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Screening BVOCs in Cypress Cones to Improve Anxiety and Insomnia and Target Prediction

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Abstract The Cypress (*Platycladus orientalis* (L.) Franco) cones have a unique aroma and are commonly used in pillow fillings to alleviate anxiety and insomnia. In this study, gas chromatography-mass spectrometry (GC-MS) was employed to extract and analyze the biogenic volatile organic compounds (BVOCs) from Cypress cone shells, identifying a total of 28 components, with terpenoids comprising over 99% of the total. α-Pinene was the predominant component, accounting for 67% of the total content (176.328 μg/g). Target prediction identified significant interactions between 14 BVOCs and 19 protein targets, with (-)-α-Pinene, limonene, bornyl acetate, and 3-carene being potential key components for alleviating anxiety and insomnia. The primary targets were VDR (Vitamin D receptor), AchE (Acetylcholinesterase), CTSD (Cathepsin D), TRPV1 (Transient Receptor Potential Cation Channel Subfamily V Member 1), CNR2 (Cannabinoid receptor 2), and PPARα (Peroxisome proliferator-activated receptor alpha), which are mainly involved in neurotransmission and circadian rhythm regulation. Molecular docking simulations showed that the binding of α-Pinene and β-caryophyllene to PPARα and CNR2 proteins was primarily driven by hydrophobic forces, with binding energies ranging from -5.17 to -7.83 kcal/mol, suggesting that these BVOCs might alleviate anxiety and insomnia by influencing related functional proteins. This study reveals the potential mechanisms by which Cypress cone shells may help alleviate anxiety and insomnia.

Keywords Cypress cones (*Platycladus orientalis* (L.) Franco); Biogenic volatile organic compounds; Target protein; Anxiety; Insomnia

1 Introduction

In recent years, the incidence of anxiety and insomnia has been steadily increasing, with estimates suggesting that by 2030, approximately 27% of the global population will suffer from sleep disorders (Ren et al., 2019). This issue has become a significant global health challenge. Modern medicine primarily relies on sedative-hypnotic drugs for treatment, but long-term use can lead to drug dependence and other adverse effects, highlighting the urgent need for effective alternative therapies. Traditional Chinese medicine (TCM) has a long history of using aromatic therapies to treat insomnia and anxiety. Through methods such as inhalation, washing, fumigation, and oral administration, aromatic medicines are absorbed by the human body and regulate bodily functions (Yang, 2018).

Modern research has found that inhaling plant aromas can effectively alleviate tension and anxiety, while also improving circulation and endocrine functions, thereby promoting sleep (Knasko et al., 1990; Moss et al., 2010). The primary components of these aromas are volatile organic compounds (VOCs) such as hydrocarbons, alcohols, and aldehydes, which are highly lipophilic and can quickly penetrate mucous membranes and the blood-brain barrier to reach the brain (Miao et al., 2013). Studies have shown that anxiety and insomnia are often closely linked to abnormalities in nervous system function. After being absorbed, aroma molecules can influence brain regions such as the hippocampus, amygdala, and hypothalamus, modulating neurotransmitter transmission to produce calming and relaxing effects, thereby improving anxiety and insomnia (Li et al., 2015; Lin and Zhang, 2017). In drug development, target screening is a crucial step in studying the mechanisms of action. Structure-based target prediction allows for the efficient identification of disease-related active targets from a multitude of molecular structures. Current research indicates that volatile compounds can interact with synaptic transmission-related targets, inhibiting neurotransmission and thereby affecting brain function (Kress, 1992;

Kaech et al., 1999).

Cypress (*Platycladus orientalis* (L.) Franco), also known as the incense cedar, is a fragrant woody plant native to China. In traditional Chinese medicine, the aromatic emissions from Cypress cone shells are believed to have detoxifying, dampness-drying, insecticidal, wind-dispelling, and calming properties. Modern medical research has shown that inhaling the aroma of Cypress can alleviate anxiety (Editorial Committee of "Chinese Materia Medica" by the State Administration of Traditional Chinese Medicine, 1999; Wu et al., 2010). For example, terpenoids released by Japanese cedar have been found to reduce tension in humans (Matsubara and Kawai, 2014). Additionally, pillows filled with dried Cypress shells are known to improve sleep quality (Liu et al., 2018). The biogenic volatile organic compounds (BVOCs) in Cypress cone shells are the source of their aroma. These compounds are typically gaseous and are easily released into the environment, where they may alter the surrounding microenvironment (Ho et al., 2011). It is hypothesized that the BVOCs from the Cypress shells in the pillow cores disperse into the environment and, being small molecules, are easily absorbed through nasal inhalation, alveolar respiration, and skin penetration, subsequently binding to target molecules within the body and affecting physiological functions.

