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Network-Based Pharmacology Study Reveals Protein Targets for Medical Benefits and Harms of Cannabinoids in Humans

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Abstract: This network-based pharmacology study intends to uncover the underlying mechanisms of cannabis leading to a therapeutic benefit and the pathogenesis for a wide range of diseases claimed to benefit from or be caused by the use of the cannabis plant. Cannabis contains more than 600 chemical components. Among these components, cannabinoids are well-known to have multifarious pharmacological activities. In this work, twelve cannabinoids were selected as active compounds through text mining and drug-like properties screening and used for initial protein-target prediction. The disease-associated biological functions and pathways were enriched through GO and KEGG databases. Various biological networks [i.e., protein-protein interaction, target-pathway, pathway-disease, and target-(pathway)-target interaction] were constructed, and the functional modules and essential protein targets were elucidated through the topological analyses of the networks. Our study revealed that eighteen proteins (CAT, COMT, CYP17A1, GSTA2, GSTM3, GSTP1, HMOX1, AKT1, CASP9, PLCG1, PRKCA, PRKCB, CYCS, TNF, CNR1, CNR2, CREB1, GRIN2B) are essential targets of eight cannabinoids (CBD, CBDA, Δ^9-THC, CBN, CBC, CBGA, CBG, Δ^8-THC), which involve in a variety of pathways resulting in beneficial and adverse effects on the human body. The molecular docking simulation confirmed that these eight cannabinoids bind to their corresponding protein targets with high binding affinities. This study generates a verifiable hypothesis of medical benefits and harms of key cannabinoids with a model which consists of multiple components, multiple targets, and multiple pathways, which provides an important foundation for further deployment of preclinical and clinical studies of cannabis.

Keywords: *Cannabis*; cannabinoids; cannabidiol; tetrahydrocannabinol; network-based pharmacology; molecular docking simulation

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1. Introduction

Cannabis is an annual herbaceous flowering plant in the Cannabaceae family. While there are differences in chemical contents and plant domestication phases (e.g., *C. sativa* L, *C.* Lam, and *C. ruderalis* Janisch), this plant is often considered as a single undivided species, *C. sativa* or cannabis [1,2].

Chemically, cannabis can be grouped into three types according to the contents of its main cannabinoids, tetrahydrocannabinol (THC) and cannabidiol (CBD): Type I, high THC (>0.3%) and low CBD (<0.5%); Type II, high THC (>0.3%) and high CBD (>0.5%); and Type III, low THC (<0.3%) and high CBD (>0.5%) [3]. As commodity products, *Cannabis* can be produced in two major categories: marijuana (Types I and II) and hemp (Type III) [4]. Marijuana is used recreationally or medicinally for its intoxicating properties, but its usage remains illegal in many countries [5]. Hemp is valued for its metabolic compounds, fiber, and seed, which have been used in more than 25,000 products worldwide [6]. *Cannabis*-based products are documented to have both beneficial and adverse effects. The beneficial effects include treating various diseases, such as cancers, inflammation, pains, epilepsy,

Parkinson's, Alzheimer's, multiple sclerosis, chronic spasticity, etc., (Table 1(A)); whereas the adverse effects include respiratory and cardiovascular diseases, psychiatric comorbidities, addiction, and impairment of brain development, etc., (Table 1(B)).

Table 1. (**A**) Clinical conditions/symptoms that may be benefited from the use of cannabis. (**B**). Adverse effects in humans from the use of cannabis.

(A)		
NO.	**Disease**	**References**
1	Alzheimer's disease	[7]
2	Amyotrophic lateral sclerosis (ALS)	[8]
3	Anorexia	[7,9]
4	Cancer	[8,10]
5	Chronic spasticity	[11]
7	Crohn's disease	[8]
8	Cutaneous treatment (dermatitis, Sebum's excess & acne, epidermolysis bullosa, Kaposi sarcoma, metastatic melanoma)	[7]
9	Epilepsy	[8,9,11,12]
10	Glaucoma	[7,9,13]
11	HIV	[8,11]
12	Huntington disease	[11]
13	Infectious Diseases	[7]
14	Inflammation	[8,9,11,13]
15	Inflammatory bowel syndrome (IBS)	[8]
16	Insomnia	[7,11,14]
17	Ischemic stroke	[8,15]
18	Malaria	[15]
19	Multiple sclerosis	[7–9,11,13]
20	Nausea and vomiting	[7–9,11,14]
21	Anxiety disorders and obsessive-compulsive disorders	[8]
22	Osteoarthritis	[16]
23	Pain	[7–9,11,13]
24	Parkinson's disease	[7,8]
25	Post-traumatic stress disorder (PTSD)	[7]
26	Tourette's Syndrome	[7,11]
27	Uremic pruritus	[14]
28	Respiratory diseases: airflow obstruction, bronchitis, airway injury	[13,17]
(B)		
NO.	**Disease**	**References**
1	Impairment of brain development of fetus and adolescence, impaired brain connectivity, cognitive and motor functions, learning ability, and memory	[8,9,11,13]
2	Psychiatric comorbidities: depression, anxiety, dysphoria, delusions, bipolar disorder	[9,11,13]
3	Schizophrenia: hallucinations, paranoia, and disorganized thinking	[8,9,11,17]
4	*Cannabis* use disorders and withdrawal symptoms: dizziness, dry mouth, somnolence, and confusion; restless, irritability, mild agitation, insomnia, nausea, and cramping	[9,10,14]
5	Cannabinoid hyperemesis syndrome: nausea, vomiting, and dehydration	[18]
6	Addiction/substance dependence	[8,11,13]

Since the legalization of human consumption of hemp products by the U.S. Agriculture Improvement Act of 2018 (Available at https://www.congress.gov/115/bills/hr2/BILLS-115hr2enr.pdf, accessed on 4 October 2021), the hemp industry experienced a seismic shift and hemp-derived products are rushing into the consumer market even before the US-FDA can determine whether they are safe to be used [19], and consumers' interests in medical uses of *Cannabis* are skyrocketing [20]. However, little clinical research has been conducted with rigorous scientific evidence to prove the health benefits or harms of *Cannabis* in humans.

Up to date, more than 600 compounds in 18 different chemical classes have been isolated and characterized from *Cannabis* [21]. Among 120 cannabinoids from cannabis, the

psychoactive compound Δ^9-THC and the non-psychoactive compound CBD have become synonymous with *Cannabis* and received much attention, which based-production has been used as pharmaceutical grade products in the United States, Europe, and some Caribbean countries (e.g., Epidiolex® and Sativex®) [22]. However, the activities of other cannabinoids are not well understood, and even the non-toxic CBD may also have a negative effect [23,24]. Besides, cannabinoids can undergo mutual transformation to generate more bioactive forms under certain conditions, such as enzyme catalysis, heat, light, etc., [25] and these cannabinoids and derived cannabinoids may interact with various targets, including but not limited to endocannabinoid receptors, such as CNR1 and CNR2 to exert their individual pharmacologic effects along with any possible entourage effects [26].

