Investigation on the active ingredient and mechanism of Cannabis sativa L. for treating epilepsy based on network pharmacology

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ABSTRACT

Cannabis sativa L. (cannabis) is a medicinal plant and has been used for many years for the treatment of epilepsy (EP), which is a common neurological disease. This study aimed to investigate the mechanism of cannabis action in EP, with emphasis on the leading compounds, targets and pathways. In this study, systematic pharmacology and bioinformatics approaches were employed to identify the active ingredients and potential targets of cannabis for treating EP. Furthermore, network construction, Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis, and molecular docking were used to elucidate the mechanism of cannabis against EP. A total of 360 compounds were collected in this work. Among them, 226 active compounds and 116 predicted targets were obtained based on absorption, distribution, metabolism and excretion (ADME) screening and databases, respectively. Among the 226 active compounds, most were cannabinoids. The topological analysis showed that cannabinoid receptor 1, albumin and glycogen synthase kinase-3 beta (CNR1, ALB and GSK3B) were the key targets with intense interaction. The GO and KEGG enrichment analysis suggested cannabis might produce the antiepileptic effects by regulating many pathways, including calcium signalling pathway, MAPK signalling pathway, GABAergic synapse, etc. Additionally, cannabinol methyl ether (M54) might be the leading compound based on molecular docking. Consequently, this study holistically illuminates the active constituents and mechanism of cannabis based on network pharmacology, which contributes to searching for leading compounds and development of new drugs in the treatment of EP.

Abbreviations: EP: epilepsy; Cannabis: Cannabis sativa L.; CBD: cannabidiol; C-T: compound-target; T-P: target-pathway; TCM: traditional Chinese medicine; ADME: absorption, distribution, metabolism and excretion; OMIM: online Mendelian inheritance in man; TTD: therapeutic target database; GO: gene ontology; KEGG: Kyoto encyclopedia of genes and genomes; TCMSP: traditional Chinese medicines for systems pharmacology database and analysis platform; GI: gastrointestinal; TCM: traditional Chinese medicine; THC: tetrahydrocannabinol; HCN4: potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4; CACNA1B: voltage-dependent N-type calcium channel subunit alpha-1B; CACNA1C: voltage-dependent L-type calcium channel subunit alpha-1C; CACNA1G: voltage-dependent T-type calcium channel subunit alpha-1G; CHRNA7: neuronal acetylcholine receptor subunit alpha-7; GRB2: growth factor receptor-bound protein 2; FGFR1: fibroblast growth factor receptor 1; FGFR3: fibroblast growth factor receptor 3; SOS: son of sevenless; Mek: mitogen-activated protein kinase; MEKK1: mitogen-activated protein kinase kinase kinase 1.

Introduction

Epilepsy (EP) is a nervous system disease with transient, stereotyped, recurrent and repetitive characteristics, which is caused by sudden abnormal discharge of brain neurons. According to the meta-analysis of international studies, the prevalence of EP is 6.4 per 1,000 people, and the annual incidence is 67.8 per 100,000 people [1]. There are more than 50 million EP patients in the world, and the mortality rate is 3 times that of the general population [2]. Due to the causes above, EP has increasingly attracted people’s attention recently. The pathogenesis of EP has been
intensively studied, as relating signalling pathways include AK signalling pathway, BDNF signalling pathway, mTOR signalling pathway, TGF signalling pathway, calcium signalling pathway and MAPK signalling pathway [3].

*Cannabis sativa* L. (cannabis), an annual dioecious herb of the genus *Cannabis* of the Cannabaceae family, has been extensively used as a traditional herbal medicine for the treatment of EP and neuropathic pain. It mainly contains various chemical components such as cannabinoids, flavonoids, terpenoids and so on, leading to a mass of pharmacological effects with a wide range. Most importantly, the cannabidiol (CBD) component of cannabis has been reported to treat EP [4, 5]. However, other active components and mechanisms of cannabis therapy for EP have not been fully elucidated.

Network pharmacology, an attractive concept put forward by Hopkins [6], integrates the ideas of systems biology and multi-directional pharmacology. It analyzes the action mechanism of drugs by constructing a complex network relationship of compound–target–disease. Recently, pharmacological research has shifted from the traditional search for a single target to multiple targets multi-level comprehensive network research [7, 8]. It promotes further research on Traditional Chinese Medicine (TCM) and expands the development of modern drugs. In addition, molecular docking is a theoretical simulation method based on receptor characteristics and molecular interaction [9]. It can predict the binding sites and affinity between drug molecules and target proteins, so as to verify the experimental results of network pharmacology [10].

To explore the pharmacological and molecular mechanism of antiepileptic activity of cannabis at the overall level, the network pharmacology analysis was employed (Figure 1). Firstly, the chemical database of cannabis was constructed, and the active compounds were screened according to SwissADME. Second, the targets of compounds and EP were predicted from five databases, involving SwissTargetPrediction, PharmMapper, GeneCards, Therapeutic Target Database (TTD) and Online Mendelian Inheritance in Man (OMIM). The common targets were considered as the candidate antiepileptic targets of cannabis. Furthermore, networks were constructed by Cytoscape, and Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) were performed to clarify the mechanism of the cannabis compounds against EP. Finally, the interaction between key compounds and targets was analyzed using molecular docking.

**Materials and methods**

**Chemical ingredients database building**

All chemical ingredients from cannabis were collected from Traditional Chinese Medicines for Systems Pharmacology database and analysis platform (TCMSP, available online: http://lsp.nwsuaf.edu.cn/) [11] and related literatures.

**Active ingredients screening**

The molecular properties of compounds were determined using SwissADME database (available online: http://www.swisstargetprediction.ch/) and PharmMapper (available online: http://www.lilab-ecust.cn/pharmmapper/) databases were used to retrieve and validate related targets of compounds. Then, the target name–gene name standard conversion was performed using the UniProt database (available online: https://www.uniprot.org/), so that the names of each target were corrected to the official names. After that, the candidate targets for the treatment of EP were screened by searching the GeneCards (available online: https://www.genecards.org/), TTD (available online: http://db.idrblab.net/ttd/) and OMIM (available online: https://omim.org/) databases [13]. The Venny 2.1 platform was used to draw a Veen diagram showing the overlapping of cannabis targets and EP targets. The overlapping was considered for potential therapeutic targets of cannabis against EP.

