamily 3 group C member 1 [NR3C1], and CYP19A1) did not interact with other targets. According to the STRING database, dopamine receptor (DR) D2 and solute carrier family 6 member 3 (SLC6A3) had the strongest combination ability with a combination score of 0.98. DRD2 was located at the center of the target and had the largest degree value, followed by opioid receptor mu 1

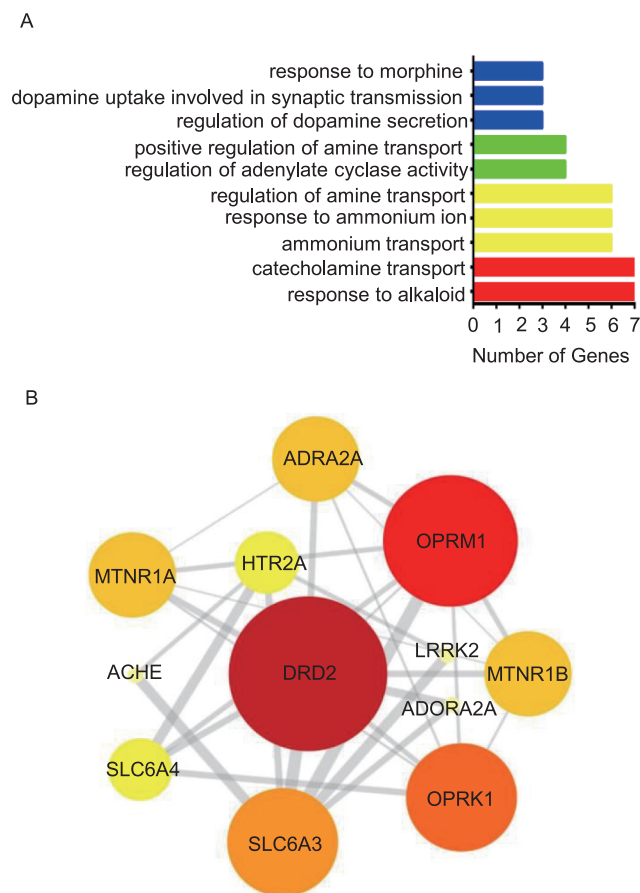


Fig. 4 The Gene Ontology (GO) enrichment analysis (A) and Protein-protein Interaction network (B) of 17 genes.

(OPRM1), SLC6A3, opioid receptor kappa 1 (OPRK1), melatonin receptor (MTNR) 1A, MTNR1B, and ADORA2A.

3.4.5 Active ingredient-target protein molecular docking analysis

The top five ingredients in BEO were borneol (16.4%), β -caryophyllene (10.7%), camphor (10.6%), limonene (8.2%), and α -pinene (7.5%) (Table S1); the network pharmacological screening "degree" ranking of the top five ingredients were borneol (12), limonene (7), linalool (7), α -selinene (4), methyl eugenol (4), bornyl acetate (4), juniper camphor (4), α -cubebene (4), α -cadinol (4), and α -ylangene (4) (Table S2). Molecular docking was performed for the 17 targets related to sedation-hypnosis for these components above (Table S3); the lowest docking energy system of each component to the target is shown in Table 1. The docking energy values of all components were below -5 kcal/mol, thereby indicating that the components have good binding activity to the target proteins; more than 50% of the components had strong binding activity to the corresponding proteins¹⁷, thereby indicating that the above components and targets are the active components and targets of the sedative-hypnotic effect of BEO.

Table 1 Molecular docking score.

Molecular name	Content (%)*	Degree	Target	Score (kcal/mol)
Borneol	16.4	12	P2RX7	−6.5
β-Caryophyllene	10.7	3	ADORA2A	−8.8
Camphor	10.6	2	LRRK2	−6.6
Limonene	8.2	7	ACHE, LRRK2	−7.1
α-Pinene	7.5	3	LRRK2	−6.6
α-Selinene	0.7	4	ADORA2A	−9.0
Linalool	0.5	7	ADORA2A	−6.4
Methyl eugenol	0.3	4	CYP19A1	−7.2
Bornyl acetate	0.2	4	LRRK2, OPRK1	−7.3
Juniper camphor	0.2	4	OPRK1	−8.5
α-Cubebene	0.1	4	ADORA2A	−8.9
α-Cadinol	0.1	4	ADORA2A	−8.3
α-Ylangene	0.1	4	OPRK1	−8.4

