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ORIGINAL ARTICLE

Exploring the anti-inflammatory effect of Fructus *Gleditsia sinensis* Lam., Fructus *Gleditsiae abnormalis*, and Gymnocladus *chinensis* Baill. using SPME-GC-MS, network pharmacology, and molecular docking

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Fructus *Gleditsiae abnormalis*;
Gymnocladus chinensis Baill.;
Solid phase micro extraction-gas chromatography-mass spectrometry;
Anti-inflammatory;
Network pharmacology;
Molecular mechanism

Abstract Fructus *Gleditsia sinensis* Lam. (FGSL), Fructus *Gleditsiae abnormalis* (FGA), and *Gymnocladus chinensis* Baill. (GCB) are fruits of leguminous plants that are used in traditional medicine. Among them, FGSL and FGA are developed to different degrees, and GCB is related to them. The literature records indicate their use in the external treatment of carbuncle. Modern pharmacological studies have shown that the formation of a carbuncle is closely related to the occurrence and development of inflammation, and the volatile components contained in the FGSL/FGA drugs have significant anti-inflammatory effects. The solid phase micro extraction-gas chromatography-mass spectrometry (SPME–GC–MS) method was used to analyze the volatile components contained in FGSL, FGA, and GCB. Moreover, the molecular mechanism underlying the anti-inflammatory effects was explored based on network pharmacology and molecular docking. The SPME-GC-MS demonstrated significant differences in the chemical constituents and percentage contents among FGSL, FGA, and GCB. 13 common volatile components were identified in FGSL, FGA, and GCB. Through network pharmacology and molecular docking, the differences in the anti-inflammatory mechanism of FGSL, FGA, and GCB were initially revealed. This study laid

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the foundation for further study of FGSL, FGA, and GCB. Simultaneously, it also provided a reference for the correct use of FGSL, FGA, and GCB in the clinic.

1. Introduction

Fructus *Gleditsia sinensis* Lam. (FGSL) and Fructus *Gleditsiae abnormalis* (FGA) are derived from the leguminous plant *Gleditsia sinensis* Lam. Where in the FGSL comprises mature fruits, and the FGA comprises sterile fruits. GCB is the fruit of the leguminous plant *Gymnocladus chinensis* Baill. The FGSL, FGA, and GCB have been used in traditional Chinese medicine and reported to possess the same effect, application, and dosage; the decoction can be used internally to expel phlegm and resuscitate, and externally to treat the swollen toxin in carbuncles (National Pharmacopoeia Committee, 2020a, 2020b; An Editorial Committee of the Administration Bureau of Traditional Chinese Medicine, 1999). In addition, modern research has shown that the saponins in FGSL, FGA, and GCB have anti-inflammatory and antiviral pharmacological effects. Furthermore, FGSL can regulate immune function and prevent cardiovascular disease (Sheng, 1997; Liu and Yuan,1996). FGA can resist allergies (Dai, et al., 2002; Hou et al., 2006a, 2006b; Xia et al., 2005). GCB has the effects of inhibiting enzyme activity, anti-AIDS, etc. (Zhu et al., 2011; Chen et al., 2000). Modern research revolves around the saponins of FGSL, FGA, and GCB. However, there are few studies on their volatile components.

Carbuncle is a common clinical disease in traditional Chinese medicine. It refers to the evil poison trapped in *Qi* and blood and results in the blockage of *Qi* and blood. It is mostly characterized by local redness, swelling, heat, pain, and dysfunction caused by inflammation of hair follicles, skin tissue abscesses, infections, etc. In addition, the main manifestations of heat toxicity in traditional Chinese medicine also include inflammatory reactions (Liu et al., 2017). In the clinic, inflammation is treated orally or by injecting anti-inflammatory drugs. In recent years, with in-depth research, traditional Chinese medicine has the characteristics of treating small side effects and offering effective symptomatic relief. According to the efficacy and clinical application, FGSL, FGA, and GCB display obvious anti-inflammatory effects. However, their mechanism of action is not clear.

The basic research on active substances of traditional Chinese medicine has received extensive attention in recent years. Network pharmacology is a new method that combines bioinformatics, pharmacology, computer science, and other disciplines to study the mechanism of drug action. Through multiple computer software and databases, it interprets the potential targets and mechanism of action of drugs in the treatment of diseases in a multi-directional manner. It is consistent with the characteristics of traditional Chinese medicine in reflecting the integrity and systematic role of drugs (Zhang, 2020; Zhang et al., 2020; Hou et al., 2019). Studies have shown that the gas chromatography-mass spectrometry (GC–MS) method is often combined with headspace solid-phase microextraction. It combines sample extraction, enrichment, and injection and thus, improves the analysis speed. It displays high detection sensitivity and good separation effect and is widely used in the analysis of volatile components and effective components of drugs. Therefore, this study employed the SPME–GC–MS method to analyze the active volatile components in FGSL, FGA, and GCB. Moreover, network pharmacology and molecular docking research ideas were incorporated to conduct information mining on the identified chemical components. The key active ingredients, key targets, and potential signal pathways involved in the anti-inflammatory action of FGSL, FGA, and GCB were screened. The study aims to provide a reference for the in-depth analysis and clinical use of the anti-inflammatory effects of FGSL, FGA, and GCB.

2. Materials and methods

2.1. Materials

The FGSL, FGA, and GCB were collected from Kaili city, Guizhou Province (China). They were identified by Prof. Xiangpei Wang (Guizhou Minzu University). The voucher specimens were deposited at the Herbarium of Guizhou University of traditional Chinese Medicine. Helium gas was purchased from Guizhou Yagang Gas Co., Ltd. (Guizhou, China).

2.2. SPME–GC–MS analysis

Analyses were carried out with the HP6890/5975C GC/MS combination instrument (Agilent Company, USA). 1 g of DZJ and ZYZ, 3 g of FZJ were crushed and placed in a 25-mL sample bottle of solid-phase microextraction. Next, a manual sampler equipped with a 2 cm Stable Flex fiber of 50/30 μm DVB/CAR/PDMS (divinylbenzene/Carboxen/polydimethylsiloxane) was inserted into the injection port of the gas chromatograph (250 °C) instrument after 60 min of headspace extraction under the heating condition of a flat plate at 60 °C; the sample was introduced to the GC/MS by thermal desorption. The volatile compounds were separated on an Agilent HP–5MS capillary column (60 m × 0.25 mm × 0.25 μm). High-purity helium (99.999%) served as the carrier gas. The temperature of the vaporization chamber was set at 250 °C, the column front pressure was15.85 psi, and the carrier gas flow rate was 1.0 mL/min. The column temperature was held at 40 °C for 2 min and then increased to 180 °C at the rate of 3.5 °C/min; this was followed by an increase to 260 °C at 10 °C/min. The running time was 50 min. The injection method was splitless. The solvent delay time was 3 min.

A full scan mode was applied to identify all target compounds. The parameters were as follows: ion source: EI, ion source temperature: 230 °C, quadrupole temperature: 150 °C, electron energy: 70 eV, emission current: 34.6 μA, multiplier voltage: 1847 V, interface temperature: 280 °C, and the mass scan range: 29–500 amu. The samples were analyzed under the above-mentioned GC–MS conditions, and the chromatograms and mass spectral data were recorded. The chromatographic peaks were identified based on MS computer data system retrieval, collating Nist2005 and Wiley 275 standard mass spectra. The peak area normalization method was used to determine the relative mass fraction of each chemical component.