However, the specific aroma molecules in Cypress shells and their potential targets remain unknown. Therefore, this study employs headspace extraction combined with gas chromatography-mass spectrometry (GC-MS) to extract BVOCs from Cypress shells, identifying compounds such as β-caryophyllene, D-limonene, α-pinene, and bornyl acetate. Target prediction suggests that these compounds may interact with key proteins such as CNR2 (Cannabinoid receptor 2), PPARα (Peroxisome proliferator-activated receptor alpha), VDR (Vitamin D receptor), and AChE (Acetylcholinesterase), which are closely associated with anxiety and sleep regulation. This research aims to provide a scientific basis for the application of Cypress cone shells in alleviating anxiety and insomnia and to promote the use of natural plant medicines in modern medicine. The findings are expected to lay the foundation for further exploration of the bioactivity of plant-derived volatile organic compounds and their potential applications in neurological disorders, revealing the mechanisms of key compounds in Cypress cone shells and offering new directions for developing natural therapies for anxiety and insomnia.

2 Results and Analysis

2.1 Analysis of BVOCs components in Cypress shells

The total ion flow diagram of BVOCs from Cypress branches and leaves is shown in Figure 1. The results indicate that 28 BVOCs were extracted and identified from the Cypress cone shells, including 22 terpenoids, 2 hydrocarbons, and one each of benzene, alcohol, aldehyde, and ester. Among these, terpenoids were the most abundant, accounting for 99.7% of the total volatile components, with other components making up only 0.3%. The major terpenoids identified include α-Pinene, 3-Carene, and Camphene. Among these, α-Pinene had the highest relative content, accounting for over 67.313%, with a concentration of 176.328 μg/g, making it the predominant component in the BVOCs of Cypress cone shells.