Therefore, to increase our understanding of cannabinoid actions and the underlying molecular mechanisms of a wide range of diseases claimed to be benefited from or induced by the use of the plant, a network-based pharmacology study on multiple compounds, multiple targets, and multiple pathways were performed to investigate the effects of *Cannabis* on human health. The workflow of this study was shown in Figure 1, where a group of 12 cannabinoids was first selected from herbal medicine databases and literature search, and screened by in silico prediction of drug-like properties; then, the protein targets of the cannabinoids were identified using a pharmacophore database for the construction of the protein-protein-interaction (PPI) network to explore the connectivity among the targets; GO biological functions and KEGG pathways were enriched to create the target-pathway (T-P) network, and the pathway-associated diseases were collected to construct the pathway-disease (P-D) network. Following that, the target-target (T-T) network was created based on T-P interactions, where targets sharing the same pathway were connected, and the network nodes were partitioned into various functional modules based on their biological pathways. Each functional module was evaluated based on its contribution scores (CSs) to various diseases, and the essential protein targets within each module were chosen according to their integrated centrality (IC) values. Finally, the modules with high CSs to relevant beneficial or adverse effects of *Cannabis* and the protein targets with high IC values within each module, as well as the key cannabinoids binding to the protein targets were identified to explore the effects of *Cannabis* in humans.

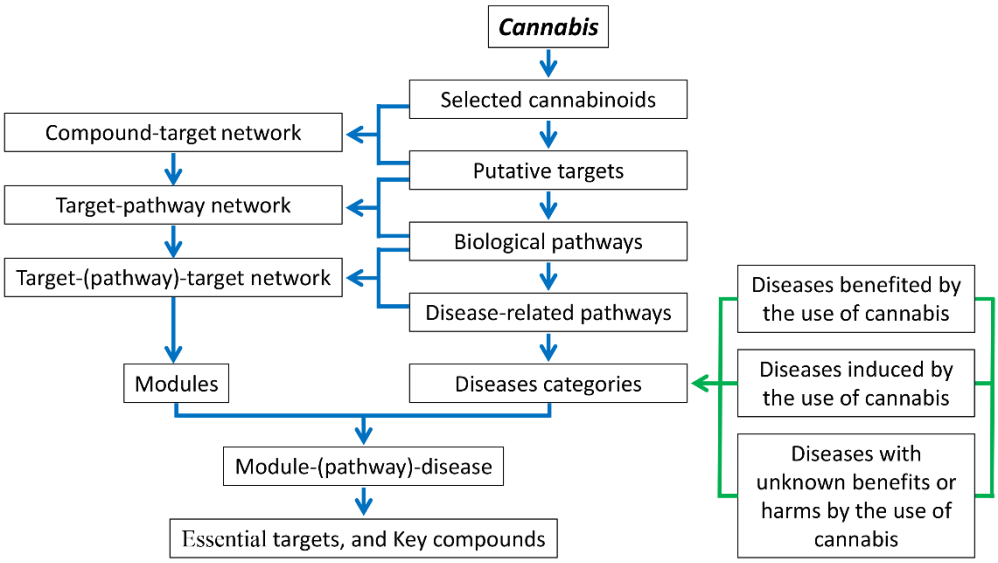

Figure 1. The network pharmacology workflow for this study.

2. Materials and Methods

2.1. Data Acquisition and Processing

Twelve cannabinoids Δ^9-THC, Δ^8-THC, 11-OH-Δ^9-THC, Δ^9-THCA, Δ^9-THCV, CBN, CBD, CBDA, CBDV, CBC, CBG, and CBGA were selected for this study from herbal

medicine databases including the Traditional Chinese Medicine Systems Pharmacology Database and Analysis Platform (TCMSP v.2.3) (Available at http://tcmspw.com/, accessed on 4 October 2021), the Encyclopedia of Traditional Chinese Medicine (ETCM) (Available at http://www.tcmip.cn/ETCM/, accessed on 4 October 2021), and literature search. The systematic evaluation of drug-likeness, physicochemical, and ADMET properties of the 12 selected cannabinoids was carried out using ADMETlab (Available at http://admet.scbdd.com, accessed on 4 October 2021) [27]. The two-dimensional (2-D) and three-dimensional (3-D) structures of these cannabinoids were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/, accessed on 4 October 2021).

The putative protein targets of the twelve selected cannabinoids were retrieved from PharmMapper, an integrated pharmacophore matching platform with a statistical method for potential target identification (Available at http://www.lilab-ecust.cn/pharmmapper/, accessed on 4 October 2021) with a "fit score" >4 [28]. These targets were used to construct the protein-protein interaction (PPI) network using the online tool STRING v.11.0 (Available at https://string-db.org/, accessed on 4 October 2021). The protein targets with an overall confidence score >0.4 [29] were further enriched for relevant diseases and disease-associated pathways using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database (Available at https://www.kegg.jp/, accessed on 4 October 2021), as well as biological functions and processes using the Gene Ontology (GO) knowledgebase (Available at http://geneontology.org/, accessed on 4 October 2021) with a p-value < 0.01.

The reported diseases benefited from or induced by the use of *Cannabis* were searched on ClinicalTrials.gov (https://www.clinicaltrials.gov/, accessed on 4 October 2021), GeneCards® (Available at https://www.genecards.org/, accessed on 4 October 2021), Web of Science (Available at https://www.webofknowledge.com/, accessed on 4 October 2021), and Google Scholar (Available at https://scholar.google.com/, accessed on 4 October 2021) using a search string "*cannabis*" OR "marijuana" OR "hemp" OR "phytocannabinoid" OR "cannabinoid" OR "cannabidiol" OR "tetrahydrocannabinol". Publications included were limited to the English language.

2.2. Network Construction and Module Identification

The networks of compound-target (C-T) and protein-protein interaction (PPI) were constructed and visualized using Cytoscape v.3.7.2 (Available at https://cytoscape.org/, accessed on 4 October 2021) [30].

For functional module identification, the two-mode T-P relationships were first transformed into the one-mode target-target (T-T) relationships using Excel2Pajek (Available at http://vlado.fmf.uni-lj.si/pub/networks/pajek/howto/excel2Pajek.htm, accessed on 4 October 2021) [31]. The target-pathway-disease (T-P-D) and T-T network were constructed using Gephi v.0.92 (Available at https://gephi.org/, accessed on 4 October 2021); and the modularity classes were analyzed and identified using the Louvain algorithm with a resolution of 1.0 [32].

2.3. Contribution Score Calculation

The contribution score (CS) of a functional module (M_i) to a particular disease (D_j) through a set of common pathways (X_{ij}) can be calculated as follows:

$$C_{M_iD_j} = \sum_{P_w \in X_{ij}} C_{M_iP_w} C_{P_wD_j} = \sum_{P_w \in X_{ij}} \frac{1}{\mu_{iw} \times \nu_{wj}} \quad (i = 1, 2, 3 \ldots \mathrm{I};\ j = 1, 2, 3 \ldots \mathrm{J};\ w = 1, 2, 3 \ldots \mathrm{W}) \tag{1}$$

where X_{ij} refers to a set of pathways that are relevant to M_i and D_j simultaneously; $C_{M_iP_w} = \frac{1}{\mu_{iw}}$ refers to the contribution of $\mathrm{M_i}$ to a particular pathway (P_w), where μ_{iw} is the number of the module(s) related to P_w, each relevant module contributes $\frac{1}{\mu_{iw}}$ fraction to P_w; $C_{P_wD_j} = \frac{1}{\nu_{wj}}$ refers to the contribution of P_w to D_j, where ν_{wj} is the number of the pathway(s) related to D_j, each relevant pathway contributes $\frac{1}{\nu_{wj}}$ fraction to D_j; and $C_{M_iD_j}$

refers to the CS of M_i to D_j, which is the sum of the contribution of M_i to D_j through all the relevant P_w in X_{ij}. The value of $C_{M_iD_j}$ ranged from 0 to 1, the higher the CS value, the greater the contribution of M_i to D_j. The sum of all $C_{M_iD_j}$ to a particular disease is equal to unity [33].