2.3. Network pharmacology and molecular docking

2.3.1. Screening active ingredient

The compounds were imported into the Traditional Chinese Medicine System Pharmacology Analysis Platform (TCMSP,

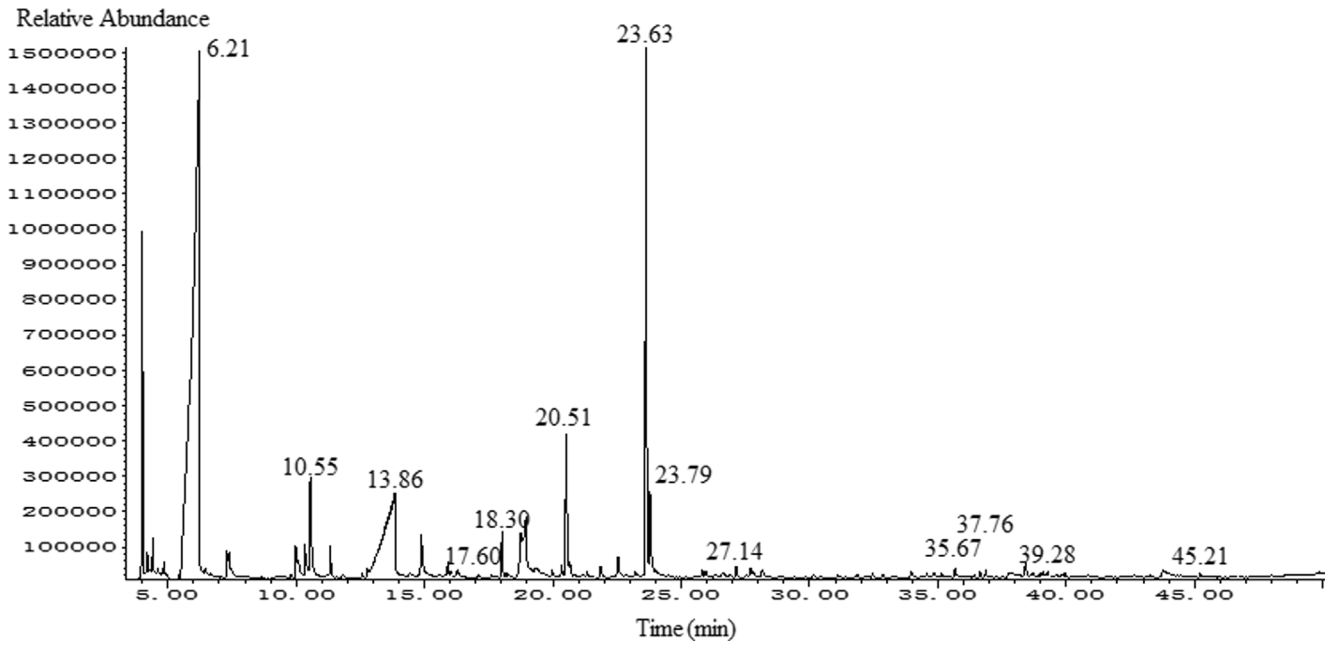

Fig. 1 The total ion chromatogram of GC–MS of FGSL.

https://tcmspw.com/tcmsp.php) to screen the active ingredients with oral availability (OB > 30% (Bryan et al., 2014)) from FGSL, FGB, and GCB.

2.3.2. *Retrieval of target proteins*

The target proteins of these active ingredients in FGSL, FGB, and GCB were predicted using the TCMSP and PharmMapper (https://www.lilab-ecust.cn/pharmmapper/) database. The target was searched using "Homo sapiens" as the limited condition. Using "Inflammation" as the keyword, inflammation-related targets in Gene Cards (https://www.genecards.org/), OMIM (https://mirror.omim.org/), and other databases were retrieved after deduplication. The above targets were converted and queried into the UniProtID format with "Homo sapiens" as the qualifying condition in the UniProt database (https://www.uniprot.org/).

2.3.3. *Target analysis and network diagram construction*

The above targets were imported into the STRING database (https://string-db.org/) for protein interaction network analysis and with the species to "Homo sapiens." The Degree, Betweenness centrality, and Closeness centrality for each node

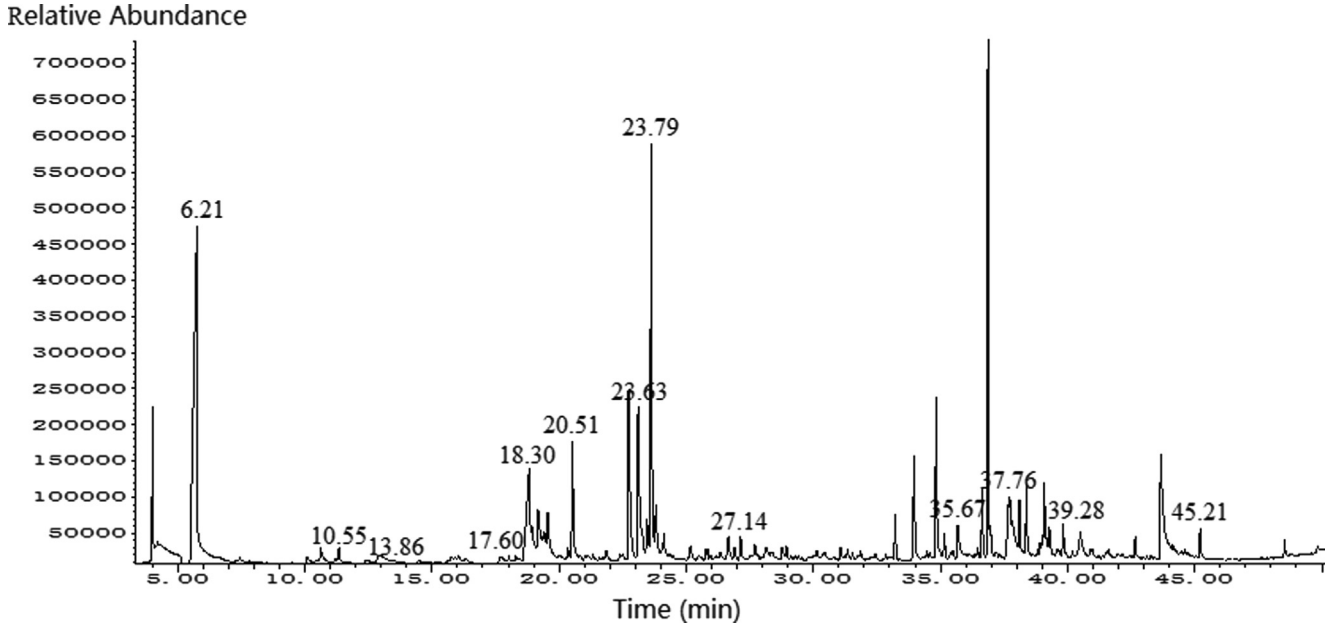

Fig. 2 The total ion chromatogram of GC–MS of FGA.