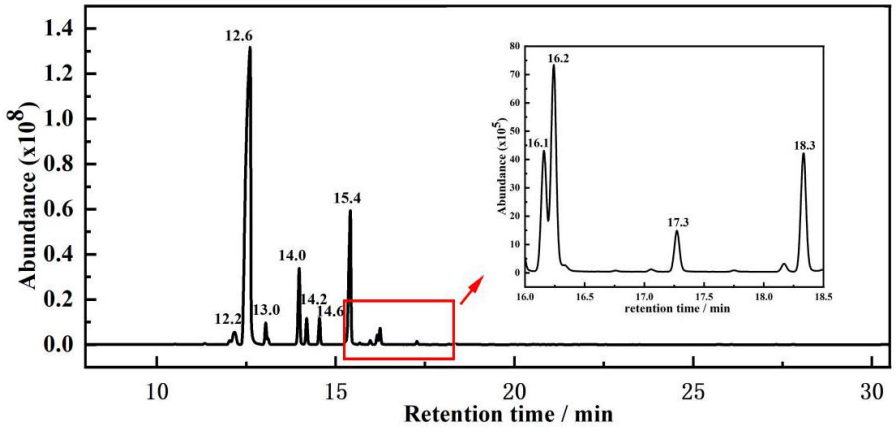

Figure 1 Total ion flow of BVOCs components in Cypress cones

α-Pinene is an effective inhibitor of acetylcholinesterase activity (Miyazawa and Yamafuji, 2005), possessing neuroprotective properties that can calm the central nervous system and thus inhibit the onset of anxiety (Khoshnazar et al., 2020; Allenspach and Steuer, 2021). Limonene has a positive impact on relieving psychological tension, mood fluctuations, and sleep disorders caused by stress, with inhalation of limonene essential oil blends shown to alleviate anxiety in patients (Eddin et al., 2021). Modern pharmacology has proven that 3-Carene has sleep-inducing and antidepressant effects, as demonstrated by reduced sleep latency and increased sleep duration in mice treated with 3-Carene (Woo et al., 2019). Bornyl acetate has a central nervous system inhibitory effect, reducing neuronal excitability, thereby exerting a sedative effect that prolongs sleep duration, reduces the number of awakenings, and improves sleep quality (Matsubara et al., 2011). Preparations containing bornyl acetate, such as valerian volatile oil, are widely used in Europe and America to treat mild to moderate insomnia (Chandra Shekhar et al., 2024). These studies suggest that α-Pinene, limonene, bornyl acetate, and 3-Carene in the BVOCs of Cypress cone shells can improve anxiety, indicating that these small volatile molecules are likely the main active components responsible for the anxiety-relieving effects of Cypress cone shells (Table 1).

2.2 Target prediction of BVOCs from Cypress shells

Current research indicates that the vast majority of targets are proteins, including various receptors and enzymes. According to predictions from SwissTargetPrediction, using "Probability > 0.05" as the screening criterion, 14 BVOCs were identified from the 28 BVOCs, including 3-Carene, (+)-2-Carene, Cyclofenchene, α-Thujene, and (-)-α-Pinene, which interact with 19 target protein molecules such as Alcohol dehydrogenase alpha chain (Figure 2). These targets belong to 10 categories, including Oxidoreductase, Nuclear receptor, and Lyase (Table 2). Among these, the Oxidoreductase category had the most targets, followed by Nuclear receptors and Lyases, while the remaining categories had only 1-2 targets each.

2.3 Interaction between BVOCs from Cypress shells and their targets

The predicted interaction relationships between BVOCs and target molecules show that among the 14 interacting BVOCs, 10 small molecules, including β-Caryophyllene, D-Limonene, and α-Pinene, interact with CNR2 and PPARα (Table 2). Among these, β-Caryophyllene had the highest interaction probability with PPARα and CNR2, both at 0.56; D-Limonene had the next highest probability, both at 0.39; and the remaining 9 small molecules had interaction probabilities of 0.05 with PPARα and CNR2 proteins. Bornyl acetate showed interaction probabilities of 0.11 and 0.09 with VDR and AchE proteins, respectively. (-)-trans-Pinocarveol interacted with six proteins, including PTGS1 (Prostaglandin-endoperoxide synthase 1), NR1H3 (Nuclear receptor subfamily 1 group H member 3), CA4 (Carbonic anhydrase IV), CA1 (Carbonic anhydrase I), CA2 (Carbonic anhydrase II), and UGT2B7 (UDP-glucuronosyltransferase 2B7), while α-Campholenal interacted with eight proteins, including CTSD (Cathepsin D), ADH1C (Alcohol dehydrogenase gamma chain), ADH1B (Alcohol dehydrogenase beta chain), ADH4 (Alcohol dehydrogenase 4), SRD5A1 (Steroid 5-alpha-reductase 1), CYP19A1 (Cytochrome P450 19A1), ADH1A (Alcohol dehydrogenase alpha chain), and TRPV1 (Transient receptor potential cation channel subfamily V member 1), and Cyclofenchene interacted solely with SHBG (Sex hormone binding globulin), with interaction probabilities of 0.05 for all these interactions.

Among the proteins interacting with bornyl acetate, VDR encodes the Vitamin D3 receptor (Salem et al., 2023). Research has shown that a lack of Vitamin D may lead to anxiety and other mental health issues, with individuals having low Vitamin D levels more prone to anxiety symptoms, which can be significantly alleviated by Vitamin D supplementation (Zhu et al., 2020; Kouba et al., 2022). Since VDR is a target of bornyl acetate in Cypress cone BVOCs, it indicates that bornyl acetate released from Cypress shells may influence the activity of the Vitamin D3 receptor, VDR. Among the proteins interacting with bornyl acetate, AchE acetylcholinesterase can degrade acetylcholine, terminating the excitation of the postsynaptic membrane and ensuring normal nerve signal transmission, thereby relieving excessive neural excitation (Scacchi et al., 2009). As AchE is a target of bornyl acetate in Cypress cone BVOCs, it suggests that bornyl acetate released from Cypress shells may influence nerve signal transmission, exerting a calming and soothing effect.