*Preliminary work acquisition

In the docking system, for the P2RX7 protein, the amino acids that make up the small molecule binding cavity are C176, W102, F312, F308, K309, and F86. Borneol is able to enter the binding cavity and occupy the entire active pocket and to form weak hydrogen bonds with C176 (Fig. 5A). The adenosine A2a receptor (ADORA2A) protein binding cavity is large and contains more amino acids, including L249, I274, F168, V84, I66, and A63, all of which are hydrophobic amino acids, and some polar amino acids, H278 and S67. β-caryophyllene is a hydrophobic compound with a larger body size and a higher number of carbon atoms, so it can form better hydrophobic bonds with hydrophobic amino acids in the protein binding cavity (Fig. 5B). In the leucine-rich repeat kinase 2 (LRRK2) protein, polar amino acids include R1412, S1508, and K1336, while hydrophobic amino acids include L1414, L1390, L1388, and F1511. α-pinene can display hydrophobic interactions with hydrophobic amino acids (Fig. 5C). In addition, α-selinene and α-cubebene also have high affinity with ADORA2A targets (Table S3 and Fig. S1). ADORA2A has been widely reported to be closely associated with sleep-wake regulation¹⁹⁾, thereby suggesting that ADORA2A is an important active target for the sedative-hypnotic action of BEO.

4 Discussion

The results showed that BEO at a dose of 600 mg/kg significantly reduced pentobarbital sodium-induced sleep latency by about 40.2% and increased sleep duration by about 53.3%, when compared with the control group (Fig. 2). The most abundant component of borneol (16.4% in BEO, Table S1), has been demonstrated to significantly in-

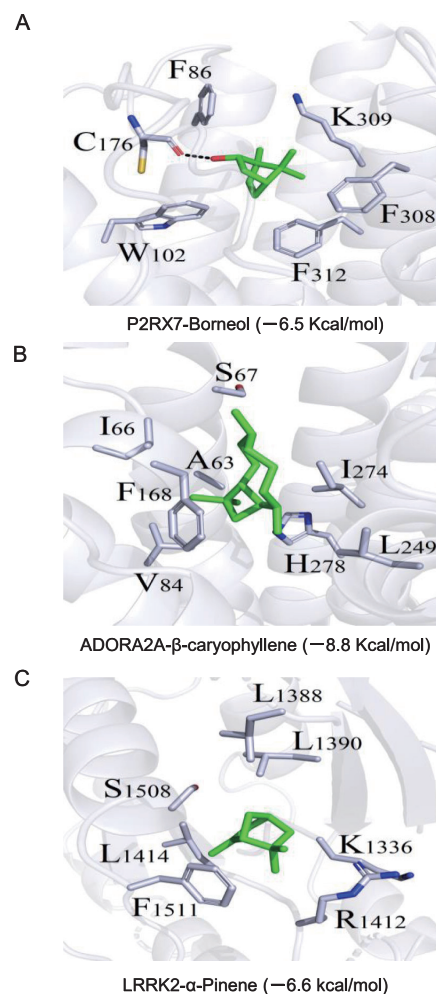


Fig. 5 The molecular docking diagram of borneol (A), β-caryophyllene (B), and α-pinene (C).

crease the level of γ -aminobutyric acid in the cerebrospinal fluid of rats gavage at a dose of 270 mg/kg, thus showing a sedative effect⁸⁾. The main compound of BEO, β -caryophyllene (10.7% in BEO, **Table S1**), by gavage at a dose of 400 mg/kg could reduce the sleep latency induced by pentobarbital sodium in mice by about 19%, and could increase the sleep duration induced by pentobarbital sodium by about 94%⁹⁾, and its dose in this study was 64 mg/kg. Another compound, α -pinene (7.5% in BEO, **Table S1**) at a dose of 100 mg/kg by gavage in mice reduced pentobarbital sodium-induced sleep latency, about 19%, and increased pentobarbital sodium-induced sleep duration, about 63%¹¹⁾. Its dose in the present study was 44 mg/kg. These results suggested that the active components in BEO were derived not only from β -caryophyllene and α -pinene, but also from other components in BEO, with each other possibly playing a synergistic role. These results also suggested that β -caryophyllene, α -pinene, and borneol were the active components that mediated the sedative-hypnotic effect of BEO, and BEO could exert better sedative-hypnotic effects at lower levels of its components, showing its superior potential for application. To verify the active components and targets of BEO action, further analysis was performed using network pharmacology and molecular docking.

