Original Article

Identification of *in vitro-in vivo* components of Caoguo using accelerated solvent extraction combined with gas chromatography-mass spectrometry integrated with network pharmacology on indigestion

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Background: Caoguo (*Tsaoko Fructus*), a traditional Chinese medicine, is widely used as medicine and dietary spices. Volatile components are among its important bioactive constituents used to treatment of abdominal distension and pain, but the mechanism is not clear up to now. The purpose of this study was to develop a simple, sensitive, and accurate method to analyze and identify components of Caoguo *in vitro* and *in vivo*, and further investigate the therapeutic mechanism of Caoguo on indigestion using network pharmacology.

Methods: Caoguo were extracted by accelerated solvent extraction (ASE) and n-hexane:ethyl acetate (1:1, v/v) was selected as the extraction solvent. Gas chromatography-mass spectrometry (GC-MS) was adopted to analyze and identify the volatile components *in vitro* and *in vivo*. Network pharmacology including protein-protein network construction, Gene Ontology (GO) enrichment and pathway enrichment analysis and component-target-pathway network construction was applied.

Results: By comparing the retention times and mass spectrometry data, as well as retrieving the reference literature, a total of 169 components were tentatively identified in Caoguo extract and 43 components were identified in rats plasma samples for the first time. The results of network pharmacology analysis indicated that the potential mechanism was mainly associated with regulation of lipolysis in adipocytes and serotonergic synapse signaling pathway, which might be responsible for the effect of indigestion.

Conclusions: Caoguo was first extracted by ASE and the volatile chemical components *in vivo* were first identified by GC-MS. Coupled with network pharmacology analysis, a network of component-target-pathway was constructed to reveal the possible mechanism of Caoguo in treatment of indigestion. This study provided a new reference method for the extraction and analysis of Caoguo, laid a chemical basis for in-depth studies on pharmacodynamics and pharmacology, and revealed an updated understanding of the therapeutic effects of Caoguo on indigestion.

Keywords: Traditional Chinese medicine; gas chromatography-mass spectrometry (GC-MS); accelerated solvent extraction (ASE); Caoguo; network pharmacology

Submitted Jun 04, 2021. Accepted for publication Jul 07, 2021.
doi: 10.21037/atm-21-3245
View this article at: https://dx.doi.org/10.21037/atm-21-3245

Introduction

Caoguo (*Tsaoko Fructus*), is the dried and mature fruit of *Amomum tsao-ko Crevost et Lemaire*, exhibits a broad biological activities such as anti-infectious, anti-oxidant, anti-proliferation and α-glucosidase inhibitory activity (1-5). As a herb, Caoguo can used as medicine and dietary spices, which has pharmacological effects including reduce plasma and liver lipids, reduce plasma glucose levels, adjust gastrointestinal metabolic function, anti-inflammatory, analgesic and neuroprotective effects (6-8). Caoguo Zhimu Ddecoction with CaoGuo as the monarch was used in clinical for treatment of epilepsy and chronic renal failure (9,10). Mongolian medicine containing twenty-one Chinese medicines was used in chronic aplastic anemia therapy (11).

Volatile oil is the main components in Caoguo, many methods such as reflux extraction, steam distillation, supercritical fluid CO_2 extraction, ultrasonic extraction and microwave extraction have been adopt for volatile oil extraction from Caoguo in recent years (12-16). Different extraction methods and solvents both impact on the components and contents of volatile oil, causing distinct extraction efficiency and yields. Traditional extraction methods such as soaking extraction and ultrasonic extraction are easy to operate, but they are both time-consuming process and a large amount of medicinal materials and solvents are always required, causing difficult to extract the chemical components that are unstable and easily oxidized. While supercritical fluid CO_2 extraction method don't need organic solvents in the whole extraction process and has a higher extraction efficiency for oil, but it is generally suitable for extraction of lipophilicity and small molecular substances and cost much than other extraction methods due to expensive equipment. Accelerated solvent extraction (ASE) technique, as a fast and efficient extraction method with low solvent consumption, has been adopt for active components extraction in many traditional Chinese medicine herbs such as *Xanthii Fructus*, *Salvia miltiorrhiza*, *Aucklandia lappa Decne* (17,18).

The network pharmacology can construct a network model to explain the relationship among the compounds-targets-pathways-diseases by combining the system pharmacology and systems biology. As a paradigm, network pharmacology has been used more and more in traditional Chinese medicine research for the purpose to predict the multi-targets of the mechanism of diseases treatment (19-21).

