RESEARCH ARTICLE

Mechanism of peppermint oil within the therapy for irritable bowel syndrome based on network pharmacology and gas chromatography-mass spectrometry

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Abdominal pain or malaise is a usual symptom of irritable bowel syndrome (IBS), a functional bowel illness. One of the primary active elements within peppermint oil (PO), which has been used to treat IBS, is thought to be Menthol and L-Menthol. It's unclear what PO's fundamental mechanism of action is while treating IBS. The purpose of this study was to explore the main compounds in PO for the treatment of IBS and their mechanisms of action. The constituents of volatile oil were analyzed by gas chromatography-mass spectrometry (GC-MS), and the mechanism of action of volatile oil in IBS treatment was investigated by network pharmacology. Methods from network pharmacology were applied to investigate PO's potential central pathways and core targets in the management of IBS. Additionally, molecular docking simulation was employed to assess the binding activity among key therapeutic targets and the relevant drugs. The results of the molecular docking simulation demonstrated a robust interaction between the eight main therapeutic targets as well as the matching drugs. In conclusion, this research clarified that the G-protein coupled receptor signaling process, which is related to the cyclic nucleotide second messenger and is activated by phospholipase C, might be responsible for the effectiveness of PO during the treatment of IBS.

Keywords: gas chromatography-mass spectrometry; peppermint oil; network pharmacology; irritable bowel syndrome.

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Introduction

In the gastrointestinal tract, irritable bowel syndrome (IBS) is a prevalent functional condition. Although the gut does not appear to have any pathological conditions, there is also a risk of damage to people's health [1, 2]. IBS is a persistent digestive system malfunction. Individuals typically present with diarrhea, as well as constipation along with discomfort in the stomach and altered bowel habits [3]. Although IBS is benign in nature, it can cause a lot of distress to patients because its etiology is complex and appears to be multifactorial [4]. Therefore, it is important to understand the pathogenesis of IBS. Among them, intestinal motility disorder is the key to its multifactorial pathogenesis. Since many years ago, a diverse class of medications known as "antispasmodics" including calcium channel blockers and direct smooth muscle relaxants such as papaverine, mebeverine, and peppermint oil are commonly employed to treat IBS [5].

Peppermint is a well-known plant that comes in numerous forms including oil, leaves, leaf

extracts, and leaf water [6]. Peppermint's ability to treat IBS may be related to its anti-inflammatory, antioxidant, immunomodulatory, and anesthetic activities [7]. This article focused on oil because it has the highest usage and is the most used in IBS. The oil is extracted by steam distillation from the aboveground plant just before the plant flowers. It is a clear or light yellow liquid having a strong taste and a bright, new menthol smell. When applied to the skin, it causes a cooling sensation [8]. Peppermint oil (PO) is widely used in the treatment of IBS. Some experiments have shown that peppermint oil is more effective than other drugs in improving symptoms in IBS [9]. PO is used as an ingredient in oils and enteric-coated capsules (Mintec) and other intestinal medications [10]. PO is considered safe for culinary and medicinal purposes. Thus, it may be considered a drug of choice for treating patients with IBS symptoms. Notably, peppermint oil contains more than 30 known components. The main components are menthol and menthone, and it also contains various flavonoids, which contribute to its overall activity as an antioxidant. Among them, menthol has been the most widely studied in treating IBS. Menthol and mentholone of PO are thought to induce desensitization of intestinal smooth muscle relaxation and nociceptive neuroafferents and are rapidly absorbed from the proximal gut to the abdomen to reduce pain in IBS patients.

There are several methods for removing active ingredients through essential oils [11]. The primary method used in this study is steam distillation (SD) to extract PO. Over time, several methods were established to investigate essential oils. Gas chromatography-mass spectrometry (GC-MS) peppermint oil approach is the recommended technique [12]. GC-MS analyzers can qualitatively analyze multi-component mixtures in a relatively short period of time. It is perfect for figuring out how mixes, like essential oils with volatile parts, are put together. A comprehensive understanding of the connections among medications, particular proteins, as well as illnesses is the goal of network pharmacology. The perspective comes from network analytical tools for predicting targets or mechanism evaluation, or from high-throughput screening research [13]. One important avenue in structural molecular biology as well as computer-aided medication creation for novel therapeutics is molecular docking [14]. Although PO is commonly used to treat IBS, the mechanism remains unclear. Therefore, the purpose of this study was to explore the main compounds in PO for the treatment of IBS and their mechanisms of action. It would play a supporting role in the subsequent treatment of intestinal diseases with peppermint oil. In this study, SD method and GC-MS technology were employed to extract PO and analyze the chemical composition of PO. Network pharmacology and molecular docking were applied to anticipate possible targets, signaling networks, and investigate the connection between PO's active compounds and IBS.