Table 1 Analysis of BVOCs in Cypress branches and leaves

No.	Retention time (tR/min)	Compound name	CAS number	Relative content (%)	Concentration (μg/g)
1	10.6	3-Carene	13466-78-9	0.057	0.15
2	11.3	(+)-2-Carene	4497-92-1	0.155	0.317
3	12.0	Cyclofenchene	488-97-1	30.994	1.136
4	12.2	α-Thujene	2867-05-2	1.703	4.46
5	12.6	(-)-α-Pinene	7785-26-4	67.313	176.328
6	13.0	α-Fenchene	471-84-1	1.722	4.512
7	13.1	Camphene	79-92-5	0.522	1.073
8	13.2	2,4(10)-Thujadiene	36262-09-6	0.013	0.034
9	14.0	4(10)-Thujene	3387-41-5	6.422	16.876
10	14.2	(-)-β-Pinene	18172-67-3	2.082	5.454
11	14.6	Myrcene	123-35-3	2.091	5.477
12	15.0	4-Carene; (+)-4-Carene	29050-33-7	0.024	0.062
13	15.4	(+)-3-Carene	498-15-7	14.046	36.795
14	15.7	α-Terpinene	99-86-5	0.143	0.376
15	15.8	O-Cymene	527-84-4	0.031	0.082
16	16.0	M-Cymene	535-77-3	0.228	0.598
17	16.1	D-Limonene	5989-27-5	0.752	1.969
18	16.2	β-Phellandrene	555-10-2	1.225	3.209
19	16.3	(E)-β-ocimene	3779-61-1	0.029	0.076
20	17.1	b-Ocimene (>90%)	13877-91-3	0.014	0.037
21	17.3	γ-Terpinene	99-85-4	0.247	0.648
22	18.2	(-)-2-carene	554-61-0	0.045	0.118
23	18.3	Terpinolene	586-62-9	0.736	1.927
24	18.5	m-Isopropenyltoluene	1124-20-5	0.012	0.03
25	19.9	α-Campholenal	4501-58-0	0.014	0.038
26	20.5	(-)-trans-Pinocarveol	547-61-5	0.029	0.077
27	25.7	(-)-Bornyl acetate	5655-61-8	0.024	0.062
28	30.4	(E)-2-epi-β-caryophyllene	87-44-5	0.012	0.031