This study is aimed to apply ASE combined gas chromatography-mass spectrometry (GC-MS) method to extract and identify the *in vivo* and *in vitro* volatile constituents of Caoguo. Based on the identified components, network pharmacology analysis was proceeded to predict the protein/gene targets and signaling pathways of Caoguo in the treatment of indigestion. We present the following article in accordance with the ARRIVE reporting checklist (available at https://dx.doi.org/10.21037/atm-21-3245).

Methods

Reagents and materials

Dried Caoguo, derived from the Yunnan province (China), was purchased from Beijing Tongrentang of Chinese medicinal material in Shenyang, Liaoning, China. Reference standards (purity >98%) of α-pinene, β-pinene, eucalyptol, nerolidol, and α-terpineo were purchased from SinoStandards (Chengdu, Sichuan, China) for GC-MS analysis. Distilled water was provided by Wahaha (Hangzhou, Zhejiang, China). Methanol (chromatographic grade) was purchased from Sigma-Aldrich Company (Shanghai, China). Ethyl acetate and hexane of chromatographic grade were obtained separately from Tianjin Fuyu Chemical Limited Company (Tianjin, China) and Shandong Yuwang Industrial (Yucheng, Shandong, China).

Preparation of Caoguo extract

The Caoguo samples were extracted by ASE, which was carried out with a Dionex ASE 350 (Thermo Fisher Scientific, Waltham, MA, USA). All extractions were equipped with 34 mL capacity stainless steel cells. A cellulose filter was placed at the bottom of the extraction cell, and then 5 g Caoguo powder mixed with a small amount of diatomaceous earth was poured into the extraction cell. Then, the remaining void was filled with diatomaceous earth, with a cellulose filter placed on the top. Mixture solvent of n-hexane:ethyl acetate (1:1, v/v) was used as extraction solvent. Three static cycles of each 15 min under pressure of 1,500 psi (10.3 MPa) and temperature of 110 ℃, then the extraction cell was flushed with solvent (100% of the cell volume) and purged with nitrogen for 90 s. All the extracts were collected in 250 mL bottles and filtered.

Animals dosing and sampling

A total of 12 male pathogen-free Sprague-Dawley (SD) rats weighing 180–220 g were provided by the Experimental

Animal Center of Shenyang Pharmaceutical University. Rats were housed and bred under a 12 h dark-light cycle at suitable room temperature (20–25 ℃) and humidity (40–70%). Rats were placed in this conditions for one week with free access to water and standard rodent food before experiment. Experiments were performed under a project license (No.: SYPU-IACUC-C2018-12-26-103) granted by institutional ethics board of Shenyang Pharmaceutical University, in compliance with Shenyang Pharmaceutical University institutional guidelines for the care and use of animals. All the rats were randomly divided into two groups, with six in each group, and fasted overnight prior to the experiments. Caoguo extract was redissolved with 0.5% carboxymethyl cellulose sodium (CMC-Na) solution and diluted to an appropriate concentration (0.1 g/mL). A volume of 10 mL/kg (equivalent to raw medicine 100 g/kg) was intragastrically administrated to rats in the treatment group and the same volume of 0.5% CMC-Na was intragastrically administrated to rats in the vehicle group, doses in both groups were once every two hours for a total of three times. After the last dosing, plasma samples (~0.5 mL) were collected into heparinized tubes from the post-orbital venous plexus at 0.25, 0.5, 075, 1, 1.5, 2, and 3 h. Then, the samples were centrifuged with speed of 4,000 rpm for 10 min. The samples were stored at −80 ℃ before analysis.

Sample preparation of Caoguo extract and rats plasma for GC-MS analysis

The Caoguo extract processed by ASE was dissolved in an appropriate amount of n-hexane/ethyl acetate (1:1, v/v) and vortex-mixing for 5 min, after stand still for 3 min and then was centrifuged at 4,000 rpm for 10 min. The supernatant was transferred to sampler vials and an aliquot of 1 μL was injected into the GC-MS system for analysis.

Plasma samples of 200 μL were mixed with 200 μL n-hexane, vortexed for 5 min and then rested for 5 min. Then, the samples were centrifuged at 4,000 rpm for 10 min. The supernatant was transferred to sampler vials and an aliquot of 1 μL of the organic extracts supernatant was injected into the GC-MS system for analysis.