Materials and Methods

Valuable chemical separation *via* steam distillation

Chinese medicinal peppermint (*Lamiaceae*) was purchased from Huawei Pharmacy (Jiamusi City, Heilongjiang, China). Dried leaves were obtained from the aerial part of peppermint and were grinded into a coarse powder. 50 g of leaf powder was weighted and mixed with 500 mL of water before distilled with steam for 5 hours by using a heating jacket. The yellow oily liquid with an aromatic smell was collected as volatile peppermint oil. The oil was then rinsed with hexane and dried by adding 0.5 g of sodium sulfite.

Component analysis

The Agilent 7890A/5975C GC-MS instrument (Agilent Technologies, Santa Clara, California, USA) was used to identify the PO components. Each chromatographic peak separated by GC-MS was analyzed and screened through NIST08 spectrum library (National Institute of Standards and Technology, Gaithersburg, MD, USA). The

compounds contained in the volatile oil were then determined and the percentage of each component was calculated.

Network pharmacology

(1) Target protein retrieval and molecular structure

The pharmacological targets were predicted by using Swiss Target Prediction software (http://swisstargetprediction.ch/), while the chemical structures of the volatile substances in two-dimensional were obtained from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The target genes associated with IBS were obtained through OMIM (http://www.omim.org/) and GeneCards (https://www.genecards.org/). The intersection targets between the disease targets of IBS and the active ingredients in peppermint volatile oil were extracted using Venny 2.1.0 program (https://bioinfogp.cnb.csic.es/tools/venny/). The potential uses of peppermint oil's volatile oil for treating IBS was explored.

(2) Construction of the network model

The intersection targets from the Venny investigation were imported into the STRING database (https://string-db.org/) to determine the interaction between IBS targets and PO targets. The tissue type was specified as "homo sapiens" to generate the Protein-Protein Interaction (PPI) networks. The file was kept in TSV format, and Cytoscape 3.8.1 (https://cytoscape.org/) was used to upload and visually evaluate the intersection target interaction data. The degree of every node and the topological characteristics of mediation centrality were assessed by the network analyzer. Proteins that possessed the aforementioned two topological parameters in excess of the mean value across all nodes had been deemed plausible candidates for anti-inflammatory targets. The STRING database was utilized to analyze the possible targets' protein interactions.

(3) Analysis of Gene Ontology (GO) function and KEGG pathway enrichment

The DAVID (https://david.ncifcrf.gov/) database's KEGG pathway and GO biological process investigation were employed to assess the possible targets.

Molecular docking

The two-dimensional (2D) structures of the Menthol were downloaded *via* the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). Chem3D was used to analyze and convert the 2D structure to PDB format. AutoDock Tools 1.5.6 (https://autodocksuite.scripps.edu/adt/) was then used to store the PDBQT formatted structure as docking ligands. From the RCSB PDB library (https://www.rcsb.org/), the three-dimensional (3D) structure was retrieved. Gasteiger charges were computed using AutoDock Tools 1.5.6 program, while proteins were isolated, nonpolar hydrogen was added, and the structure was saved within a PDBQT file. Proteins served as ligands and were active as receptors. The ligand coordinate in the target complex served as the basis for determining the molecular docking active site. The receptor had been rigid, and the ligand was set to be flexible. Protein was docked with small molecules using AutoDock Vina 1.1.2 (https://vina.scripps.edu/). Each of the docking results produced 20 conformations. The ultimate docking conformation was chosen based on its highest affinity, and Pymol 2.3 (https://pymol.org/2/) was used to visualize it.