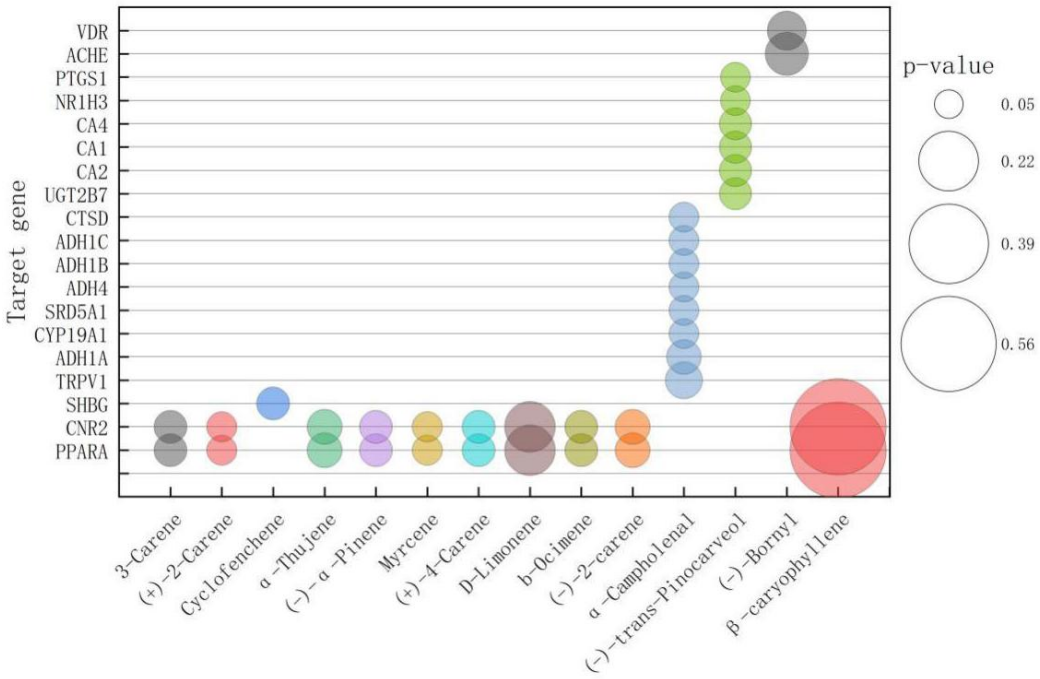

Figure 2 Prediction of interaction relationships between BVOCs and target molecules

Table 2 Target gene prediction

No.	Target	Common name	Uniprot ID	Target Class
1	Alcohol dehydrogenase alpha chain	ADH1A	P07327	Oxidoreductase
2	Steroid 5-alpha-reductase 1	SRD5A1	P18405	Oxidoreductase
3	Alcohol dehydrogenase beta chain	ADH1B	P00325	Oxidoreductase
4	Alcohol dehydrogenase gamma chain	ADH1C	P00326	Oxidoreductase
5	Prostaglandin-endoperoxide synthase 1	PTGS1	P23219	Oxidoreductase
6	Peroxisome proliferator-activated receptor alpha	PPARα	Q07869	Nuclear receptor
7	Nuclear receptor subfamily 1 group H member 3	NR1H3	Q13133	Nuclear receptor
8	Vitamin D receptor	VDR	P11473	Nuclear receptor
9	Carbonic anhydrase II	CA2	P00918	Lyase
10	Carbonic anhydrase I	CA1	P00915	Lyase
11	Carbonic anhydrase IV	CA4	P22748	Lyase
12	Alcohol dehydrogenase 4	ADH4	P08319	Enzyme
13	UDP-glucuronosyltransferase 2B7	UGT2B7	P16662	Enzyme
14	Cytochrome P450 19A1	CYP19A1	P11511	Cytochrome P450
15	Cannabinoid receptor 2	CNR2	P34972	Family A G protein-coupled receptor
16	Acetylcholinesterase	ACHE	P22303	Hydrolase
17	Cathepsin D	CTSD	P07339	Protease
18	Sex hormone binding globulin	SHBG	P04278	Secreted protein
19	Transient receptor potential cation channel subfamily V member 1	TRPV1	Q8NER1	Voltage-gated ion channel

Among the proteins interacting with α-Campholenal, CTSD deficiency is associated with the pathogenesis of neuronal lipidosis and may be related to the mechanism of Alzheimer's disease (Siintola et al., 2006). The TRPV1 protein is the receptor for capsaicin, selectively activating sensory neurons that transmit nociceptive signals to the central nervous system, causing a burning sensation (Caterina et al., 1997). This indicates that CTSD and TRPV1, as targets of α-Campholenal in Cypress cone BVOCs, may participate in nerve signal transmission, thereby influencing central nervous system function.

Among these interacting proteins, CNR2 and PPARα are particularly noteworthy as they interact with multiple BVOCs from Cypress shells, including α-Pinene and D-Limonene. CNR2 is involved in the central nervous system effects induced by cannabinoids, such as changes in mood and cognition (Onaivi et al., 2008; Ishiguro et al., 2010). It is hypothesized that multiple components of Cypress cone BVOCs, including α-Pinene, may interact with the CNR2 target protein, influencing central nervous system functions through the modulation of CNR2. PPARα is a member of the nuclear receptor superfamily involved in the expression of genes related to cell proliferation, differentiation, and immune and inflammatory responses (Tai et al., 2002). Modern clinical studies have found that patients with delayed sleep phase syndrome (DSPS) have significant difficulty falling asleep, and DSPS is associated with circadian clock gene polymorphisms and PPARα (Mezhnina et al., 2022). The interaction between multiple BVOCs from Cypress cone shells, including α-Pinene, and the PPARα target protein suggests that Cypress shells may alleviate insomnia symptoms by influencing circadian rhythm proteins.