Further network pharmacological analysis results showed that the 24 active components of BEO acted on 17 targets. Among them, CYP19A1 was mainly involved in steroid hormone biosynthesis and metabolism of drugs²⁰⁾. SLC6A4 had been reported to be closely associated with sleep disorders²¹⁾. ACHE degraded acetylcholine, and an increase in acetylcholine led to a prolongation of rapid eye movement (REM) sleep phase²²⁾. Molecular docking between 13 components of BEO obtained by screening and 17 targets obtained by network pharmacology analysis was performed, and the binding energy between the BEO components and targets are shown in **Table S3**. Based on the sedative-hypnotic activity reported in the previous literature and the content of BEO, we found that the docking energy of borneol, β -caryophyllene, and α -pinene with multiple targets was lower than -5 kcal/mol (**Table S3**), showing good binding activity^{15, 17)}, such as CYP19A1, SLC6A4, ACHE, ADORA2A, DRD2, LRRK2, OPRK1, P2RX7, and 5-hydroxytryptamine receptor 2A (HTR2A). Among these targets, ADORA2A has been reported to be closely associated with sleep-wake regulation and interacts with DRD2¹⁹⁾, LRRK2, OPRK1²³⁾, P2RX7, and 5-hydroxytryptamine receptor 2A (HTR2A)²¹⁾, and they have also been reported to be associated with sleep disorders. It has also been shown that α -pinene, β -pinene, camphor, and limonene inhibited ACHE activity; limonene also inhibited butyrylcholinesterase (BCHE) activity²⁴⁾, thereby suggesting that these abovementioned components and targets were important for the regulation of insomnia by BEO.

Based on analysis of the KEGG signaling pathway (**Fig.**

3B), among them, the neuroactive ligand-receptor interaction signaling pathway controlled and regulated many important biological functions, such as learning and memory, sleep-wake cycle regulation, etc.²⁵⁾. Astrocytic calcium signaling pathway played an important role in the regulation of slow wave sleep. Circadian entrainment imbalance lead to sleep disorders²⁶⁾. In addition, acetylcholinesterase inhibitors (ACHEIs) increase REM sleep in patients with Alzheimer's disease (AD), and the SLC6A4 polymorphism has been shown to be associated with tremor and rigidity in Parkinson's disease²⁷⁾. The analysis of the relevant targets and pathways in this study was essentially consistent with the literature reports, indicating that the potential active ingredients and targets obtained were reliable and could provide a theoretical basis for an in-depth understanding of the regulation of insomnia by BEO. Based on the GO enrichment analysis (**Fig. 4A**), among them, alkaloids²⁸⁾ and catecholamine²⁹⁾ have been shown to be associated with sedation-hypnosis. Ammonium ions excite histaminergic neurons, thereby controlling arousal and sleep³⁰⁾. Pituitary adenylate cyclase-activating peptide affects homeostatic sleep regulation in healthy young men³¹⁾. All of the above results were associated with sedation-hypnosis, thereby suggesting that regulation of insomnia by BEO was achieved through these biological processes. Among the results of the PPI network, DRD2 has been shown to be associated with insomnia and anxiety³²⁾. SLC6A3³³⁾ and OPRM1³⁴⁾ have been associated with sleep-wake regulation in humans. MTNR1A was associated with sleep in patients with schizophrenia³⁵⁾. It has also been shown that DRD2 and SLC6A3 interacted with each other³⁶⁾. ADORA2A has been reported to be closely related to sleep-wake regulation and synergistically interacted with DRD2¹⁹⁾. The present study was consistent with the above studies, and not only confirmed the reliability of PPI analysis, but also provided a basis for studying the mechanism of action of BEO in the treatment of insomnia, although the components and targets of BEO exerting sedative and hypnotic effects need to be further confirmed.

In summary, this study provided new clues for further research on the pharmacological basis of BEO in the treatment of insomnia, and may also facilitate the design of targeted drugs for insomnia basic research.

5 Conclusion

In this paper, we demonstrated that BEO can significantly reduce the locomotor activity, shorten the sleep latency, and prolong sleep duration, and has a sedative-hypnotic effect, through the locomotor activity test and pentobarbital sodium-induced sleep test in mice. Network pharmacology was used to construct the "components-targets-signaling pathways" and "PPI" networks of BEO in regulating

insomnia. Twenty-four active components of BEO were found to regulate 17 targets, which were involved in the pathways of response to alkaloid, catecholamine transport, and neuroactive ligand-receptor interaction in insomnia. Molecular docking results preliminarily verified the good binding activity between these active components and targets. This study provides a theoretical basis for the mechanism of action of BEO in the treatment of insomnia and a new way to explore safe adjuvant and alternative therapies for insomnia.

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Conflict of Interest

The authors declare no conflicts of interest related to this work.

Supporting Information

This material is available free of charge via the Internet at doi: 10.5650/jos.ess21278

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