Results

Chemical components within PO

A total of 46 chemical substances from PO had been detected through GC-MS analysis (Figure 1). The chemical substances having quality values higher than 90 was listed in Table 1. With an area proportion of 78.96% compared to the other ten components, menthol and menthone represented the two main ingredients in the mint oil.

Building networks and identifying possible target proteins

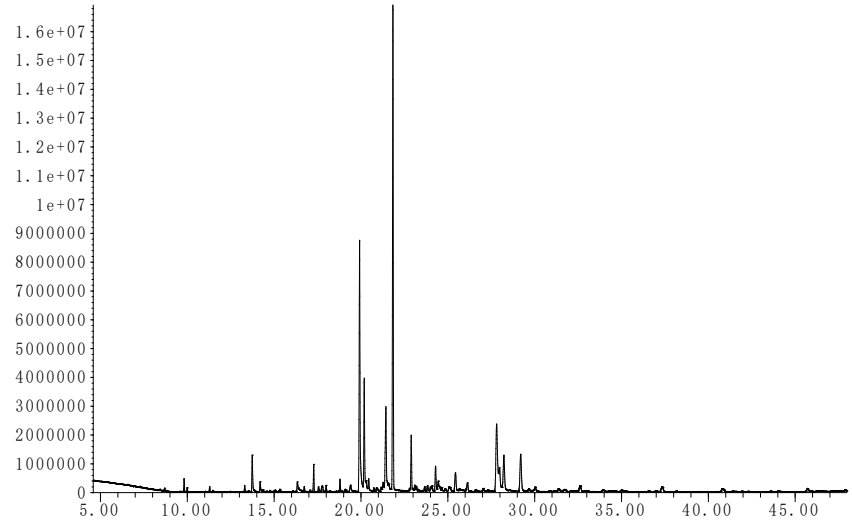

Figure 1. GC-MS chromatogram for mint oil.

Table 1. Information on PO components.

NO.	Retention time (min)	Compound	Molecular formula	Molecular weight	Percentage content (%)	Quality (g)
1	19.9	Eugenol	$C_{10}H_{12}O_2$	164.201	16.2	98
2	21.8	Methyl Eugenol	$C_{11}H_{14}O_2$	178.228	2.41	98
3	21.4	Menthone	$C_{10}H_{18}O$	154.249	24.3	97
4	22.9	L-Menthol	$C_{10}H_{20}O$	156.265	25.9	98
5	23.9	β-Caryophyllene	$C_{15}H_{24}$	204.351	7.23	99
6	29.2	Pulegone	$C_{15}H_{22}$	202.335	0.9	93
7	45.7	α -Pinene	$C_{15}H_{24}$	204.351	2.41	95
8	47.9	(-)-Cedrene	$C_{15}H_{24}$	204.351	4.06	98
9	50.4	Hexahydrofarnesyl acetone	$C_{18}H_{36}O$	268.478	0.09	98
10	54	Palmitic acid	$C_{16}H_{32}O_2$	256.421	3.06	94

Swiss Target Prediction and PubChem had been utilized to search the targets of ten different chemical substances. OMIM 90 possible targets had been discovered by intersecting the targets of chemical substances and IBS targets. IBS-related targets were identified utilizing Gene Cards. The target network composed of components was built (Figure 2). The network contained 101 nodes with a total of 90 target nodes, 1 IBS node, and 10 compound nodes. The action intensity was indicated by the "degree" and the degree value by the size of the nodes. It contained 314 edges with each of them connecting between a target and a component. The bioactive component was by the dark red nodes, whereas the possible target was symbolized by the pink nodes. The degree value of a color increased with darkness. Eight genes generated centrality, and their degrees of freedom values were higher than the average (Table 2). Based on the results, the following genes were not considered to be encoded essential proteins including NOS2, PGE2, MAPK3, CYP2A6, AKT1, EGFR, TNF, and AR. In the entire network, the degree of correlation between L-menthol and possible targets were 26. The created network of the 90 possible targets' PPI was displayed in Figure 3.