2.4. Integrated Centrality Calculation

The integrated centrality (IC) of a protein target was calculated using the following equation.

$$IC_i = \frac{1}{4}\left(\frac{DC_i - DC_{min}}{DC_{max} - DC_{min}} + \frac{BC_i - BC_{min}}{BC_{max} - BC_{min}} + \frac{CC_i - CC_{min}}{CC_{max} - CC_{min}} + \frac{EC_i - EC_{min}}{EC_{max} - EC_{min}}\right) \quad (i = 1,\ 2,\ 3,\ 4,\ 5,\ 6 \ldots \mathrm{I}) \tag{2}$$

where IC_i refers to the integrated centrality of target i; DC_i, BC_i, CCi, and EC_i refer to the degree, betweenness, closeness, and eigenvector centralities of target i; DC_{min}, BC_{min}, $CC_{min,}$ and EC_{min} refer to the minimum degree, betweenness, closeness, and eigenvector centralities of the functional module; and DC_{max}, BC_{max}, CC_{max}, and EC_{max} refer to the maximum degree, betweenness, closeness, and eigenvector centralities of the functional module. The value of IC ranged from 0 to 1. The higher the IC value of a target, the more important the target was in its functional module from the topological perspective.

2.5. Molecular Docking

To confirm the binding affinity of an essential protein target to a cannabinoid ligand, molecular docking simulation was performed using AutoDock Vina (Available at http://vina.scripps.edu/, accessed on 4 October 2021) [34].

The 3-D protein structure was downloaded as a pdb file from the PDB database (Available at https://www.rcsb.org/, accessed on 4 October 2021) and uploaded to PyMOL v.2.3 (Available at https://pymol.org/2/, accessed on 4 October 2021) [35] to remove water molecules and other ligands from the structure before it was saved as a pdb file; then polar hydrogens and charges were added to the protein structure using Mgltools (Available at http://mgltools.scripps.edu/, accessed on 4 October 2021) [36] and saved as a pdbqt file. The protein grid box was set to cover up the entire protein molecule with a spacing 1 angstrom (Å) in Mgltool and the grid box coordinates were saved as a text file.

The 3-D cannabinoid structure was downloaded as an sdf file from PubChem (Available at https://pubchem.ncbi.nlm.nih.gov/, accessed on 4 October 2021) and converted to a pdb file using Openbabel (Available at http://openbabel.org/wiki/Main_Page, accessed on 4 October 2021) [37]; Then, charges were added and the torsion tree was constructed using Mgltools before it was saved as a pdbqt file.

The blind docking [38] with the AutoDock vina was performed where the protein structure in the pdbqt format was set as the receptor, the cannabinoid structure in the pdbqt format was set as the ligand, and the grid box coordinates were copied from the txt file of the protein grid box. Once the docking was performed, the ligand configurations in the protein structure were generated and saved as a pdbqt file, and the corresponding binding free energy changes (ΔG) of these configurations were calculated and saved as a log.txt file. The visualization of the docking structures was achieved in PyMOL by uploading both protein structure and ligand configurations in the pdbqt format. The images of molecular docking were exported from PyMOL as png files.

3. Results and Discussion

3.1. Key Cannabinoids

There are increasing pieces of evidence that the medicinal properties of *Cannabis* mainly come from cannabinoids [39]. In living and raw *Cannabis* plants, both cannabigerolic acid (CBGA) and cannabigevarolic acid (CBGVA) are found to be the primary metabolite precursors that can be converted into smaller and more stable cannabinoid acids and

cannabinoids by enzyme reactions, decarboxylation by heating or smoking, and oxidation by light and air exposures [25,40].

As shown in Figure 2, through enzyme reactions, CBGA turns into tetrahydrocannabinolic acid (THCA), cannabidiolic acid (CBDA), and cannabichromenic acid (CBCA), whereas CBGVA breaks down into cannabichromevarinic acid (CBCVA), cannabidivarinic acid (CBDVA), and tetrahydrocannabivarinic acid (THCVA); by decarboxylation, CBGA becomes cannabigerol (CBG), THCA becomes Δ^9-tetrahydrocannabinol (Δ^9-THC), CBDA becomes cannabidiol (CBD), CBCA becomes cannabichromene (CBC), CBGVA becomes cannabigerivarin (CBGV), CBCVA becomes cannabichromevarin (CBCV), CBDVA becomes cannabidivarin (CBDV), and THCVA becomes Δ^9-tetrahydrocannabinolic acid (Δ^9-THCA). Furthermore, Δ^9-THC can be converted to cannabinol (CBN) by oxidation and Δ^8-tetrahydrocannabinol (Δ^8-THC) by isomerization, as well as 11-Hydroxy-Δ^9-tetrahydrocannabinol (11-OH-Δ^9-THC) by metabolization. The acidic and neutral forms of phytocannabinoids can undergo further transformation to generate over one hundred different cannabinoids.

Among the cannabinoids discovered, the psychoactive Δ^9-THC and the non-psychoactive CBD are the two most abundant cannabinoids, and all other cannabinoids are in trace amounts. The health effects of twelve key cannabinoids including Δ^9-THC, Δ^8-THC, 11-OH-Δ^9-THC, Δ^9-THCA, Δ^9-THCV, CBN, CBD, CBDA, CBDV, CBC, CBG, and CBGA have been investigated, the therapeutic benefits of these cannabinoids range from analgesics, anorectic, anxiolytic, appetite stimulant, anti-bacterial, anticonvulsant, anti-diabetic, antiemetic, antiepileptic, anti-fungal, anti-inflammatory, anti-insomnia, anti-ischemic, anti-proliferative, antipsoriatic, antipsychotic, and antispasmodic to bone-stimulant, immunosuppressive, intestinal antiprokinetic, and neuroprotective, etc. [39]. Hence, these cannabinoids were adopted as the chemical markers of the *Cannabis* potency test (expect 11-OH-Δ^9-THC) (Medical Marijuana SCF Technical Guide, Version 5.0, Available at https://www.michigan.gov/documents/lara/FINAL.TESTING.GUIDE_634575_7.pdf, accessed on 4 October 2021).

3.2. Drug-Like Properties of the Selected Cannabinoids

Table S1 presented the drug-like properties of the twelve selected cannabinoids obtained by in silico prediction [27]. As shown in Table S1, the selected cannabinoids possess most of the preferable drug-like properties for candidates in drug development; for example, the drug-likeness (DL), blood-brain ratio (BBB), and oral bioavailability (F-20) of these cannabinoids were ranged 0.50–0.87 ($\geq$0.180), 0.196–0.931 ($\geq$0.100), and 20.6–51.5% ($\geq$20.0%), respectively.

3.3. C-T Network Construction and Analysis

A total of 234 proteins were retrieved as the putative targets of the twelve selected cannabinoids from PharmMapper (Table S2), and the C-T network was constructed which consisted of 247 nodes and 688 edges with a median degree centrality (DC) of 17 (Figure 3). The CBD had the highest DC and connecting to 126 protein targets, the second one was CBDA with a DC of 115, and the third one was Δ^9-THC with a DC of 82, followed by CBN (73), CBC (55), CBGA (44), Δ^8-THC (42), CBG (40), Δ^9-THCA (37), CBDV (27), 11-OH-Δ^9-THC (25), and Δ^9-THCV (22).

3.4. PPI Network Construction and Analysis

The 234 putative protein targets were uploaded to the STRING database for network analysis and the 223 protein targets with overall confidence scores > 0.4 (Szklarczyk et al., 2019) were used to construct the PPI network which consisted of 223 nodes and 2308 edges with a median DC of 26 (Figure 4). The protein targets with the higher values of DC were INS (105), ALB (104), AKT1 (97), TNF (76), PTGS2 (72), EGFR (72), CXCL8 (64), CAT (64), BDNF (59), and ESR1 (58), respectively, indicating their important roles in the PPI network.