2.4 Molecular interaction modeling of BVOCs and protein targets

The binding site between a target and small molecule is the specific region where the two interact, often possessing a high degree of stereoconfiguration specificity, ensuring that the binding between the small molecule and target is specific and reversible. At the binding site, the small molecule interacts with the target through various forces, including electrostatic interactions, hydrogen bonds, and hydrophobic interactions, enabling specific recognition and binding of the target.

The study illustrated the interaction models between α-Pinene, β-Caryophyllene, and the PPARα and CNR2 protein molecules (Figure 3), highlighting the significant amino acid residues surrounding the binding site. Six amino acid residues of the PPARα protein, Phe94, Phe106, Lys109, His95, Ile110, and Phe91, form hydrophobic

interactions and Pi-Sigma bonds with α-Pinene (Figure 3A), with a binding energy of -5.17 kcal/mol. Eight amino acid residues of PPARα, Val113, Ile110, Phe105, Phe91, His95, Phe183, Phe94, and Pro184, form Alkyl hydrophobic interactions with β-Caryophyllene (Figure 3B), with a binding energy of -6.56 kcal/mol. Five amino acid residues of the CNR2 protein, Val324, Ile317, Met320, Phe218, and Leu321, form Alkyl hydrophobic interactions with α-Pinene, with a binding energy of -5.61 kcal/mol (Figure 3C). Six amino acid residues of CNR2, Met320, Phe218, Val324, Leu321, Tyr334, and Met220, form Alkyl hydrophobic interactions with β-Caryophyllene, with a binding energy of -7.83 kcal/mol (Figure 3D).

Molecular interaction simulations indicate that except for the formation of a Pi-Sigma bond between α-Pinene and PPARα, the other binding sites predominantly involve hydrophobic interactions. Although hydrophobic interactions are relatively weaker compared to hydrogen bonds and other intermolecular forces, Autodock simulations show that the binding energies between these molecules range from -5.17 to -7.83 kcal/mol, suggesting that these interactions can form relatively stable BVOC small molecule-protein complexes. Since the hydrophobic effects on the amino acid chains of proteins play a crucial role in the formation and stabilization of protein tertiary and quaternary structures, the modeled interaction between the BVOCs and these amino acids suggests that hydrophobic interactions may influence the conformation of interacting proteins, thereby affecting protein function. Additionally, these interactions rely mainly on hydrophobic forces, forming a relatively stable but weak binding that is reversible, aligning with the traditional Chinese medicine concept of "flexibility and adaptability," offering a therapeutic advantage in alleviating anxiety and insomnia without causing drug dependence or adverse effects.

3 Concluding Remarks

This study identified 28 volatile organic compounds (BVOCs) through GC-MS analysis, with terpenoids accounting for over 99% of the total content, notably α-Pinene (67.313%) and 3-Carene (14.046%) which were significantly higher than other components. These high-content terpenoids are likely the primary active substances responsible for the bioactivity of Cypress cone shells. α-Pinene has been shown in several studies to have sedative and anxiolytic effects (Miyazawa and Yamafuji, 2005), while 3-Carene has been found to improve sleep quality (Woo et al., 2019). Therefore, these key components in Cypress cone shells may play a critical role in alleviating anxiety and insomnia.