**Compound-target (C-T) network construction**

For a scientific explanation of the pharmacological mechanism of cannabis, two networks were established as follows: (1) the C–T network was established by linking active ingredients with their targets that were related to EP; (2) the C-T network of cannabinoids and their related targets was constructed.
GO and KEGG enrichment

In organisms, genes cannot perform their functions independently. Different genes coordinate with each other to finish a series of biochemical reactions to perform their biological functions. Therefore, GO and KEGG pathway enrichment analysis were used to provide a more systematic and comprehensive understanding of the mechanism of action. The Metascape platform [14] was applied to thoroughly analyze enrichment information. GO/KEGG terms with $p < 0.05$ were considered significantly enriched. The top 20 pathways containing the number of targets were selected. The Target–Pathway (T–P) network was
constructed to link compounds and pathways with related targets by Cytoscape [15], and the network topological properties were analyzed by Network Analyzer. In addition, the KEGG Mapper tool (available online: https://www.kegg.jp/kegg/mapper.html) was used for the construction of the pathway map.

**Molecular docking**

Active ingredients that were in the top ten and the top five targets in terms of degree value were screened based on the C–T network. The structures of the target proteins and the compounds were obtained from the PDB (available online: http://www.rcsb.org/) and PubChem (available online: http://www.ncbi.nlm.gov/pccompond) databases, respectively. Both of them were introduced into Schrodinger for molecular docking, and the docking score was analyzed to predict and evaluate the interaction between compounds and targets (the lower the docking score, the more negative number, and the better the binding affinity).

**Results**

**Classification of active compounds and predicted targets**

In this research, a total of 360 compounds were found in cannabis from the literatures and TCMSP database. Among them, 235 active compounds were selected through SwissADME database. The screening criteria were GI absorption shown as ‘high’, and at least two rules being ‘yes’ in the drug likeness rules (Table 1). In addition, a total of 1117 targets were predicted through Swiss TargetPrediction and PharmMapper databases using these active compounds, and 1403 EP-related targets were retrieved from the GeneCards, TTD and OMIM databases. By comparing these targets with the predicted cannabis targets, 116 common targets were filtered as the key targets for researching on the antiepileptic activity of the cannabis compounds (Figure 2). Nine ingredients that did not have any relevant targets were removed. The detailed information of the 116 candidate targets is displayed in Table 2.

**C–T Network construction and analysis**

To understand the interaction between compounds and targets, the C–T network (Figure 3(A)) was constructed by mapping 226 candidate ingredients and their related 116 targets, which consisted of 342 nodes and 1668 edges. The possible interactions between natural products and target proteins were assessed by degree, an important topological parameter. The results showed that cannabinoids displayed more intimate association to most targets, when docking with Cannabinoid receptor 1 (CNR1), Albumin (ALB) and Glutamate receptor ionotropic, NMDA 2A (GRIN2A), etc. To further identify the active ingredients of cannabis for the treatment of EP, the C-T network (Figure 3(B)) of cannabinoids was also constructed, which embodied 199 nodes (106 cannabinoid-compounds and their related 93 targets) and 996 edges. A network analysis showed that cannabicitran (M117, degree = 20), cannabichromanone D (M85, degree = 20), (±)-6,7-trans-epoxycannabigerolic (M100, degree = 19) and cannabiol methyl ether (M54, degree = 18) had the highest number of connections to different, which might play a key role in the treatment of EP. What is more, it could be observed that CNR1 (degree = 113), Androgen receptor (AR, degree = 97), Glycogen synthase kinase-3 beta (GSK3B, degree = 59), ALB (degree = 53) and Mitogen-activated protein kinase 10 (MAPK10, degree = 51), which corresponded to multiple compounds, might be the key targets of the network.

**GO and KEGG enrichment analysis**

The Metascape database was used to analyze the 116 potential targets of cannabis for EP by using GO and KEGG enrichment analysis. The threshold was set at \( p < 0.05 \), and the previous GO annotation results and KEGG pathway results were filtered out, as shown in Figure 4. GO enrichment results showed that the main biological processes involved in the active ingredients of cannabis were chemical synaptic transmission, anterograde trans-synaptic signalling and so on. The main cellular components of cannabis were synaptic membrane, post-synapse, postsynaptic membrane and so on. The major molecular functions were neurotransmitter receptor activity, postsynaptic neurotransmitter receptor activity and so on. KEGG pathway analysis displayed that cannabis could play an overall regulatory role through multiple pathways, including neurotransmitter receptor activity, nicotinic addiction and so on.

**Pathway construction and analysis**

Based on the prediction results of KEGG enrichment, the pathways and targets were shown in Supplemental Table S1. The results showed an average degree of
Table 1. 235 potential active ingredients and ADME parameters of *Cannabis sativa* L.

