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ORIGINAL ARTICLE

Data Mining and Network Pharmacology Study on Dried Tangerine Peel and Immature Tangerine Peel Essential Oil as a Treatment for Liver Depression and Insomnia

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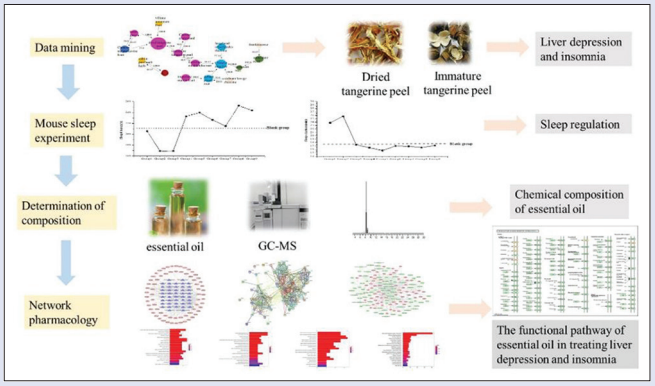
ABSTRACT

Background: With the variation of modern medical mode from "disease medicine" to "health medicine," the traditional Chinese medicine (TCM) aromatherapy befits an important part of the exclusive health service resource intended for the prevention of diseases, rehabilitation and health care in China. Liver depression and insomnia are among the human health conditions that are apparently treated by TCMs since the ancient Chinese period. **Objectives:** To study the mechanism of volatile oil in treating liver depression and insomnia. **Materials and Methods:** Through data mining, we found dried and immature tangerine peel as a prescription for treating liver depression and insomnia. Gas chromatography-mass spectrometry was achieved to control the chemical composition of the essential oil. The effects of dried and immature tangerine peel essential oil on sleep latency and sleep duration were also explored using a pentobarbital-induced sleep test in mice. Network pharmacology tactics was executed to determine the potential active ingredients and molecular mechanism of the dried and immature tangerine peel essential oil in treating liver depression and insomnia. **Results:** Dried and immature tangerine peel essential oil has both calming and tranquilizing effects. Based on the network pharmacology, it was foretold that essential oil were mostly employed to treat liver depression and insomnia with predicted functions convoluted in regulating several neurotransmitters, such as the gamma-aminobutyric acid in the neuroactive receptor-ligand signaling pathway. **Conclusion:** In this study, the effect of volatile oil from dried tangerine peel and immature tangerine peel on mice sleep was established, and the mechanism of action for treating liver depression and insomnia was preliminarily deliberated.

Key words: Data mining, dried tangerine peel and immature tangerine peel, essential oil, liver depression and insomnia, network pharmacology

SUMMARY

- In this study, the volatile oil of dried tangerine peel and immature tangerine peel was dogged to be employed in the treatment of liver depression and insomnia through data mining. The volatile oil of dried tangerine peel and immature tangerine peel has positive effect on sleep through the experiment of pentobarbital sodium and sleep in mice. Finally, based on the network pharmacology, it was anticipated that the volatile oil of dried tangerine peel and immature tangerine peel was primarily used to treat liver depression and insomnia by regulating the neurotransmitter such as gamma-aminobutyric acid in the neurolig and-receptor interaction pathway.



Abbreviations used: TCM: Traditional Chinese medicine; GC-MS: Gas chromatography-mass spectrometry; BP: Biological process; MF: Molecular function; CC: Cell component; KEGG: Kyoto Encyclopedia of Genes and Genomes; GABA: γ-aminobutyric acid; GABAAα1: Gamma-aminobutyric acid receptor subunit alpha-1; GABAAγ2: Gamma-aminobutyric acid receptor subunit gamma-2.

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INTRODUCTION

Traditional Chinese Medicine (TCM) has been part of China's medical practice for thousands of years, generating plentiful prescriptions with stated effects on specific diseases and conditions. Chinese medicine aromatherapy has been extensively used to manage any conditions allied with the central nervous, cardiovascular, respiratory, digestive, and other major systems. Meanwhile, many TCMs were employed to treat and accomplish insomnia, depression, Alzheimer's disease, coronary heart disease, asthma, colds, diarrhea, and many other health circumstances.

Using a data mining tactic, researchers can now extract the evidence from a large number of databases of TCM prescriptions, thus providing a more operative and suitable way to study the use of Chinese medicine.[1] Data mining was completed to excavate all available data on TCM volatile oils that are seemingly useful in the treatment of liver depression and insomnia, thus providing the theoretical basis and data support for the selection of aromatic Chinese medicine.

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In this study, we employed data mining to classify dried tangerine peel and immature tangerine peel as drugs for treating liver depression and insomnia. To further validate the role of dried tangerine peel and immature tangerine peel essential oil in the treatment of insomnia, this paper changed a pentobarbital-induced sleep experiment in mice and discovered the guidance of essential oil inhalation in the brain of sleep-induced mice using the mouse turn-over reflex. Gas chromatography-mass spectrometry (GC-MS) was employed to determine the chemical constituents in the volatile oil. Network pharmacology was used to forecast the mechanism of volatile oils from dried tangerine peel and immature tangerine peel in the treatment of liver depression and insomnia. Molecular docking was used to envisage the affinity between the target and chemical components. The flowchart of the experimental procedures is revealed in Figure 1.

MATERIALS AND METHODS

Reagents and Instruments

The GC-MS machine model used was the Agilent 7890A/5975C (Palo alto, California, USA). The compressed air atomizer originated from Jilin Dingxiang Technology Development Co., Ltd., (Jilin, China). Pentobarbital sodium comes from Merck Company (Darmstadt, Germany), Diazepam comes from Shandong Xinyi Pharmaceutical Co., Ltd., (Dezhou, China).

Data sources and screening

The data employed in this paper were derived from the database of essential oil of TCM (http://39.106.34.47:8848/wiki), which widely and systematically collects and sorts out the prescriptions comprising volatile oil of TCM in ancient and modern prescriptions of TCM. This database embraces ancient prescriptions, containing prescription names, prescription sources, drug composition, indications, efficacy and other contents, with ample data information. Using the TCM oil database rescue functions, the following keywords were searched: "wakefulness," "sleepless," "incapability of supination" and "insomnia with restlessness." The exploration in the database also encompassed the ingredients of the TCM prescriptions that comprehend the volatile oil for the treatment of insomnia. To consider the compatibility of Chinese medicine applications, the statistics of the high frequency of use of Chinese medicine were also measured.