GC-MS conditions and compound identification

The volatile compounds were analyzed using a GC-MS system, comprising a Trace 1300 GC system combined with a Triplus RSH autosampler, an ISQ MS system, and X calibur software for data analysis (all components manufactured by Thermo Fisher). Separation was accomplished on a TS-5MS column (30 m × 0.25 mm, 0.25 μm; Thermo Fisher). Helium was used as carrier gas at a flow rate of 1.0 mL/min, and split injection was used with a ratio of 10:1. Temperature programming conditions for qualitative analysis were as follows: the initial oven temperature was set at 40 ℃ for 3 min, increased to 130 ℃ by increments of 10 ℃/min keeping for 5 min, finally increased to 280 ℃ at a rate of 6 ℃/min and retained for 15 min. The inlet temperature was set at 280 ℃. Electron ionisation mode (EI) with ionization energy at 70 eV was used for mass analysis operating in full scan mode (m/z 60-500).

The chemical components were identified by comparing to the reference spectra from the National Institute of Standards and Technology (NIST) library combined with literature, reference standards and manual analysis. Information of volatile chemical components in Caoguo was also searched from the literature and collected to conduct an Excel database for compound identification and comparison.

Active components screening and target genes prediction

The chemical compounds were obtained from the traditional Chinese medicine systems pharmacology database (TCMSP) database (http://tcmspw.com/) coupled with identification in this study. Then bioactive compounds with oral bioavailability (OB) ≥30% and drug-likeness (DL) ≥0.18 were selected for further study. The targets of the bioactive components above were collected from the TCMSP database.

Target genes for indigestion

Indigestion-related target proteins were collected from the biological targets related to indigestion which were selected from the GeneCards (https://www.genecards.org/) database and then put them into UniProt databases (http://www.uniprot.org/) to search the reviewed target genes of human species

Protein-protein interaction (PPI) network

The acquired intersection target genes were submitted to the Search Tool for the Retrieval of Interacting Genes/Proteins (http://string-db.org/) with the organism set to "homo sapiens", the PPIs with the confidence score of >0.4 were reserved and then ranked in network by Analyze

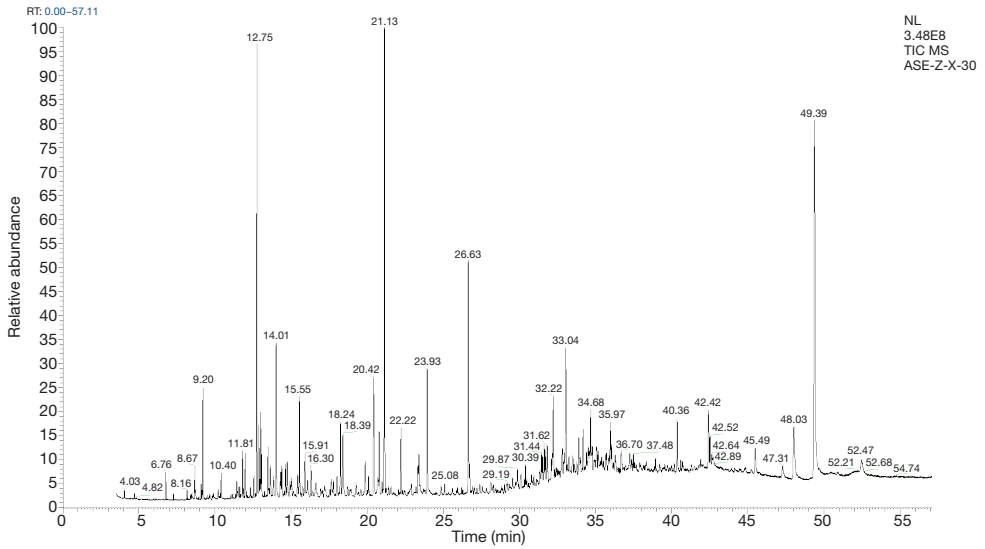

Figure 1 The GC-MS chromatogram of Caoguo extract processed by ASE. GC-MS, gas chromatography-mass spectroscopy; ASE, accelerated solvent extraction.

Network tool in Cytoscape. Furthermore, a PPI network of closely related proteins was made by using the network visualization software Cytoscape (http://cytoscape.org/).

Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis and the compound-target-pathway network construction

The GO enrichment analysis and KEGG pathway enrichment analysis were proceeded. The species were set to "homo sapiens" to predict the potential biological mechanisms in biological process, cell component, molecular function and illustrate the target genes expressed in the pathways.

The network composed of the bioactive compounds, target genes and enriched pathways were constructed by importing data into Cytoscape software.

Results

Volatile components identification in vitro and in vivo

The typical total ion chromatogram of the Caoguo extract is shown in *Figure 1*. All the main components were completely separated within 57 min. The identification results of the GC-MS analysis of Caoguo extract including compound names, molecular weight, molecular formula, retention time and content are presented in *Table 1*. A total of 169 components were identified in Caoguo extract processed by ASE. Their relative contents were calculated by peak area normalization method. The compounds in Caoguo extract were classified into 18 categories. The prevailing compounds found in Caoguo extract were 39 alcohols (37.1%), 21 aldehydes (11.71%), 5 sterols (10.37%), 21 esters (10.27%), 19 ketones (6.21%), 11 alkanes (4.56%), 16 organic acids (4.27%), 3 terpenes (3.23%), 6 alkylene oxides (3.20%) and 11 olefins (3.01%).