| No. | Name                                                | Gl absorption | Lipinski | Ghose | Veber | Egan | Muegge |
|-----|-----------------------------------------------------|---------------|----------|-------|-------|------|--------|
| M1  | (-)-Δ⁸-trans-Tetrahydrocannabinol                    | High          | Yes      | No    | Yes   | Yes  | No     |
| M2  | (-)-Δ⁸-trans-Tetrahydrocannabinolic acid A           | High          | Yes      | Yes   | Yes   | Yes  | No     |
| M3  | (-)-Δ⁸-trans-Tetrahydrocannabinolic acid B           | High          | Yes      | Yes   | Yes   | Yes  | No     |
| M4  | (-)-Δ⁸-trans-Tetrahydrocannabinol -C4                | High          | Yes      | Yes   | Yes   | Yes  | No     |
| M5  | (-)-Δ⁸-trans-Tetrahydrocannabinolic acid A-C4        | High          | Yes      | Yes   | Yes   | Yes  | No     |
| M6  | (-)-Δ⁸-trans-Tetrahydrocannabinol                    | High          | Yes      | Yes   | Yes   | Yes  | No     |
| M7  | (-)-Δ⁸-trans-Tetrahydrocannabinavirin acid           | High          | Yes      | Yes   | Yes   | Yes  | No     |
| M8  | (-)-Δ⁸-trans-Tetrahydrocannabinorol                  | High          | Yes      | Yes   | Yes   | Yes  | Yes    |
| M9  | (-)-Δ⁸-trans-Tetrahydrocannabinolic acid             | High          | Yes      | Yes   | Yes   | Yes  | Yes    |
| M10 | 8a-Hydroxy-Δ⁸-trans-tetrahydrocannabinolate          | High          | Yes      | Yes   | Yes   | Yes  | No     |
| M11 | 8b-Hydroxy-Δ⁸-trans-tetrahydrocannabinol             | High          | Yes      | Yes   | Yes   | Yes  | No     |
| M12 | 11-Acetoxy-Δ⁸-trans-tetrahydrocannabinolic acid A    | High          | Yes      | Yes   | Yes   | Yes  | No     |
| M13 | Δ⁸-THC aldehyde                                      | High          | Yes      | Yes   | Yes   | Yes  | Yes    |
| M14 | 8-Oxo-Δ⁸-trans-tetrahydrocannabinol                  | High          | Yes      | Yes   | Yes   | Yes  | Yes    |
| M15 | β-Fenchyl-Δ⁸-trans-tetrahydrocannabinolate          | High          | Yes      | No    | Yes   | Yes  | No     |
| M16 | α-Fenchyl-Δ⁸-trans-tetrahydrocannabinol              | High          | Yes      | No    | Yes   | Yes  | No     |
| M17 | α-Terpenyl-Δ⁸-trans-tetrahydrocannabinol             | High          | Yes      | No    | Yes   | Yes  | No     |
| M18 | (-)-D⁹-cis-(6aS, 10aR)-Tetrahydrocannabinol          | High          | Yes      | No    | Yes   | Yes  | No     |
| M19 | Cannabidiol                                          | High          | Yes      | Yes   | Yes   | Yes  | Yes    |
| M20 | Cannabidiolic acid                                   | High          | Yes      | Yes   | Yes   | Yes  | Yes    |
| M21 | Cannabidiol- c4                                      | High          | Yes      | Yes   | Yes   | Yes  | Yes    |
| M22 | Cannabidiol- c3                                      | High          | Yes      | Yes   | Yes   | Yes  | Yes    |
| M23 | (-)-trans-10-Ethoxy-9-hydroxy-D6a(10a)-tetrahydrocannabinol | High       | Yes      | Yes   | Yes   | Yes  | Yes    |
| M24 | (-)-trans-11-Acetoxy-9-hydroxy-D6a(10a)-tetrahydrocannabinol | High   | Yes      | Yes   | Yes   | Yes  | Yes    |
| M25 | (-)-trans-3″-Hydroxy-D4″-cannabichromene              | High          | Yes      | No    | Yes   | Yes  | No     |
| M26 | 2-Methyl-2-(4-methyl-2-pentyl)-7-propyl-2H-1-benzopyran-5-ol | High     | Yes      | Yes   | Yes   | Yes  | Yes    |