Regulating effects of dried and immature tangerine peel essential oil on the sleep of pentobarbital sodium in mice

A total of 80 mice were alienated into 10 groups with 8 mice in each group. The experimental conditions comprised the control group and treatment groups 1–9. The control group did not receive any stimulation or medication. In treatment groups, the experimental mice expected fluctuating doses of dried and immature tangerine peel essential oil as follows: The doses of groups 1 to 9 are: 10 μL, 25μL, 40μL, 55μL, 70μL, 85μL, 100μL, 115μL, 130μL. The administration of essential oil was accomplished using an aromatherapy smelling device, which is made of a large transparent plexiglass box (80 cm × 80 cm × 65 cm) and four small transparent plexiglass boxes (30 cm × 30 cm × 30 cm). The small boxes were calmly located at the bottom of the big box, while the aromatherapy atomizer for storing essential oils was positioned in the middle of the big box. The essential oil was added to the spray pot of the atomizer with water added to the highest scale of 8 ml. The mice were sited in a small box and then the aromatherapy atomization was carried out. The sniffing time of each group was 1 h, once a day. After the last administration, each group was intraperitoneally injected with pentobarbital sodium solution (50 mg/kg) and the sleep latency and sleep duration of each mouse were inspected.[2,3]

Determination of the volatile oil chemical composition

The volatile oils of dried tangerine peel and immature tangerine peel were mined and determined by GC-MS. The GC conditions were as follows: HP-5MS capillary quartz string; carrier gas: Helium; column flow rate: 1.0 ml/min; sample quantity: 1.0 ul; shunt ratio: 40:1; inlet temperature: 250°C, heating procedure: 40°C, holding for 2 min, 10°C/min to 220°C, 20°C/min to 300°C, holding for 5 min. The MS conditions were as follows: Electron source is EI; electron energy: 70eV; ion source temperature: 230°C; the temperature of four-stage bar: 150°C and quality scan range: Full scan.

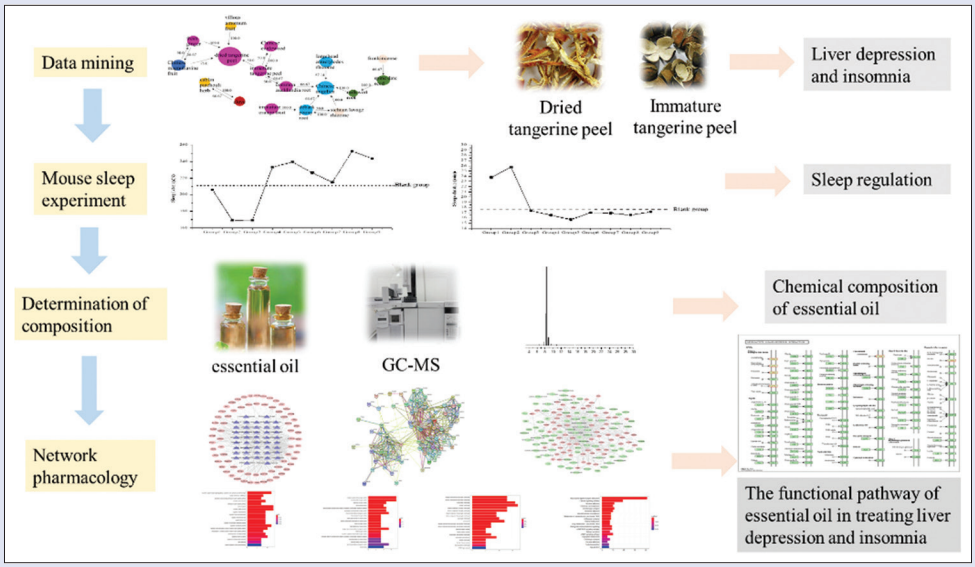

Figure 1: Flowchart of the experimental procedures

Collection of chemical composition targets and disease targets

The TCMSP, PubChem, STITCH, and Swiss target prediction databases were employed to pursuit for protein targets of chemical composition. Several database search targets were also unified. The keywords of insomnia and depression were input into TTD, OMIM, TCMSP, Drug bank and DisGeNET databases to search for the action targets of insomnia and depression. The two targets were combined to get the targets of liver depression insomnia.[4-6]

Construction of chemical composition-action target network diagram

The potential targets of the dried tangerine peel and immature tangerine peel in the treatment of liver depression and insomnia were attained by the intersection of the chemical ingredients of the target and the disease targets. The target of each chemical component was plotted to the potential target and the relationship between each chemical component and the potential target was achieved.[7] The relationship between components and potential targets was presented into Cytoscaper3.7.0 software to paradigm the network diagram of chemical components-action target.[8] Finally, the main targets and chemical constituents of the essential oil of dried and immature tangerine peel in the treatment of liver depression and insomnia were prophesied.

Construction of target interaction network diagram

The potential targets were introduced into the STRING database to attain the network diagram of the interrelation among latent targets. Next, the network was examined to predict the targets with vital positions in the potential targets.[9,10]

Construction of action target distribution network diagram

Using the BioGPS database, all organs and tissues with the target distribution were divided for the top ten gene expression levels.[11] The potential Target and Organ distribution network (T-O map) was created to envisage the main functional tissues/organs.

Analysis of target enrichment pathway

R language and RStudio software were employed to enrich and examine the biological process (BP), molecular function (MF), cell component (CC), and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways of potential action targets. The KEGG database was used to map the target action pathways.[12] The combination of all the analyses was intended to predict the mechanism of the essential oil of dried and immature tangerine peel to treat liver depression and insomnia.

Molecular docking

Through the PubChem database to download chemical structure (SDF), PDB database to download the structure of the target. The target structure was preserved with the Discovery Studio 4.5 software, comprising hydrogenation and removal of crystal water. Each binding site of each target was docked with all the chemical components and the value of libdockScore was occupied as the assessment standard of docking degree.

RESULTS AND DISCUSSION

Screening and analysis of prescriptions for treating liver depression and insomnia

The proportion of TCM inclosing volatile oil accounted for more than 50% after the screening, of which 35 prescriptions for insomnia were nominated from the database of the Chinese medicine essential oil and a total of 59 drugs were documented. The most frequently used prescriptions were Chinese angelica, dried tangerine peel, spine date seed, large head atractylodes rhizome, ginseng, immature tangerine peel, Chinese magnolia vine fruit, etc.[13,14] An connotation rule for the mining method was employed to examine the high-frequency core combination of the 35 prescriptions. The "networked presentation" of the associations between drugs is accessible in Figure 2. The larger the node is, the higher the frequency of use of the drug. The nodes of the same color signify the drugs with the same efficacy. The arrow shows the compatibility of the drug with another drug and the number on the arrow symbolizes confidence. This analysis naked that tangerine peel, angelica, and jujube kernel were the most regularly used in the network.