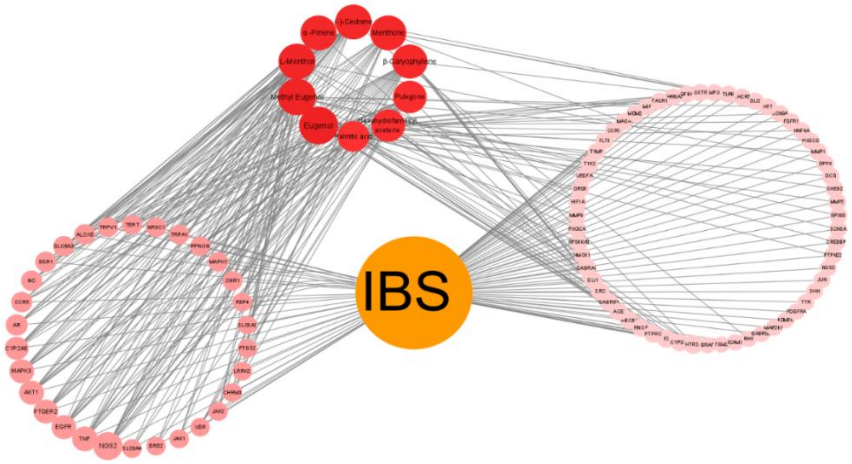

Figure 2. Compound-target-IBS network.

Table 2. Information of 8 genes.

NO.	Gene abbreviation	Betweenness centrality	Degree
1	NOS2	0.529100529	11
2	PTGER2	0.523560209	10
3	MAPK3	0.523560209	10
4	CYP2A6	0.512820513	8
5	AKT1	0.512820513	8
6	EGFR	0.512820513	8
7	TNF	0.507614213	7
8	AR	0.507614213	7

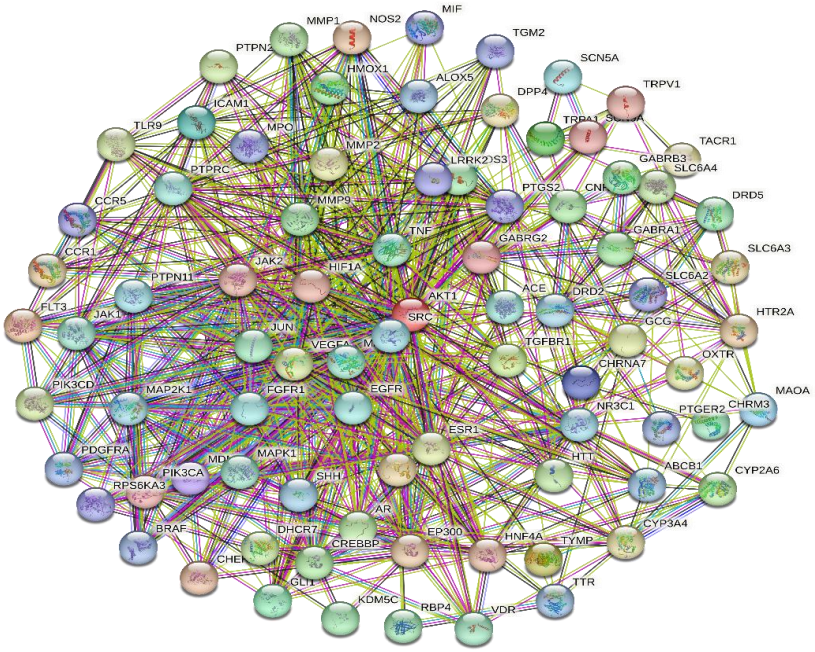

Figure 3. The 90 possible targets' network for protein-protein interactions (PPI).