The total ion chromatograms of Caoguo extract in rat plasma is depicted in *Figure 2*. The results of GC-MS analysis of the volatile components of Caoguo extract processed by ASE in rat plasma is shown in *Table 2*. Comparing to the plasma sampled from the vehicle group, 43 constituents were identified in rats plasma after dosing Caoguo extract. Among the 43 components, the prevailing compounds were attributed to 15 alkanes (30.37%), 6 esters (20.13%), 4 olefins (15.37%), 5 alcohols (6.57%) and 6 organic acids (3.52%). The following five compounds including bis(6-methylheptyl) benzene-1,2-dicarboxylate (13.72%), cholesta-3,5-diene (8.61%), 11-decyltetracosane (5.12%), 11-pentan-3-ylhenicosane (4.88%) and methyl (E)-octadec-6-enoate (3.85%) were higher in the plasma.

Among the chemical components, β-pinene, nonanal, eucalyptol, ethyl iso-allocholate, oleic acid, hexadec-9-enoic acid, (5Z,8Z,11Z,14Z,17Z)-icosa-5,8,11,14,17-pentaenoic acid, (8Z,11Z,14Z)-Icosa-8,11,14-trienoic acid and bis(6-methylheptyl) benzene-1,2-dicarboxylate were identified

Table 1 The identified compounds of Caoguo processed by ASE

No.	Retention time (min)	Compound name	Molecular formula	Molecular weight	Content (%)
1	4.53	2-Methyl-2-propan-2-yloxirane	$C_6H_{12}O$	100.09	0.03
2	4.65	Hexan-3-ol	$C_6H_{14}O$	102.10	0.01
3	4.71	Hexanal	$C_6H_{12}O$	100.09	0.15
4	4.83	Hex-3-enal	$C_6H_{10}O$	98.07	0.02
5	6.48	13-Heptadecyn-1-ol	$C_{17}H_{32}O$	252.25	0.08
6	6.59	2-Butylfuran	$C_8H_{12}O$	124.09	0.02
7	6.77	Heptanal	$C_7H_{14}O$	114.10	0.77
8	8.26	3-Methylpentanoic acid	$C_6H_{12}O_2$	116.08	0.02
9	8.39	6-Methylhept-5-en-2-one	$C_8H_{14}O$	126.10	0.08
10	8.48	α-Pinene	$C_{10}H_{16}$	136.13	1.23
11	8.58	3-Ethenylhex-2-en-1-ol	$C_8H_{14}O$	126.10	0.01
12	8.67	Octanal	$C_8H_{16}O$	128.12	0.55
13	8.94	β-Pinene	$C_{10}H_{16}$	136.13	1.44
14	9.08	1-Methyl-2-propan-2-ylbenzene	$C_{10}H_{14}$	134.11	0.20
15	9.20	Eucalyptol	$C_{10}H_{18}O$	154.14	3.81
16	9.30	(3Z)-3,7-Dimethylocta-1,3,6-triene	$C_{10}H_{16}$	136.13	0.01
17	9.48	Z,Z,Z-1,4,6,9-Nonadecatetraene	$C_{19}H_{32}$	260.25	0.04
18	9.57	1,1-Dimethyl-2-(2-methylbut-3-en-2-yl)cyclopropane	$C_{10}H_{18}$	138.14	0.02
19	9.63	(E)-Oct-2-enal	$C_8H_{14}O$	126.10	0.05
20	9.67	3,7,7-Trimethylbicyclo[4.1.0]hept-3-ene	$C_{10}H_{16}$	136.13	0.56
21	9.91	Heptanoic acid	$C_7H_{14}O_2$	130.10	0.14
22	10.21	2-Hexyl-furan	$C_{10}H_{16}O$	152.12	0.19
23	10.29	3-Methyl-2-(2-methyl-2-butenyl)-furan	$C_{10}H_{14}O$	150.10	0.06
24	10.34	1-Methyl-4-prop-1-en-2-ylcyclohexan-1-ol	$C_{10}H_{18}O$	154.14	0.12
25	10.40	Nonanal	$C_9H_{18}O$	142.14	0.61
26	10.48	(Z)-Icos-11-enoic acid	$C_{20}H_{38}O_2$	310.29	0.08
27	11.17	2,7,7-Trimethyl-3-oxatricyclo[4.1.1.02,4]octane	$C_{10}H_{16}O$	152.12	0.15
28	11.36	(8Z,11Z,14Z)-icosa-8,11,14-trienoic acid	$C_{20}H_{34}O_2$	306.26	0.07
29	11.44	Oleic Acid	$C_{18}H_{34}O_2$	282.26	0.37
30	11.52	1,3-Dimethylcyclohexene	C_8H_{14}	110.11	0.17
31	11.62	4,7,7-Trimethylbicyclo[4.1.0]hept-4-en-3-ol	$C_{10}H_{16}O$	152.12	0.22
32	11.69	2-Pentadec-12-ynoxyoxane	$C_{20}H_{36}O_2$	308.27	0.10
33	11.81	α-Terpineol	$C_{10}H_{18}O$	154.14	3.27
34	11.91	Benzene-1,2-diol	$C_6H_6O_2$	110.04	0.11
35	11.98	[(1S,3S,5S)-4-Methylidene-1-propan-2-yl-3-bicyclo[3.1.0]hexanyl] acetate	$C_{10}H_{16}O$	152.12	0.63
36	12.12	2-Methylheptan-2-ol	$C_8H_{18}O$	130.14	0.06