The stress-inducing culture of work in modern society has been concerned with numerous human health conditions, which contain liver depression and insomnia. In TCM, the Qi is vigorous energy or life force that mingles in balance so that its deficiency may cause human disease. Results of the data mining presented that the therapeutic principles of using tangerine peels were to pacify the liver, regulate the Qi for mental balance and the relief of depression. Fallouts also displayed that dried tangerine peel and immature tangerine peel are the most regularly employed drugs in regulating Qi balance in illness allied with insomnia. It was also stated to promote circulation and strengthen the spleen, as well as convoluted in removing dampness and relieving phlegm. Immature tangerine peel may dismiss the stagnation of Qi by soothing the liver, promoting digestion and resolving food retention, thereby treating liver depression and insomnia. Of note, although mature dried tangerine peel and young immature tangerine peel are both derived from the orange fruit, the two may have diverse efficacy due to variable active compounds as both may differ in physicochemical properties. Essentially, drugs for regulating Qi are categorized by its warmth and fragrance and are mostly invented to improve the functions of the liver, spleen, and stomach meridian. These drugs are employed to promote Qi circulation, treat Qi stagnation and manage the Qi adverse syndrome.[15]

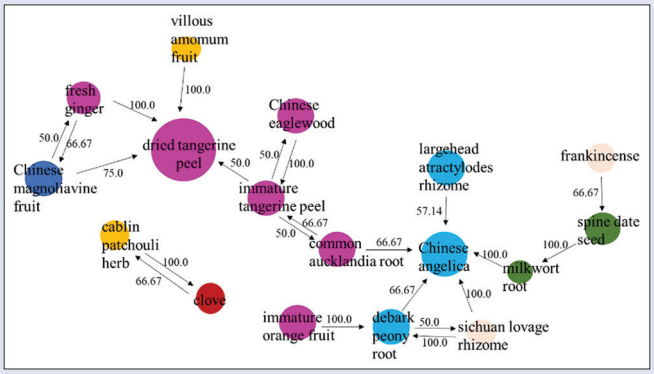

Figure 2: Network diagram of drug combination

The effects of essential oil on the sleep regulation of pentobarbital sodium in mice

The results on the effect of dried and immature tangerine oil on the sleep parameters of pentobarbital-induced mice are accessible in Table 1 and Figures 3, 4. Compared with the control group, the sleep latency of treatment groups 2 and 3 was meaningfully shortened ($P < 0.05$). This recommends that at these doses the tangerine essential oil holds a calming effect. By contrast, mice under the treatment group 8 showed a relatively protracted sleep latency ($P < 0.05$), signifying a certain excitatory effect. These findings designate that the essential oil of dried tangerine peel and immature tangerine peel might have a tranquilizing effect in low doses and stimulating effect in high doses. Compared to the control group, the sleep duration of treatment group 1 and group 2 was suggestively extended ($P < 0.05$), telling a calming effect. There was no difference detected in sleep duration among treatment groups. The low dose of dried tangerine peel and immature tangerine peel essential oil exposed a calming effect while the high doses presented little effect on sleep duration.

Determination of chemical constituents of volatile oil from dried tangerine peel and immature tangerine peel

There are 38 chemical components perceived in the essential oil of Tangerine peel, including beta-Thujene, alpha-Pinene, beta-Pinene, alpha-Terpinene, D-limonene, gamma-Terpinene, etc., among which, D-limonene is the most copious, accounting for more than 80% of the whole essential oil. Therefore, D-limonene is the most vital chemical component in the essential oil of dried tangerine peel and immature tangerine peel [Figure 5].

Construction and analysis of chemical composition-target network diagram

A total of 45 chemical compositions of the volatile oil were distinguished from dried tangerine peel and immature tangerine peel using the GC-MS method. The chemical composition and target evidence are revealed in Tables 2 and 3. The highest content was D-limonene, which created more than 80% of the chemical compositions. The gamma-Terpinene, Cymene, Myrcene were also among the most plentiful compounds found in tangerine peel volatile oil. In data mining, we attained 617 targets of volatile oil chemical composition, 203 targets for insomnia, and 1538 targets for depression. A total of 69 intersections of component targets, insomnia targets, and depression targets were also attained. Of note, the intersection target is where the latent target for the treatment of liver depression and insomnia by the volatile oil of tangerine peel and green peel.[16]

Table 1: Sleep latency and sleep duration of each group ($\bar{X}$±S, n=8)

Group	Sleep latency (s)	Sleep duration (min)
Blank group	211.50±23.76	17.63±2.08
Group 1	206.00±31.40	23.81±9.13*
Group 2	169.00±19.55*	25.74±15.55**
Group 3	169.33±9.95*	17.36±4.96
Group 4	233.25±43.99	16.53±4.75
Group 5	239.50±44.05	15.69±8.54
Group 6	226.44±57.71	17.02±6.82
Group 7	215.00±19.53	16.91±10.49
Group 8	252.43±41.14*	16.56±3.45
Group 9	243.78±34.11	17.14±6.14

Compared with the control group, *P<0.05, **P<0.01

Figure 6 displays the network diagram of volatile oil chemical constituents and the targets of liver depression and insomnia. Here, 113 nodes were produced comprising the blue triangle node that signifies the volatile oil chemical compositions, of which topological parameters are publicized in Table 3. The pink oval node represents the potential action target for liver depression and insomnia and its topological parameters are presented in Table 3. Each side indicates the interaction between the chemical composition and the target.[17]

The greater the degree of nodes, the more biological functions they contribute in and the greater their biological significance. As shown in

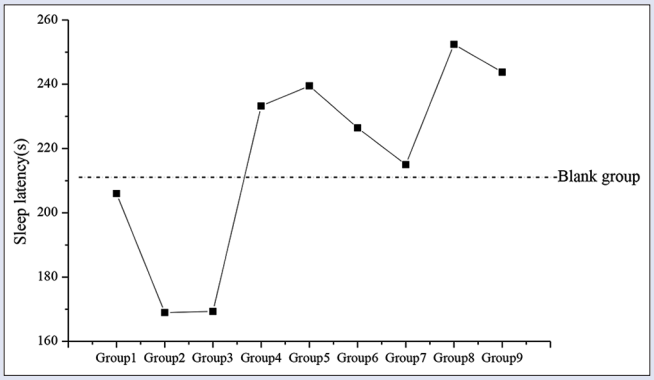

Figure 3: Comparison of sleep latency in each group

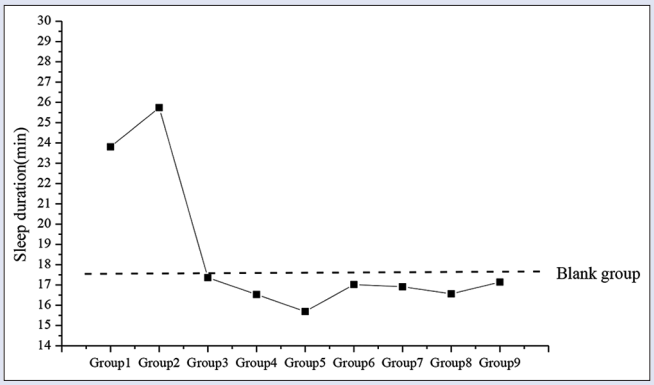

Figure 4: Comparison of sleep duration in each group

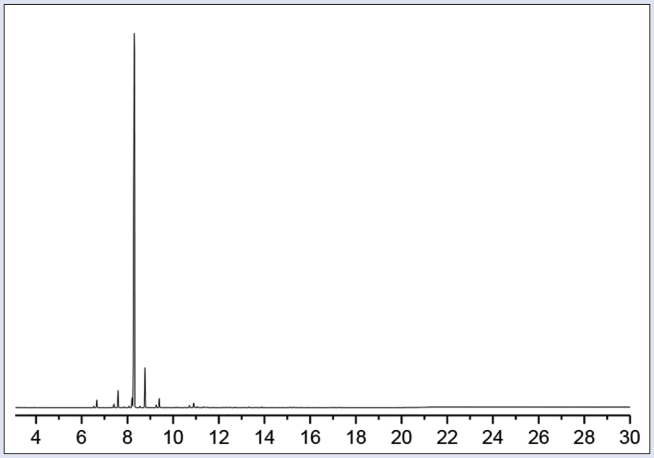

Figure 5: TIC chart of chemical constituents of volatile oil

Table 2: Topological parameter analysis of chemical constituents in the network diagram