(continued)
| Code | Compound                                      | Activity 1 | Activity 2 | Activity 3 | Activity 4 | Activity 5 | Activity 6 |
|------|-----------------------------------------------|------------|------------|------------|------------|------------|------------|
| M81  | Cannabichromane-C5                            | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M82  | Cannabichromane-C3                            | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M83  | Cannabichromane B                             | High       | Yes        | Yes        | Yes        | No         | No         |
| M84  | Cannabichromane C                             | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M85  | Cannabichromane D                             | High       | Yes        | Yes        | Yes        | No         | No         |
| M86  | Cannabigerol                                  | High       | No         | Yes        | No         | No         | No         |
| M87  | Cannabigerolic acid                           | High       | Yes        | No         | Yes        | No         | No         |
| M88  | Cannabigerol monomethylether                  | High       | No         | Yes        | No         | No         | No         |
| M90  | Cannabigerovarinic acid A                     | High       | Yes        | Yes        | Yes        | No         | No         |
| M91  | Cannabigerovarin                              | High       | Yes        | Yes        | Yes        | No         | No         |
| M95  | Cannabigerolic acid A                         | High       | Yes        | Yes        | Yes        | No         | No         |
| M96  | Camagerol                                     | High       | Yes        | Yes        | Yes        | No         | No         |
| M98  | (±)-6,7-trans-Epoxycannabigerolic acid        | High       | Yes        | Yes        | Yes        | No         | No         |
| M99  | (±)-6,7-cis-Epoxycannabigerolic acid          | High       | Yes        | Yes        | Yes        | No         | No         |
| M100 | (±)-6,7-trans-Epoxycannabigerolic             | High       | Yes        | Yes        | Yes        | No         | No         |
| M101 | (±)-6,7-cis-Epoxycannabigerolic               | High       | Yes        | Yes        | Yes        | No         | No         |
| M102 | Cannabielsoic acid A                          | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M103 | Cannabielsoin                                 | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M104 | Cannabielsoic acid B                          | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M105 | C7-Cannabielsoic acid B                       | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M106 | C6-Cannabielsoin                              | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M107 | Dehydrocannabifuran                           | High       | No         | Yes        | No         | No         | No         |
| M108 | Cannabinol                                    | High       | Yes        | No         | Yes        | No         | No         |
| M109 | 8-Hydroxy-isohexahydrocannabivirin             | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M110 | Cannabicycloc                                  | High       | Yes        | No         | Yes        | No         | No         |
| M111 | Cannabicyclolic acid                          | High       | Yes        | No         | Yes        | No         | No         |
| M112 | Cannabicyclovarin                             | High       | Yes        | No         | Yes        | No         | No         |
| M114 | (-)-(7R)-Cannabicoumaronic acid               | High       | Yes        | No         | Yes        | No         | No         |
| M115 | 2-Geranyl-5-hydroxy-3-n-pentyl-1,4-benzoquinone| High       | Yes        | Yes        | Yes        | No         | No         |
| M116 | S-Acetoxy-6-geranyl-3-n-pentyl-1,4-benzoquinone| High       | Yes        | No         | Yes        | No         | No         |
| M117 | Cannabicitran                                  | High       | Yes        | No         | Yes        | Yes        | Yes        |
| M118 | 4-Actoxy-2-geranyl-5-hydroxy-3-n-pentylphenol  | High       | Yes        | No         | No         | Yes        | No         |
| M119 | Cannabinomone                                  | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M120 | Cannabioxidepene                              | High       | Yes        | Yes        | No         | No         | No         |
| M121 | Vitexin                                       | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M122 | Isovitexin                                    | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M123 | Apigenin-6,8-di-C-β-D-glucopyranoside         | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M125 | Cannflavin B                                  | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M129 | Orientin                                      | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M130 | Orientin-7-O-rhamnogluosid                    | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M131 | 6-Geranylapigenin                             | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M132 | Apigenin                                      | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M133 | Chrysoeriol                                    | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M134 | Apigenin-7-O-glucoside                        | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M135 | Luteolin                                      | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M136 | Luteolin-7-O-a-D-glucoside                    | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M137 | 2″-O-Glucopyranosylvitexin                    | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M138 | 2″-O-Glucopyranosylorientin                   | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M139 | Kaempferol                                    | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M140 | Kaempferol 3-O-sophoroside                    | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M142 | Quercetin                                     | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M143 | Quercetin-0-glucoside(s)                      | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M144 | Quercetin-3-O-sophoroside                     | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M145 | trans-Anethol                                  | High       | Yes        | No         | Yes        | No         | No         |
| M146 | cis-Anethol                                    | High       | Yes        | No         | Yes        | No         | No         |
