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Exploring the mechanism of Cinnamomi Cortex against morphine addiction using network pharmacology and molecular docking analyses

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Abstract: *Cinnamomi Cortex* is a commonly used herb with a variety of pharmacological effects. We investigated the molecular mechanisms by which *Cinnamomi Cortex* antagonises morphine addiction (MA) using network pharmacology and molecular docking techniques in a morphine-dependent rat withdrawal model. The antagonistic effect of *Cinnamomi Cortex* was observed by inducing withdrawal symptoms in morphine-dependent rats through a dose-escalation method. Network pharmacology and molecular docking techniques were further employed to analyze the substance basis and mechanism of *Cinnamomi Cortex* in antagonizing MA. *Cinnamomi Cortex* was screened to contain 10 active ingredients, 127 active targets and 1724 MA-related targets. Among them, 52 targets overlapped between *Cinnamomi Cortex* and MA and 13 core targets were identified by metric analysis. *Cinnamomi Cortex* had a significant inhibitory effect on withdrawal symptoms in MA rats, with the most pronounced effect at a moderate dose. The active ingredients of *Cinnamomi Cortex* (including oleic acid) can act on multiple targets related to MA and regulate multiple pathways to treat MA. The present study reveals the material basis and mechanism of cinnamon's action on MA, and provides insights and references for subsequent experiments exploring the potential therapeutic approach of *Cinnamomi Cortex* on MA.

Keywords: *Cinnamomi Cortex*, morphine addiction, network pharmacology, gene targets, molecular docking.

INTRODUCTION

Morphine addiction (MA) is a chronic relapsing brain disorder characterized by dependence on a drug resulting from habitual intake (Cozzoli *et al*., 2021). According to the World Drug Report 2016, there were approximately 247 million drug addicts worldwide as of 2014. Cannabis ranked highest among addictive drugs, followed closely by amphetamines, opioids and prescription opioids (Wang *et al*., 2023). Methadone and buprenorphine are currently used as alternative maintenance and withdrawal treatments for drug addiction (DA). However, they are ineffective in reducing psychological cravings and carry the risk of abortion. Due to the lack of specific treatment for synthetic drugs, symptomatic approaches are used. Researchers worldwide are exploring new methods, drugs, and administration forms for effective DA treatment (Everitt *et al*., 2013). For over 200 years, China has been using herbal medicines to treat opioid addiction. Herbal medicine for DA treatment has the advantages of being mild, non-toxic and having few side effects. Therefore, investigating the use of the herbal medicine *Cinnamomi Cortex* for DA treatment through bioinformatics is highly important as a clinical guide.

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Cinnamomi Cortex is the dried bark of *Cinnamomum cassia* Presl, a member of the camphor family. It has the ability to expel cold from the body, warm the spleen and stomach, promote the recovery of organ function and relieve pain (Seibel *et al*., 2021). A study by Hamidpour *et al.* (2015) revealed various biological activities of *Cinnamomi Cortex*, including neuromodulatory, antitumor, antibacterial, anti-inflammatory, antioxidant, hypoglycemic, lipid-lowering and uric acid-lowering effects. *Cinnamomi Cortex* also has a regulatory effect on the nervous system and shows potential in treating neurological diseases such as Parkinson's disease, Alzheimer's disease and chronic cerebral ischemia. Administration of *Cinnamomi Cortex* improved memory in Alzheimer's mice and enhanced synaptic transmission in the hippocampus, resulting in improved learning and memory functions. These findings suggest that *Cinnamomi Cortex* has therapeutic potential for Alzheimer's disease (Momtaz *et al*., 2018).

Network pharmacology, which explores the relationship between drugs and diseases, is frequently employed to predict targets of herbal medicine and investigate the principles and interventions of herbal medicine in diseases (LI *et al*., 2013; Gao *et al*., 2021; Jiashuo *et al*., 2022). In this study, we established a rat model of MA withdrawal to observe the therapeutic effect of

Cinnamomi Cortex and explore its molecular mechanism of antagonism against MA using network pharmacology and molecular docking. This approach will help elucidate the underlying substance basis and potential mechanisms of *Cinnamomi Cortex's* antagonistic effects against MA, providing valuable insights for DA treatment. We analyzed the mechanism of action of *Cinnamomi Cortex* against MA using bioinformatics tools, various databases, and mapping analysis software to identify its components, targets, biological functions, and signaling pathways. The flow chart depicting the study methodology is presented in fig. 1.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats, 8-week old and weighing 180-220g, were obtained from the Experimental Animal Center of the Southern Medical University (animal license number: SCXK (Guangdong) 2016-0041). The laboratory maintained a controlled environment at (20± 2)°C, (60±5)% humidity and a 12-h light-dark cycle. The rats were individually housed in cages and provided with ad libitum access to water and food. This study was approved by the Experimental Animal Ethics Committee of the Southern Medical University (Approval No.20210024).

Reagents and instruments

Morphine hydrochloride (10 mg per vial, purity ≥98%, lot number: 710303) was obtained from the Drug Supply Station of the General Logistics Department of the People's Liberation Army. Naloxone hydrochloride (Approval No.: State Pharmacopoeia H20046295, lot number: 20050415) was purchased from Beijing Sihuan Pharmaceutical Company Limited. Buprenorphine (lot number: 20210012) was manufactured by the Qinghai Pharmaceutical Factory. Sterile saline (Approval No. State Pharmacopoeia H20046295) was acquired from Guangdong Kellen Pharmaceutical Co., Ltd.

Source of material

Cinnamomi Cortex was purchased from Kangmei Pharmaceutical Co., Ltd, batch number: KMZH0522668, and was identified as the dried bark of *Cinnamomum cassia* Presl by Professor Liu Chuanming of the Department of Chinese Medicine Identification, School of Traditional Chinese Medicine, Southern Medical University.

Preparation of cinnamomi cortex as an experimental drug

The drug was extracted using a reflux method, where it was subjected to two extractions with 75% ethanol. The extract was then recovered under pressure and concentrated to obtain a solution with a concentration of 1g/mL. Pre-testing was conducted to determine the low, medium and high doses of *Cinnamomi Cortex*, which were determined to be 10g/kg, 20g/kg and 40g/kg, respectively. As a positive control the drug buprenorphine was administered orally at a dose of 0.4 mg/kg.

Model construction

The experiments conducted in the morphine-dependent rat withdrawal model comprised three phases and lasted for 12 days (Kourosh-Arami *et al*., 2020). During days 1-3, a baseline measurement was performed, wherein the behavior of the rats was observed and recorded in a transparent Plexiglas container for 30min. Rats displaying abnormal behavioral symptoms were excluded from the study. From days 4-9, the morphine dependence training phase took place. Rats were subcutaneously injected with increasing doses of morphine once daily at 8:00 a.m. The doses administered were 5, 10, 20, 40, 60 and 80mg/kg, with a volume of 0.2mL/100g. From days 10-12, the withdrawal phase commenced. Morphine administration was ceased on day 10 and the rats were orally gavaged with an equal volume of 0.9% NaCl at 8:00 a.m. daily for three days. On days 10 and 12, naloxone (4.0mg/kg) was intraperitoneally administered 2 h after the oral gavage of 0.9% NaCl. The withdrawal symptoms of the rats were recorded within 30 min following withdrawal.

Animal grouping and dosing method

From days 1-3, rats were selected and randomly assigned to the following groups: Normal group, model group, *Cinnamomi Cortex* low-, medium- and high-dose groups, and buprenorphine-positive group, each comprising 10 rats. From days 4-9, the model group underwent morphine dependence training as described in the model construction section. The normal group received subcutaneous injections of an equal volume of 0.9% NaCl, while the *Cinnamomi Cortex* low-, medium- and high-dose groups, as well as the buprenorphine-positive group, were orally administered the corresponding drug concentrations. From days 10-12, all groups received the same drug dose, and withdrawal was induced following the procedure outlined in the model construction section. Withdrawal symptoms were recorded on days 10 and 12 for each group and quantified using Chichiji Yanagida's withdrawal scoring criteria (Nakagawa *et al*., 2000). Additionally, the body mass of the rats was measured before and after withdrawal.

Cinnamomi Cortex's effect on component screening and component target prediction

Information regarding *Cinnamomi Cortex* compounds was acquired by conducting a search in the TCM Systematic Pharmacology Database and Analysis Platform (TCMSP, old.tcmsp-e.com) database (Ru *et al*., 2014). The screening process for effector ingredients of *Cinnamomi Cortex* involved the use of qualifying values such as oral bioavailability (OB) ≥30% and drug-forming properties (DL) ≥0.18 (Tong *et al*., 2022). The PubChem platform (Organics Bioactivity Database, https://pubchem.ncbi.nlm.nih.gov/) was utilized to

identify the name or Cid of the active ingredient Cid (Wang *et al*., 2012). Subsequently, the SMILES number of the active ingredient was obtained from PubChem and used to retrieve target and gene information for potential active ingredients of *Cinnamomi Cortex* through the online small molecule target prediction platform (Swiss Target Prediction, https://swisstargetprediction.ch/).