Number	Chemical constituents	Average shortest path length	Betweenness centrality	Closeness centrality	Degree
1	Alpha-Terpineol	2.3304	0.0942	0.4291	26
2	Terpinolene	2.4375	0.0289	0.4103	13
3	Gamma-Terpinene	2.5804	0.0074	0.3875	5
4	Beta-Ocimene	3.6696	0.0000	0.2725	1
5	D-Limonene	2.4732	0.0229	0.4043	11
6	Cymene	2.5268	0.0158	0.3958	7
7	Alpha-Terpinene	2.5625	0.0294	0.3902	14
8	Myrcene	2.5446	0.0120	0.3930	7
9	Beta-Pinene	2.5089	0.0350	0.3986	17
10	Alpha-Pinene	2.4554	0.0244	0.4073	12
11	Beta-Thujene	3.2946	0.0015	0.3035	3
12	Alloisolongifolene	4.5804	0.0000	0.2183	1
13	Spathulenol	3.1161	0.0037	0.3209	3
14	(-)-gamma-Elemene	3.6696	0.0000	0.2725	1
15	(+)-delta-Cadinene	3.6696	0.0000	0.2725	1
16	Alpha-Farnesene	2.7946	0.0002	0.3578	3
17	Beta-Farnesene	2.8125	0.0034	0.3556	3
18	Germacrene B	3.6518	0.0012	0.2738	2
19	Beta-Caryophyllene	2.7768	0.0007	0.3601	4
20	(-)-Germacrene D	2.5982	0.0113	0.3849	4
21	(-)-alpha-Copaene	2.5446	0.0104	0.3930	7
22	Humulene	3.6696	0.0000	0.2725	1
23	Lavandulyl acetate	2.4732	0.1045	0.4043	19
24	Neryl propionate	2.2768	0.1145	0.4392	20
25	2,6-Dimethylocta-2,6-diene	3.6696	0.0000	0.2725	1
26	Beta-Elemene	3.7589	0.0000	0.2660	1
27	4'-Hydroxy-3'-methylacetophenone	3.3661	0.0201	0.2971	3
28	4-Isopropyl-3-methylphenol	2.6518	0.0743	0.3771	11
29	DELTA-Elemene	3.6696	0.0000	0.2725	1
30	Perillaldehyde	2.7589	0.0039	0.3625	5
31	Carvone	2.7054	0.0101	0.3696	8
32	d-Carvone	2.4375	0.0623	0.4103	23
33	Thymol methyl ether	2.7589	0.0038	0.3625	4
34	2-Methoxy-4-vinylphenol	2.7232	0.0546	0.3672	7
35	Thymol	2.2946	0.1160	0.4358	28
36	Nerol	2.6875	0.0353	0.3721	7
37	Citronellol	2.4018	0.0554	0.4164	24
38	(-)-cis-Carveol	2.8482	0.0018	0.3511	5
39	Decanal	2.5446	0.0117	0.3930	6
40	Terpinen-4-ol	2.7054	0.0048	0.3696	8
41	Beta- Terpineol	2.7054	0.0172	0.3696	6
42	Linalool	2.3304	0.1019	0.4291	28
43	Alpha-phellandrene	2.5804	0.0158	0.3875	5
44	Beta-Phellandrene	2.6161	0.0040	0.3823	3

Table 2, the highest degree value was detected for thymol and linalool. The alpha-Terpineol, Citronellol, d-Carvone, and D-limonene also exposed large degree values, representing that these components are closely connected to the targets of liver depression and insomnia. In particular, the D-limonene disclosed the highest content of more than 80% in the volatile oil of tangerine and green peels. Furthermore, linalool showed the most targets in the treatment of liver depression and insomnia. Indeed, earlier reports showed that Linalool and limonene were concerned to have a calming effect on sleep. Inhaling a certain concentration of linalool can delay the sleep time induced by pentobarbital sodium, demonstrating its potential in improving sleep efficiency, curtail the time to fall asleep, extend the time of deep sleep (REM) and improve mental anxiety.[18,19] Limonene can also protract the total sleep time and relieve daytime dysfunction and can usually improve somatic anxiety.

As shown in Table 3, the members of the γ-aminobutyric acid (GABA) were the budding target receptors of the compounds from the tangerine peel volatile oil that are related to liver depression and insomnia. These receptors incorporated GABRA3, GABRA6, GABRA2, GABRA1, GABRB3, GABRG2, and GABRB2, with the combined degree value of these receptors as high as 36. This result infers that the GABA receptor is thoroughly related to liver depression and insomnia. GABA is the main inhibitory neurotransmitter in the mammalian brain in which one-third of the synapses in the whole brain use GABA as a transmitter. GABA has a sedative and hypnotic effect and is closely linked to sleep.

Construction and analysis of target interaction network diagram

The network diagram of impending targets of liver depression and insomnia interaction achieved using the STRING software is shown in Figure 7. The network nodes signify the proteins with the inclusive score for the interaction between potential targets in liver depression and insomnia. The correlation between the targets with a comprehensive score of >0.95 is revealed in Table 4. As shown in Figure 7 and Table 4,