Table 1 (*continued*)

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No.	Retention time (min)	Compound name	Molecular formula	Molecular weight	Content (%)
37	12.18	1-Ethenyl-4-ethoxybenzene	$C_{10}H_{12}O$	148.09	0.03
38	12.32	(R)-3,7-Dimethyl-6-octen-1-ol	$C_{10}H_{20}O$	156.15	0.15
39	12.42	3-Methyl-3-(4-methylpent-3-enyl)oxirane-2-carbaldehyde	$C_{10}H_{16}O_2$	168.12	0.04
40	12.75	Geraniol	$C_{10}H_{18}O$	154.14	4.88
41	12.88	(Z)-Dec-2-enal	$C_{10}H_{18}O$	154.14	0.80
42	12.99	Dec-2-en-1-ol	$C_{10}H_{20}O$	156.15	0.77
43	13.05	(2E)-3,7-Dimethylocta-2,6-dienal	$C_{10}H_{16}O$	152.12	0.97
44	13.16	Hexadec-9-enoic acid	$C_{16}H_{30}O_2$	254.22	0.03
45	13.34	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.32	0.03
46	13.48	α-Ethylbenzeneacetaldehyde	$C_{10}H_{12}O$	148.09	0.82
47	13.63	2,6-Dimethyl-octa-2,6-diene-1,8-diol	$C_{10}H_{18}O_2$	170.13	0.49
48	13.73	2-[(Z)-Octadec-9-enoxy]ethanol	$C_{20}H_{40}O_2$	312.30	0.02
49	13.83	Tetracyclo[5.2.1.01,5.05,9]decan-8-one	$C_{10}H_{12}O$	148.09	0.34
50	14.02	(2S,3R)-2,6,6-Trimethylbicyclo[3.1.1]heptane-2,3-diol	$C_{10}H_{18}O_2$	170.13	2.34
51	14.31	11,12-Dioxatetracyclo[4.3.1.1(3,10).1(2,5)]dodecane	$C_{10}H_{14}O_2$	166.10	0.79
52	14.39	1a,2,3,3a,4,5,6,7-Octahydroindeno[1,7a-b]oxirene	$C_9H_{14}O$	138.10	0.96
53	14.55	1,2-Dimethylcyclooctane	$C_{12}H_{24}$	168.19	0.21
54	14.63	2,3-Dihydro-1H-indene-4-carbaldehyde	$C_{10}H_{10}O$	146.07	0.28
55	14.75	3-Ethyl-4-methyl-3-hepten-2-on	$C_{10}H_{18}O$	154.14	0.56
56	14.91	2-Propenoic acid octyl ester	$C_{11}H_{20}O_2$	184.15	0.45
57	15.06	n-Decanoic acid	$C_{10}H_{20}O_2$	172.15	0.46
58	15.17	1,4-Cyclododecanedione	$C_{12}H_{20}O_2$	196.15	0.15
59	15.29	5-Methyl-2-prop-1-en-2-ylhex-4-enal	$C_{10}H_{16}O$	152.12	0.06
60	15.42	2-Methyl-3-phenylprop-2-enal	$C_{10}H_{10}O$	146.07	0.25
61	15.55	Geranyl acetate	$C_{12}H_{20}O_2$	196.15	0.60
62	15.66	2-Cyclopentylcyclopentan-1-one	$C_{10}H_{16}O$	152.12	0.10
63	15.77	(2Z)-3,7-Dimethyl-2-octen-1-yl 2-methylpropanoate	$C_{14}H_{26}O_2$	226.19	0.40
64	15.93	Bicyclo(3.1.1)heptane-2,3-diol,2,6,6-trimethyl	$C_{10}H_{18}O_2$	170.13	0.53
65	16.07	Vanillin	$C_8H_8O_3$	152.05	0.49
66	16.15	(E)-2-Dodecenoic acid	$C_{10}H_{18}O_2$	170.13	0.21
67	16.42	Cis-bicyclo[4.4.0]decan-1-ol-3-one	$C_{10}H_{16}O_2$	168.12	0.13
68	16.63	6-Methyl-7-oxabicyclo[4.1.0]heptan-2-one	$C_{10}H_{16}O_2$	168.12	0.73
69	16.95	p-Menth-1-en-3-one	$C_{10}H_{16}O_2$	168.12	0.25
70	17.05	1-(2-Nitro-2-propen-1-yl)cyclohexene	$C_9H_{13}NO_2$	167.09	0.31
71	17.20	Tricyclo[7.1.0.01,3]decane-2-carbaldehyde	$C_{11}H_{16}O$	164.12	0.60
72	17.46	2-Methylene-5α-cholestan-3α-ol	$C_{28}H_{48}O$	400.37	0.12
73	17.54	(E)-Docos-13-enoic acid	$C_{22}H_{42}O_2$	338.32	0.11