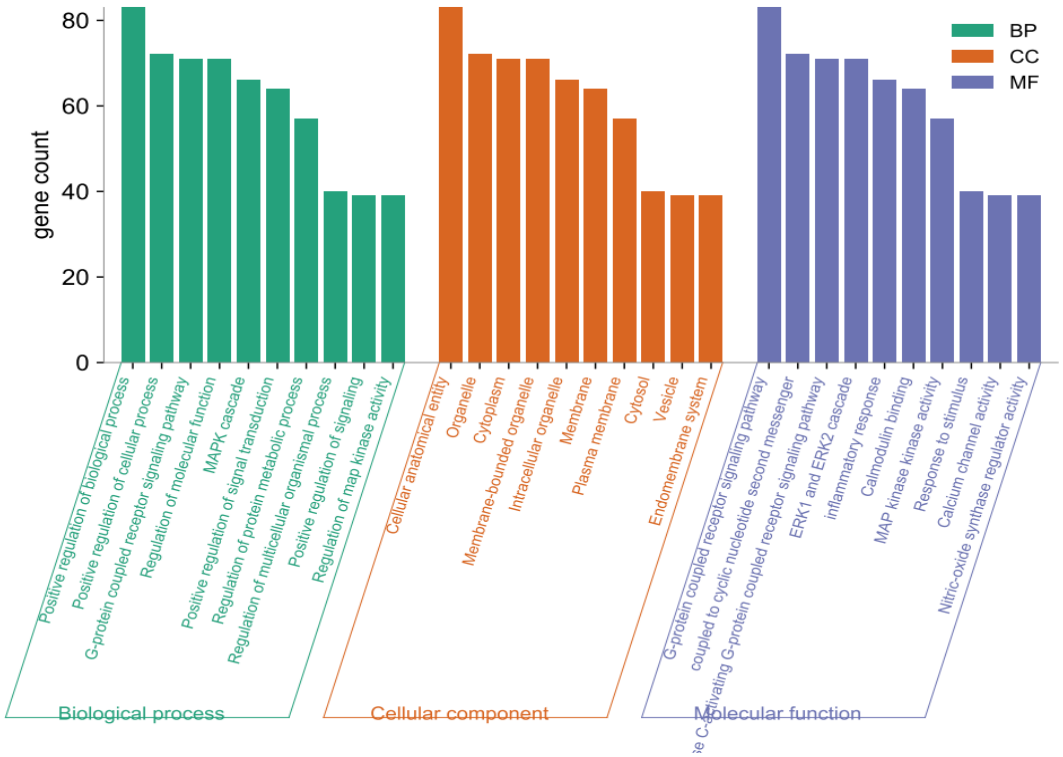

Figure 4. GO enrichment analysis.

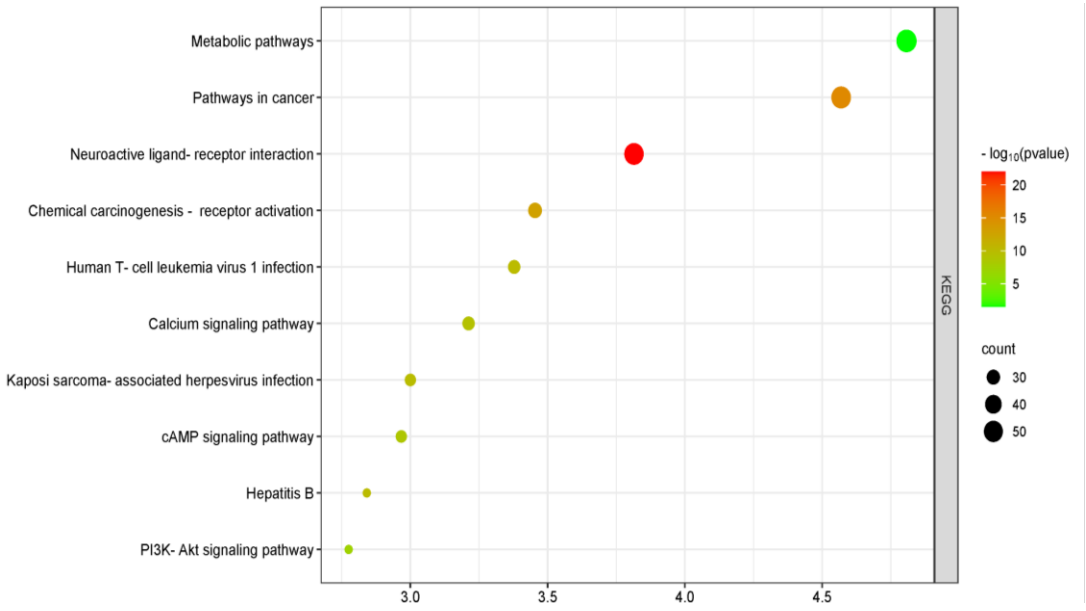

Figure 5. KEGG pathway analysis.