Table 3: Topological parameter analysis of target in the network diagram

Number	Gene name	Average shortest path length	Betweenness centrality	Closeness centrality	Degree
1	ADRB1	3.2679	0.0001	0.3060	2
2	ADRA1B	3.2857	0.0001	0.3043	2
3	GABRA3	3.0714	0.0006	0.3256	4
4	GABRA6	3.0714	0.0006	0.3256	4
5	GABRA2	3.0357	0.0042	0.3294	6
6	CHRNA7	3.4643	0.0000	0.2887	1
7	ABCC1	3.4643	0.0000	0.2887	1
8	ADRA1D	3.4643	0.0000	0.2887	1
9	ADRA2A	3.2500	0.0006	0.3077	2
10	TSPO	3.2679	0.0000	0.3060	1
11	GRIN2B	3.2679	0.0000	0.3060	1
12	GABRA5	2.7500	0.0064	0.3636	6
13	GABRA1	2.6607	0.0220	0.3758	11
14	CHRM3	2.7679	0.0049	0.3613	5
15	CNR1	4.3571	0.0000	0.2295	1
16	GABRB3	3.6429	0.0000	0.2745	1
17	GABRG2	3.3214	0.0016	0.3011	2
18	GABRB2	3.3214	0.0016	0.3011	2
19	ADRB3	3.6429	0.0000	0.2745	1
20	DRD5	3.6429	0.0000	0.2745	1
21	HTR6	3.1071	0.0021	0.3218	2
22	CYP2A6	3.0179	0.0031	0.3314	4
23	CHRNA4	2.9464	0.0008	0.3394	2
24	HTR7	3.4107	0.0001	0.2932	2
25	HTR2A	2.7679	0.0216	0.3613	5
26	CYP1B1	3.7143	0.0000	0.2692	1
27	CYP1A1	3.7143	0.0000	0.2692	1
28	CYP1A2	3.7143	0.0000	0.2692	1
29	ALB	3.0893	0.0084	0.3237	2
30	HTR2C	2.7679	0.0030	0.3613	3
31	HTR2B	3.1429	0.0018	0.3182	2
32	UGT2B15	3.2857	0.0000	0.3043	1
33	DDC	3.6786	0.0000	0.2718	1
34	CYP2C19	2.8571	0.0020	0.3500	5
35	SLC6A2	2.6607	0.0092	0.3758	10
36	SLC6A4	2.7679	0.0034	0.3613	6
37	CHRM2	2.5179	0.0172	0.3972	12
38	UGT2B7	3.5893	0.0187	0.2786	4
39	PTGS1	2.2679	0.0691	0.4409	13
40	HRH4	2.7500	0.0029	0.3636	3
41	LRRK2	2.7500	0.0029	0.3636	3
42	ADRA1A	2.9643	0.0014	0.3373	4
43	OPRK1	3.3214	0.0000	0.3011	1
44	ADRA2C	2.9286	0.0032	0.3415	6
45	IDO1	2.8214	0.0091	0.3544	4
46	SIGMAR1	2.9286	0.0027	0.3415	5
47	SLC6A3	2.7321	0.0075	0.3660	8
48	ABCB1	2.9643	0.0061	0.3373	5
49	PRNP	3.0357	0.0007	0.3294	4
50	TARDBP	3.0357	0.0007	0.3294	4
51	UGT1A1	3.0893	0.0004	0.3237	3
52	CHRM4	2.5357	0.0144	0.3944	8
53	CHRM5	2.5714	0.0096	0.3889	6
54	ABCB6	3.0357	0.0007	0.3294	4
55	KCNH2	2.3571	0.0252	0.4242	10
56	CHRM1	2.4821	0.0179	0.4029	11
57	SMPD1	3.0357	0.0007	0.3294	4
58	DRD1	3.2679	0.0001	0.3060	2
59	OPRD1	3.0357	0.0007	0.3294	4
60	OPRM1	3.0357	0.0007	0.3294	4
61	ADRB2	2.7857	0.0036	0.3590	6
62	DRD2	2.6071	0.0188	0.3836	9
63	HCRTR1	3.0357	0.0007	0.3294	4
64	CYP3A4	2.6250	0.0074	0.3810	8
65	ADORA2A	3.5357	0.0011	0.2828	3
66	NR1I2	2.1607	0.0928	0.4628	25
67	CNR2	2.6786	0.1649	0.3733	22
68	ESR1	2.1250	0.1094	0.4706	27
69	CYP19A1	1.9286	0.1801	0.5185	30

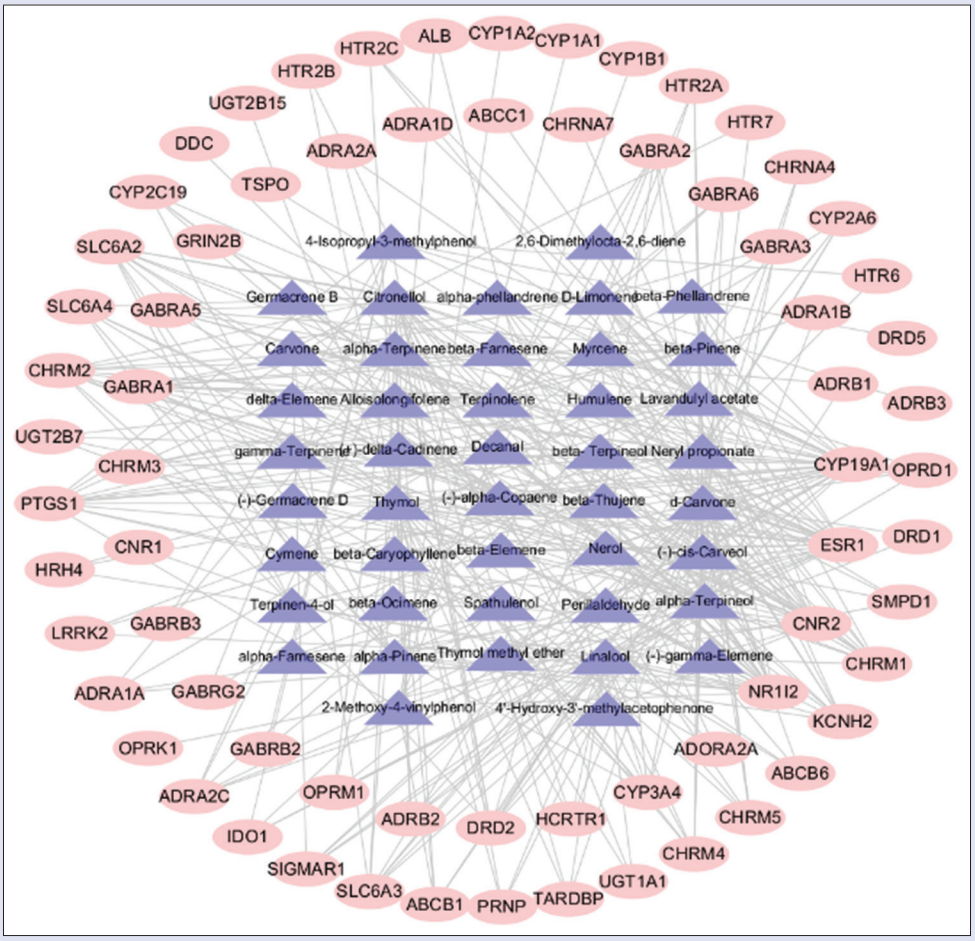

Figure 6: Network diagram of volatile oil chemical constituents - potential targets of liver depression and insomnia

the complete score of the correlation between GABRG2 and GABRA1 was the highest at 0.991. The comprehensive scores of interaction between DRD2 and SLC6A3, GABRG2 and GABRB2, GABRA1 and GABRB2 and GABRG2 and GABRA5 were also high, representing that these targets are meticulously related to each other. In Table 4, the highest comprehensive score of the interaction between targets pointed to all receptors of GABA subtypes. GABA seemed to be the most important target in the potential interaction between targets in liver depression and insomnia.[20] About 20% of the neurons in the CNS are GABA neurons, which play a main role in brain function. GABA neurons are the foremost inhibitory neurons that inhibit the excitatory neurons, playing an important role in the brain's self-balance.[21] The downregulation of GABA levels in the brain has been connected to emotional problems, such as insomnia, anxiety, tension, and depression.