| M147 | iso-Eugenol                                   | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M148 | Eugenol                                       | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M149 | Methyleugenol                                 | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M150 | N-p-coumaroyltyramine                         | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M151 | N-trans-caffeoyltyramine                      | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M152 | N-trans-feruloyltyramine                      | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M174 | Coumaroylaminobutanol glucopyranoside         | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M175 | Cannithrene 1                                 | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M176 | Cannithrene 2                                 | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M177 | 4,5-Dihydroxy-2,3,6-trimethoxy-9,10-dihydropentanethrene | High     | Yes        | Yes        | Yes        | Yes        | Yes        |
| M178 | 4-Hydroxy-2,3,6,7-tetramethoxy-9,10-dihydropentanethrene | High     | Yes        | Yes        | Yes        | Yes        | Yes        |
| M179 | Dihydroresveratrol                            | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M180 | 3,4″-Dihydroxy-5-methoxy bibenzyl              | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M181 | 3,3″-Dihydroxy-5,4″-dimethoxy bibenzyl         | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M182 | Cannabistilbene Iib                           | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M183 | Cannabistilbene ila                           | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M184 | Cannabistilbene I                            | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M185 | 3,4″-Dihydroxy-5,3″-dimethoxy-5″-isoprenyl     | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M186 | Canniprene                                    | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M187 | Cannabipirone                                  | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M188 | iso-Cannabipirone                             | High       | Yes        | Yes        | Yes        | Yes        | Yes        |
| M | Name | High | Yes | Yes | Yes | Yes | Yes |
|---|------|------|-----|-----|-----|-----|-----|
| M189 | Cannabispirl | High | Yes | Yes | Yes | Yes | Yes |
| M190 | Acetylcanbispirl | High | Yes | Yes | Yes | Yes | Yes |
| M191 | 7-Hydroxy-5-methoxyindan-1-spiro-cyclohexane | High | Yes | Yes | Yes | Yes | Yes |
| M192 | 5-Hydroxy-7-methoxyindan-1-spiro-cyclohexane | High | Yes | Yes | Yes | Yes | Yes |
| M193 | 5,7-Dihydroxyindan-1-spiro-cyclohexane | High | Yes | Yes | Yes | Yes | Yes |
| M194 | α-Cannabispiranol 401-O-β-D-glucopyranose | High | Yes | Yes | Yes | Yes | Yes |
| M195 | Cannabispirenone-A | High | Yes | Yes | Yes | Yes | Yes |
| M196 | Cannabispirenone-B | High | Yes | Yes | Yes | Yes | Yes |
| M197 | Cannabispiradene | High | Yes | Yes | Yes | Yes | Yes |
| M200 | Cannabispentetal | High | Yes | Yes | Yes | Yes | Yes |
| M222 | 1,4-Cineol | High | Yes | No | Yes | Yes | No |
| M223 | 1,8-Cineol | High | Yes | No | Yes | Yes | No |
| M226 | Borneol | High | Yes | No | Yes | Yes | No |
| M227 | Bornyl acetate | High | Yes | No | Yes | Yes | No |
| M229 | Camphenehydrate | High | Yes | No | Yes | Yes | No |
| M230 | Camphor | High | Yes | No | Yes | Yes | No |
| M231 | Carvacrol | High | Yes | No | Yes | Yes | No |
| M232 | Carvone | High | Yes | No | Yes | Yes | No |
| M233 | cis-Linalool oxide | High | Yes | No | Yes | Yes | No |
| M234 | cis-α-Ocimene | High | Yes | No | Yes | Yes | No |
| M235 | Citral B | High | Yes | No | Yes | Yes | No |
| M236 | Citronellol | High | Yes | No | Yes | Yes | No |
| M237 | Dihydrocarvaryl acetate | High | Yes | No | Yes | Yes | No |
| M238 | Dihydrocarvone | High | Yes | No | Yes | Yes | No |
| M239 | Fenchone | High | Yes | No | Yes | Yes | No |
| M240 | Fenchyl alcohol | High | Yes | No | Yes | Yes | No |
| M241 | Geraniol | High | Yes | No | Yes | Yes | No |
| M242 | Geranyl acetone | High | Yes | No | Yes | Yes | No |
| M243 | Ipsidinol | High | Yes | No | Yes | Yes | No |
| M245 | Linalool | High | Yes | No | Yes | Yes | No |
| M247 | Nerol | High | Yes | No | Yes | Yes | No |
| M248 | Nerolidol | High | Yes | No | Yes | Yes | No |
| M250 | p-Cymene-8-ol | High | Yes | No | Yes | Yes | No |
| M251 | Perillene | High | Yes | No | Yes | Yes | No |
| M252 | Pinocarveol | High | Yes | No | Yes | Yes | No |
| M253 | Pinocarvone | High | Yes | No | Yes | Yes | No |
| M254 | Piperitenone oxide | High | Yes | No | Yes | Yes | No |
| M255 | Piperitenone | High | Yes | No | Yes | Yes | No |
| M256 | Piperitone oxide | High | Yes | No | Yes | Yes | No |
| M257 | Pulegone | High | Yes | No | Yes | Yes | No |
| M259 | Sabinol | High | Yes | No | Yes | Yes | No |
| M260 | Safranal | High | Yes | No | Yes | Yes | No |
| M261 | Thuyl alcohol | High | Yes | No | Yes | Yes | No |
| M262 | trans-Linalool oxide | High | Yes | No | Yes | Yes | No |
| M263 | trans-Sabinene hydrate | High | Yes | No | Yes | Yes | No |
| M265 | α-Pinene oxide | High | Yes | No | Yes | Yes | No |
| M266 | α-Terpinene-4-ol | High | Yes | No | Yes | Yes | No |
| M269 | α-Terpinol | High | Yes | No | Yes | Yes | No |
| M272 | β-Cyclocitrinal | High | Yes | No | Yes | Yes | No |
| M273 | β-Fenchol | High | Yes | No | Yes | Yes | No |
| M276 | β-Terpineol | High | Yes | No | Yes | Yes | No |
| M278 | m-Menth-1.8(9)-dien-5-01-ol | High | Yes | No | Yes | Yes | No |
| M282 | Eucalyptol | High | Yes | No | Yes | Yes | No |
| M283 | 4-Allylanisole | High | Yes | No | Yes | Yes | No |
| M284 | (-)-Verbenone | High | Yes | No | Yes | Yes | No |
| M285 | α-Pulegone | High | Yes | No | Yes | Yes | No |
| M286 | (-)-Isouleegone | High | Yes | No | Yes | Yes | No |
| M287 | Linalylacetate | High | Yes | No | Yes | Yes | No |
| M288 | cis-Sabinene hydrate | High | Yes | No | Yes | Yes | No |
| M289 | DL-Citronellyl acetate | High | Yes | No | Yes | Yes | No |
| M290 | Carvyl acetate | High | Yes | No | Yes | Yes | No |
| M294 | Caryophyllene oxide | High | Yes | No | Yes | Yes | No |
| M297 | Famesol | High | Yes | No | Yes | Yes | No |
| M298 | Famesyl Acetone | High | Yes | No | Yes | Yes | No |
| M299 | Humulene epoxide I | High | Yes | No | Yes | Yes | No |
| M300 | Humulene epoxide II | High | Yes | No | Yes | Yes | No |
| M302 | Ledol | High | Yes | No | Yes | Yes | No |
| M308 | α-Bisabolol | High | Yes | No | Yes | Yes | No |
| M309 | α-Caryophyllene alcohol | High | Yes | No | Yes | Yes | No |
| M314 | α-Eudesmol | High | Yes | No | Yes | Yes | No |
| M324 | β-Eudesmol | High | Yes | No | Yes | Yes | No |
| M333 | γ-Eudesmol | High | Yes | No | Yes | Yes | No |
| M334 | trans-Nerolidol | High | Yes | No | Yes | Yes | No |
| M335 | Guaiol | High | Yes | No | Yes | Yes | No |
| M339 | (+)-Cedrol | High | Yes | No | Yes | Yes | No |
| M344 | β-Bisabolol | High | Yes | No | Yes | Yes | No |
Table 2. Information on 116 potential targets of *Cannabis sativa* L.