GO and KEGG pathway investigation

These putative target proteins were analyzed for enrichment across GO biological processes, cell components, molecular functions, and KEGG pathways using DAVID database (Figures 4 and 5). A *P* value of less than 0.01 was found for 225 biological processes, cell components, and molecular functions according to GO enrichment

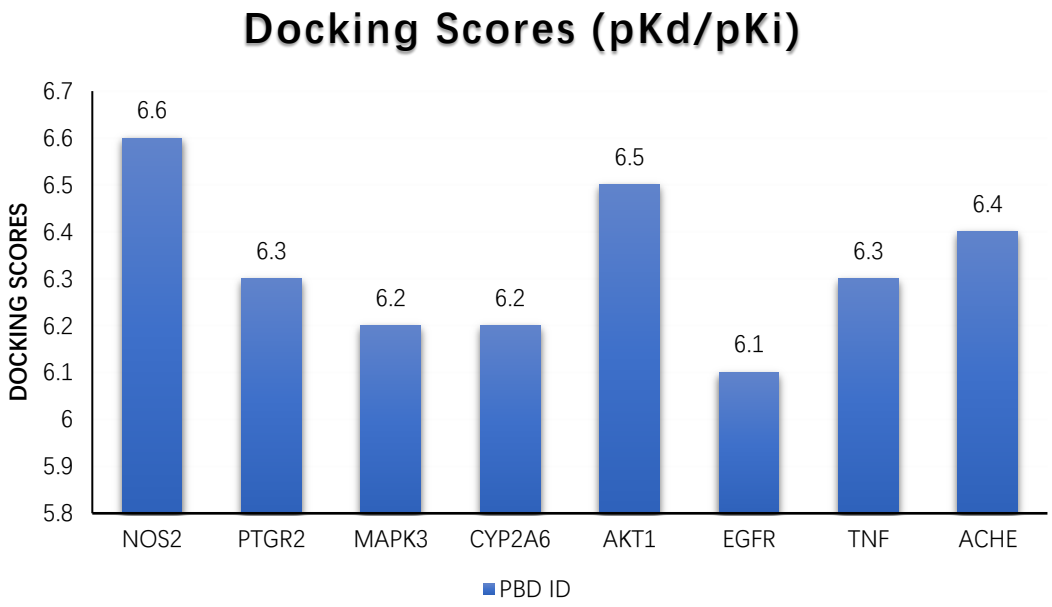

Figure 6. Molecular docking results.

investigation. The results suggested that the targets had been connected to multiple biological processes such as the G-protein coupled receptor signaling process, phospholipase C-activating G-protein coupled receptor signaling process, inflammatory response, and the beneficial control of ERK1 and ERK2 cascade. The occurrence and progression of IBS might be intimately linked to these biological processes. These proteins' cellular components included zinc ion binding, enzyme binding, G-protein-coupled serotonin receptor function, and sequence-specific DNA binding. Molecular processes including protein binding, metal ion binding, ATP binding, zinc ion binding, and protein homodimerization activity were mediated by genes encoding proteins that celastrol targets.

Molecular docking verification

The practical chemical component of PO in treating IBS was Menthol. In order to confirm the binding ability for Menthol proteins with two tiny-molecule drugs, molecular docking was carried out utilizing the putative protein and L-Menthol as study objects. The L-Menthol molecule's docking results was shown in Figure 6. The common consensus is that, when the docking percentage is higher above 4.25, the docking molecule is actively attaching to the target protein. Greater binding activity is indicated by values above 5.00, while substantial binding activity is indicated by values above 7.00 [15]. The compound's targeted binding activity increases with increasing molecular docking percentage. Although a score is not absolute, generally speaking, a higher score is preferable. According to the findings for molecular docking, these substances and two target proteins had system docking scores above 5, indicating that these substances had a higher probability of interacting with the projected target protein. These targets might have a tight relationship with the impact of volatile chemicals from PO on IBS. The top four in scores outcomes had been displayed in Figure 7.