Construction and analysis of target distribution network diagram

The BioGPS database was employed to attain the potential target of liver depression and insomnia – organ distribution (T-O) diagram. In Figure 8, the pink diamond nodes denote the potential targets and the green oval nodes represent the organs/tissues where these potential targets are disseminated. The edges represent the interactions between targets and organs. There were 146 nodes in the T-O diagram, of which 51 targets were highly articulated in Burkett's lymphoma cells, 49 targets were highly expressed in the liver, 43 targets were conveyed in the heart and more targets were expressed in cardiomyocytes, skeletal muscles, pineal gland, and prefrontal cortex.

One of the functions of the liver is the filtration and storage of blood that later moves to the cardiopulmonary system for oxygenation and body organ distribution. In the TCM notion, when the human moves, the blood flows through all the channels. When the human is still, the blood returns to the liver. The heart controls the blood, so it stores the spirit and it controls the thinking activities of spiritual perception. When the spirit is peaceful, Yang will enter the Yin and contribute in regulating the sleep cycle of human beings. The liver stores blood, so it stores the soul. The liver's blood is Yin. The blood volume is adequate to nourish the mind and the liver soul. The liver stores blood, so the soul lives. The liver regulates blood volume. If the blood is infused into the heart, the heart is nurtured and the spirit lives. Only when the liver has a normal sleep, can Yin and Yang play a major role to have a harmonious and stable spirit. The results of the T-O diagram are reliable with the conclusion of Chinese medicine that sleep is thoroughly connected to the liver and heart.

Target enrichment pathway analysis

A target enrichment pathway analysis was carried out on the three levels of BP, MF, CC, and KEGG pathway. In particular, the enrichment analysis of the KEGG pathway would foretell the mechanism of the treatment of liver depression and insomnia by the volatile oil of the dried tangerine peel and immature tangerine peel. The BP with the smallest *P* value was the G protein-coupled receptor signaling pathway that is coupled to cyclic nucleotide second messenger, which is mostly correlated to

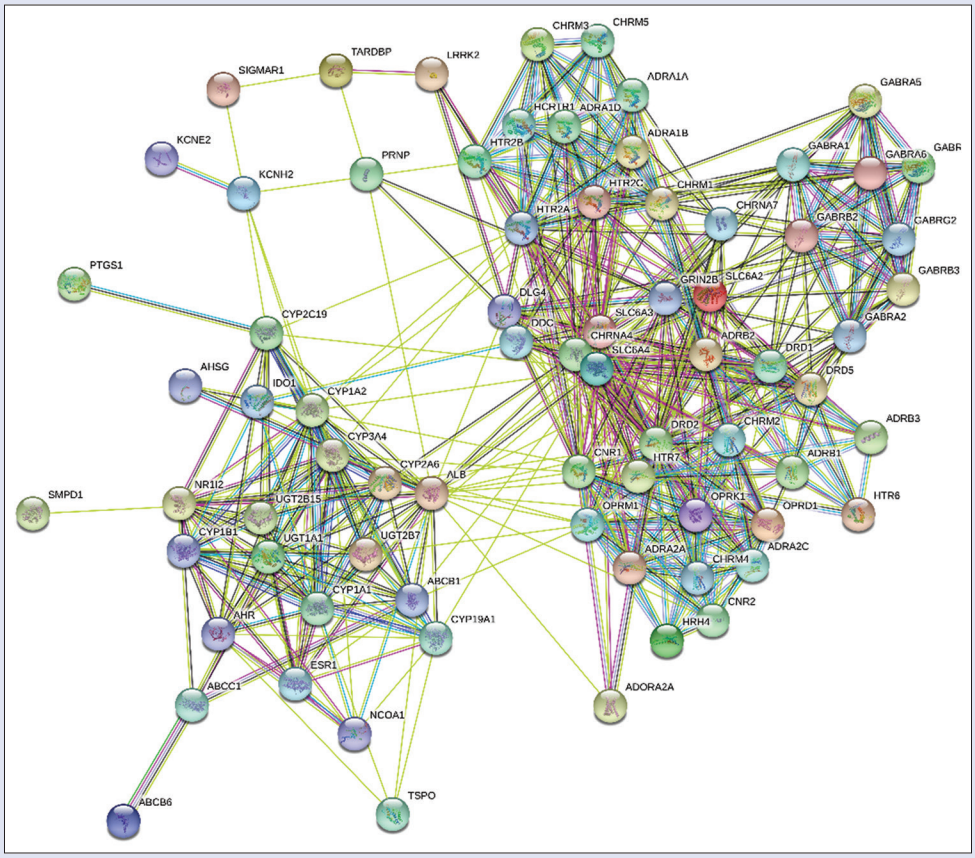

Figure 7: Diagram of protein-protein interaction (diagram)

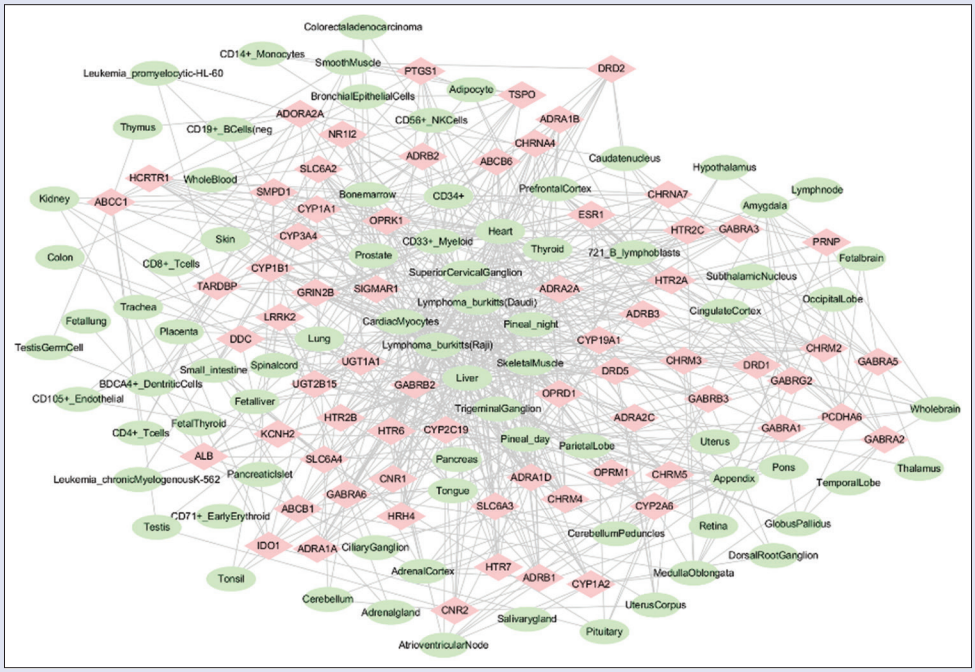

Figure 8: Distribution of target-organ/tissue (T-O diagram)

the aminergic acetylcholine receptor, adrenergic receptor, cannabinoid receptor, dopamine receptor, and serotonin receptor. Meanwhile, the second BPs were allied with responses on ammonium ion [Figure 9].