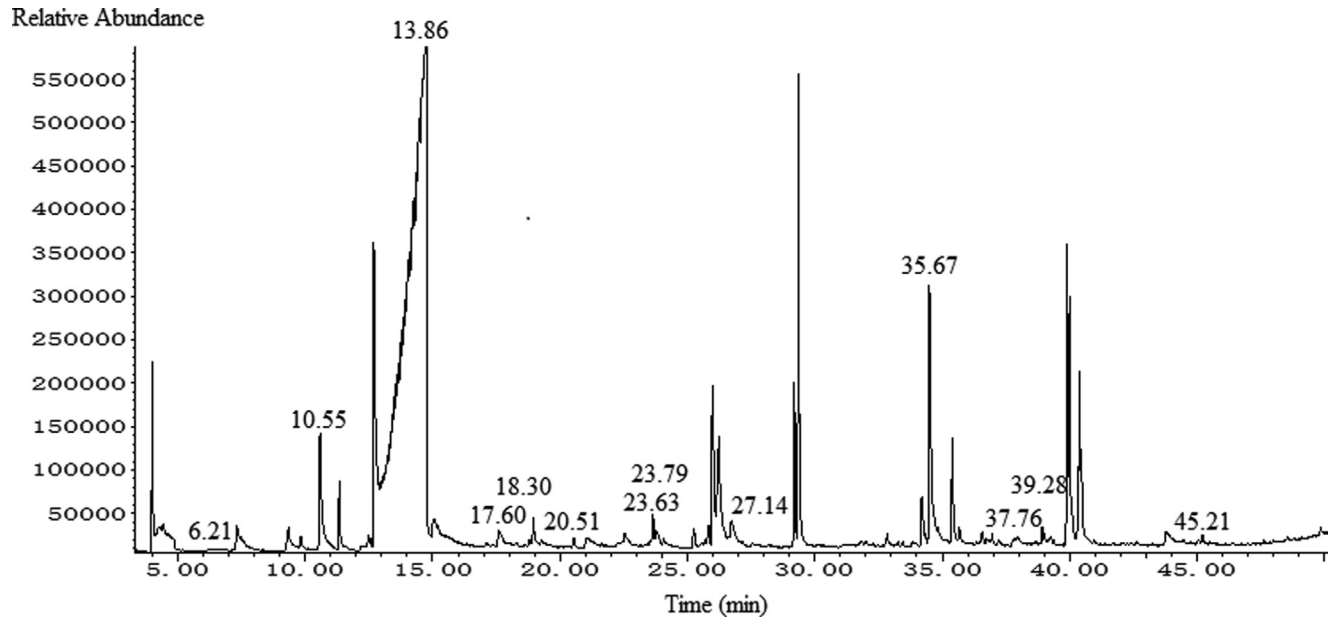

Fig. 3 The total ion chromatogram of GC–MS of GCB.

were evaluated by the Cytoscape 3.6.1 software, and the anti-inflammatory target points of FGSL, FGB, and GCB were obtained. The active ingredients, targets, and diseases of FGSL, FGB, and GCB were imported into the Cytoscape 3.6.1 software to construct a ''drug–component–disease–targe t'' network.

2.3.4. GO and KEGG enrichment analysis

The target points selected above were subjected to the Kyoto Encyclopedia of Genes and Genome (KEGG) pathway enrichment analysis and the Gene Ontology (GO) biological process analysis in the DAVID database (https://david.ncifcrf.gov/). The signal pathways closely related to the targets were imported into the Cytoscape 3.6.1 software to construct a ''target-signal pathway'' network diagram.

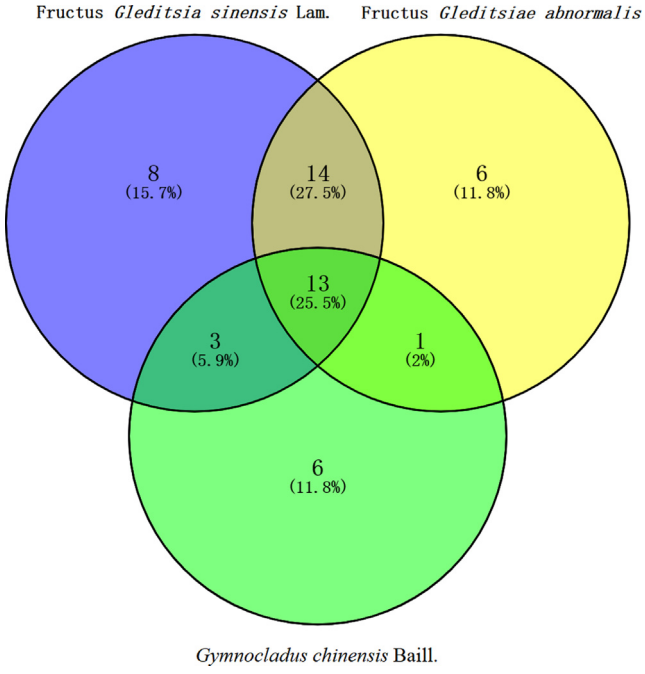

Fig. 4 The venn diagram of the target points of FGSL, FGA, and GCB.

2.3.5. Molecular docking

The crystal structures of the targets and the chemical structures of the composition were obtained from the PDB (https://www.rcsb.org) and the ZINC database (https://zinc.docking.org/). Molecular docking was performed using the AutoDock software. The water molecules and atoms were removed from the target receptors by the PyMOL software. Docking was enabled using the AutoGrid and AutoDock modules to obtain the affinities.

3. Results

3.1. SPME–GC–MS analysis

The SPME–GC–MS total ion chromatograms of FGSL, FGA, and GCB are shown in Figs. 1–3. A total of 60 volatile components were isolated from FGSL, and 38 components were identified, 61 volatile components were isolated from FGA, and 34 components were identified, 38 volatile components were isolated from GCB, and 23 components were identified. There were eight terpenes and 26 other compounds such as aldehydes, alkanes, and alcohols in FGA. There were five aldehyde compounds, five esters, and 12 other compounds such as alcohols, alkanes, ethers, and terpenes in GCB. There were 13 common compounds in FGSL, FGA, and GCB, such as hexanal, linalool, limonene, etc. And there were eight unique ingredients in FGSL, six unique ingredients in FGA, and six unique ingredients in GCB. The Venn diagram of the ingredients of FGSL, FGA, and GCB is shown in Fig. 4. A normalization method was used to determine the relative mass

Table 1 Volatile components and percentage content of FGSL, FGA and GCB.