| No. | Gene names       | Protein names                                      | Uniprot ID |
|-----|------------------|----------------------------------------------------|------------|
| 1   | CASR             | Extracellular calcium-sensing receptor             | P41180     |
| 2   | CNR1             | Cannabinoid receptor 1                             | P21554     |
| 3   | CTSD             | Cathepsin D                                        | P07339     |
| 4   | GLRA1            | Glycine receptor subunit alpha-1                   | P23415     |
| 5   | GPR55            | G-protein coupled receptor 55                      | Q9Y2T6     |
| 6   | GRM5             | Metabotropic glutamate receptor 5                  | P41594     |
| 7   | TACR3            | Neuromedin-K receptor                              | P29371     |
| 8   | Tspo             | Translocator protein                               | P30536     |
| 9   | DUSP3            | Dual specificity protein phosphatase 3             | P51452     |
| 10  | GRIN1            | Glutamate receptor ionotropic, NMDA 1              | Q05586     |
| 11  | mTor             | Serine/threonine-protein kinase mTOR               | P42345     |
| 12  | GPHN             | Gephyrin                                           | Q9NQK3     |
| 13  | KCNMA1           | Calcium-activated potassium channel subunit alpha-1| Q12791     |
| 14  | SCN2A            | Sodium channel protein type 2 subunit alpha        | Q99250     |
| 15  | FOLH1            | Glutamate carboxypeptidase 2                       | Q04609     |
| 16  | SCN5A            | Sodium channel protein type 5 subunit alpha        | Q14524     |
| 17  | GSK3B            | Glycogen synthase kinase-3 beta                    | P49841     |
| 18  | TPS3             | Cellular tumor antigen p53                         | P04637     |
| 19  | GSR              | Glutathione reductase, mitochondrial               | P00390     |
| 20  | SOD2             | Superoxide dismutase [Mn], mitochondrial           | P04179     |
| 21  | Adora1           | Adenosine receptor A1                              | P30542     |
| 22  | APP              | Amyloid-beta precursor protein                     | P50567     |
| 23  | CACNA2D2         | Voltage-dependent calcium channel subunit alpha-2/delta-2 | Q9NYG7 |
| 24  | SCN9A            | Sodium channel protein type 9 subunit alpha        | Q15858     |
| 25  | Gck              | Hexokinase-4                                       | P35557     |
| 26  | Gabra1           | Gamma-aminobutyric acid receptor subunit alpha-1   | P14867     |
| 27  | Gabra2           | Gamma-aminobutyric acid receptor subunit alpha-2   | P47869     |
| 28  | CYP2C9           | Cytochrome P450 2C9                                | P11712     |
| 29  | CYP3AA           | Cytochrome P450 3A4                                | P08684     |
| 30  | CYP2C19          | Cytochrome P450 2C19                               | P33261     |
| 31  | Pkcska           | Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha isoform | P42336 |
| 32  | DAB1             | Disabled homolog 1                                 | Q75553     |
| 33  | ME2              | NAD-dependent malic enzyme, mitochondrial          | P23368     |
| 34  | Slc6a3           | Sodium-dependent dopamine transporter              | Q01959     |
| 35  | Gabrb3           | Gamma-aminobutyric acid receptor subunit beta-3    | P28472     |
| 36  | Ntrk2            | BDNF/NT-3 growth factors receptor                  | Q16620     |
|   | Protein Name | Description | Entrez ID |
|---|--------------|-------------|-----------|
| 37 | MAPK10       | Mitogen-activated protein kinase 10 | P53779 |
| 38 | SLC2A1       | Solute carrier family 2, facilitated glucose transporter member 1 | P11366 |
| 39 | TRPV1        | Transient receptor potential cation channel subfamily V member 1 | Q8NER1 |
| 40 | GRM1         | Metabotropic glutamate receptor 1 | Q13255 |
| 41 | AR           | Androgen receptor | P10275 |
| 42 | HTR2A        | 5-hydroxytryptamine receptor 2A | P28223 |
| 43 | MR1          | Methylthioribose-1-phosphate isomerase | Q9BV20 |
| 44 | MT-CO3       | Cytochrome c oxidase subunit 3 | P00414 |
| 45 | ALB          | Albumin | P02768 |
| 46 | RORA         | Nuclear receptor ROR-alpha | P35398 |
| 47 | SNX27        | Sorting nexin-27 | Q96L92 |
| 48 | VRK2         | Serine/threonine-protein kinase VRK2 | Q86Y07 |
| 49 | CACNA1B      | Voltage-dependent L-type calcium channel subunit alpha-1B | Q00975 |
| 50 | MECP2        | Methyl-CpG-binding protein 2 | P51608 |
| 51 | PPOX         | Protoporphyrinogen oxidase | P50336 |
| 52 | CASP1        | Caspase-1 | P29466 |
| 53 | DHFR         | Dihydrofolate reductase | P00374 |
| 54 | GABRG2       | Gamma-aminobutyric acid receptor subunit gamma-2 | P18507 |
| 55 | MTNR1B       | Melatonin receptor type 1B | P49286 |
| 56 | GRM2         | Metabotropic glutamate receptor 2 | Q14416 |
| 57 | FGFR1        | Fibroblast growth factor receptor 1 | P11362 |
| 58 | CTSA         | Lysosomal protective protein | P10619 |
| 59 | BRD2         | Bromodomain-containing protein 2 | P25440 |
| 60 | ABCB1        | ATP-dependent translocase ABCB1 | P08183 |
| 61 | GABRA5       | Gamma-aminobutyric acid receptor subunit alpha-5 | P31644 |
| 62 | GYS1         | Glycogen [starch] synthase, muscle | P13807 |
| 63 | ITGA4        | Integrin alpha-4 | P13612 |
| 64 | GABBR2       | Gamma-aminobutyric acid type B receptor subunit 2 | Q75899 |
| 65 | CTSB         | Cathepsin B | P07858 |
| 66 | OPRM1        | Mu-type opioid receptor | P35372 |
| 67 | ABCG2        | Broad substrate specificity ATP-binding cassette transporter ABCG2 | Q9UNQ0 |
| 68 | IL1B         | Interleukin-1 beta | P01584 |
| 69 | CACNA1C      | Voltage-dependent L-type calcium channel subunit alpha-1C | Q13936 |
| 70 | SHBG         | Sex hormone-binding globulin | P04278 |
| 71 | HTR1A        | 5-hydroxytryptamine receptor 1A | P08908 |
| 72 | KCNQ3        | Potassium voltage-gated channel subfamily IQT member 3 | Q04325 |
| 73 | GBA          | Lysosomal acid glucosidase | P04684 |
| 74 | CHRNA7       | Neuronal acetylcholine receptor subunit-7 | P36544 |
| 75 | GRIN2A       | Glutamate receptor ionotropic, NMDA 2A | Q12879 |
| 76 | CHRNA4       | Neuronal acetylcholine receptor subunit-4 | P43681 |
| 77 | AKT3         | RAC-gamma serine/threonine-protein kinase | P93243 |
| 78 | ADK          | Adenosine kinase | P55263 |
| 79 | IRR1         | Interleukin-1 receptor-associated kinase 1 | P51617 |
| 80 | HTR1D        | 5-hydroxytryptamine receptor 1D | P28221 |
| 81 | TK2          | Thymidine kinase 2, mitochondrial | P00142 |
| 82 | SMARC2       | Probable global transcription activator SNF2L2 | P15131 |
| 83 | GRM4         | Metabotropic glutamate receptor 4 | Q14833 |
| 84 | CASK         | Peripheral plasma membrane protein CASK | O14936 |
| 85 | CSN1K1G1     | Casein kinase I isoform gamma-1 | Q99CH0 |
| 86 | SCN1A0       | Sodium channel protein type 10 subunit alpha | Q9JY59 |
| 87 | GABRB6       | Gamma-aminobutyric acid receptor subunit alpha-6 | Q16445 |
| 88 | ADRA2B       | Alpha-2B adrenergic receptor | P18089 |
| 89 | ICK          | Serine/threonine-protein kinase ICK | Q99UP7 |
| 90 | FGFR3        | Fibroblast growth factor receptor 3 | P22607 |
| 91 | RORB         | Nuclear receptor ROR-beta | Q92753 |
| 92 | FOS          | Proto-oncogene c-Fos | P01100 |
| 93 | ASAH1        | Acid ceramidase | Q13510 |
| 94 | MAPT         | Microtubule-associated protein tau | P10636 |
| 95 | CASA         | Carbonic anhydrase 5A, mitochondrial | P35218 |
| 96 | GABBR1       | Gamma-aminobutyric acid type B receptor subunit 1 | Q9UBS5 |
| 97 | KCNJ11       | ATP-sensitive inward rectifier potassium channel 11 | Q14654 |
| 98 | PLAA2G6      | 85/88 kDa calcium-independent phospholipase A2 | O60733 |
| 99 | GLUL         | Glutamine synthetase | P15104 |
| 100 | ACP1         | Low molecular weight phosphotyrosine protein phosphatase | P24666 |
| 101 | ALDH5A1      | Succinate-semialdehyde dehydrogenase, mitochondrial | P51649 |
| 102 | ABAT         | 4-aminobutyrate aminotransferase, mitochondrial | P80404 |
| 103 | SNCA         | Alpha-synuclein | P37840 |
| 104 | TNF          | Tumor necrosis factor | P01375 |
| 105 | IL6          | Interleukin-6 | P05231 |
| 106 | CTSB         | Dipetidyl peptidase 1 | P35364 |
| 107 | PRNP         | Major prion protein | P04156 |
| 108 | HTR3A        | 5-hydroxytryptamine receptor 3A | P46098 |
| 109 | HNMT         | Histamine N-methyltransferase | P50135 |
| 110 | CACNA1G      | Voltage-dependent T-type calcium channel subunit alpha-1G | O43497 |
| 111 | SLC1A1       | Excitatory amino acid transporter 3 | P43005 |
| 112 | GRIA4        | Glutamate receptor 4 | P48058 |
| 113 | DNMT1        | Dmnt1 | Q05193 |
| 114 | TGFBI        | Transforming growth factor beta-1 proprotein | P01137 |
| 115 | CSN1K1E      | Casein kinase I isoform epsilon | P49674 |
| 116 | CTSF         | Cathepsin F | Q9UBK1 |
Figure 3. C-T network. (A) The compound – target network of all compounds. (B) The compound – target network of cannabinoids.
9.71 per target and 5.57 per pathway, and several target proteins (82 out of 98) mapped to multiple pathways. The T–P network of the top 20 pathways with 73 targets mapped to 20 pathways was constructed as shown in Figure 5. The results displayed that the candidate ingredients were intensively associated with the pathways as follows: neuroactive ligand-receptor interaction, MAPK signalling pathway, taste transduction, GABAergic synapse, retrograde endocannabinoid signalling and cAMP signalling pathway. An integrated ‘EP pathway’ was constructed using 15 EP-related signalling pathways extracted from KEGG pathway as shown in Figure 6(A). The targets of the integrated ‘EP pathway’ exhibited a close functional relationship with those related to EP. The calcium signalling pathway, MAPK signalling pathway and synaptic connections were the main antiepileptic pathways in cannabis, which were displayed in Figure 6(B)–(D), respectively.