DA disease-related target acquisition

The GeneCards database (https://www.genecards.org/) and the DisGeNET database (https://www.disgenet.org/dbinfo) were utilized to retrieve information on MA-associated targets using the key term morphine addiction. (Barshir *et al*., 2021) Information on MA-associated targets was obtained from the GeneCards database (genecards.org/) and the DisGeNET database. The number of targets obtained from the searches was combined and duplicates were removed.

Network construction and analysis of cinnamomi cortex for MA

The Venny 2.1 platform (https://bioinfogp.cnb.csic.es/tools/venny/) was employed to establish associations between *Cinnamomi Cortex* genes and MA, generate a Venn diagram illustrating shared genetics between drugs and diseases, and identify potential targets for *Cinnamomi Cortex's* effects on MA. Cytoscape 3.9.0 was utilized to construct a visual representation of the network encompassing *Cinnamomi Cortex* active ingredient common gene. The various active ingredients were subjected to numerical analysis using the CytoNCA plugin, and node sizes were determined by the degree value, with larger nodes corresponding to higher degree values (Tang *et al*., 2015).

Protein–protein interaction (PPI) network mapping and analysis of Cinnamomi Cortex anti-MA targets

The intersecting target genes between the *Cinnamomi Cortex* component and MA were retrieved and analyzed using the String information database (https://cn.string-db.org/). The parameters were set accordingly (Szklarczyk *et al*., 2014), with the biological species specified as human and the confidence level set to the default value of 0.4. The results were exported as a tsv file, which was subsequently imported into Cytoscape software version 3.9.0 for further analysis. In Cytoscape, a PPI network was plotted and the network was sorted based on the degree values of the shared targets. The key targets were then filtered to obtain a PPI sub-network using the MCODE plug-in.

Biofunctional and pathway enrichment analysis

The DAVID platform (Human Genome Annotation Database, https://david.ncifcrf.gov/) was utilized to enrich the target genes of *Cinnamomi Cortex* against dopamine addiction (DA) in terms of Gene Ontology (GO) biological function and KEGG signaling pathway (Sato, Taku *et al*., 2020; Jiao *et al*., 2012). The outcomes of the enrichment analysis were then incorporated into the bioinformatics website (www.bioinformatics.com.cn), where the parameters were adjusted to generate enrichment bubble maps. These visual representations were used to illustrate the potential biological processes and signaling pathways through which *Cinnamomi Cortex* may act against MA.

Molecular docking validation

To validate the molecular docking, mol2 format files of the three most significant active ingredients of *Cinnamomi Cortex*, namely oleic acid, EIC and alpha-cedrene, were downloaded from the TCMSP database. The 3D structures of the top six proteins, IL6, MAPK3, PPARG, PPARA, CNR1 and ESR1, in the PPI network with high degree values were obtained from the RCSB PDB database (https://www.rcsb.org/) and Uniprot (Berman HM *et al*., 2006; 'Uniprot: A worldwide hub of Protein knowledge', 2018; 'Uniprot: A hub for protein information', 2014; Mohamed *et al*., 2018) using "Homo sapiens" as the selected species. High-resolution versions of the protein structures were downloaded. The proteins were pre-processed using Pymol and AutoDock tools software (Song *et al*., 2020, Clyne *et al*., 2020). Molecular docking was conducted using AutoDock tools, and the docking results were visualized using Pymol software (Seeliger *et al*., 2010; Zhao *et al*., 2021).

STATISTICAL ANALYSIS

Experimental data were expressed as mean ± standard deviation (SD). Between-group differences were analyzed using one-way analysis of variance. All statistical analyses were performed using Service Solutions 22.0 (International Business Machines Corporation) and GraphPad Prism 8.0 (GraphPad Software Corporation). A p-value < 0.05 was considered statistically significant.

RESULTS

Morphine-dependent rat withdrawal experiments

The model group exhibited symptoms of diarrhea on days 2 and 3 following subcutaneous morphine injection, which resolved by day 4. Six days after receiving increasing doses of morphine, the model group displayed typical signs of morphine dependence, including reduced activity, remaining in a standing or lying position, unresponsiveness to touch and depression.

Excitement was mainly characterized by heightened activity, scurrying, standing up, hair licking, biting of front paws and cage knocking. The duration of excitement lasted for approximately 1.5h or more. These abnormalities were not observed in the normal group, indicating the successful induction of morphine dependence in rats using the dose-escalation method.

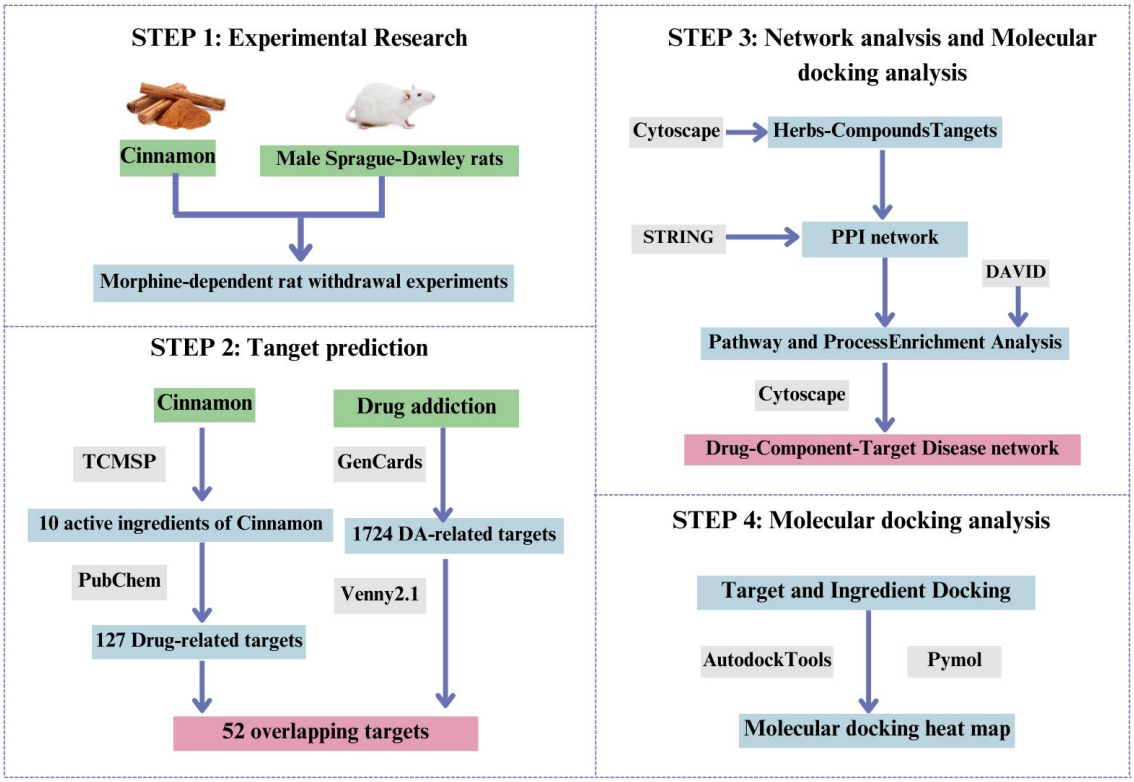


Fig. 1: Experimental Procedure Illustrated Guide.