Table 1 (*continued*)

Table 1 (*continued*)

No.	Retention time (min)	Compound name	Molecular formula	Molecular weight	Content (%)
74	17.64	2-Hydroxy-6-methyl-3-(1-methylethyl)-2-Cyclohexen-1-one	$C_{10}H_{16}O_2$	168.12	0.28
75	17.78	(E)-1-Ethoxy-4,4-dimethylpent-2-ene	$C_9H_{18}O$	142.14	0.50
76	18.01	(3-Cyclopropyl-7-bicyclo[4.1.0]heptanyl)methanol	$C_{11}H_{18}O$	166.14	0.59
77	18.16	Ethyl 8-[3-[(3-pentyloxiran-2-yl)methyl]oxiran-2-yl]octanoate	$C_{20}H_{36}O_4$	340.26	0.65
78	18.25	2-Dodecenal	$C_{12}H_{22}O$	182.17	0.74
79	18.41	Trans-2-dodecen-1-ol	$C_{12}H_{24}O$	184.18	1.42
80	18.59	3-(4-Hydroxyphenyl) propanal	$C_9H_{10}O_2$	150.07	0.19
81	19.59	3-Cyclohexene-1-methanol,2-hydroxy-α, α,4-trimethyl	$C_{10}H_{18}O_2$	170.13	0.38
82	19.70	9,12,15-Octadecatrienoic acid	$C_{25}H_{40}O_6$	436.28	0.03
83	19.86	(3S,3aR,3bR,4S,7R,7aR)-3,7-Dimethyl-4-(propan-2-yl)octahydro-1H-cyclopenta[1,3]cyclopropa[1,2]benzen-3-ol	$C_{15}H_{26}O$	222.20	0.68
84	20.02	1-[(Z)-3-Ethoxy-1-Propenyl]-1-Cyclohexene	$C_{11}H_{18}O$	166.14	0.24
85	20.10	5-Tert-butylpyrogallol	$C_{10}H_{14}O_3$	182.09	0.25
86	20.44	Cyclopentaneacetaldehyde,2-formyl-3-methyl-α-methylene	$C_{10}H_{14}O_2$	166.10	3.71
87	20.72	(4Z)-11-Oxabicyclo[8.1.0]undec-4-ene	$C_{10}H_{16}O$	152.12	2.06
88	20.78	[s- (Z, Z)]-α, α,4,8-Tetramethyl-3,7-Cyclodecadiene-1-methanol	$C_{15}H_{26}O$	222.20	1.48
89	20.99	(3-Cyclopropyl-7-bicyclo[4.1.0]heptanyl)methanol	$C_{11}H_{18}O$	166.14	0.57
90	21.13	Nerolidol	$C_{15}H_{26}O$	222.20	4.46
91	21.61	Bicyclo[3.3.1]nonane-2,9-diol	$C_9H_{16}O_2$	156.12	0.46
92	21.88	Methyl octadeca-2,5-diynoate	$C_{19}H_{30}O_2$	290.22	0.08
93	22.09	2-Dodecenoic acid	$C_{12}H_{22}O_2$	198.16	0.36
94	22.22	[(E)-Dec-2-enyl] acetate	$C_{12}H_{22}O_2$	198.16	0.61
95	22.31	(2E,4Z,8Z,10E)-N-(2-methylpropyl)dodeca-2,4,8,10-tetraenamide	$C_{16}H_{25}NO$	247.19	0.14
96	22.47	Methyl (8E,11E,14E)-docosa-8,11,14-trienoate	$C_{23}H_{40}O_2$	348.30	0.21
97	22.76	2-Methyl-5-propan-2-yl-7-azabicyclo[4.1.0]heptane	$C_{10}H_{19}N$	153.15	0.18
98	22.89	Guaia-1(10),11-diene	$C_{15}H_{24}$	204.19	0.46
99	23.12	3,7-Dioxatetracyclo[6.4.0.02,6.04,9]dodecane	$C_{10}H_{14}O_2$	166.10	0.14
100	23.33	2-[(2R,4aR,8aS)-4a-Methyl-8-methylidene-1,2,3,4,5,6,7,8a-octahydronaphthalen-2-yl]propan-2-ol	$C_{15}H_{26}O$	222.20	0.39
101	23.40	α-Acorenol	$C_{15}H_{26}O$	222.20	2.06
102	23.47	4-Hydroxy-3,5-dimethoxybenzaldehyde	$C_9H_{10}O_4$	182.06	0.31
103	23.72	Pentadec-2-yn-1-ol	$C_{15}H_{28}O$	224.21	0.15
104	23.79	Caryophyllene oxide	$C_{15}H_{24}O$	220.18	0.09
105	23.85	3-Cyclohex-3-en-1-ylpropanal	$C_9H_{14}O$	138.10	0.14
106	23.95	Tricyclo[5.2.2.1(2,6)]dodecan-12-ol	$C_{12}H_{20}O$	180.15	1.41
107	24.18	1,4-Benzenedicarboxaldehyde,2,5-dimethyl	$C_{10}H_{10}O_2$	162.19	0.16
108	24.39	Methyl (E)-heptadec-10-en-8-ynoate	$C_{18}H_{30}O_2$	278.22	0.06
109	25.08	3-Ethoxy-1,4,4a,5,6,7,8,8a-octahydroisoquinoline	$C_{11}H_{19}NO$	181.15	0.35