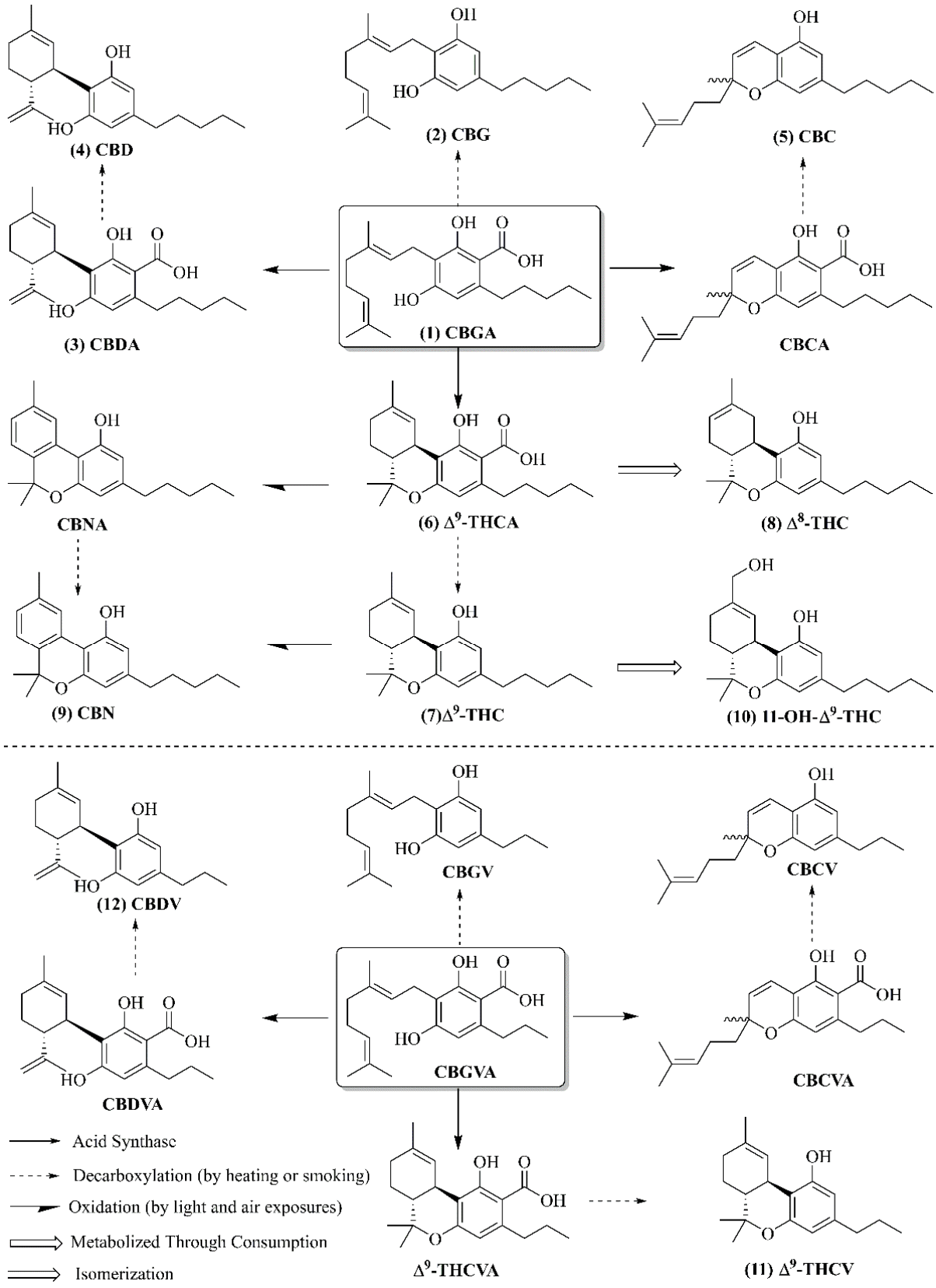

Figure 2. The chemical structures of twelve key cannabinoids and their biosynthesis pathways [25,40].

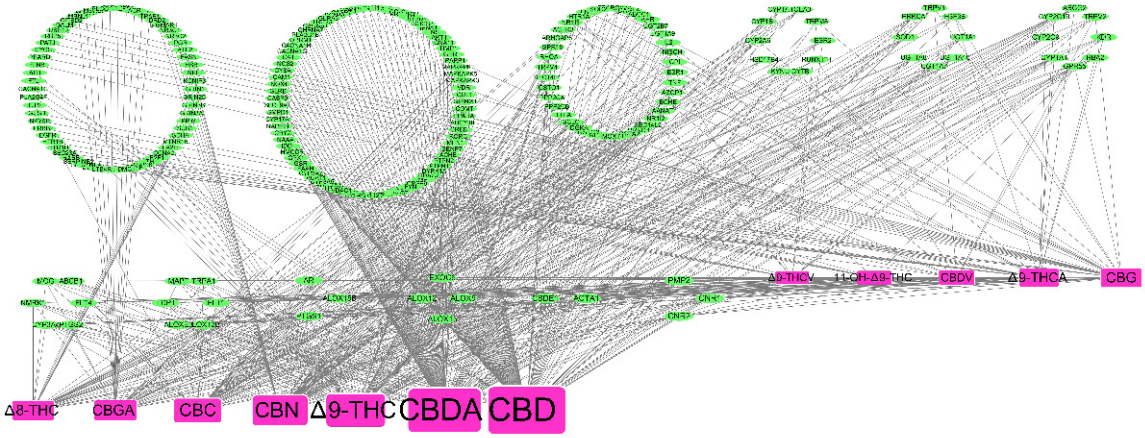

Figure 3. In the compounds-targets interaction network, the green ovals represent the targets, the red rectangles represent the cannabinoids, and the node size is proportional to its degree centrality.

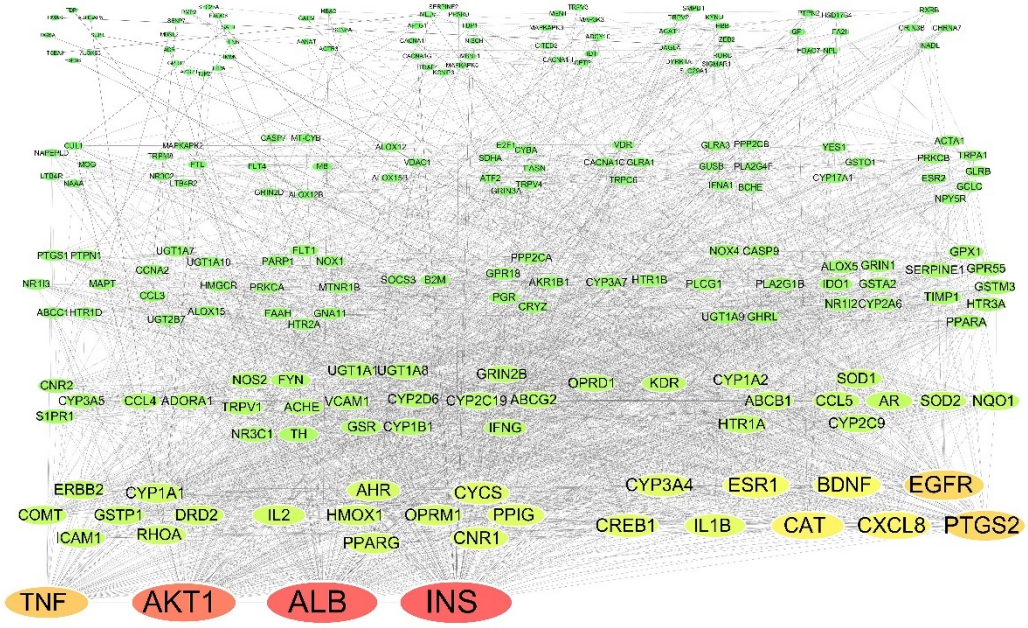

Figure 4. Protein-protein interaction network, the green oval represents the target, the size of the node is proportional to its degree centrality, and the color of the node gradually changes from red, yellow, and then green as the degree centrality decreases. Table S1. The putative targets of the twelve selected cannabinoids were predicted using PharmMapper.