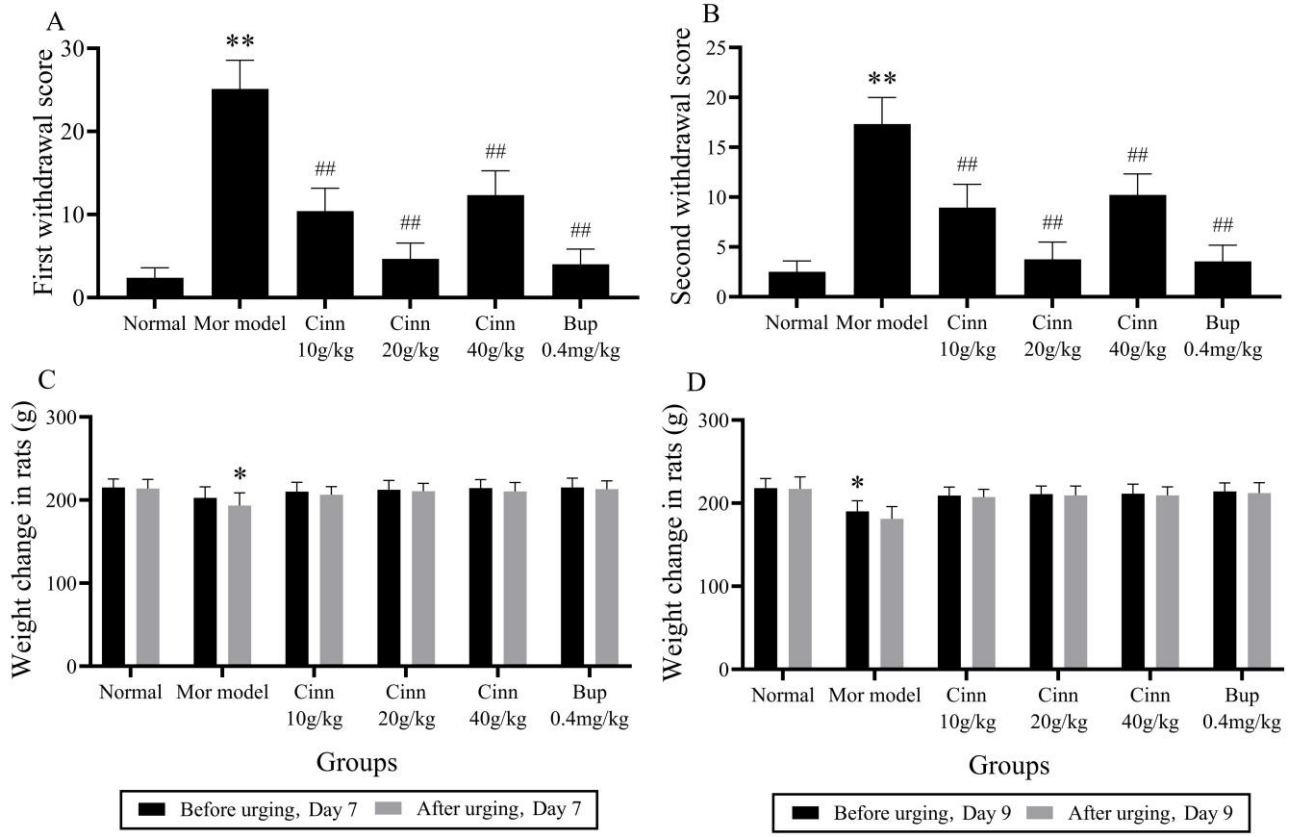


Fig. 2: A: Comparison of first withdrawal symptom scores in rats; B: Comparison of second withdrawal symptom scores in rats; C, D: Comparison of body weight changes before and after naloxone-induced withdrawal in rats. Compared with Normal group, **P<0.01, *P<0.05; compared with Morphine model group, $^{\#\#}$P<0.01, $^{\#}$P<0.05.

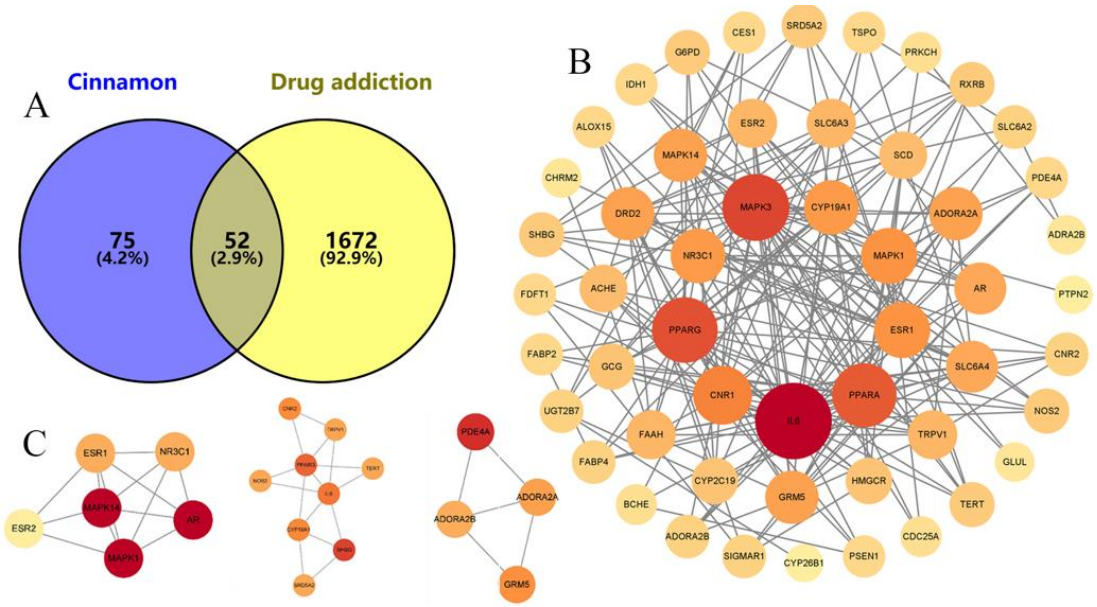


Fig. 3: Construction of a network diagram related to the treatment of MA by cinnamon. (A) Venn diagram construction of the intersection of cinnamon and DA-related genes. (B) PPI network diagram illustrating the interaction between cinnamon and DA. (C) Summary of gene cluster modules.

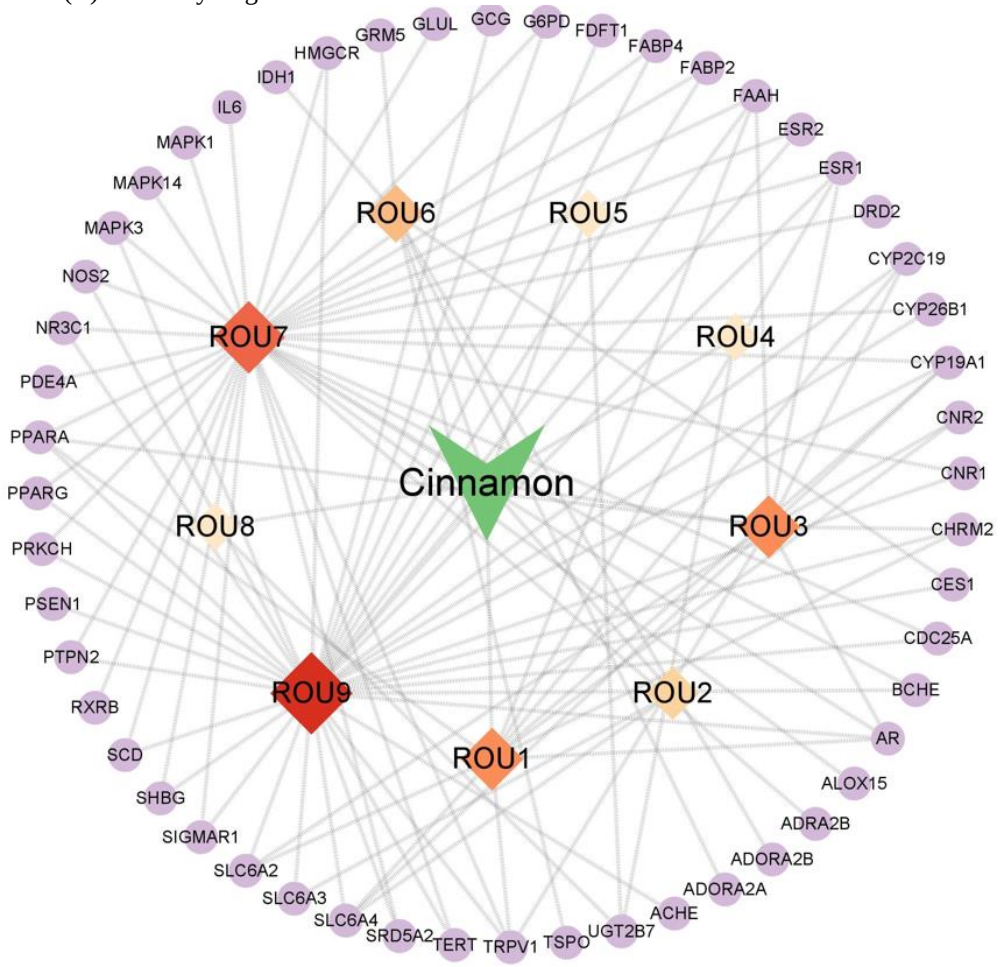


Fig. 4: Network diagram showing the shared targets of cinnamon and its active ingredients. ROU1, (-)-alpha-cedrene; ROU2, (-)-Caryophyllene oxide; ROU3, (+)-Ledene; ROU4, (+)-Sativene; ROU5, beta-Cubebene; ROU6, DIBP; ROU7, EIC; ROU8, junipene; ROU9, oleic acid.