The molecular process with the smallest *P* value was the neurotransmitter receptor activity, which is largely interrelated to the receptors of aminobutyric acid, muscarinic acetylcholine, adrenergic,

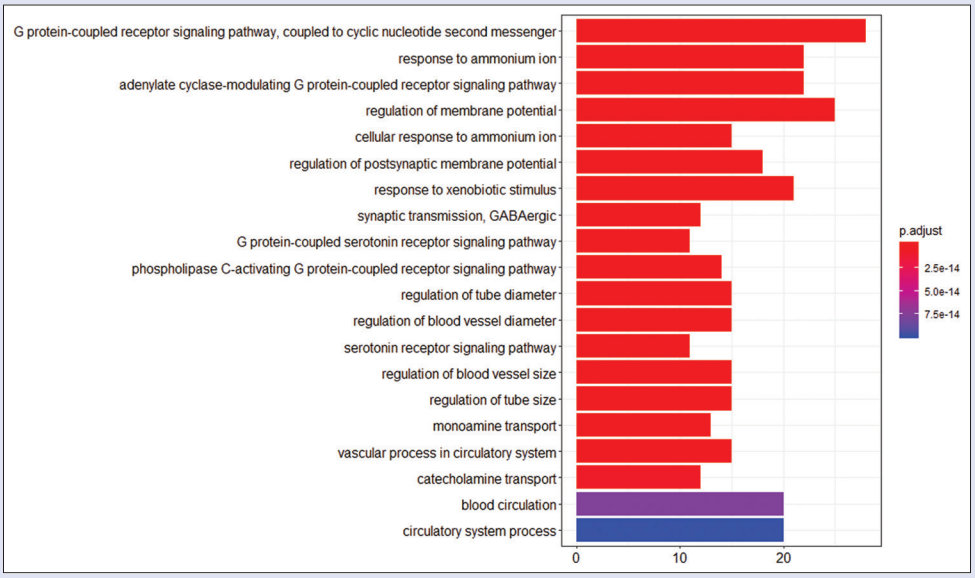

Figure 9: Enrichment analysis of biological process

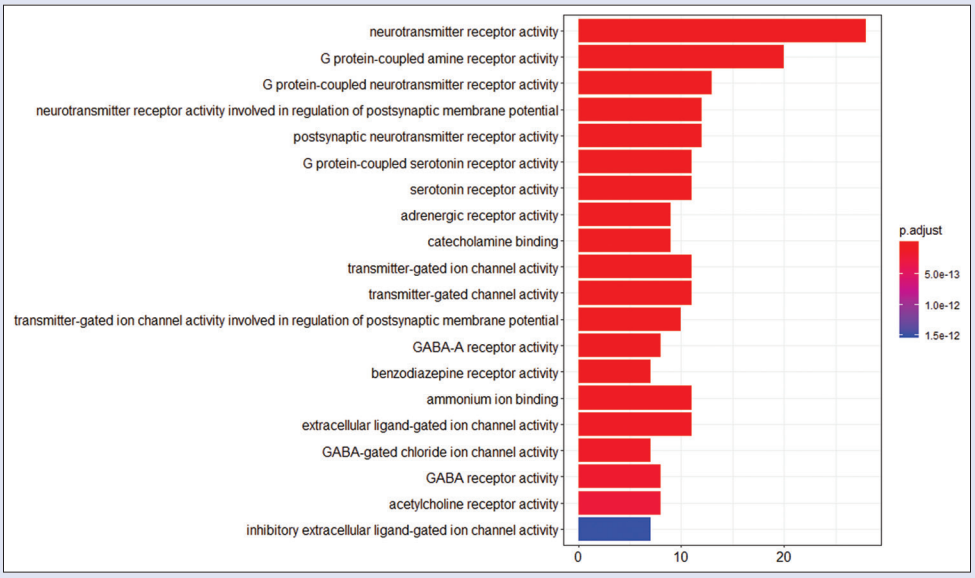

Figure 10: Enrichment analysis of molecular function

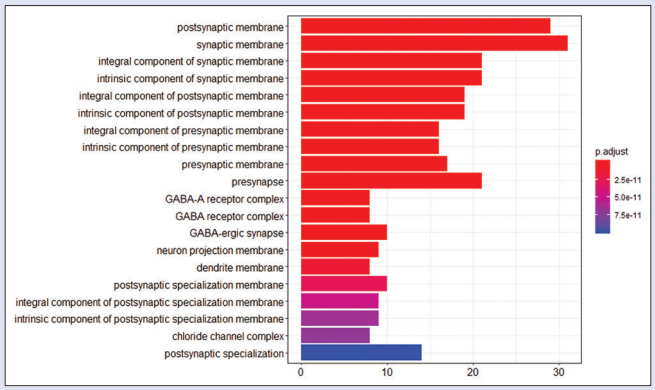

Figure 11: Enrichment analysis of cell component

dopamine, and serotonin [Figure 10]. The second molecular process convoluted the MF of G protein-coupled amine receptor activity.

The cell element with the smallest *P* value was the postsynaptic membrane, which was related to the aminobutyric acid receptor, muscarinic acetylcholine receptor, adrenergic receptor, sodium-dependent serotonin transporter, dopamine D2 receptor, and serotonin receptor [Figure 11]. The second progressions under the CCs were the synaptic membrane, integral component of synaptic membrane, etc.

The KEGG pathway with the smallest *P* value was the neuroactive ligand-receptor interaction, followed by the calcium signaling pathway and nicotine addiction [Figure 12]. The neuroactive ligand-receptor interaction pathway was planned using the KEGG database as exposed in Figure 13. In the diagram of the neuroactive receptor-ligand signaling pathway, the red targets characterize the gravest targets in the diagram, which are GABA, 5-hydroxytryptamine, dopamine, acetylcholine,

epinephrine, norepinephrine, and cannabinoid. This result directs that these targets play a key role in the neuroactive receptor-ligand signaling pathway.

Molecular docking

The Discovery Studio 4.5 software was employed to conduct molecular docking for each site of each target and all the chemical constituents of the essential oil. The target with the highest docking fraction value and its conforming chemical components were designated. The chemical components such as Neryl propionate, alpha-Farnesene, beta-Farnesene, Lavandulyl acetate had higher docking value with the target. The butt fraction values of the targets were higher, comprising DDC, CYP3A4, HTR2A, HTR2C, HRH4, CNR1, CHRM1, GABRB3, and GABRG2. The partial molecular docking diagram is revealed in Figure 14.