3.5. GO Biological Function and KEGG Pathway Enrichment Analysis

Enrichment analysis of GO biological functions and KEGG pathways on the 223 protein targets resulted in 140 GO terms (Table S3) whose top 10 were shown in Figure 5A, and 101 KEGG pathways (Table S4) whose top 10 were shown in Figure 5B. All 101 KEGG pathways were mapped by five pathway categories that were cellular processes, environmental information processing, human diseases, metabolism, and organismal systems (Figure 5C). There were 65 KEGG disease entries found to be associated with some of these pathways (Table S5) which were classified into 10 disease categories (Figure 5D), including one cardiovascular disease, one urinary system disease, two nervous system diseases, one congenital malformation, thirteen cancers, six endocrine and metabolic diseases, six immune system diseases, six mental and behavioral disorders, six neurodegenerative diseases, and 23 infectious diseases. Among the 65 disease entries, 60 diseases had pathways associated with human diseases, three diseases had pathways related to environmental

information processing, and two diseases had pathways related to organismal systems and metabolism, respectively.

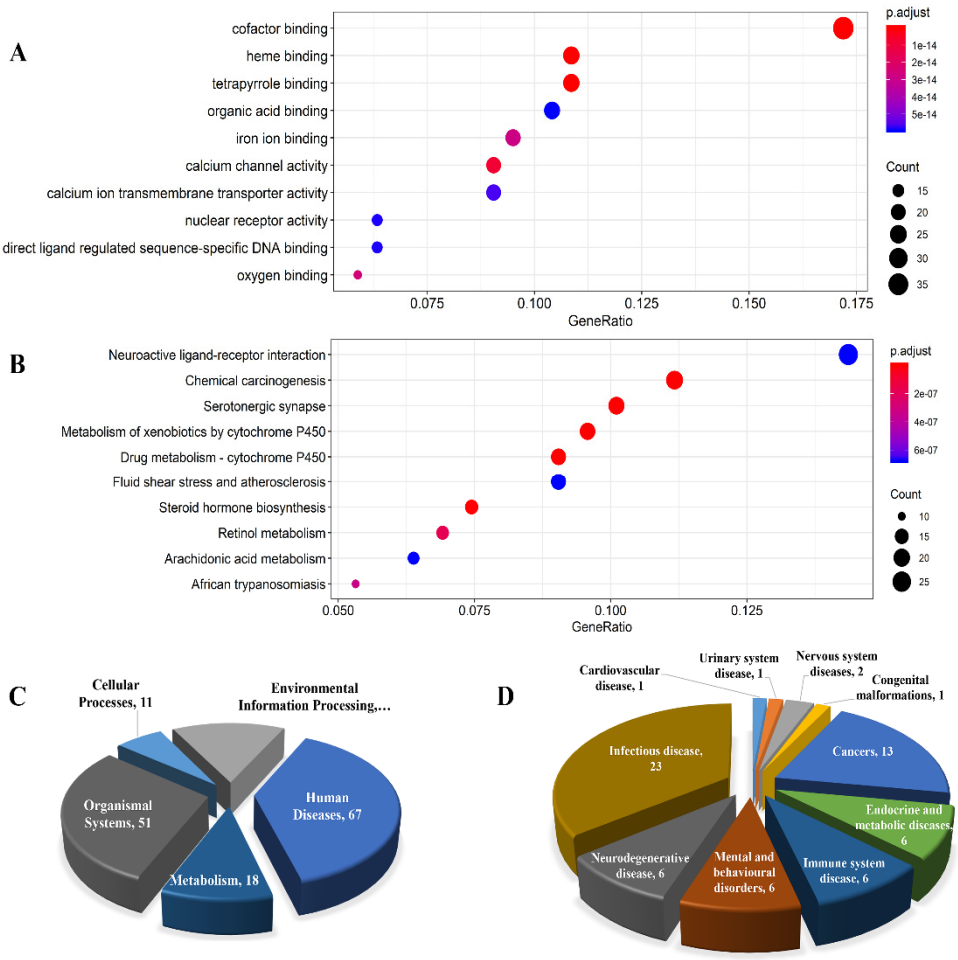

Figure 5. (**A**) The top 10 items of GO biological function enrichment; (**B**) the top 10 items of KEGG pathway enrichment; (**C**) the categories of the pathways enriched; and (**D**) the disease categories of KEGG enrichment.

According to the documented beneficial and adverse effects of *Cannabis* use in humans (Table 1), the 65 disease entries were divided into three groups (Table S5): Group I (beneficial) consisted of 32 diseases that were reported to be benefited from the use of *Cannabis* [41,42]; Group II (adverse) included six diseases that were reported to be induced by the use of *Cannabis* [43]; and Group III (unknown) had 27 diseases that had not been reported to be affected by the use of *Cannabis*.

3.6. Network Construction and Module Identification

The T-P-D network of the Group I and Group II diseases was constructed using Gephi, which consisted of 196 nodes (i.e., 122 targets, 36 pathways, and 38 diseases) and 392 edges (Figure 6A). As shown in Figure 6A, a target in the network was connected to either one or multiple pathways and a pathway was connected to either one or multiple diseases. To extract the relationship among the targets, the T-T network was constructed through the corresponding relationships between targets and pathways, where the edges of the network represent the common pathways between every two targets. A functional module of targets was identified through a set of shared pathways using Gephi with the Louvain algorithm (Figure 6B).

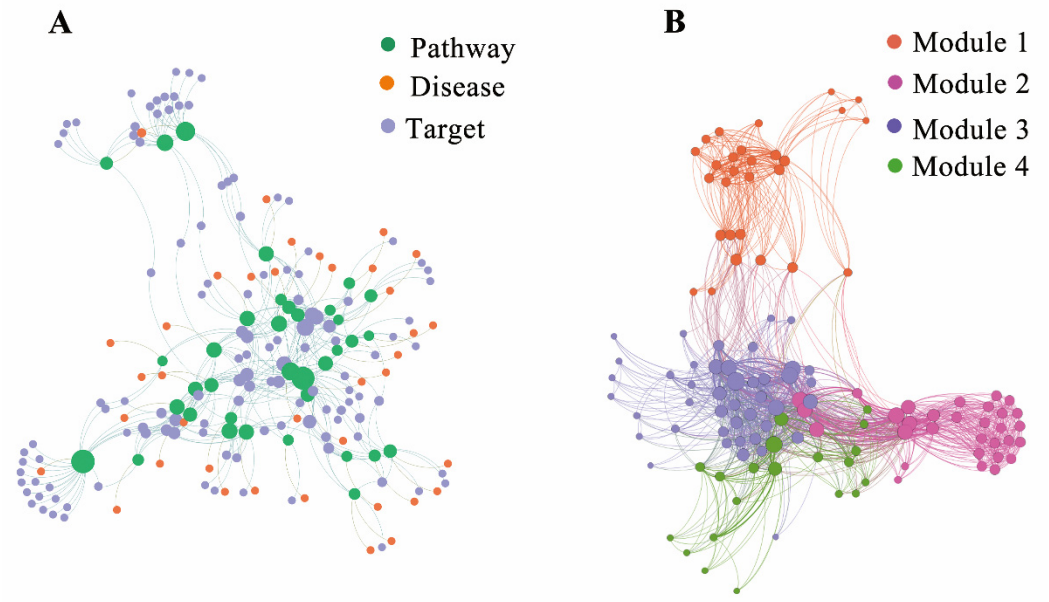

Figure 6. (**A**) The target-pathway-disease interaction network and (**B**) The target-(Pathway)-Target interaction network with modularity partition by Gephi with Louvain algorithm, where the nodes were targets and the edges were the shared pathways of these targets.