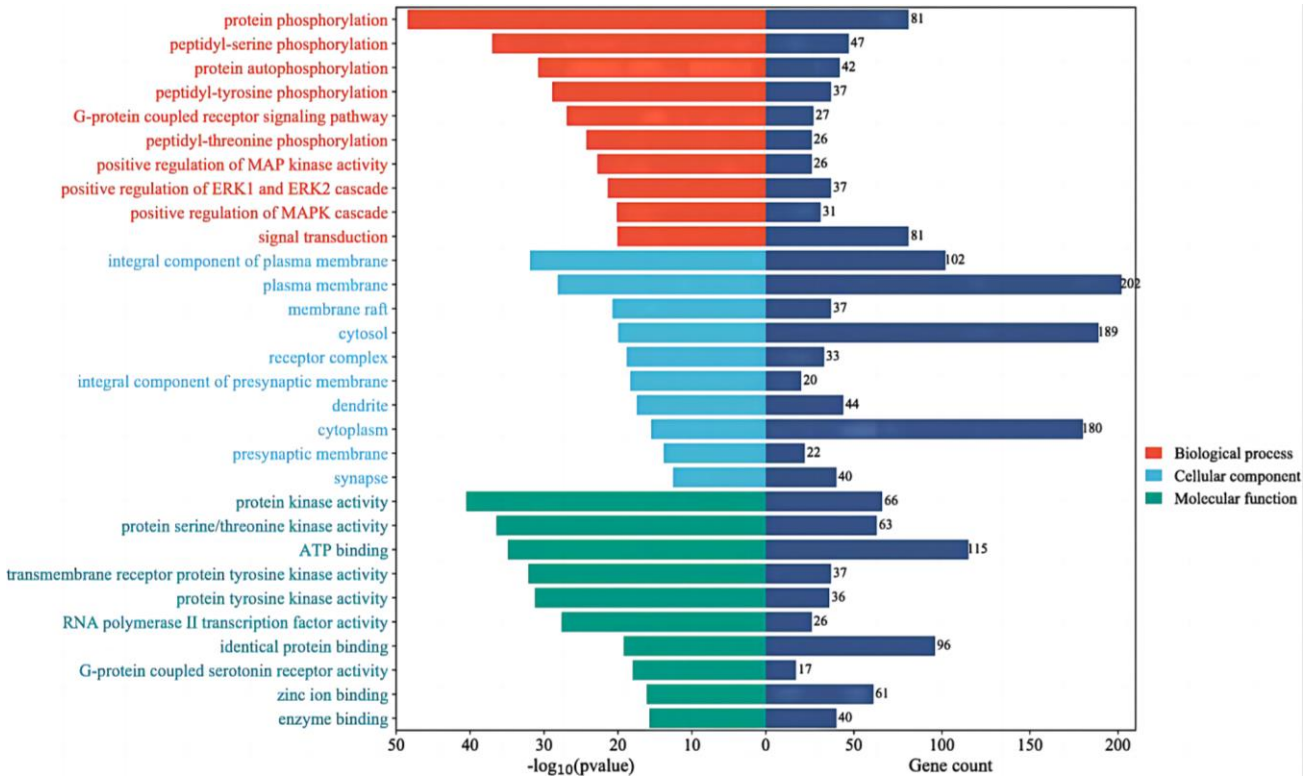


Fig. 5: Enriched GO term BP, CC, MF 3-in-1 histograms. Triad diagram representing Gene Ontology (GO) Biological Process (BP), Cellular Component (CC) and Molecular Function (MF).

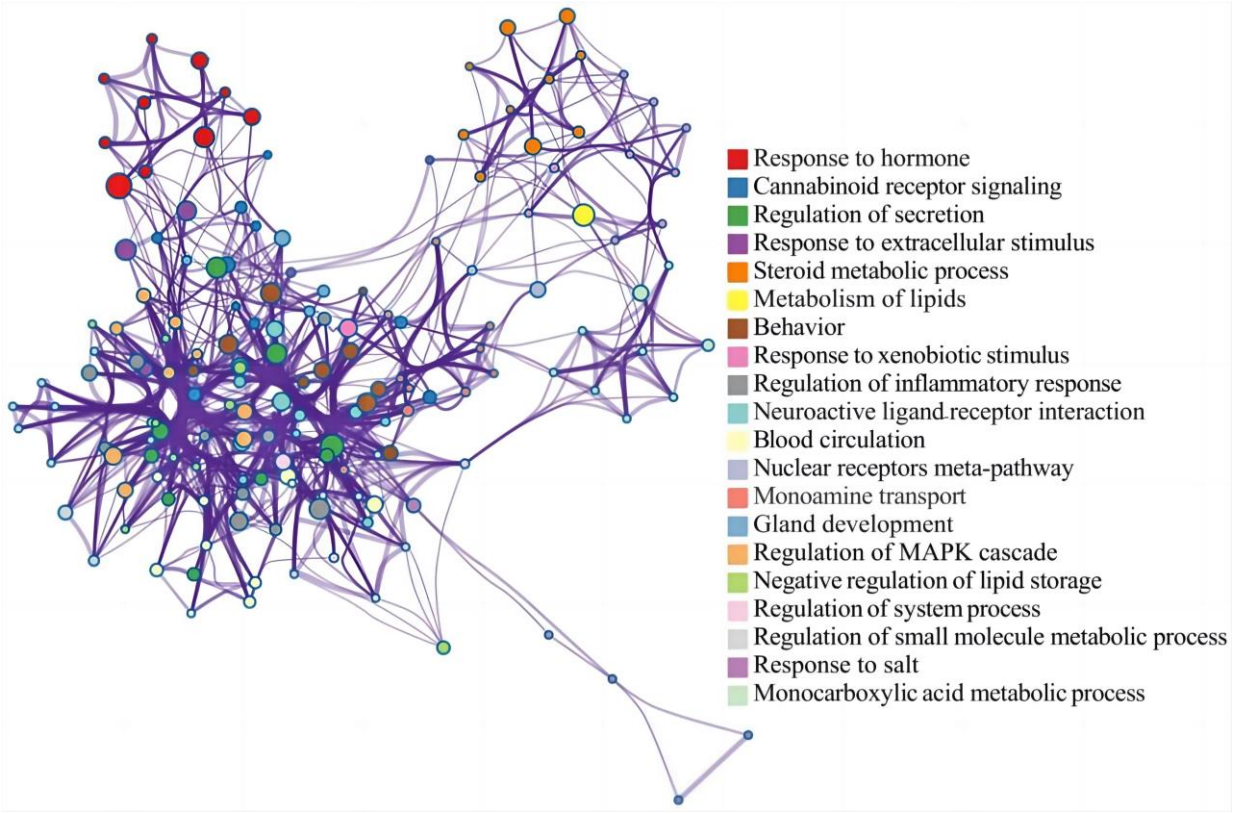


Fig. 6: Pathway and Process Enrichment Analysis. The larger the point and the darker the colour, the more meaningful the path is.

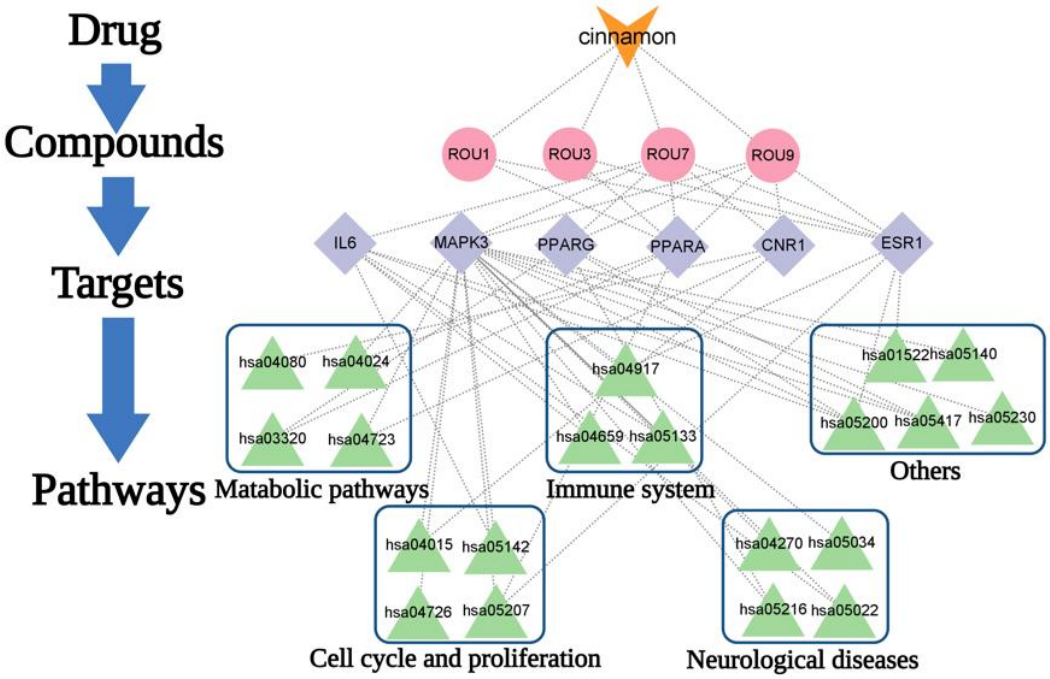


Fig. 7: Network diagram illustrating the core targets, pathways, and active ingredients of cinnamon. hsa04024, cAMP signaling pathway; hsa03320, PPAR signaling pathway; hsa04723, Retrograde endocannabinoid signaling; hsa04015, Rap1 signaling pathway; hsa05142, Chagas disease; hsa04726, Serotonergic synapse; hsa05207, Chemical carcinogenesis-receptor activation; hsa04917, Prolactin signaling pathway; hsa05133, Pertussis; hsa05216, Thyroid cancer; hsa01522, Endocrine resistance; hsa05200, Pathways in cancer; hsa04659, Th17 cell differentiation; hsa05034, Alcoholism; hsa05022, Pathways of neurodegeneration-multiple diseases; hsa04270, Vascular smooth muscle contraction; hsa05230, Central carbon metabolism in cancer; hsa05417, Lipid and atherosclerosis; hsa05140, Leishmaniasis.