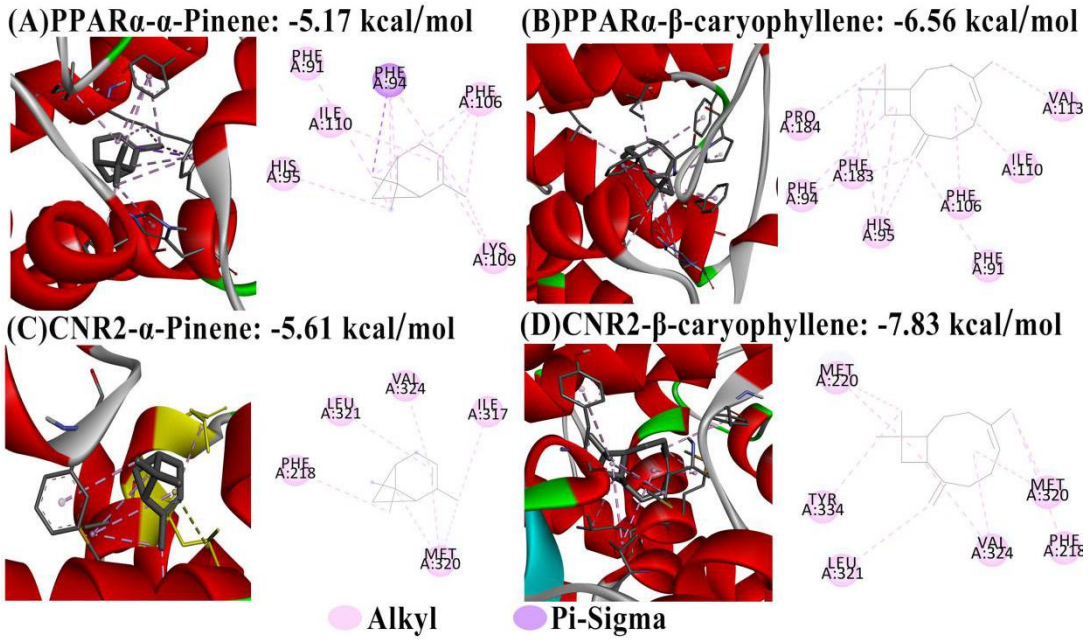

Figure 3 Docking conformations and binding sites of two compounds with proteins
Note: A: PPARα-α-Pinene; B: PPARα-β-caryophyllene; C: CNR2-α-Pinene; D: CNR2-β-caryophyllene

Target prediction results suggest that 14 BVOCs may interact with 19 protein targets, including CNR2, PPARα, and VDR, which are closely related to neurotransmission and circadian rhythm regulation. CNR2 is closely linked to the regulation of the central nervous system (Ishiguro et al., 2010), while PPARα is involved in the regulation of sleep and the biological clock (Mezhnina et al., 2022). These findings provide important clues for further research into the mechanisms by which Cypress cone shells may affect anxiety and insomnia, though further experimental validation is needed to confirm the accuracy of these predictions.

Compared to previous studies, this research systematically identified the volatile organic components in Cypress cone shells and predicted their potential targets. Previous research has hypothesized that these specific small aromatic molecules may be inhaled through the respiratory tract or absorbed through the skin via direct contact with a pillow, thereby influencing the function of the central nervous system (Li et al., 2023; Dong, 2024). In contrast, this study not only identified the key BVOCs but also predicted their potential protein targets, providing new perspectives and evidence for the pharmacological mechanism of Cypress cone shells.

Despite revealing the potential mechanisms by which Cypress cone shells may alleviate anxiety and insomnia through GC-MS and target prediction, this study has certain limitations. The GC-MS analysis was conducted under in vitro conditions, and the absorption, metabolism, and bioavailability of BVOCs in actual applications may be influenced by various factors. Although target prediction offers valuable insights, these results have not yet been validated through in vivo experiments, and the actual biological effects require further investigation. Future research should focus on validating the biological activity of BVOCs through in vivo studies and exploring their clinical application potential in different populations.

4 Materials and Methods

4.1 Experimental materials

The Cypress (*Platycladus orientalis* (L.) Franco) cones were collected from the Dabie Mountains in Anhui Province during the autumn when the cones were mature. Random sampling was conducted in typical Cypress forests, with sample trees selected every 50 meters, resulting in a total of 50 trees being sampled. The maturity of the cones was determined based on the color and hardness of the shells, with all selected trees being at least five years old. Each tree produced no fewer than 50 mature cones to ensure the representativeness of the sample. The cones were air-dried naturally for 7 days, with the ambient temperature controlled at (25±2) ℃ and relative humidity at (40±5)%, ensuring the stability of the volatile components. After air-drying and natural seed shedding, the dry Cypress shells were collected.

4.2 BVOCs extraction and detection

The collected Cypress shells were sampled using the quartering method, with 2.419 7 g of the sample placed in a 20 mL headspace vial to allow the volatile components to naturally disperse. After equilibrating the sample for 2 hours, 1 mL of the headspace was sampled and analyzed using GC-MS (Agilent, GC QQQ8890-7000) to determine the BVOCs composition.