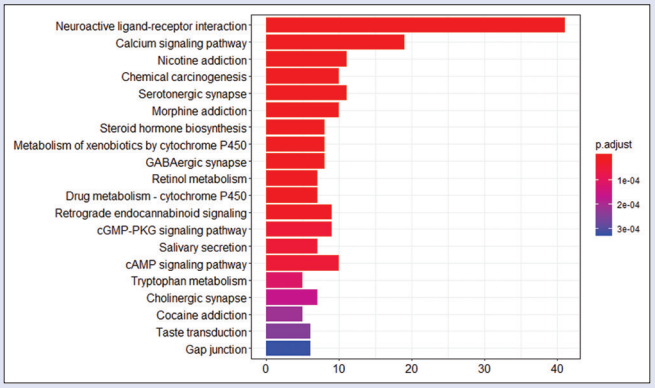

Figure 12: Enrichment analysis of Kyoto Encyclopedia of Genes and Genomes

CONCLUSION

Liver dysfunction causes liver depression and insomnia that are considered by poor mood, depression, insomnia, and anger. In TCM, if the liver Qi is not balanced, liver depression befalls, and induces insomnia. According to the TCM principle of liver treatment, the key treatment of liver depression and insomnia involves soothing the liver to regulate the Qi, thereby regulating the spirit. Immature tangerine peel is concerned to soothe the liver and thus eradicate Qi accumulation and stagnation. Dried tangerine peel is occupied to regulate the Qi of the spleen by strengthening the spleen, drying the dampness, and resolving the phlegm. The combination of the two drugs can not only regulate the liver and spleen, but can also control the spleen and stomach, leading to Qi balance and relief of depression. Therefore, this study picks the dried tangerine peel and immature tangerine peel as drugs for the treatment of liver depression and insomnia.

The pentobarbital-induced sleep experiment in mice displayed that low-dose tangerine essential oil aromatherapy may have both calming and tranquilizing effects, signifying that it may not only curtail the sleep incubation period but also extend the duration of sleep. Meanwhile, high-dose aromatherapy may only extend the sleep latency but not the duration of sleep. From the chemical composition of the volatile oil-potential target network diagram and protein-protein interaction diagram, it can be detected that γ-aminobutyric acid plays an important role in liver depression and insomnia. The results of the pathway enrichment analysis disclosed that the neuroactive receptor-ligand signaling pathway was most expected related to the mode of action of essential oil of dried tangerine peel and immature tangerine peel on liver depression and insomnia. The most vital targets of this pathway were GABA, acetylcholine, epinephrine, dopamine, and 5-hydroxytryptamine. The data excavating tactic indicated that the dried and immature

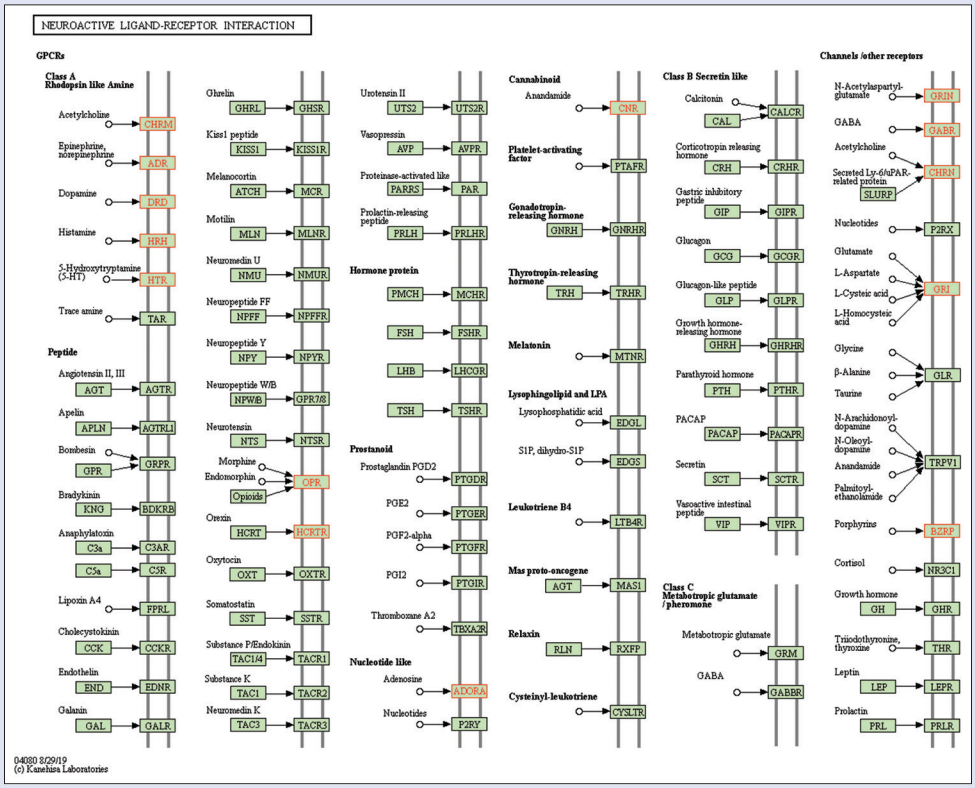

Figure 13: Diagram of the neuroactive receptor-ligand signaling pathway

Table 4: Target interaction relationship

Node 1	Node 2	Combined score
GABRG2	GABRA1	0.991
DRD2	SLC6A3	0.99
GABRG2	GABRB2	0.989
GABRA1	GABRB2	0.988
GABRG2	GABRA5	0.986
GABRG2	GABRB3	0.984
CNR1	DRD2	0.984
GABRG2	GABRA6	0.984
CNR1	OPRD1	0.983
GABRA5	GABRB3	0.981
GABRA5	GABRB2	0.98
GABRA1	GABRB3	0.979
GABRA2	GABRG2	0.978
GABRG2	GABRA3	0.977
GABRB2	GABRA6	0.976
UGT1A1	CYP3A4	0.975
CYP3A4	UGT2B7	0.969
GABRB3	GABRA6	0.967
CNR2	OPRD1	0.965
GABRA2	GABRB2	0.963
ESR1	CYP19A1	0.962
CYP3A4	NR1I2	0.962
CNR1	ADRA2A	0.962
ADRA2C	CNR1	0.961
GABRA3	GABRB2	0.961
GABRA3	GABRB3	0.96
GABRA2	GABRB3	0.96
GABRA1	GABRA6	0.954
CYP1A2	CYP3A4	0.952
CYP2C19	CYP3A4	0.951
CNR2	DRD2	0.951
CYP1A1	CYP3A4	0.95

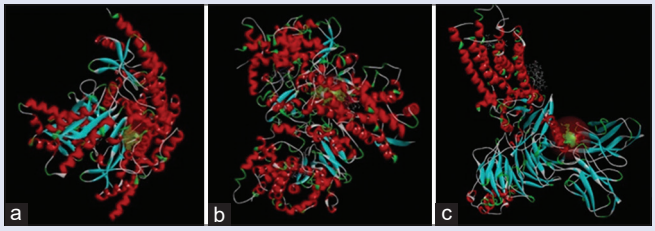

Figure 14: Molecular docking diagram of DDC and Neryl propionate (a); Molecular docking diagram of CYP3A4 and alpha-Farnesene (b); Molecular docking diagram of CNR1 and Neryl propionate (c)

tangerine peel was formerly recommended for insomnia, especially in the case of liver depression and insomnia. Treatment with dried and immature tangerine oil was stated to regulate the Qi balance in the liver, thereby adjusting the spirit to relieve depression. The network pharmacology method prophesied that the essential oil of dried and immature tangerine peel was largely used to treat liver depression and insomnia by regulating the neurotransmitters such as GABA on the neuroactive receptor-ligand signaling pathway.

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Conflicts of interest

There are no conflicts of interest.

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