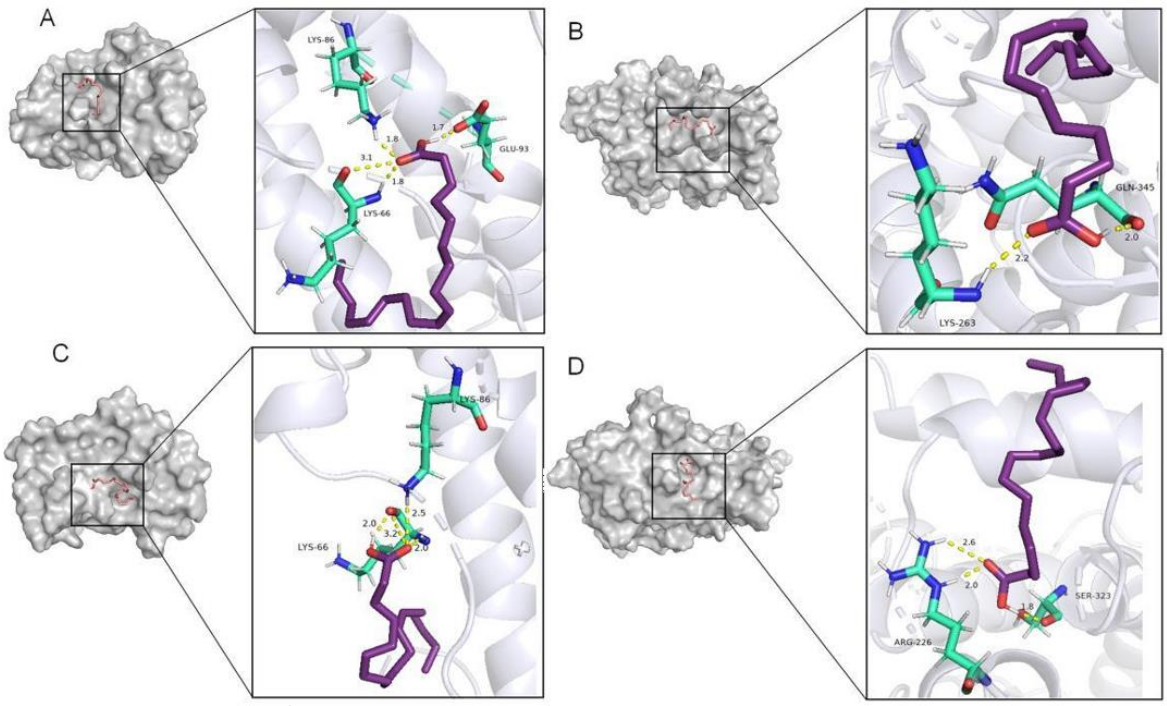


Fig. 8: Molecular docking of selected active ingredients with core targets. A: Oleic acid and 1alu; B: Oleic acid and 7awc; C: EIC and 1alu; D: EIC and 6lxa.

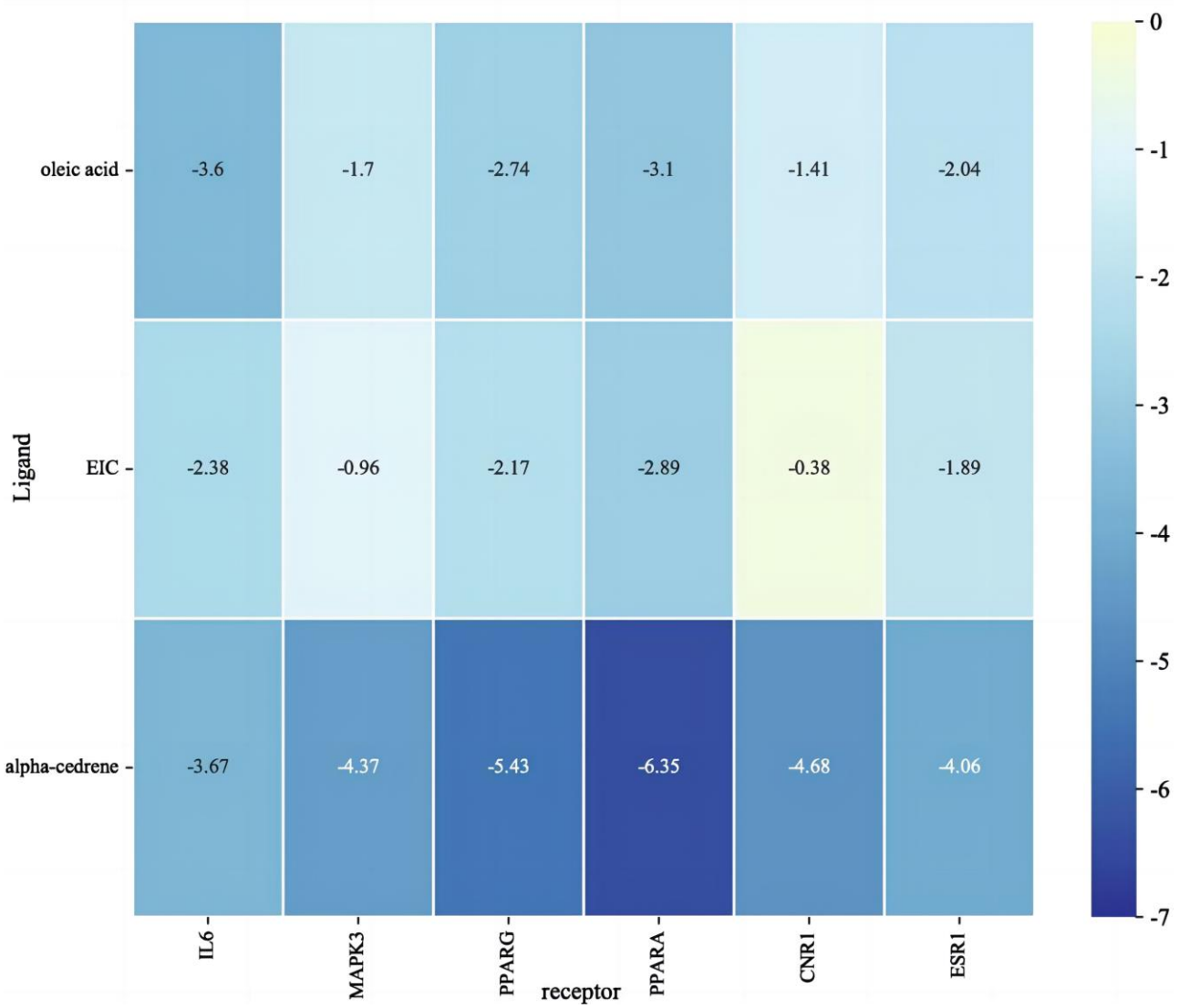


Fig. 9: Thermogram depicting the results of molecular docking. The darker the tabular area of this chart, the more free-bonding ability the two have.

Table 1: Information on the active ingredients of cinnamon. The fraction is therapeutically significant when OB% (Oral bioavailability) is ≥30 and DL (Drug-likeness) is ≥0.18.

MOL ID	Compound	Molecular weight	OB (%)	DL
MOL000131	EIC	280.5	41.90	0.14
MOL000208	(+)-Aromadendrene	204.39	55.74	0.10
MOL000266	beta-Cubebene	204.39	32.81	0.11
MOL002697	junipene	204.39	44.07	0.11
MOL003522	(+)-Sativene	204.39	37.41	0.10
MOL003538	(+)-Ledene	204.39	51.84	0.10
MOL002003	(-)-Caryophyllene oxide	220.39	32.67	0.13
MOL000057	DIBP	278.38	49.63	0.13
MOL000612	alpha-cedrene	204.39	55.56	0.10
MOL000675	Oleic acid	282.52	33.13	0.14

Table 2: Basic information on cinnamon therapeutic MA compounds. The larger the value of degree, the more important the component is.

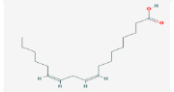
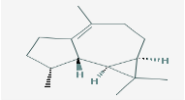
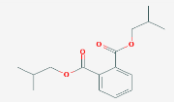
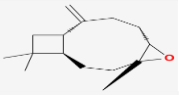
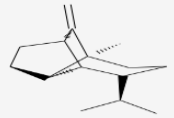
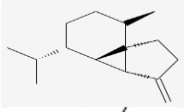
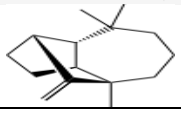
MOL ID	Compound	Structure	Degree
MOL000675	oleic acid		37.0
MOL000131	EIC		36.0
MOL000612	(-)-alpha-cedrene		12.0
MOL003538	(+)-Ledene		12.0
MOL000057	DIBP		7.0
MOL002003	(-)-Caryophyllene oxide		3.0
MOL003522	(+)-Sativene		2.0
MOL000266	beta-Cubebene		2.0
MOL002697	junipene		2.0

Table 3: Active ingredient and core target docking results. The smaller the value of affinity, the stronger the intermolecular binding ability.