NO.	Retention time (min)	Name of compound	Molecular formula	Molecular weight	Retention index	Percentage content (%)		
						Fructus *Gleditsia sinensis* Lam.	Fructus Gleditsiae Abnormalis	*Gymnocladus chinensis* Baill.
1	4.78	Dimethyl sulfide	C2H6S	62	520	0.09	–	–
2	6.21	Acetic acid	C2H4O2	60	610	49.89	18.61	0.24
3	6.46	3-Methylbutanal	C5H10O	86	652	0.12	–	–
4	7.38	Pentanal	C5H10O	86	699	0.51	–	–
5	7.47	Furan, 2-ethyl-	C6H8O	96	703	0.23	–	0.60
6	9.81	methyl 2-methylbutanoate	C6H12O2	116	775	0.08	–	0.23
7	9.98	2,3-Butanediol	C4H10O2	90	788	0.80	0.39	–
8	10.55	Hexanal	C6H12O	100	800	2.49	0.89	2.07
9	12.44	Furfural	C5H4O2	96	833	–	0.34	0.15
10	12.57	Butanoic acid, 2-methyl-, ethyl ester	C7H14O2	130	849	0.11	–	0.10
11	13.86	Butanoic acid, 2-methyl-	C5H10O2	102	861	13.67	1.00	62.16
12	14.47	Styrene	C8H8	104	893	0.09	0.24	–
13	14.78	Heptanal	C7H14O	114	901	0.05	–	–
14	15.78	Pyrazine, 2,3-dimethyl-	C6H8N2	108	926	–	0.26	–
15	15.79	Hexanoic acid, methyl ester	C7H14O2	130	925	0.04	–	–
16	17.60	Benzaldehyde	C7H6O	106	962	0.17	0.49	0.46
17	18.02	sabinene	C10H16	136	974	0.79	0.19	–
18	18.30	1-Octen-3-ol	C8H16O	128	980	0.09	0.24	0.07
19	18.80	Furan, 2-pentyl-	C9H14O	138	993	–	–	0.10
20	18.95	Hexanoic acid	C6H12O2	116	990	3.35	4.85	–
21	19.33	Octanal	C8H16O	128	1003	0.23	–	–
22	19.39	Pyrazine, trimethyl-	C7H10N2	122	1004	–	1.18	–
23	20.34	p-Cymene	C10H14	134	1025	0.25	0.23	–
24	20.51	Limonene	C10H16	136	1030	3.09	2.52	0.14
25	20.66	Eucalyptol	C10H18O	154	1032	0.28	–	–
26	21.05	Benzyl alcohol	C7H8O	108	1036	–	–	0.51
27	21.87	γ-Terpinene	C10H16	136	1060	0.30	0.28	–
28	23.11	2,3-Dimethyl-5-ethylpyrazine	C8H12N2	136	1090	–	4.77	–
29	23.63	Linalool	C10H18O	154	1099	9.16	7.24	0.32
30	23.77	Butanoic acid, 2-methyl-, 2-methylbutyl ester	C10H20O2	172	1105	–	–	0.11
31	23.79	Nonanal	C9H18O	142	1104	1.97	1.31	0.12
32	25.76	(+)-2-Bornanone	C10H16O	152	1144	–	0.16	–
33	25.95	(+)-Citronellal	C10H18O	154	1152	0.19	–	–
34	26.66	*endo*-Borneol	C10H18O	154	1167	0.22	0.76	–
35	26.91	dl-Menthol	C10H20O	156	1174	0.08	0.34	–
36	27.14	Terpinen-4-ol	C10H18O	154	1177	0.28	0.55	0.07
37	27.82	Dodecane	C12H26	170	1200	0.13	0.16	–
38	28.15	Decanal	C10H20O	156	1206	0.27	0.51	–
39	28.94	β-Cyclocitral	C10H16O	152	1220	0.06	0.30	–
40	29.19	*cis*-3-Hexenyl-.alpha.-methylbutyrate	C11H20O2	184	1234	–	–	1.31
41	29.35	Butanoic acid, 2-methyl-, hexyl ester	C11H22O2	186	1236	–	–	3.93
42	30.14	Linalyl acetate	C12H20O2	196	1257	0.08	0.20	–
43	31.86	Tridecane	C13H28	184	1300	0.12	0.17	–
44	34.49	2(3H)-Furanone, dihydro-5-pentyl-	C9H16O2	156	1363	–	–	3.58
45	35.15	Copaene	C15H24	204	1376	0.11	0.55	–
46	35.67	Tetradecane	C14H30	198	1400	0.23	1.29	0.23
47	36.86	Caryophyllene	C15H24	204	1419	0.13	8.36	–
48	37.76	Ethanone, 1-(2-hydroxy-4-methoxyphenyl)-	C9H10O3	166	1438	0.42	1.14	0.13

(*continued on next page*)

Table 1 (*continued*)

NO.	Retention time (min)	Name of compound	Molecular formula	Molecular weight	Retention index	Percentage content (%)		
						Fructus *Gleditsia sinensis* Lam.	Fructus Gleditsiae Abnormalis	*Gymnocladus chinensis* Baill.
49	38.38	Alloaromadendrene	C15H24	204	1461	–	1.34	–
50	39.28	Pentadecane	C15H32	212	1500	0.16	0.50	0.07
51	42.66	Caryophyllene oxide	C15H24O	220	1581	–	0.38	–
52	45.21	Heptadecane	C17H36	240	1700	0.08	0.58	0.11
Total						90.42	62.34	76.85

fraction of each chemical component, and the information of the volatile components contained in the FGSL, FGA, and GCB was obtained. The results are shown in Table 1.

It can be seen from Table 1 that 40, 34, and 23 volatile components have been identified for FGSL, FGA, and GCB, respectively. Among them, there were 13 common components, mainly acetic acid, hexanal, butanoic acid, 2-methyl-, limonene, linalool. The content of acetic acid in FGSL, FGA, and GCB is 49.892%, 18.606%, 0.244% respectively. The content of hexanal in FGSL, FGA, and GCB is 2.489%, 0.894%, 2.073% respectively. The content of butanoic acid, 2-methyl- in FGSL, FGA, and GCB is 13.675%, 0.998%, 62.164% respectively. The content of limonene in FGSL, FGA, and GCB is 3.088%, 2.520%, 0.145% respectively. The content of linalool in FGSL, FGA, and GCB is 9.159%, 7.236%, 0.316% respectively.

FGSL mainly contain acetic acid (49.892%), butanoic acid, 2-methyl- (13.675%), linalool (9.159%), Among them, acetic acid is mainly present in the form of esters in the fruit, indicating that the FGSL are mainly esters and alcohol compounds. FGA mainly contain acetic acid (18.606%), caryophyllene (8.361%), linalool (7.236%), illustrating that FGA mainly contains esters, terpenes and alcohol compounds. GCB mainly contain butanoic acid,2-methyl- (62.164%), butanoic acid,2-methyl-, hexyl ester (3.930%), 2(3H)-Furanone, dihydro-5-pentyl- (3.580%), illustrating that GCB mainly contains esters, terpenes and alcohol compounds esters and aldehydes.

3.2. Network pharmacology and molecular docking

3.2.1. Construction of active components

There were 15, 11, and 7 active ingredients in FGSL, FGA, and GCB, respectively. They contained five common ingredients such as hexanal, linalool, nonanal, limonene, and benzaldehyde. The results are shown in Table 2.

3.2.2. Retrieval of target proteins

A total of 166 targets related to the volatile active ingredients of FGSL, 27 for FGA, and 29 for GCB, were obtained. From GeneCards and OMIM databases, 10,272 inflammation-related targets were obtained by deduplication. There were 128, 21, and 20 anti-inflammatory targets for the volatile active ingredients of FGSL, FGA, and GCB, respectively.

3.2.3. Target analysis and network diagram construction

The protein interaction analysis of the above targets was analyzed by the STRING database. The analysis results were

Table 2 Active components of FGSL, FGA and GCB.

NO.	MolID	Name of compound	OB (%)	Number of targets		
				Fructus *Gleditsia sinensis* Lam.	Fructus Gleditsiae Abnormalis	*Gymnocladus chinensis* Baill.
1	MOL002046	Hexanoic acid	73.08	3	3	–
2	MOL001335	Benzyl alcohol	58.68	–	–	10
3	MOL005970	Eucalyptol	60.62	26	–	–
4	MOL001604	Linalool	58.18	8	8	8
5	MOL002379	Pentanal	59.53	1	–	–
6	MOL000666	Hexanal	55.71	6	6	6
7	MOL000066	Alloaromadendrene	50.62	–	5	–
8	MOL000116	Nonanal	40.28	4	4	4
9	MOL004627	Hexanoic acid, methyl ester	52.44	2	–	–
10	MOL001193	Caryophyllene oxide	45.75	–	7	–
11	MOL000023	Limonene	39.84	13	13	13
12	MOL005315	(+)-Citronellal	50.78	1	–	–
13	MOL000722	β-Cyclocitral	40.36	0	0	–
14	MOL000172	Furfural	34.35	–	4	4
15	MOL000708	Benzaldehyde	32.63	5	5	5
16	MOL002484	3-Methylbutanal	44.71	87	–	–
17	MOL000202	γ-Terpinene	33.02	7	7	–
18	MOL002025	Acetic acid, methyl ester	40.17	37	–	–
19	MOL002850	Butylated Hydroxytoluene	40.02	19	–	–

Table 3 Related parameters of potential target proteins of FGSL, FGA and GCB.