Discussion

Peppermint is a perennial flowering member of the mint family and is grown worldwide for its flavor, smell, potential medical, and pharmacological uses [16]. Being a homologous herb in Chinese medicine and food, peppermint has the efficacy of treating a variety of diseases,

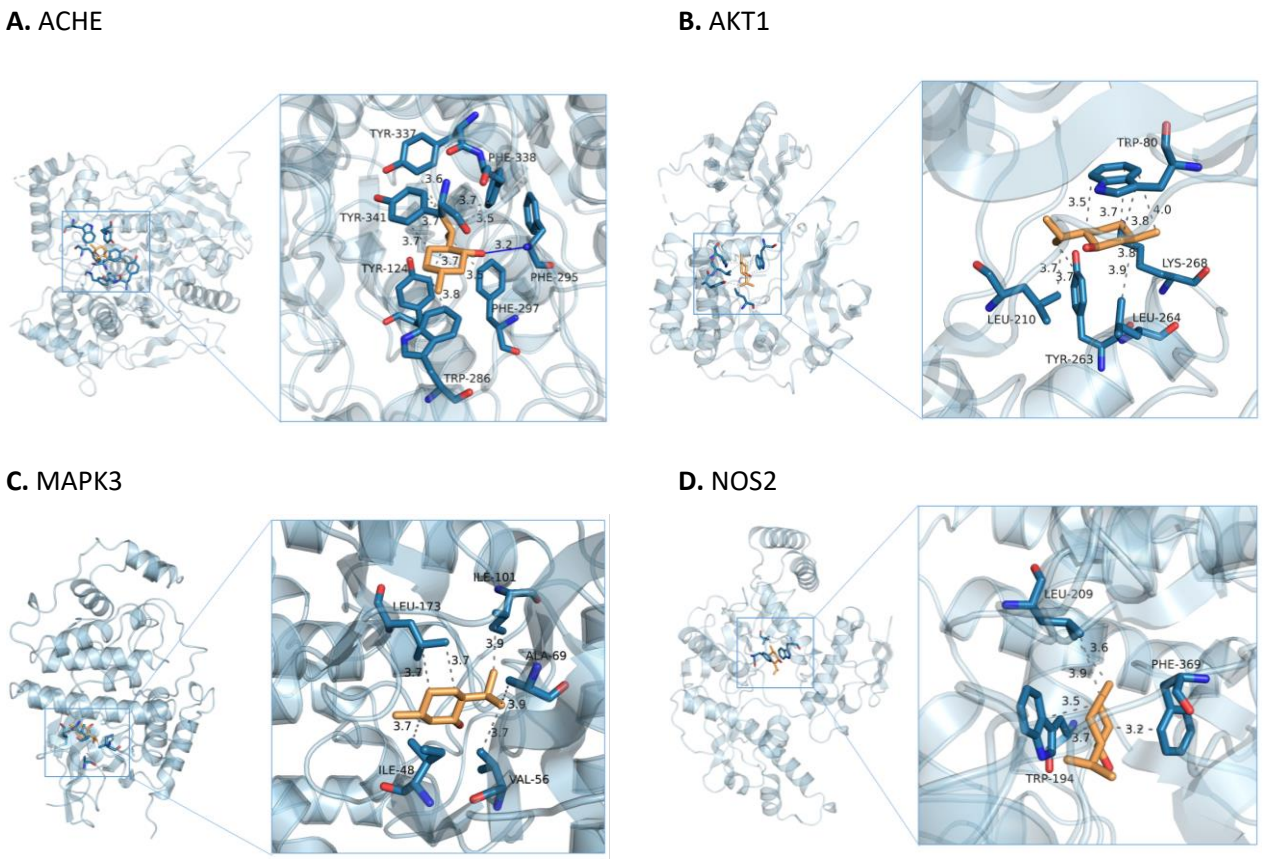

Figure 7. Molecular docking visual analysis.