Compound (ligand)	Targets (receptor)	PDB ID	Affnity (kcal/mol)
Oleic acid	IL6	1alu	-3.60
Oleic acid	MAPK3	2zoq	-1.70
Oleic acid	PPARG	7awc	-2.74
Oleic acid	PPARA	6lxa	-3.10
Oleic acid	CNR1	5u09	-1.41
Oleic acid	ESR1	4xi3	-2.04
EIC	IL6	1alu	-2.38
EIC	MAPK3	2zoq	-0.96
EIC	PPARG	7awc	-2.17
EIC	PPARA	6lxa	-2.89
EIC	CNR1	5u09	-0.38
EIC	ESR1	4xi3	-1.89
alpha-cedrene	IL6	1alu	-3.67
alpha-cedrene	MAPK3	2zoq	-4.37
alpha-cedrene	PPARG	7awc	-5.43
alpha-cedrene	PPARA	6lxa	-6.35
alpha-cedrene	CNR1	5u09	-4.68
alpha-cedrene	ESR1	4xi3	-4.06

In the model group, diarrhea, decreased body mass, lacrimation and jumping were the prominent symptoms following naloxone injection to induce withdrawal. This was followed by stereotyped movements and shaking similar to a wet dog. In contrast, the normal group did not exhibit any response to naloxone injection and their activities and rest remained normal. The first and second withdrawal symptom scores were significantly higher in the model group compared to the normal group, with all differences being statistically significant (all $P<0.01$), confirming the successful establishment of the morphine dependence withdrawal model.

Compared to the model group, rats in the low, medium, and high doses of *Cinnamomi Cortex* groups, as well as the buprenorphine-positive control group, only displayed minimal abnormal behaviors such as hair licking, biting front paws, and cage knocking within the first 10 min. Furthermore, the first and second withdrawal symptom scores were significantly reduced and all differences were statistically significant (all $P<0.01$). These findings indicate that *Cinnamomi Cortex* has a significant effect on the withdrawal symptoms of morphine-dependent rats, with the most notable effect observed at the medium dose of *Cinnamomi Cortex.*

The body mass of the model group was (202.56±13.24) g before the first prod and (193.43±15.22) g after, respectively. Similarly, it was (190.11±12.64) g before the second prod and (181.23±14.76) g after. These differences were statistically significant (all $P<0.05$). Rats in the model group experienced varying degrees of diarrhea, urination, lacrimation and salivation after naloxone withdrawal, resulting in severe dehydration and fasting. In contrast, the normal group, low-, medium- and high-dose *Cinnamomi Cortex* groups, as well as the buprenorphine-positive control group, did not exhibit any of the aforementioned abnormalities (fig. 2).

Screening for potential effector components and prediction of target genes in Cinnamomi Cortex

After collecting and organizing data from the TCMSP database, we obtained 100 chemical components related to *Cinnamomi Cortex*. Applying two criteria of OB ≥ 30% and DL ≥0.18, we screened and identified 10 active ingredients in *Cinnamomi Cortex* (table I). It should be noted that no target information was predicted for the compound aromadendrene. The remaining compounds exhibited target genes associated with 127 drug targets, including CNR2, AR and FAAH.

Acquisition of MA disease targets

We collected MA-related genes from the GeneCards and DisGenet comprehensive databases. After integrating and removing duplicates, we obtained a total of 1724 target genes associated with MA. By employing the Venny2.1 platform, we mapped the 127 targets predicted for *Cinnamomi Cortex* to the 1724 MA-related targets. Consequently, we identified 52 potential targets of *Cinnamomi Cortex* that may have an effect on MA, including CNR2, CES1, AR and GRM5 (refer to fig. 3A).

Construction and analysis of PPI networks for shared targets

The 52 genes shared by *Cinnamomi Cortex* and MA were analyzed using the STRING online database and a PPI network for *Cinnamomi Cortex* in DA disease was created using Cytoscape 3.9.0 (fig. 3B). The network graph comprised 52 nodes connected by 210 edges. The nodes were visualized based on their degree values, with larger node diameters and darker node colors (ranging from yellow to red) indicating higher degree values. A screening condition of degree value≥12 was applied, resulting in the identification of 13 core target genes. These genes, listed in descending order of degree value, are IL6, MAPK3, PPARG, PPARA, CNR1, ESR1, MAPK1, CYP19A1, NR3C1, MAPK14, GRM5, ADORA2A and DRD2. The high degree values of these targets suggest their potential key role in the anti-DA mechanism of *Cinnamomi Cortex*. Furthermore, the PPI network diagram was enhanced using MCODE, resulting in the identification of three sub-networks (fig. 3C). Sub-network 1 was centered around the core target MAPK14, sub-network 2 around SHBG and sub-network 3 around PDE4A. These sub-networks provide valuable insights for studying the pathogenesis of MA.

Cinnamomi Cortex - major component - shared target network construction

The nine *Cinnamomi Cortex* components obtained and the 52 common targets of *Cinnamomi Cortex* and DA were compiled into a table according to the rules of use. This table was then imported into Cytoscape to construct a network diagram representing the interactions between *Cinnamomi Cortex* components and their target proteins, as depicted in fig. 4. In the diagram, target proteins are represented by circles. By utilizing the CytoNCA plugin, the degree values of the *Cinnamomi Cortex* components were analyzed and ranked in descending order as follows: oleic acid, EIC, (-)-alpha-cedrene, (+)-Ledene, DIBP, (-)-Caryophyllene oxide, (+)-Sativene, beta-Cubebene and junipene. Detailed information about these components can be found in table II. The higher degree values of oleic acid and EIC indicate their greater importance in the network and their key roles in the anti-MA process.

GO functional enrichment analysis of Cinnamomi Cortex therapeutic MA targets

GO enrichment analysis was performed using the DAVID database to investigate the *Cinnamomi Cortex* targets associated with MA, resulting in the identification of 290 GO terms (197 for biological pathways [BP], 38 for cellular components [CC] and 55 for molecular functions [MF]). The top 10 entries from the GO analysis (sorted in ascending order based on P value, $P<0.05$) for BP, CC,

and MF are depicted in a unified bar chart on the bioinformatics platform (fig. 5). The BP category primarily involves intracellular steroid hormone receptor signaling pathways and positive regulation of gene expression, among others. The CC category encompasses components of glutamatergic synapses, presynaptic membranes, and post-synaptic membranes, among others. In the MF category, notable functions include protein binding and steroid binding, among others.

Analysis of the KEGG pathway of the Cinnamomi Cortex anti-MA target

KEGG analysis of *Cinnamomi Cortex's* therapeutic targets for MA using the DAVID database revealed that the common targets for *Cinnamomi Cortex* and MA were concentrated in 61 pathways. The enrichment bubble diagram on the bioinformatics platform presents the top 20 target-enriched KEGG pathways, ordered by the smallest to largest P-value ($P<0.05$). These pathways include signaling pathways such as neuroactive receptor-ligand interactions, cAMP, PPAR and others. In the graph, darker circles indicate pathways with larger P-values, while larger circles represent higher counts for that pathway (fig. 6).

Cinnamomi Cortex - active ingredient - target - pathway network construction

Using Cytoscape, a *Cinnamomi Cortex*-active-component-target-pathway network was generated. fig. 7 presents three active components of *Cinnamomi Cortex*, 52 common targets associated with *Cinnamomi Cortex* and the disease and the top 20 pathways identified through KEGG analysis (ranked by P-value from smallest to largest, $P<0.05$). The interrelationships among the components are depicted in the diagram. The drug *Cinnamomi Cortex* is represented by an orange "V" the active ingredient by a pink circle, the compound/disease target by a purple diamond and the predicted target KEGG enrichment pathway by a green triangle. The three components of *Cinnamomi Cortex* can act on multiple shared targets and modulate various pathways involved in MA, thereby offering a therapeutic effect for MA. Node size and color in the visualization correspond to the degree values. Targets with higher degree values, such as LI6 and MAPK3, are associated with four *Cinnamomi Cortex* active ingredients, namely EIC, oleic acid, alpha-cedrene, and (+)-Ledene, which also exhibit higher degree values (ROU7, ROU9, ROU1 and ROU3, respectively). The network diagram suggests that these active ingredients, particularly oleic acid (ROU9), EIC (ROU7), (-)-alpha-cedrene (ROU1),= and (+)-Ledene (ROU3), may impact signaling pathways related to core targets IL6, MAPK3, PPARG, PPARA, CNR1 and ESR1-related signaling pathways, such as neuroactive receptor–ligand interactions, PPAR, cAMP and neurodegenerative pathways associated with multiple diseases. Consequently, they have the potential to treat MA.

Molecular docking results

The binding energies obtained after docking are presented in table 3. A lower value of the binding energy indicates a stronger binding ability between the molecule and the target. As demonstrated in table III, the binding energy of each active ingredient with the core was below 0 kcal/mol, suggesting the presence of free binding activity between them (Aravind M *et al*., 2022). A partial visualization of the docking results can be seen in fig. 8, while fig. 9 illustrates a molecular docking heat map. However, it is crucial to acknowledge that factors beyond binding energy should be taken into consideration when evaluating the molecule's binding capability.