**Molecular docking analysis**

In order to further explore the active mechanism, the interactions between the potential active compounds (M10, M11, M30, M34, M46, M54, M85, M100, M116 and M117) and the targets proteins were elucidated using Schrodinger, the docking scores of targets with the active compounds are listed in Table 3. The interaction between the active site residues and the target protein is shown in Figure 7. The results indicated that M10 and M46 produced hydrogen bonds with GSK3B (LYS-85, ASP-200 and GLC-185) and MAPK10 (H 2O), respectively (Figure 7(A) and (B)). In addition, M54 showed a PI–PI stacking interaction with CNR1 (PHE-379 residue) and AR (TYR–210 and TRP–21), respectively (Figure 7(C) and (D)). According to the docking result of ALB, there was not only conventional PI-PI stacking interaction (TYR-138) but also a Pi–anion interaction with ABG-117 residue (Figure 7(E)).

**Discussion**

Network pharmacology has caught more and more attention and becomes an effective tool in identifying alternative targets for traditional medicines and developing new drugs. In the present study, the action mechanism of cannabis against EP was analyzed at the overall level based on the network pharmacology. As a result, a total of 116 potential targets and 226 active compounds in cannabis were obtained, suggesting a potential comprehensive treatment strategy based on TCM featured by multiple compounds, targets and pathways by applying a variety of methods, including C-T and T-P network construction, GO and KEGG pathway enrichment analysis and molecule docking.
Firstly, we predicted the active compounds in cannabis which might be the leading compounds for further drug development, indicating that cannabinoids have better performance corresponding to the potential targets, such as CNR1, GSK3B and MAPK10. As shown in Figure 3(B), a total of 106 active cannabinoids and 93 potential targets of cannabinoids against EP were obtained. It is interesting to note that in addition to CBD and tetrahydrocannabinol (THC) [16], other cannabinoids might have the antiepileptic effect that has not been confirmed, such as M117, M85, M100 and M54. In these cannabinoids, the oxygen of the hydroxyl and keto groups prefer to interact with the targets by forming hydrogen bonds with the active site residues, while the benzene rings and hexatomic rings prefer to form Pi–Pi stacking interaction or Pi–anion interaction with the active site residues, which might have better performance in the treatment of EP. CNR1, AR, GSK3B, ALB and MAPK10 acted on most cannabinoids, which might be the key targets for the treatment of EP. Already accumulated evidence indicated that CNR1 is abundant in the hippocampus of the central nervous system, and activation of CNR1 inhibits nerve transmission, reduces nerve excitability and regulates the intrinsic excitability of neurons, and most cannabinoids act on CNR1 [17]. GSK3B plays a key role in maintaining intracellular homeostasis. When GSK3B is activated, the expression of Potassium/
sodium hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4) increases and the phosphorylation of AMPA receptor subunit GluA1 at Serine 831 is inhibited, which protects the central nervous system and achieves antiepileptic effect [18]. Therefore, active cannabinoids might play a key role in the process of EP treatment.