The gas chromatography conditions were as follows: the silica capillary column was Agilent HP-5MS (250 μm × 0.25 μm, 30 m); the carrier gas was high-purity helium (purity not less than 99.999%) with a constant flow rate of 1.0 mL/min. The injection port temperature was set at 250 ℃, with a splitless injection. The temperature program started at 40 ℃ for 1 minute, then increased at 4 ℃/min to 230 ℃, and finally ramped at 100 ℃/min to 260 ℃, held for 11.7 minutes.

Mass spectrometry conditions were as follows: the ion source was an electron impact (EI) source, with the ion source temperature at 230 ℃, quadrupole temperature at 150 ℃, and interface temperature at 280 ℃. The electron energy was set at 70 eV, and the scan mode was full scan (SCAN) with a mass range of m/z 50-500. The solvent delay was set to 8 minutes.

4.3 Quantification of BVOCs components

The total ion chromatogram was matched against the NIST 2.0 standard library for similarity, retention index, and

CAS number to identify the compounds. The relative content of each component was calculated using the total ion peak area normalization method. Based on the component analysis results, β-caryophyllene in the sample was quantified using an external standard method, with chromatographic grade β-caryophyllene (Sigma, 99.9%) as the standard. A standard curve was plotted based on the concentration and peak area to determine the release amount of β-caryophyllene per gram of sample. The absolute content of trans-caryophyllene in the Cypress shells was determined using the standard curve. The content of other BVOCs components in the Cypress shells was semi-quantified using trans-caryophyllene as a reference (Koziel et al., 2017), with the calculation formula as follows:

$$c_0 = \frac{m_0 \times A_1 \times 1000000}{A_2}$$

Where: m_0 is the injection amount of the standard sample (g); A_1 is the peak area of the sample; A_2 is the peak area of the standard; C_0 is the sample content (μg/g); 1 000 000 is the conversion factor.

4.4 BVOCs target prediction

Based on the GC-MS detection results of Cypress BVOCs, the molecular information of the BVOCs was obtained. The CAS numbers of the small molecules were queried on the PubChem website (https://pubchem.ncbi.nlm.nih.gov) to obtain the SMILES information of the small molecules. The SMILES information was then input into the SwissTargetPrediction website (http://www.swisstargetprediction.ch/) to obtain the target prediction results. The prediction results for all small molecules of BVOCs were screened using the interaction probability >0.05 as the criterion, and the obtained results were considered as effective target molecules of Cypress BVOCs.

4.5 BVOCs-protein interaction modeling

Based on the target prediction results, the predicted major proteins were used as receptor proteins, and α-pinene, a highly abundant component of BVOCs, as well as β-caryophyllene, which has a high probability of interaction with the proteins, were used as drug small molecules. The interaction between the small molecules and proteins was simulated using Autodock software. The main process involved three steps (Sarkar et al., 2024). First, the preparation of the macromolecular protein receptor: the 3D structure of the protein molecule was downloaded from the National Center for Biotechnology Information (NCBI) protein database (https://www.ncbi.nlm.nih.gov/protein), and the downloaded 3D model was converted to the mol2 (Molecular file) chemical file format containing molecular structure and related property information using OpenBabel software. Second, the preparation of the small molecule receptor file: the 3D structures of α-pinene (CID: 440968) and β-caryophyllene (CID: 5281515) were retrieved from the PubChem database, and the SDF (Solvent Description File) files containing information on molecular structure, charge, and interactions were downloaded. Finally, Autodock software was used: first, the macromolecular mol2 file was opened, dewatered, hydrogenated, and the charge calculated, and atomic types were added before being saved as a pdbqt format file, which was used as the macromolecular receptor. Then, the small molecule was opened, hydrogenated, the ligand root was determined, and flexible torsion was set before being saved as a pdbqt format file, which was used as the small molecule ligand. Blind docking was used to set the docking site and docking times, and semi-flexible docking was performed. The conformation with the lowest binding free energy was selected for model evaluation, and the docking results were visualized using Discovery Studio (https://www.3ds.com/products/biovia/discovery-studio).

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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