DISCUSSION

Long-term use of morphine can result in drug resistance and physical dependence, where drug resistance leads to a decrease in effectiveness with repeated use, and physical dependence leads to withdrawal symptoms upon abrupt cessation (Contet C *et al*., 2008). Animal experiments conducted in this study showed that both withdrawal scores of the model group were significantly higher than those of the normal group, indicating the successful establishment of a morphine-dependent rat withdrawal model. After naloxone administration, the body mass of the rats was significantly lower compared to before administration, which may be attributed to withdrawal symptoms such as lacrimation, vomiting, and diarrhea.

Traditional Chinese medicine has been studying drug treatment methods for over 200 years, with a rich history and a distinctive theoretical system (Lu *et al*., 2009) According to ancient Chinese traditional medical theory, morphine addiction is primarily caused by the accumulation of a substance called tobacco toxins in the body. This substance leads to dysfunction of internal organs, blood stagnation, insufficient vital energy, and fluctuating body temperature (Tang and Hao 2007). Huang *et al*. conducted studies demonstrating that Yian Hui Sheng Oral Liquid, which contains red ginseng, Radix et Rhizoma and *Cinnamomi Cortex*, can effectively control heroin addiction (Nakagawa *et al*., 2000). Pharmacological studies have confirmed the neuroprotective biological activity of *Cinnamomi Cortex*, suggesting its potential for treating certain neurological disorders (Almatroodi *et al*., 2020). These findings indicate that *Cinnamomi Cortex* has palliative and therapeutic effects on MA.

This study aimed to investigate the material basis and mechanism of action of *Cinnamomi Cortex* in the treatment of MA using bioinformatics. Relevant databases were used to obtain 9 effector ingredients, 127 targets of these ingredients, 1724 genes associated with MA, and 52 common targets of *Cinnamomi Cortex* and MA. PPI and core network diagrams were constructed to visualize the

interactions between *Cinnamomi Cortex*, its active components and the target pathways. The network diagram revealed that three effector components in *Cinnamomi Cortex*, including oleic acid, could act synergistically with 52 anti-morphine addiction targets. Moreover, these targets were mainly enriched in cAMP and other related signaling pathways.

The intersectional network of *Cinnamomi Cortex*, its active ingredients, and the targets showed that *Cinnamomi Cortex* treats MA through a multi-component, multi-target approach. The PPI network identified 13 potential key targets of *Cinnamomi Cortex* against MA, including MAPK3, PPARG, PPARA, and CNR1. GO analysis demonstrated that *Cinnamomi Cortex's* anti-morphine addiction effects involve response to drugs, positive regulation of gene expression, and other biological processes, primarily associated with cellular components such as pre- and post-synaptic membrane components, glutamatergic synapses, identical protein binding, and steroid binding. This indicates that *Cinnamomi Cortex's* anti-morphine addiction action is intricate and complex. KEGG analysis revealed that the common targets of *Cinnamomi Cortex* and MA are primarily enriched in signaling pathways such as neuroactive receptor ligand interactions, prolactin, PPAR and cAMP.

Based on the results of GO analysis, it is evident that *Cinnamomi Cortex's* treatment of MA may be closely related to presynaptic and postsynaptic membrane components, as well as glutamatergic synapses and other cellular components. Changes in synaptic plasticity in relevant brain regions (ventral tegmental area, hippocampus, nucleus ambiguus, etc.) are neurobiological mechanisms that contribute to dopamine addiction (Wise *et al*., 2021). MA has been shown to affect glutamatergic transmission in the hippocampal region of the brain and alter synaptic plasticity in the hippocampus (Yamada, D. *et al*., 2021). The author hypothesized that the active ingredients of *Cinnamomi Cortex* may act on these cellular components to restore the neuroadaptive changes induced by MA, thereby serving as a treatment for the addiction.

KEGG enrichment analysis revealed that the neuroactive receptor–ligand interaction pathway is closely related to MA, particularly involving neurotransmitters such as dopamine and 5-hydroxytryptamine. Dopaminergic neurons and the dopamine system play crucial roles in the reward system and are considered “common pathways” in the process of morphine addiction (Buck *et al*., 2022). Similarly, 5-hydroxytryptamine (Verheij M.M.M. *et al*., 2018) is an important system involved in MA. A study by Angelopoulou *et al*. demonstrated that *Cinnamomi Cortex* extract has neuroprotective effects in a subacute Parkinson's model in mice (Angelopoulou *et al*., 2021). It is speculated that the effector components of *Cinnamomi Cortex* may act on targets such as CHRM2, CNR2, GRM5 and others in this signaling pathway to regulate the release of neurotransmitters like dopamine and inhibit the damage caused by addictive substances to nerve cells, thereby exerting an anti-morphine addiction effect.

The cAMP pathway is closely associated with MA. cAMP functions as an essential intracellular secondary messenger molecule (Arumugham *et al.*, 2017). Chronic opioid use upregulates the cAMP pathway in the central nervous system, which is a key molecular mechanism of opioid addiction (Chan and Lutfy, 2016). The nucleus accumbens is one of the brain areas most sensitive to triggering opioid withdrawal responses. In the early stages of opioid use, opioids act on the mu-opioid receptor of the nucleus accumbens, inhibiting the activity of adenylyl cyclase (AC) and leading to a decrease in cellular cAMP levels, thereby inhibiting norepinephrine release. However, the cAMP pathway remains functionally hyperactive, eventually resulting in addiction and withdrawal symptoms in humans (Chaijale *et al*., 2013, Werling, 1987). In a study by (Peng *et al*., 2021), a combination of *Cinnamomi Cortex* and Bacopa monnieri in a 1:3 ratio was used to warm kidney yang in rats, leading to a significant increase in cAMP content compared to the model group. It can be speculated that several components of *Cinnamomi Cortex* may act on targets such as MAPK1, MAPK14, ESR1, ESR2, and MAPK3 in the cAMP signaling pathway, effectively modulating cAMP levels in neuronal cells, thereby alleviating the addiction and withdrawal symptoms induced by addictive substances in the body. This is consistent with the PPI network, which suggests that MAPK3, ESR1, MAPK1, and MAPK14 are key targets of *Cinnamomi Cortex's* anti-morphine addiction action.

CONCLUSION

In this groundbreaking study, we discovered, for the first time, that *Cinnamomi Cortex* has the ability to alleviate withdrawal symptoms in morphine-dependent rats. The most significant impact was observed at medium doses of *Cinnamomi Cortex*. To delve deeper into the mechanism of action, we employed network pharmacology and molecular docking techniques. Our findings revealed that components like oleic acid present in *Cinnamomi Cortex* can target multiple addiction-related receptors and modulate various pathways to effectively treat morphine addiction. This study not only elucidates the material foundation and mechanism of *Cinnamomi Cortex* in combating drug addiction but also offers valuable insights and references for future experiments exploring the potential of *Cinnamomi Cortex* in drug addiction treatment.

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REFERENCES

Almatroodi SA, Mohammed AA, Ahmad A, Shehwaz A and Arshad HR (2020). Cinnamon and its active compounds: A potential candidate in disease and tumour management through modulating various genes activity. *Gene Reports*, **21**(10): 100966.

Angelopoulou E, Paudel YN, Piperi C and Mishra A (2021). Neuroprotective potential of cinnamon and its metabolites in Parkinson's disease: Mechanistic insights, limitations and novel therapeutic opportunities. *J. Biochem. Mol. Toxicol.*, **35**: e22720.

Aravind M, Kumaresubitha T and Ahmed N (2022). DFT, molecular docking, photocatalytic and antimicrobial activity of coumarin enriched cinnamon bark extract mediated silver nanoparticles. *Inorg. Chem. Commun.*, **146**: 110-176.

Arumugham VB and Baldari CT (2017). cAMP: A multifaceted modulator of immune synapse assembly and T cell activation. *J. Leukoc. Biol.*, **101**(6): 1301-1316.

Barshir R, Fishilevich S, Iny-Stein T, Zelig O, Mazor Y, Guan-Golan Y, Safran M and Lancet D (2021). GeneCaRNA: A comprehensive gene-centric database of human non-coding RNAs in the gene cards suite. *J. Mol Biol.*, **433**(11): 166913.

Berman HM, Westbrook J, Feng Z, Gilliland G, Bhat TN, Weissig H, Shindyalov IN and Bourne PE (2000). The protein data bank. *Nucleic Acids Res.*, **28**(1): 235-242.

Buck SA, Quincy Erickson-Oberg M, Logan RW and Freyberg Z (2022). Relevance of interactions between dopamine and glutamate neurotransmission in schizophrenia. *Mol. Psychiatry*, **27**(9): 3583-3591.

Chaijale NN, Curtis AL, Wood SK, Zhang XY, Bhatnagar S, Reyes BA, Van Bockstaele EJ and Valentino RJ (2013). Social stress engages opioid regulation of locus coeruleus norepinephrine neurons and induces a state of cellular and physical opiate dependence. *Neuropsychopharmacology*, **38**(10): 1833-1843.