Secondly, GO, the main bioinformatics approach, consists of biological process, cellular component and molecular function. KEGG, a database about pathways, was accessed to obtain not only a gene set but also to define the complex relationship between genes. Based on the results of GO and KEGG pathway enrichment analysis, the targets were associated with various biological processes and pathways, including calcium signalling pathway, MAPK signalling pathway, Synaptic connections, nicotine addiction and morphine addiction, which were interacting.

**Calcium signalling pathway**

The calcium signalling pathway (Figure 6(B)) plays an important role in epileptic seizures, and Voltage-dependent N-type calcium channel subunit alpha-1B (CACNA1B), Voltage-dependent L-type calcium channel subunit alpha-1C (CACNA1C), Voltage-dependent T-type calcium channel subunit alpha-1G (CACNA1G) and Neuronal acetylcholine receptor subunit alpha-7 (CHRNA7) in the pathway are all related to EP. These targets change the concentration of Ca^{2+} in and out of cells, which in turn affects the cell activities such as transmitter release and cell excitation, and prevents the occurrence and seizure of EP. The mutation of CACNA1B affected the calcium ion transmission and leads to the damage of the synaptic nerve, thus causing EP-related diseases [19].
Clinical studies had shown that the mutation of CACNA1C encoding L-type calcium channel was associated with Timothy and Brugada syndrome [20]. At the same time, CACNA1G mutation was closely related to the onset of cerebellar atrophy in childhood [21]. These provide evidence for the active compounds in cannabis to inhibit EP.

**MAPK signalling pathway**

The MAPK signalling pathway (Figure 6(C)), which is involved in signal transduction after activation of various growth factors, cytokines, mitogens and hormone receptors [22, 23], is closely related to the pathogenesis of EP. Growth factor receptor-bound protein 2 (GRB2), which binds Fibroblast growth factor receptor 1 (FGFR1) and Fibroblast growth factor receptor 3 (FGFR3) to Son of sevenless (SOS), Transduced rat sarcoma (Ras), which in turn activate Mitogen-activated protein kinase (Mek) and Mitogen-activated protein kinase kinase kinase 1 (MEKK1) and phosphorylate MAPK10. P38 MAPK is activated and its phosphorylation level is increased in the model of epileptic seizure in rats. Inhibition of P38 MAPK reduces neuronal damage and thus reduces the epileptic seizures [24]. This underscores the importance of the MAPK signalling pathway in preventing seizures.

**Synaptic connections**

During seizures, the synaptic connections (Figure 6(D)) in the brain are abnormal, which in turn increases brain excitability. Cannabis might be able to treat EP through GABAergic Synapse, glutamatergic synapse, dopaminergic synapse, cholinergic synapse, and so on. Studies have shown that GABA_A receptor mutation causes GABAergic synapses to be impaired, the function of GABA_A receptor to be reduced and the binding force of GABA to be reduced; and GABRA5, the subunit of GABA_A receptor, has been proposed as the pathogenic gene of epileptic encephalopathy with early onset [25, 26]. When the content of GABA is increased, excitatory neurotransmitter is inhibited, to control seizures [27]. Glutamatergic Synapse is directly related to an abnormal discharge of neurons and has always been thought to be closely related to the occurrence of EP. Hyperactivity of GLS and other targets in this pathway leads to an increase in glutamate content, which acts on ion channels and increases excitability [28–30]. Similarly, dopaminergic synapse and cholinergic synapse also facilitate epileptic seizures [31–33].

**Other signalling pathways**

The study also predicted that nicotine addiction and morphine addiction, which was also effective in EP, reduced the duration and frequency of epileptic seizures by stimulating neurocytokines and suppressing neurotransmission [34, 35]. Retrograde endocannabinoid could promote seizures by inhibiting the release of GABA at inhibitory synapses [36].

In summary, these pathways play a significant role in the treatment of EP and also provide a piece of
powerful evidence for multi-target treatment. Besides, molecular docking was used as the target and molecular interaction widely. The good molecular docking results showed that M54 (cannabinol methyl ether) had a high binding score with its corresponding targets, which could possibly become the lead compound for further research.

Conclusions
To clarify the mechanism of action of cannabis in the EP treatment systematically, the network pharmacology and molecular docking were employed to explore the active compounds, the targets of cannabis against EP, and the related signalling pathways. A total of 226 potentially active compounds in cannabis and 1117 of their targets were predicted by multiple databases. At the same time, 1403 EP-related targets were screened by multiple databases. A total of 116 common targets were obtained by statistical intersection. The GO and KEGG enrichment analysis indicated that there were multiple interactions. The results showed that compounds in cannabis, especially cannabinoids, interact with the targets (CNR1, ALB, GSK3BA, AR and MAPK10) to ameliorate EP, and the effect is due to the joint action of the calcium signalling pathway, MAPK signalling pathway, synaptic connections and so on. Moreover, molecular docking analysis confirmed the antiepileptic effect of cannabis. However, in vitro and in vivo experiments should be carried out based on this study. In sum, the mechanism of cannabis in the EP treatment was analyzed systemically based on network pharmacology. This study provides a practicable strategy for the development of new cannabis drugs for EP.

Data availability
The data supporting the findings reported in this study are available from the corresponding author upon reasonable request.

Disclosure statement
W.X. is affiliated with Kanion Pharmaceutical Co. Ltd. There is no conflict of interest to declare regarding the content of interest of this article.

Funding
This work was supported by High-level talents project of Dalian City (2016RQ064); Research Foundation of Education Bureau of Liaoning Province (2016J008); and National Natural Science Foundation (U1603285) of China.

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