	NO.	Name	Protein names	Betweenness Centrality	Closeness Centrality	Degree	UniProtID
FGSL	1	SRC	Proto-oncogene tyrosine-protein kinase Src	0.1759636	0.52892562	21	P12931
	2	TNF	Tumor necrosis factor	0.21411127	0.53781513	20	P01375
	3	F2		0.13924273	0.52892562	20	P00734
	4	ACHE		0.10423389	0.50793651	19	P22303
	5	GPT		0.16118246	0.49612403	14	P24298
	6	SLC6A4		0.03239261	0.41290323	14	P31645
	7	CHRM1		0.04586799	0.46376812	14	P11229
	8	ADRA1B		0.00617478	0.42384106	13	P35368
	9	ADRB2		0.05708121	0.48120301	13	P07550
	10	ADRA1D		0.00548488	0.42105263	12	P25100
	11	ADRA1A		0.00548488	0.42105263	12	P35348
	12	HTR2A		0.00622669	0.42105263	12	P28223
	13	PTGS2		0.0198051	0.47058824	12	P35354
	14	JUN		0.04380317	0.46376812	12	P05412
	15	SLC6A3		0.00480293	0.38554217	10	Q01959
	16	OPRM1		0.06479608	0.46715328	10	P35372
	17	CHRM2		0.04180519	0.4	10	P08172
	18	BCHE		0.01939574	0.42105263	10	P06276
FGA	1	TNF		0.41140351	0.45454545	8	P01375
	2	JUN		0.42982456	0.5	6	P05412
	3	PTGS2		0.04824561	0.41666667	5	P35354
	4	CHRM1		0.47807018	0.46511628	5	P11229
GCB	1	TNF		0.46296296	0.51351351	8	P01375
	2	JUN		0.37231969	0.5	6	P05412
	3	CHRM2		0.35282651	0.42222222	5	P08172
	4	PTGS2		0.03996101	0.41304348	5	P35354
	20	PRSS3		0	0.26760563	1	P35030

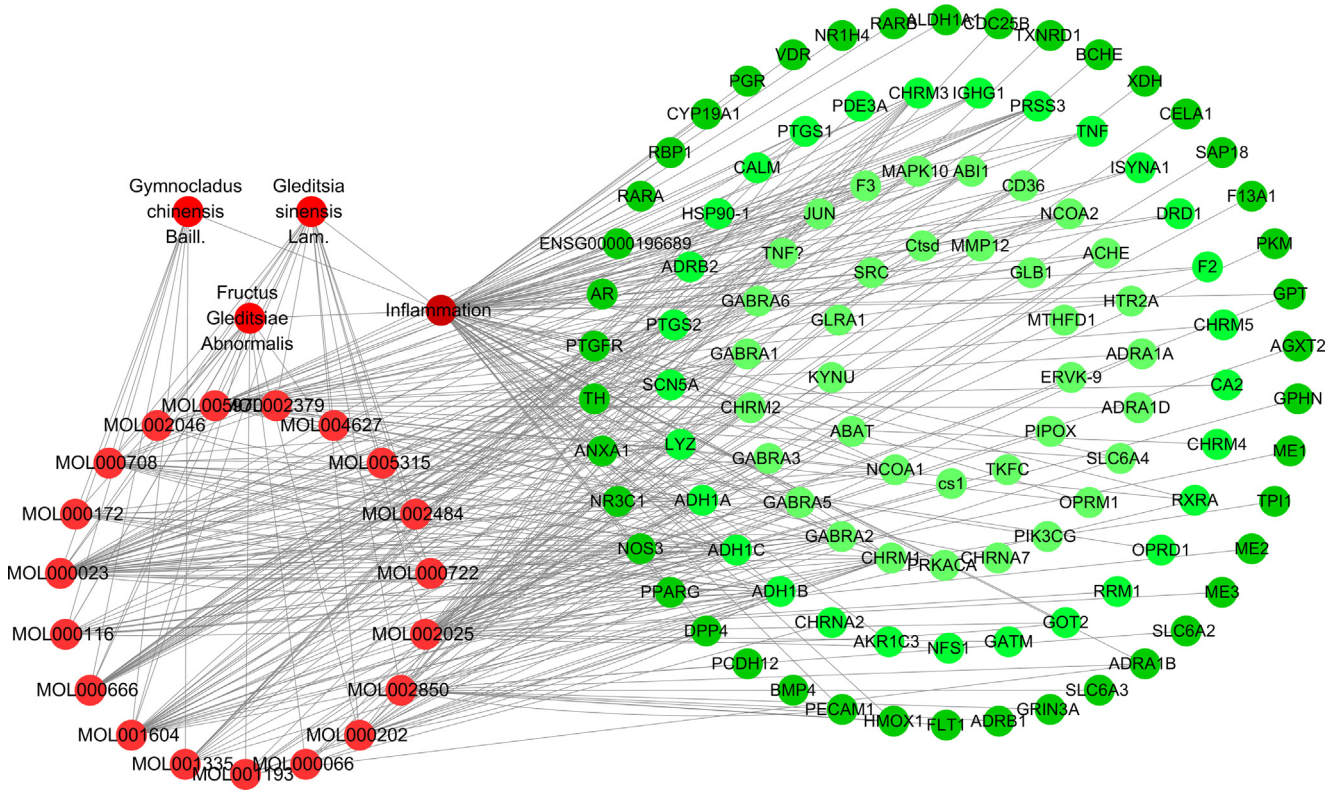

Fig. 5 The "drug-component-disease-target" network diagram of the anti-inflammatory effects of FGSL, FGA and GCB.