Chan P and Lutfy K (2016). Molecular changes in opioid addiction: The role of adenylyl cyclase and cAMP/PKA system. *Prog. Mol. Biol. Transl. Sci.*, **137**(10): 203-227.

Clyne A, Yang L, Yang M, May B and Yang A (2020). Molecular docking and network connections of active compounds from the classical herbal formula Ding Chuan Tang. *Peer J.*, **8**(3): e8685.

Contet C, Filliol D, Matifas A and Kieffer BL (2008). Morphine-induced analgesic tolerance, locomotor sensitization and physical dependence do not require modification of mu opioid receptor, cdk5 and adenylate cyclase activity. *Neuropharmacology*, **54**(3): 475-486.

Cozzoli D, Daponte A, De Fazio S, Ariano V, Quaranta MR, Leone V, Ostuni A, Casanova M, Catacchio CR, Ventura M and Montinaro F (2021). Genomic and personalized medicine approaches for substance use disorders (SUDs) looking at genome-wide association studies. *Biomedicines*, **9**(12): 1799.

Everitt BJ and Robbins TW (2013). From the ventral to the dorsal striatum: Devolving views of their roles in drug addiction. *Neurosci. Biobehav. Rev.*, **37**(9 Pt A): 1946-1954.

Gao L, Cao M, Li JQ, Qin XM and Fang J (2021). Traditional Chinese medicine network pharmacology in cardiovascular precision medicine. *Curr. Pharm. Des.* **27**(26): 2925-2933.

Hamidpour R, Hamidpour M, Hamidpour S and Shahlari M (2015). cinnamon from the selection of traditional applications to its novel effects on the inhibition of angiogenesis in cancer cells and prevention of Alzheimer's disease, and a series of functions such as antioxidant, anticholesterol, antidiabetes, antibacterial, antifungal, nematicidal, acaracidal and repellent activities. *J. Tradit. Complement. Med.*, **5**(2): 66-70.

Jiao X, Sherman BT, Huang W, Stephens R, Baseler MW, Lane HC and Lempicki RA (2012). DAVID-WS: A stateful web service to facilitate gene/protein list analysis. *Bioinformatics*, **28**(13): 1805-1806.

Jiashuo WU, Fangqing Z, Zhuangzhuang LI, Weiyi J and Yue S (2022). Integration strategy of network pharmacology in traditional Chinese medicine: A narrative review. *J. Tradit Chin. Med.*, **42**(3): 479-486.

Kourosh-Arami M, Joghataei MT, Komaki A, Gholami M, Najafi Z and Lavaie M (2022). Persistent effects of the orexin-1 receptor antagonist SB-334867 on naloxone precipitated morphine withdrawal symptoms and nociceptive behaviors in morphine dependent rats. *Int. J. Neurosci.*, **132**(1): 67-76.

Li S and Zhang B (2013). Traditional Chinese medicine network pharmacology: Theory, methodology and application. *Chin. J. Nat. Med.*, **11**(2): 110-120.

Lu L, Liu Y, Zhu W, Shi J, Liu Y, Ling W and Kosten TR (2009). Traditional medicine in the treatment of drug addiction. *Am. J. Drug Alcohol. Abuse*, **35**(1): 1-11.

Mohamed A, Shah AD, Chen D and Hill MM (2019). RaftProt V2: Understanding membrane microdomain function through lipid raft proteomes. *Nucleic Acids Res.*, **47**(D1): D459-D463.

Momtaz S, Hassani S, Khan F, Ziaee M and Abdollahi M (2018). Cinnamon, a promising prospect towards Alzheimer's disease. *Pharmacol. Res.*, **130**: 241-258.

Nakagawa T, Masuda T, Watanabe T, Minami M and Satoh M (2000). Possible involvement of the locus coeruleus in inhibition by prostanoid EP(3) receptor-selective agonists of morphine withdrawal syndrome in rats. *Eur. J. Pharmacol.*, **390**(3): 257-266.

Peng Z and University C (2011). Study on the effect of prescription for warming and recuperating the kidney-yang on the ethology and 17-OHCS, cAMP in plasma

of PTSD rat (2011). *J. Sichuan Tradit. Chin. Med.*, **29**(9): 19-21.

Ru J, Li P, Wang J, Zhou W, Li B, Huang C, Li P, Guo Z, Tao W, Yang Y, Xu X, Li Y, Wang Y and Yang L (2014). TCMSP: A database of systems pharmacology for drug discovery from herbal medicines. *J. Cheminform.*, **6**(1): 13.

Sato T, Ishikawa S, Asano J, Yamamoto H, Fujii M, Sato T, Yamamoto K, Kitagaki K, Akashi T, Okamoto R and Ohteki T (2020). Regulated IFN signalling preserves the stemness of intestinal stem cells by restricting differentiation into secretory-cell lineages. *Nat. Cell Biol.*, **22**(8): 919-926.

Seeliger D and de Groot BL (2010). Ligand docking and binding site analysis with PyMOL and Autodock/ Vina. *J. Comput. Aided Mol. Des.*, **24**(5): 417-422.

Seibel R, Schneider RH and Gottlieb MGV (2021). Effects of spices (saffron, rosemary, cinnamon, turmeric and ginger) in Alzheimer's disease. *Curr. Alzheimer Res.*, **18**(4): 347-357.

Song X, Zhang Y, Dai E, Wang L and Du H (2020). Prediction of triptolide targets in rheumatoid arthritis using network pharmacology and molecular docking. *Int. Immunopharmacol.*, **80**: 106179.

Szklarczyk D, Franceschini A, Wyder S, Forslund K, Heller D, Huerta-Cepas J, Simonovic M, Roth A, Santos A, Tsafou KP, Kuhn M, Bork P, Jensen LJ and von Mering C (2015). String v10: Protein-protein interaction networks, integrated over the tree of life. *Nucleic Acids Res.*, **43**(D1): D447-D452.

Tang Y, Li M, Wang J, Pan Y and Wu FX (2015). CytoNCA: A cytoscape plugin for centrality analysis and evaluation of protein interaction networks. *Biosystems*, **127**(11): 67-72.

Tang YL and Hao W (2007). Improving drug addiction treatment in China. *Addiction*, **102**(7): 1057-1063.

Tong T, Cheng B, Tie S, Zhan G, Ouyang D and Cao J (2022). Exploring the mechanism of Epimedii folium and notoginseng radix against vascular dementia based on network pharmacology and molecular docking analysis: Pharmacological mechanisms of EH-PN for VD. *Medicine*, **101**: e31969.

UniProt Consortium (2015). UniProt: A hub for protein information. *Nucleic Acids Res.*, **43**(D1): D204-D212.

UniProt Consortium (2019). UniProt: A worldwide hub of protein knowledge. *Nucleic Acids Res.*, **47**(D1): D506-D515.

Verheij MMM, Contet C, Karel P, Latour J, van der Doelen RHA, Geenen B, van Hulten JA, Meyer F, Kozicz T, George O, Koob GF and Homberg JR (2018). Median and dorsal raphe serotonergic neurons control moderate versus compulsive cocaine intake. *Biol. Psychiatry*, **83**(10): 1024-1035.

Wang HR, Gao XR, Zhang KG and Han JS (2003). Current status in drug addiction and addiction memory research. *Sheng Li Ke Xue Jin Zhan*, **34**(3): 202-206.

Wang Y, Xiao J, Suzek TO, Zhang J, Wang J, Zhou Z, Han L, Karapetyan K, Dracheva S, Shoemaker BA, Bolton E, Gindulyte A and Bryant SH (2012). PubChem's BioAssay Database. *Nucleic Acids Res.*, **40**(D1): D400-D412.

Werling LL, Brown SR and Cox BM (1987). Opioid receptor regulation of the release of norepinephrine in brain. *Neuropharmacology*, **26**(7B): 987-996.

Wise RA and Jordan CJ (2021). Dopamine, behavior and addiction. *J. Biomed. Sci.*, **28**(1): 83.

Yamada D, Takahashi J, Iio K, Nagase H and Saitoh A (2021). Modulation of glutamatergic synaptic transmission and neuronal excitability in the prelimbic medial prefrontal cortex via delta-opioid receptors in mice. *Biochem. Biophys. Res. Commun.*, **560**(5): 192-198.

Yang T (2017). Cinnamon improves learning memory in Alzheimer's disease rats by inhibiting hippocampal oxidative stress. *China J. Chin. Med.*, **9**(2): 8-11.

Zhang P, Xie L and Luo R (2011). Study on the effect of prescription for warming and recuperating the kidney-yang on the ethology and 17-OHCS, cAMP in Plasma of PTSD rat. *J. Sichuan Tradit. Chin. Med.*, **29**(9): 19-21.

Zhao J, Mo C, Shi W, Meng L and Ai J (2021). Network pharmacology combined with bioinformatics to investigate the mechanisms and molecular targets of astragalus radix-panax notoginseng herb pair on treating diabetic nephropathy. *Evid. Based Complement. Alternat. Med.*, **7**: 9980981.