As shown inFigure 6B, four functional modules (Modules 1–4) were identified, where Module 1 consisted of 28 targets (22.95% of total targets) that were connected by eight pathways, such as amyotrophic lateral sclerosis (hsa05014), hepatocellular carcinoma (hsa05225), prostate cancer (hsa05215), and so on. Module 2 consisted of 43 targets (35.25%) that were connected by 27 pathways, such as Huntington disease (hsa05016), Parkinson's disease (hsa05012), Alzheimer's disease (hsa05010), and so on. Module 3 consisted of 20 targets (16.39%) that were connected by 17 pathways, such as human T-cell leukemia virus 1 infection (hsa05166), type II diabetes mellitus (hsa04930), inflammatory bowel disease (hsa05321), and so on. Module 4 consisted of 31 targets (25.41%) that were connected by 17 pathways, such as alcohol dependence (hsa05034), nicotine addiction (hsa05033), cocaine addiction (hsa05030), and so on.

3.7. Contribution Scores

The contribution of each functional module of targets to a particular disease through a set of common pathways can be evaluated using the CS of each module. The CSs of the four functional modules to 38 diseases were calculated using Equation (1) (see Section 2.3) and tabulated in Figure 7. The sum of the CSs from each of the four modules for a particular disease was 1 or unity. The larger the CS value of a module, the greater the contribution of the module to a disease became.

As shown in Figure 7, the health benefits of 11 out of 32 Group I diseases (Table S5) were attributed to a single functional module (CS = 1.00) [i.e., primary congenital glaucoma (h01203) was associated with the targets in Module 1; breast cancer (H00031), endometrial cancer (H00026), gastric cancer (H00018), glioma (H00042), non-small cell lung cancer (H00014), pancreatic cancer (H00019) were connected with the targets in Module 2; malaria (H00361), inflammatory bowel disease (H01227), and ulcerative colitis (H01466) were related to the targets in Module 3; and autosomal dominant nocturnal frontal lobe epilepsy (H00807) was accredited to the targets in Module 4]; whereas the health benefits of the other 21 Group I diseases were attributed to the targets in two or three or even four functional modules. Similarly, the medical harms of six Group II diseases (Figure 7, Table S5) came from the targets of one functional module [i.e., Module 4 for alcohol dependence (H01611), cocaine addiction (H0000A), and nicotine addiction (H0000D)] or targets of two functional modules [i.e., Module 2 and Module 4 for amphetamine addiction (H0000B) and morphine

addiction (H0000C)] or targets of three functional modules [i.e., Module 1, Module 2 and Module 4 for Schizophrenia (H01649)]. Since *Cannabis* addiction has not been documented in the KEGG database and the protein targets of Module 4 are prone to cannabinoid ligands and associated with various substance dependence including alcohol, cocaine, nicotine, amphetamine, and morphine addiction, it is reasonable for one to speculate that *Cannabis* addiction shares the same pathways and protein targets of Module 4. This may explain why the use of *Cannabis* could cross-sensitize the addictive effects of nicotine and morphine [44,45].

Module 1	Module 2	Module 3	Module 4	Disease ID	Disease	Group
1.00	0.00	0.00	0.00	H01203	Primary congenital glaucoma	
0.00	1.00	0.00	0.00	H00031	Breast cancer	
0.00	1.00	0.00	0.00	H00026	Endometrial cancer	
0.00	1.00	0.00	0.00	H00018	Gastric cancer	
0.00	1.00	0.00	0.00	H00042	Glioma	
0.00	1.00	0.00	0.00	H00014	Non-small cell lung cancer	
0.00	1.00	0.00	0.00	H00019	Pancreatic cancer	
0.50	0.50	0.00	0.00	H00048	Hepatocellular carcinoma	
0.00	0.50	0.50	0.00	H00022	Bladder cancer	
0.00	0.50	0.50	0.00	H00020	Colorectal cancer	
0.00	0.50	0.50	0.00	H00013	Small cell lung cancer	
0.00	0.33	0.33	0.33	H00009	Adult T-cell leukemia	
0.00	0.33	0.33	0.33	H00041	Kaposi sarcoma	
0.33	0.33	0.00	0.33	H00024	Prostate cancer	
0.00	0.50	0.50	0.00	H00406	Acquired immunodeficiency syndrome	
0.00	0.50	0.50	0.00	H01563	HIV infection	
0.00	0.00	1.00	0.00	H00361	Malaria	Group I
0.00	0.00	1.00	0.00	H01227	Inflammatory bowel disease	
0.00	0.00	1.00	0.00	H01466	Ulcerative colitis	
0.00	0.50	0.50	0.00	H01672	Juvenile idiopathic arthritis	
0.00	0.50	0.50	0.00	H00630	Rheumatoid arthritis	
0.33	0.33	0.00	0.33	H02049	Bilateral macronodular adrenal hyperplasia	
0.00	0.50	0.50	0.00	H00408	Type 1 diabetes mellitus	
0.00	0.50	0.50	0.00	H00409	Type 2 diabetes mellitus	
0.00	0.00	0.00	1.00	H00807	Autosomal dominant nocturnal frontal lobe epilepsy	
0.00	0.33	0.33	0.33	H00808	Idiopathic generalized epilepsies	
0.00	0.33	0.33	0.33	H00056	Alzheimer disease	
0.00	0.33	0.33	0.33	H00059	Huntington disease	
0.00	0.33	0.33	0.33	H00057	Parkinson disease	
0.25	0.25	0.25	0.25	H00058	Amyotrophic lateral sclerosis	
0.25	0.25	0.25	0.25	H00970	Juvenile primary lateral sclerosis	
0.00	0.33	0.33	0.33	H01657	Nephrotic syndrome	
0.00	0.00	0.00	1.00	H01611	Alcohol dependence	
0.00	0.00	0.00	1.00	H0000A	Cocaine addiction	
0.00	0.00	0.00	1.00	H0000D	Nicotine addiction	
0.00	0.50	0.00	0.50	H0000B	Amphetamine addiction	Group II
0.00	0.50	0.00	0.50	H0000C	Morphine addiction	
0.33	0.33	0.00	0.33	H01649	Schizophrenia	

Figure 7. The contribution scores (CS) of each module to various diseases.

3.8. Integrated Centrality and Essential Protein Targets

Biological networks are heterogeneous by nature, where the connecting nodes play very different roles in structure and function, and the importance of nodes can be described by network centrality [26].However, a single centrality measure (such as degree centrality, betweenness centrality, closeness centrality, and eigenvector centrality, etc.) would not be sufficient to predict the essential nodes in biological networks; therefore, integrating various centrality measures is a preferred way to predict the essential nodes in the biological systems [46]. In this work, IC values were determined using Equation (2) (see Section 2.4) (Table S6**)** and the IC values > 0.5 were used to predict the essential protein targets in each functional module [33]. Table 2 listed eighteen essential protein targets of cannabinoids, including 16 targets selected by IC values and two targets (i.e., CNR1 and CNR2) supported by text mining of biomedical literature [39,47,48], and 8 cannabinoid ligands that bind to these targets.