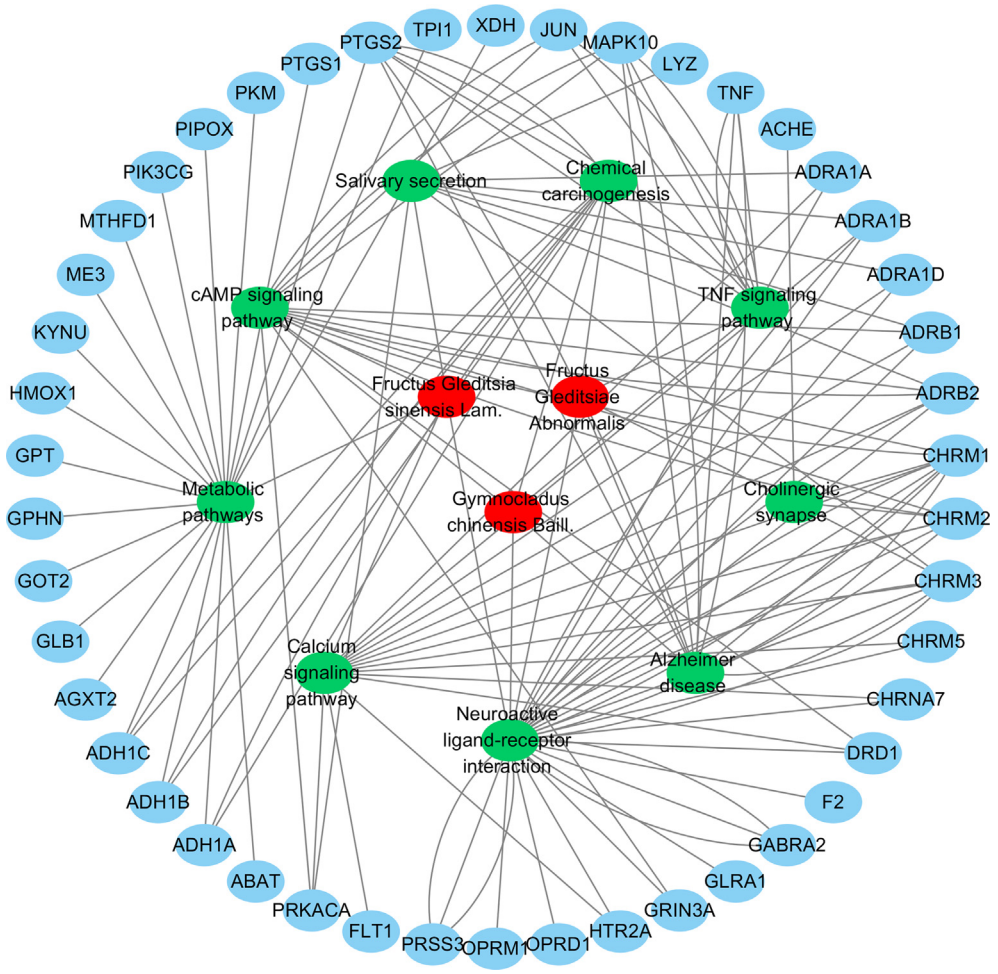

Fig. 6 The "drug-signal pathway-target" network diagram of FGSL, FGA and GCB.

imported into the Cytoscape 3.6.1 software to obtain the degree, betweenness centrality, and closeness centrality values of each target. The targets of FGSL (Degree $\geq$ 10), FGA, and GCB (Degree $\geq$ 5) are shown in Table 3. The network diagram of the "drug-component-disease-target" constructed in Cytoscape 3.6.1 is described in Fig. 5. The analysis results demonstrated the presence of 18 identical targets for FGSL, FGA, and GCB. Among these, TNF, JUN, and PTGS2 had the highest Degree value. There were 21 identical targets (TNF, ACHE, CHRM1, ADRA1B, and PTGS2) by paired comparison between FGSL and FGA, 20 identical targets (TNF, CHRM1, ADRB2, PTGS2, JUN) between FGSL and GCB, and 18 identical targets (TNF, JUN, PTGS2, CHRM1, CHRM2) between FGA and GCB. Thus, differences existed in the anti-inflammatory ingredients and targets of FGSL, FGA, and GCB.

3.2.4. GO and KEGG analysis

The target proteins were subjected to enrichment analysis of the signaling pathway and Gene Ontology biological process by the DAVID database. There were 33, 21, and 23 anti-inflammatory signal pathways for the volatile components of FGSL, FGA, and GCB ($P < 0.05$), the "drug-signal pathway-target" network diagram constructed by the Cytoscape 3.6.1 software is shown in Fig. 6. There were 37, 13, and 12 bioprocesses in FGSL, FGA, and GCB, respectively ($P < 0.005$) (Fig. 7). There were 27, 11, and 10 cell composition functions and 42, 19, and 15 molecular functions for FGSL, FGA, and GCB, respectively (Figs. 8, 9). A total of ten identical signal pathways, six biological processes, nine-cell composition, and ten molecular functions were identified in FGSL, FGA, and GCB. The targets involved in the most closely regulated signaling pathways and biological processes were "neuroactive ligand-receptor interaction" and "adenylate cyclase-activating adrenergic receptor" signaling pathways. The results indicated that the signal pathway was the same, but the number of targets involved in the signal pathway was different. There were 19, 6, and 6 targets involved in regulating the signal pathways of FGSL, FGA, and GCB.

3.2.5. Molecular docking

The six targets at the top three levels in the degree value that docked with the active ingredients of FGSL, FGA, and GCB were named SRC, TNF, ACHE, JUN, PTGS2, and F2 in the protein network analysis results (Table 4). Binding energy less than 0 indicated that the ligand molecule could spontaneously bind to the receptor target. Binding energy less than –5 indicated a good binding (Li et al., 2021). All the active components in the table were combined with the targets, and

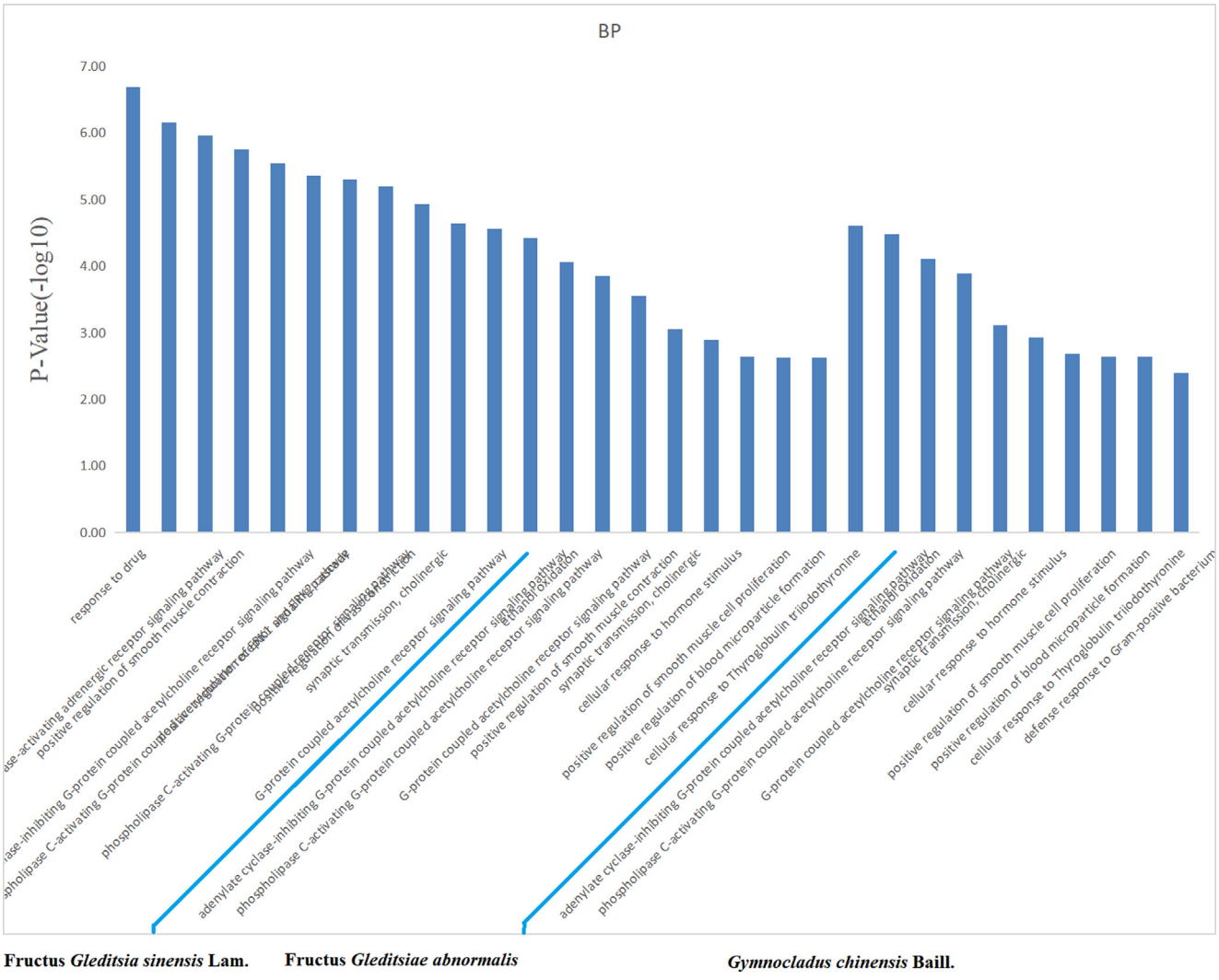

Fig. 7 Enrichment of biological processes of FGSL, FGA and GCB.

the results are shown in Table 4. The best molecular docking diagrams are shown in Fig. 10.