including biliary tract disorders, dyspepsia, enteritis, flatulence, gastritis, intestinal colic, and bile duct, gallbladder, gastrointestinal (GI) spasms [17]. The economically relevant volatile oils of menthol members are derived through distillation of freshly pulverized leaves [18]. PO comprises menthol and menthone. The processing circumstances, geographic location, and plant maturity can all affect the chemical makeup of the product [19]. There are many major pathophysiological mechanisms of IBS [20]. At present, the treatment of IBS is still a considerable challenge in clinical practice. In recent years, peppermint oil has been used to prevent irritable bowel syndrome, but the exact mechanism is unknown. This study presented a methodical distillation and analysis for the key volatile chemical constituents in PO using the approach and the Steam Distillation methodology, which were crucial in the advancement and modernization for PO compositions. In order to shed light on the potential mechanism of PO within the therapy of IBS, network pharmacology was added as well. This work expanded the understanding of PO collection and research's useful components and the foundation of its therapeutic material while offering a novel method for PO extraction and investigation employing SD in conjunction with GC-MS. In this study, GC-MS technology detected 13 chemical components from PO, among which ten quality values exceeded 90, and 8 genes were identified including NOS2, PGE2, MAPK3, CYP2A6, AKT1, EGFR, TNF, and AR.

The major ways that PO treats dysfunctional illnesses like IBS include blocking Ca^{2+} influx *via* sarcolemma L-type Ca^{2+} channels [17], affecting the enteric nervous system [18] to prevent smooth muscle contraction in the

gastrointestinal tract, or using transient receptor possible ion channels for regulating visceral sensitivity [21]. The active ingredient of PO is the cyclic terpene menthol, which can induce mucus secretion and maintain the production of prostaglandin E2, reduced levels for pro-inflammatory cytokines like interleukin IL-6 and tumor necrosis factor (TNF)-α along with elevated levels for the anti-inflammatory cytokine interleukin-10. Individuals who would like rapid relief from their IBS symptoms should consider having menthol sent directly to the small intestine [22]. Through network pharmacology, two main components of PO within the medical management about IBS were identified, and menthol represented the primary component that comprised the essential oil of peppermint, which was accountable mainly for the agent's anti-spasmolytic effects [23]. 8 potential targets associated with the medical management for IBS were identified including NOS2, PGE2, MAPK3, CYP2A6, AKT1, EGFR, TNF, and AR. The important possible targets including biological processes, molecular functions, and cellular components were examined by executing GO enriched findings in order to anticipate the way that PO works in the therapy for IBS. The G-protein coupled receptor signaling process, cyclic nucleotide second messenger, inflammatory response, beneficial control of ERK1 and ERK2 cascade, and phospholipase C-activating G-protein coupled receptor signaling process had been among the numerous biological processes that the major hubs had been shown to be significant players according to the GO terms ($P < 0.05$). The immune system, inflammatory response, gut microbiota, and other molecular processes were the primary foci of the eight active targets. According to the findings of the pathway study and KEGG pathway database investigation, NF-κB, which is controlled through mitogen-activated protein kinases (MAPKs) and stimulates gene transcription within the nucleus, was a key player within the controlling for inflammatory reactions, which suggested that immune regulation was one of the main possible mechanisms of PO within treating IBS [24]. Pro-inflammatory cytokines such as interleukin IL-6, IL-1β, and tumor necrosis factor-α (TNF-α) are secreted more frequently as a result of it. Inflammation requires the activation both chemokines and cytokines that are produced when the MAPK/NF-κB signaling pathway is activated [25]. The most powerful influence on STAT3 phosphorylation is IL-6. This cytokine storm sets off a cascade of events that result in a variety [26]. PO can modulate IBS through preventing the synthesis of pro-inflammatory cytokines and upregulating the levels of anti-inflammatory cytokines, the transcription of mRNA, and the generation of pro-inflammatory cytokines IL-6 and IL-1β. In animal trials, oral administration for PO may avoid xylene-induced inflammation of the intestines within mice as well as acetic acid-induced colitis within rats [27].

The focus of our research was on calcium signaling pathways. *In vitro*, intestinal neurons in mice and rats can be regulated by proteases released from the colonic in IBS individuals, and *in vivo*, this can result in somatic and visceral hypersensitivity. It appears that proteases PAR 1, PAR 2, and PAR 4 can influence nociception and pain by mobilizing calcium [28]. Colonic root ganglia (CRG) neurons' intrinsic activity and PAR 2-induced calcium inhibited by PAR 4 stimulation, along with general pain [29]. PO regulates the neuromotor function of the gastrointestinal tract by affecting the nervous system and acts as a blocking agent to reverse the effects of acetylcholine-induced activities by directly inhibiting contractility to counteract the of serotonin-induced contraction. This causes circular smooth muscle relaxation within the colon [30].

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