Table 2. The essential protein targets and their corresponding cannabinoid ligands.

No.	Target	Description	Uniprot	IC	Module	Cannabinoid Ligands
1	CAT	Catalase	P04040	0.63	1	CBD, CBDA
2	COMT	Catechol-O-methyltransferase	P21964	0.57	1	Δ^9-THC, CBN
3	CYP17A1	Cytochrome P450 family 17 subfamily A member 1	P05093	0.56	1	CBD, CBDA
4	GSTA2	Glutathione S-transferase alpha 2	P09210	0.51	1	Δ^9-THC, CBC, CBN
5	GSTM3	Glutathione S-transferase mu 3	P21266	0.64	1	Δ^9-THC, CBC, CBN
6	GSTP1	Glutathione S-transferase pi 1	P09211	0.72	1	Δ^9-THC, CBC, CBN
7	HMOX1	Heme oxygenase 1	P09601	0.52	1	Δ^9-THC, CBC, CBN
8	AKT1	AKT serine/threonine kinase 1	P31749	0.81	2	CBD, CBDA
9	CASP9	Caspase 9	P55211	0.67	2	CBD, CBDA
10	PLCG1	Phospholipase C gamma 1	P19174	0.51	2	CBGA
11	PRKCA	Protein kinase C alpha	P17252	0.65	2	Δ^9-THC, CBC, CBD, CBDA, CBN
12	PRKCB	Protein kinase C beta	P05771	0.56	2	Δ^9-THC, CBC, CBN
13	CYCS	Cytochrome c, somatic	P99999	0.59	3	CBG
14	TNF	Tumor necrosis factor	P01375	0.55	3	Δ^8-THC, CBD, CBDA
15	CNR1	Cannabinoid receptor 1	P21554	0.20	4	Δ^8-THC, Δ^9-THC, CBC, CBD, CBDA, CBG, CBGA, CBN
16	CNR2	Cannabinoid receptor 2	P34972	0.20	4	Δ^8-THC, Δ^9-THC, CBC, CBD, CBDA, CBG, CBGA, CBN
17	CREB1	cAMP-responsive element binding protein 1	P16220	0.63	4	Δ^9-THC, CBN
18	GRIN2B	Glutamate ionotropic receptor NMDA type subunit 2B	Q13224	0.68	4	Δ^9-THC, Δ^8-THC

These 18 essential protein targets of cannabinoids include seven from Module 1 (i.e., CAT, COMT, CYP17A1, GSTA2, GSTM3, GSTP1, and HMOX1), five from Module 2 (i.e., AKT1, CASP9, PLCG1, PRKCA, and PRKCB), two from Module 3 (i.e., CYCS and TNF), and four from Module 4 (i.e., CNR1, CNR2, CREB1, and CRIN2B). The health benefits or medical harms of cannabinoids on humans may come from the combined modulation which perturbs the functional modules of various diseases upon the binding of multiple cannabinoid ligands to the multiple essential protein targets to achieve restoration of homeostasis or induction of diseases due to the agonist or antagonist activities of the cannabinoids [49].

Many of these essential protein targets have been reported to interact with cannabinoids and are associated with Groups I and II diseases. For example, Both CNR1 and CNR2 are members of the GPCR family and are largely involved in various activities and disorders of the central nervous system, including learning and memory, appetite, anxiety, depression, stroke, schizophrenia, multiple sclerosis, neurodegenerative diseases, epilepsy, pain, and addiction [50–52]. Several variations of CNR1 have been reported to be associated with *Cannabis* dependence [53–55]. THC suppresses soluble macrophage tumoricidal activity and partially inhibits TNF signals [56], CBD prevents ischemic injury by partially inhibiting TNF [57], and CBD and its analogs had an inhibitory effect on TNF production by lipopolysaccharide-stimulated macrophages [58]. THCA has been known to suppress the expression of TNF in vitro, suggesting a mechanism for immune modulation [59]. AKT1, the serine-threonine protein kinase commonly referred to as protein kinase B (PKB), is a critical mediator of growth factor-induced neuronal survival and critical for transmitting growth-promoting signals. Gene polymorphisms in AKT1 have been shown to play a mediating role between *Cannabis* exposure and the development of psychosis, indicating the AKT1 pathway is a possible target for the prevention and treatment of *Cannabis*-related psychosis [60]. GRIN2B encoding two essential *N*-methyl-d-aspartate (NMDA) subunits have been shown to have a combined effect on the pathogenesis of Schizophrenia and are involved in regulating cortical excitability and plasticity [61]. Moreover, GRIN2B gene products play a fundamental role in many brain functions. A growing number of studies

have shown that GRIN2B is significantly associated with depression and disruptive behavior, mood disorders, and bipolar disorder [61]. Besides, various studies have shown that GRIN2B is associated with cocaine and alcohol abusers, which may be related to the shared pathway of addiction [62]. CASP9 belongs to a family of caspases that play essential roles in programmed cell death. Cannabinoids like CBD and Δ^9-THC have been proved to play role in the modulation of tumor proliferation, cell cycle, and apoptosis in various cancer types through activating CASP9 leading to apoptosis [63,64]. CREB1, which encodes cyclic adenosine monophosphate reactive element-binding protein 1, plays a core role in intracellular signal transduction and plays a significant character in a variety of cellular functions. It has been implicated in anxiety, depression [65], mood disorders [66], and drug addiction [67]. PRKCA and PRKCB have been mentioned in substance dependence [68,69].

3.9. Molecule Docking

The bindings of essential protein targets to the eight key cannabinoid ligands were further confirmed by molecular docking simulation using Autodock Vina. The binding affinity of a ligand-target complex was evaluated by the binding energy (ΔG, binding free energy change), where a more negative binding energy value indicates a stronger binding affinity or a greater binding constant for the formation of the ligand-target complex. In this work, the binding energies calculated were based on the complex conformation with the lowest docking energy. Table **??** showed the binding energies of the eight key cannabinoid ligands to the 18 essential protein targets, which had values ranging from −10.5 kcal/mol to −4.7 kcal/mol. The binding energy of ≤ -5.0 kcal/mol indicates the strong binding between a ligand and its target [70–72]. The strongest binding was observed between CBDA and TNF with a binding energy of −10.5 kcal/mol. Figure 8 illustrated the binding of CBDA to TNF at the binding pocket where hydrophobic interactions were observed between amino acid residues at GLY-121, LEU-120, LEU-57, SER-60, TYR-119, TYR-151, and TYR-59; and H-bonds were formed at GLY-121, LEU-120, and SER-60 with distances of 3.2 Å, 3.4 Å and 2.5 Å, respectively. Since the stronger the binding affinity of a ligand to its protein target, the higher the potency of the ligand, the binding affinity data can guide us to select the proper ligand-targets pairs from each functional module for experimental validation of the efficacy of cannabinoid for the aimed illnesses and therapeutic outcomes.