4. Discussion

In this study, GC–MS was combined with network pharmacology and molecular docking technology to explore the potentially active anti-inflammatory ingredients and their molecular mechanisms in FGSL, FGA, and GCB preliminarily. Through GC–MS analysis, 15 active ingredients from FGSL, 11 from FGA, and 7 from GCB were identified to possess anti-inflammatory action. After mining and analyzing databases such as TCMSP, BATMAN–TCM, STRING, DAVID, etc., 65, 21, and 20 anti-inflammatory targets of FGSL, FGA, and GCB were predicted.

There was no significant difference between FGSL and FGA in terms of efficacy and dosage, but they are still used as two drugs in clinical practice. Additionally, modern studies have shown that the total saponins and the content of different solvent extracts in the two compounds are not significantly different (Yang, 2004). GCB is related to FGA and GCB and has similar functions and applications. However, the GC–MS analysis results of this study illustrated differences in the volatile components of FGSL, FGA, and GCB. Moreover, their anti-inflammatory active ingredients and target points were also different. There are five identical active ingredients of FGSL, FGA, and GCB (Hexanal, Linalool, Limonene, Nonanal, and Benzaldehyde). Among them, the specific active components in FGSL are eucalyptol, (+)-citronellal, pentanal, etc.; those in FGA are alloaromadendrene, caryophyllene oxide; and that in GCBs is benzyl alcohol. Studies have shown that linalool has broad-spectrum antibacterial and antifungal activity (Pattnaik et al., 1997). Limonene has bactericidal and antioxidant activities. Chen (Chen et al., 2019) and others found that the main component of rosemary herb essential oil is eucalyptol, which can be used to treat arthritis. Caryophyllene oxide has a good anti-gastric ulcer effect (Duan et al., 2015).

According to the analysis results of the target points, the FGSL, FGA, and GCB have the same target points (TNF, JUN, PTGS2, etc.). In the results of protein interaction analysis, the node with the highest Degree value is regarded as the "central node" of all nodes and occupies an important position in the target interaction. Here, SRC, a unique action target of FGSL, has the largest degree among the action targets, while

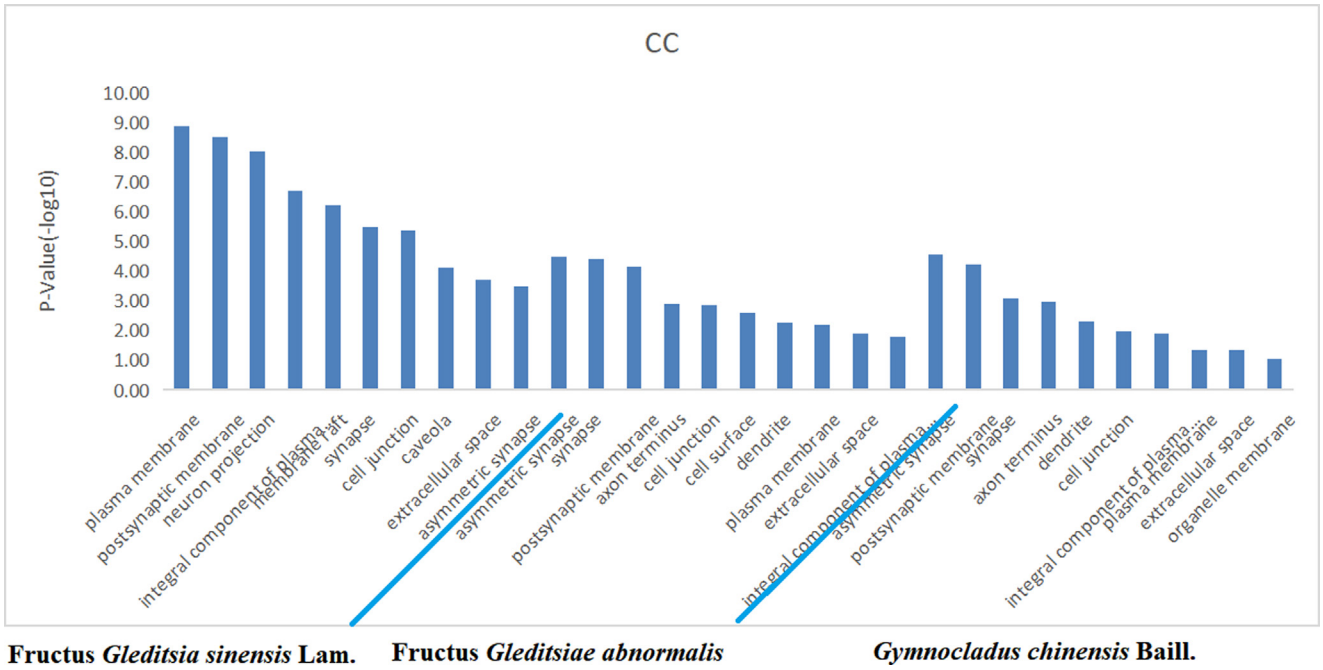

Fig. 8 Enrichment of cell composition of FGSL, FGA and GCB.