Table 1 (*continued*)

Table 1 (*continued*)

No.	Retention time (min)	Compound name	Molecular formula	Molecular weight	Content (%)
110	25.34	(2E,6E)-9-(3,3-Dimethyloxiran-2-yl)-2,7-dimethylnona-2,6-dien-1-ol	$C_{15}H_{26}O_2$	238.19	0.06
111	25.59	3-Oxatetracyclo[5.5.0.01,8.04,8]dodecan-6-one	$C_{11}H_{14}O_2$	178.10	0.28
112	25.95	2(1H)-Naphthalenone,4a,5,6,7,8,8a-hexahydro-7à-isopropyl-4aá,8aá-dimethyl	$C_{15}H_{24}O$	220.18	0.15
113	26.44	(E)-Undec-2-enoic acid	$C_{11}H_{20}O_2$	184.15	0.19
114	26.64	Tricyclo[5.2.1.04,8]decan-5-ol	$C_{10}H_{16}O$	152.12	1.55
115	26.74	(1R,4aR,7R,8aR)-7-(2-hydroxypropan-2-yl)-1,4a-Dimethyl-2,3,4,5,6,7,8,8a-octahydronaphthalen-1-ol	$C_{15}H_{28}O_2$	240.21	0.49
116	27.39	1-Ethenoxy-3,7-dimethylocta-2,6-diene	$C_{12}H_{20}O$	180.15	0.19
117	27.57	Isoaromadendrene epoxide	$C_{15}H_{24}O$	220.18	0.18
118	27.64	3,5,9-Trimethyl-2-methylidenetricyclo[6.3.0.01,5]undec-3-ene	$C_{15}H_{22}$	202.17	0.16
119	27.83	4,8a-Dimethyl-6-prop-1-en-2-yl-2,3,5,6,7,8-hexahydro-1H-naphthalen-2-ol	$C_{15}H_{24}O$	220.18	0.18
120	28.11	(2-Methyl-5-prop-1-en-2-ylcyclohex-2-en-1-yl) 2,2-dimethylpropanoate	$C_{15}H_{24}O_2$	236.18	0.38
121	28.28	5-(2,3-Dimethyl-3-tricyclo[2.2.1.02,6]heptanyl)pentan-2-one	$C_{14}H_{22}O$	206.17	0.21
122	28.42	2-Heptadec-7-ynoxyoxane	$C_{22}H_{40}O_2$	336.30	0.06
123	29.35	Hexadecanoic acid	$C_{16}H_{32}O_2$	256.24	0.78
124	29.56	Trans-Z-à-Bisabolene epoxide	$C_{15}H_{24}O$	220.18	0.39
125	29.68	[(2Z,6E)-10,11-Dihydroxy-3,7,11-trimethyldodeca-2,6-dienyl] acetate	$C_{17}H_{30}O_4$	298.21	0.20
126	29.76	Methyl pentacosa-10,12-diynoate	$C_{26}H_{44}O_2$	388.33	0.21
127	29.87	Methyl tricosa-10,12-diynoate	$C_{24}H_{40}O_2$	360.30	0.37
128	30.32	2,6-Dimethyl-3-octyl-1,2,3,4,4a,5,6,7,8,8a-decahydronaphthalene	$C_{20}H_{38}$	278.30	0.16
129	30.39	2,6,6,8-Tetramethyltricyclo[5.3.1.01,5]undec-8-en-3-one	$C_{15}H_{22}O$	218.17	0.36
130	30.49	(6E,10E)-3,7,11,15-Tetramethylhexadeca-1,6,10,14-tetraen-3-ol	$C_{20}H_{34}O$	290.26	0.12