Target (PDB-ID) \ Ligands	Δ8-THC	Δ9-THC	CBC	CBD	CBDA	CBG	CBGA	CBN
AKT1 (6S9W)	/	/	/	-8.6	−8.8	/	/	/
CASP9 (4RHW)	/	/	/	−5.8	−5.9	/	/	/
CAT (1DGF)	/	/	/	−7.5	−7.9	/	/	/
COMT (4PYI)	/	−8.0	/	/	/	/	/	−8.0
CREB1 (5ZKO)	/	−5.8	/	/	/	/	/	−6.6
CYCS (5TY3)	/	/	/	/	/	−4.7	/	/
CYP17A1 (6WR1)	/	/	/	−7.4	−7.0	/	/	/
GRIN2B (5EWM)	−7.6	−8.0	/	/	/	/	/	/
GSTA2 (5LD0)	/	−7.0	−5.4	/	/	/	/	−6.5
GSTM3 (3GTU)	/	−7.7	−6.7	/	/	/	/	−7.6
GSTP1 (5J41)	/	−8.7	−7.6	/	/	/	/	−8.1
HMOX1 (1N45)	/	−8.0	−7.4	/	/	/	/	−7.7
PLCG1 (3GQI)	/	/	/	/	/	/	−5.6	/
PRKCA (4RA4)	/	−7.5	−7.8	−6.7	−7.4	/	/	−8.2
PRKCB (2I0E)	/	−8.1	−8.1	/	/	/	/	−8.5
TNF (6OOY)	−10.4	/	/	−6.0	−10.5	/	/	/
CNR1 (5U09)	−7.9	−7.6	−7.0	−7.3	−7.1	−7.9	−6.9	−9.4
CNR2 (6KPC)	−8.0	−8.2	−7.7	−9.2	−7.3	−6.9	−7.0	−8.5

Symbol, /, means that there is no predicted binding between a cannabinoid ligand and a protein target.

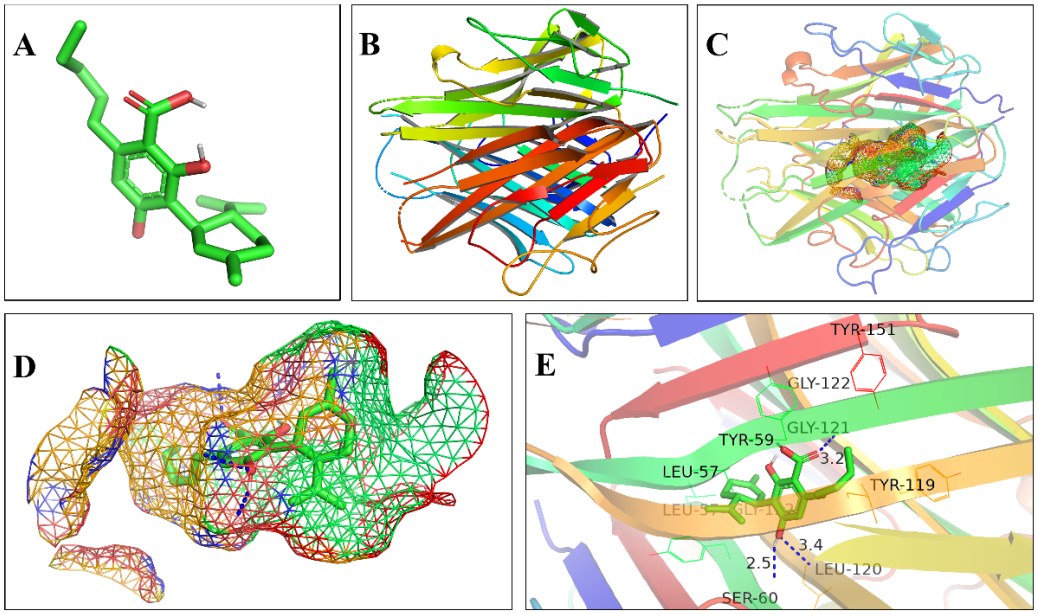

Figure 8. Molecular docking of CBDA to TNF. (**A**) 3D structure of CBDA ligand (carbon atoms and carbon-carbon bonds were green-colored, oxygen atoms were red-colored, and hydroxyl hydrogen atoms were gray-colored); (**B**) 3D structure of TNF protein targe; (**C**) The binding of CBDA to the active pocket of TNF shown in mesh surface; (**D**) the extracted and enlarged binding pocket; and (**E**) the binding sites between ligand and target (the protein target was shown as the rainbow-colored cartoon, the amino acid residues at the active sites were shown as colored ribbons with name and sequence numbers, and the hydrogen bonds were displayed by blue dashed lines with distance value in Angstrom).

4. Conclusions

We have identified the key cannabinoids from the *Cannabis* plant through text mining and screening of drug-like properties and retrieved the putative protein targets from the pharmacophore database for the building of the PPI network. Through the enrichment of GO and KEGG databases for biological functions, pathways, and diseases, various biological networks [i.e., target-pathway -disease, and target-(pathway)-target interaction] were constructed. Four functional modules with shared pathways and their association with various diseases were elucidated through the exploitation of network analysis. Eighteen essential protein targets and eight key cannabinoids were identified and verified by molecular docking simulation to be associated with the health benefits and medical harms of *Cannabis* use in humans. This study demonstrated that *Cannabis* exerted its pharmacological effects on humans through multi-components act via modulation of multiple essential protein targets to exhibit the desired disease efficacy, and the findings of this work await validation by preclinical and clinical studies.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/app12042205/s1, Table S1–S6, Table S1. The drug-like properties of the 12 selected cannabinoids obtained by in silico prediction. Table S2. The putative targets of the twelve selected cannabinoids predicted using PharmMapper. Table S3. The results of GO function enrichment analysis. Table S4. The results of KEGG pathway enrichment analysis. Table S5. The categories of diseases related to the enriched KEGG pathways. Table S6. Integrated centrality values of protein targets in the functional modules.

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Abbreviations

Δ^9-THC	Δ^9-tetrahydrocannabinol
Δ^8-THC	Δ^8-tetrahydrocannabinol
11-OH-Δ^9-THC	11-hydroxy-Δ^9- tetrahydrocannabinol
Δ^9-THCA	Δ^9-tetrahydrocannabinolic acid
Δ^9-THCV	Δ^9-tetrahydrocannabivarin
THCVA	tetrahydrocannabivarinic acid
CBN	cannabinol
CBD	cannabidiol
CBDA	cannabidiolic acid
CBDV	cannabidivarin
CBDVA	cannabidivarinic acid
CBC	cannabichromene
CBCA	cannabichromenic acid
CBCVA	cannabichromevarinic acid
CBG	cannabigerol
CBGA	cannabigerolic Acid
CBGVA	cannabigevarolic acid
CAT	catalase
COMT	catechol-O-methyltransferase
CYP17A1	cytochrome P450 family 17 subfamily A member 1
GSTA2	glutathione S-transferase alpha 2
GSTM3	glutathione S-transferase mu 3
GSTP1	glutathione S-transferase pi 1
HMOX1	heme oxygenase 1
AKT1	AKT serine/threonine kinase 1
CASP9	caspase 9
PLCG1	phospholipase C gamma 1
PRKCA	protein kinase C alpha
PRKCB	protein kinase C beta
CYCS	cytochrome c, somatic
TNF	tumor necrosis factor
CNR1	cannabinoid receptor 1
CNR2	cannabinoid receptor 2
CREB1	cAMP responsive element binding protein 1
GRIN2B	glutamate ionotropic receptor NMDA type subunit 2B
GO	the Gene Ontology
KEGG	the Kyoto Encyclopedia of Genes and Genomes
ETCM	the Encyclopedia of Traditional Chinese Medicine
TCMSP	Traditional Chinese Medicine Systems Pharmacology
ADMET	Absorption, distribution, metabolism, excretion, and toxicity
DL	drug-likeness
BBB	blood-brain ratio

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