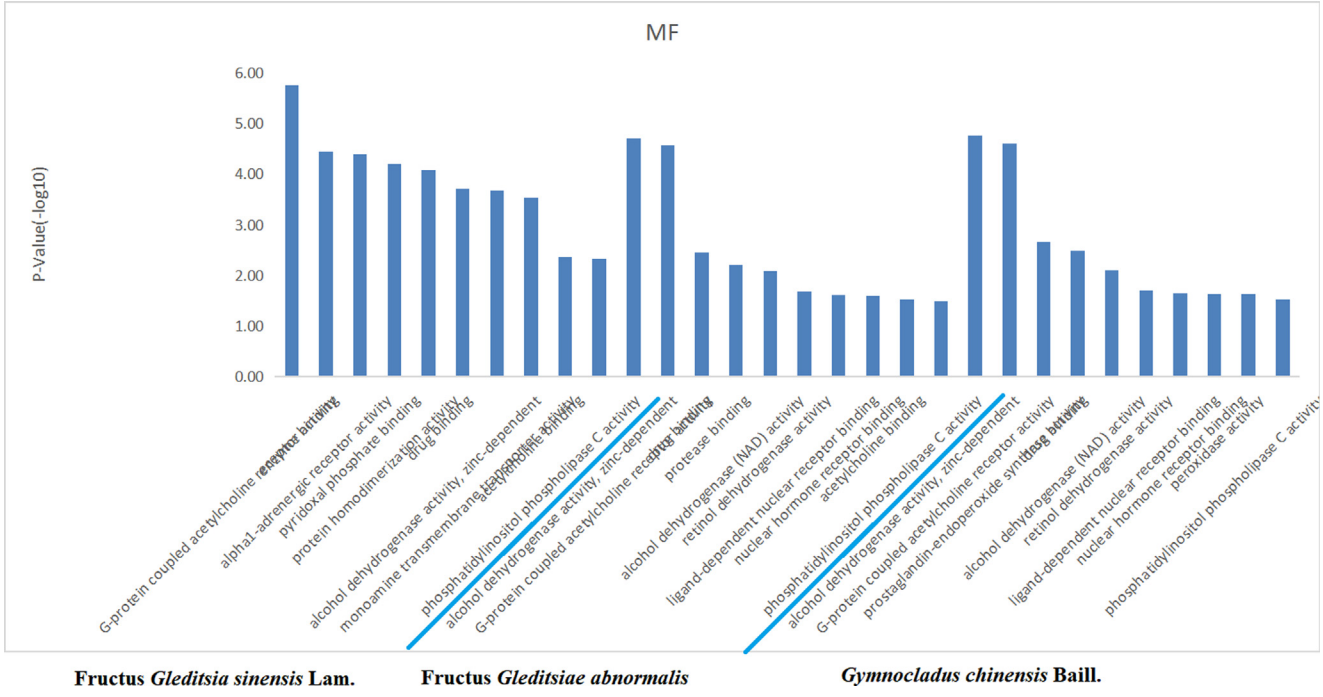

Fig. 9 Enrichment of molecular functions of FGSL, FGA and GCB.

TNF is the action target with the largest degree in FGA and GCB. SRC–1 from the steroid receptor co-activator (SRC) family acts as a transcriptional co-activator, which can simultaneously bind to the nuclear receptor GR and nuclear transcription factors NF–κB and AP–1 to play a dual role in anti-inflammation and inflammation (Leo and Chen, 2000; Tian, 2019). In addition, studies have shown that SRC–1 can also inhibit the expression of inflammatory factors such as IL–6, IL–1β, and TNF–α. TNF–α is an important pro-inflammatory cytokine. Many drugs exert anti-inflammatory effects by inhibiting the expression of TNF–α. For example, polysaccharides of the *Ginkgo biloba* leaf exert anti-inflammatory effects by inhibiting the expression of TNF–α (Martin et al., 2007).

Table 4 Binding energy of active components FGSL, FGA and GCB.

Term	Name of compound	Affinity (kcal/mol)					
		SRC	TNF	ACHE	JUN	PTGS2	F2
FGSL&FGA&GCB	Hexanal	−2.25	−2.78	−2.69	−2.72	−2.49	−3.42
	Linalool	−2.16	−2.39	−2.76	−3.45	−1.96	−2.75
	Nonanal	−1.8	−1.95	−2.25	−2.3	−2.45	−2.23
	Limonene	−3.83	−3.75	−3.96	−4.07	−6.13	−4.09
	Benzaldehyde	−2.67	−2.92	−3.05	−2.93	−3.59	−3.25
FGSL&FGA	Hexanoic acid	−0.7	−0.84	−0.37	−1.65	−1.15	−1.74
	.beta.-Cyclocitral	−3.48	−3.32	−4.7	−4.48	−3.64	−4.6
	.gamma.-Terpinene	−3.59	−3.72	−4.32	−4.31	−3.64	−4.28
FGA&GCB	Furfural	—	−2.94	−3.51	−3.24	−2.98	–
FGSL	Eucalyptol	−3.69	−3.86	−4.7	–	–	−5.13
	Pentanal	−2.01	−2.36	−2.39	–	–	−2.38
	Hexanoic acid, methyl ester	−2.12	−2.76	−2.05	–	–	−2.58
	(+)-Citronellal	−2.55	−2.76	−2.52	–	–	−3
	3-Methylbutanal	−2.32	−2.73	−2.52	–	–	−2.5
	Butylated Hydroxytoluene	−3.09	−3.09	−3.46	–	–	−3.51
	Acetic acid, methyl ester	−2.25	−2.5	−2.36	–	–	−2.08
FGA	Caryophyllene oxide	–	−4.24	−5.19	−5.87	−4.12	–
	Alloaromadendrene	–	−4.32	−5.39	−4.86	−4.22	–
GCB	Benzyl alcohol	–	−2.22	–	−2.93	−3.38	–

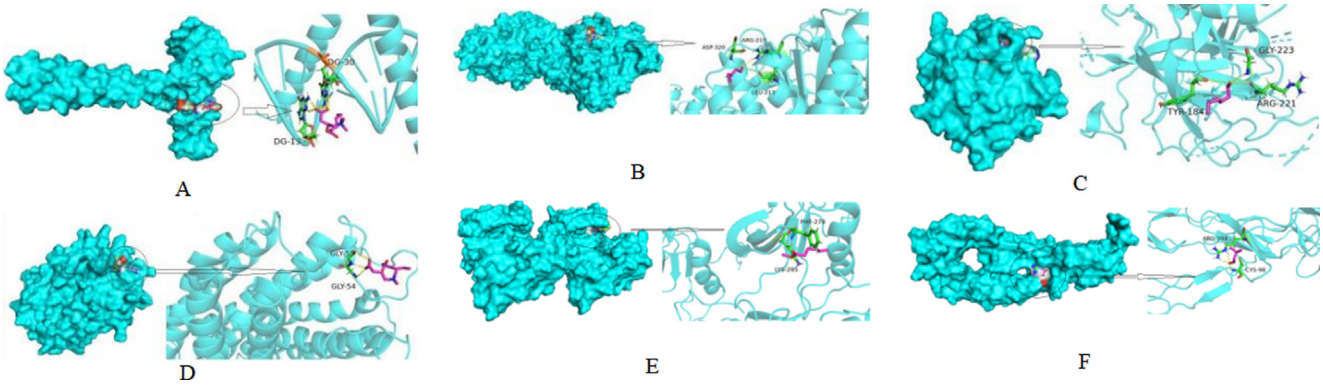

Fig. 10 Molecular docking results of FGSL, FGA and GCB. (A: Hexanoic acid-JUN; B: Pentanal-ACHE; C: Hexanal-F2; D: Hexanoic acid-PTGS2; E: Hexanoic acid, methyl ester-SRC; F: 3-Methylbutanal-TNF).

The results of GO and KEGG analysis demonstrated that the FGSL, FGA, and GCB have the same signal pathway, the neuroactive ligand-receptor interaction signal pathway, which is more closely related; the same genes Chrm1, Chrm2, and Chrm3are enriched in this pathway. Studies have shown that the neuroactive ligand-receptor interaction is a collection of all receptors and ligands related to the intracellular and extracellular signaling pathways on the plasma membrane[34]. The expression of Chrm3, DRD5, and HTR1B affects the pathway. For example, the up-regulation of Chrm3 expression enhances cholinergic function and promotes the improvement of learning and memory abilities (Pan, 2011; Wang et al., 2007). The molecular docking results demonstrated that among the targets with the higher degree value, the SRC, PTGS2, F2, TNF, ACHE have better binding properties with the active components of FGSL, FGA, and GCB.

5. Conclusion

In this study, GC–MS was used to analyze the volatile components of different varieties (FGSL, FGA, and GCB). The results indicated differences in the species and contents of the volatile components in FGSL, FGA, and GCB. Network pharmacology and molecular docking analyzed the anti-inflammatory mechanism of the volatile components of FGSL, FGA, and GCB. This explained the characteristics of the multi–component, multi-target, and multi–pathway functions of FGSL, FGA, and GCB at the molecular level. The results also illustrated differences in the anti-inflammatory targets and signal pathways of the volatile active ingredients of FGSL, FGA, and GCB. Thus, these results have provided a reference for the correct clinical use and in–depth development and utilization of FGSL, FGA, and GCB.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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