131	30.79	31,32-Dioxapentacyclo[20.8.1.17,16.01,22.07,16]dotriacontane	$C_{30}H_{52}O_2$	444.40	0.35
132	30.85	Methyl 8-(2-hexyl-5,6-dihydro-2H-naphthalen-4a-yl)octanoate	$C_{25}H_{40}O_2$	372.30	0.41
133	31.44	α-Ylangene	$C_{15}H_{24}$	204.19	0.68
134	31.52	(5Z,8Z,11Z,14Z,17Z)-Icosa-5,8,11,14,17-pentaenoic acid	$C_{20}H_{30}O_2$	302.22	0.38
135	31.70	Methyl octadeca-11,14-diynoate	$C_{19}H_{30}O_2$	290.22	0.40
136	31.82	Urs-12-en-28-ol	$C_{30}H_{50}O$	426.39	0.66
137	32.09	Cholest-22-ene-21-ol,3,5-dehydro-6-methoxy-, pivalate	$C_{33}H_{54}O_3$	498.41	0.23
138	32.15	3,7,11-Trimethyldodeca-1,6,10-trien-3-yl formate	$C_{16}H_{26}O_2$	250.19	0.34
139	32.23	(2E,6E)-1-Methoxy-3,7,11-trimethyldodeca-2,6,10-triene	$C_{16}H_{28}O$	236.21	1.34
140	32.34	Methyl (6E,9E,12E,15E)-docosa-6,9,12,15-tetraenoate	$C_{23}H_{38}O_2$	346.29	0.13
141	32.63	2,3-Dihydroxypropyl (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	$C_{21}H_{36}O_4$	352.26	0.06
142	32.83	5,6,7,8,9,10,11,12,13,14-Decahydrocyclododeca[b]pyridine	$C_{15}H_{23}N$	217.18	0.72

Table 1 (*continued*)

Table 1 (*continued*)

No.	Retention time (min)	Compound name	Molecular formula	Molecular weight	Content (%)
143	32.91	2-[4-Methyl-6-(2,6,6-trimethylcyclohexen-1-yl)hexa-1,3,5-trienyl] cyclohexene-1-carbaldehyde	$C_{23}H_{32}O$	324.25	0.14
144	33.04	4,6,6-Trimethyl-2-(3-methylbuta-1,3-dienyl)-3-oxatricyclo[5.1.0.02,4] octane	$C_{15}H_{22}O$	218.17	1.36
145	33.27	Octadec-6-ynenitrile	$C_{18}H_{31}N$	261.25	0.44
146	33.51	(3E,6E)-6-Methyl-5-propan-2-ylidenedeca-3,6,9-trien-2-one	$C_{14}H_{20}O$	204.15	0.35
147	33.90	5-Hydroxy-6-octylquinoline-7,8-dione	$C_{17}H_{21}NO_3$	287.15	0.64
148	34.19	Cyclohexanepropanol,2,2-dimethyl-6-methylene	$C_{12}H_{22}O$	182.17	0.66
149	34.53	3,7,11,15-Tetramethylhexadeca-1,6,10,14-tetraen-3-ol	$C_{20}H_{34}O$	290.26	0.45
150	34.61	3-Ethyl-3-hydroxy-5alpha-androstan-17-one	$C_{21}H_{34}O_2$	318.26	0.35
151	34.68	2-Methyl-4-(2,6,6-trimethylcyclohexen-1-yl)but-2-en-1-ol	$C_{14}H_{24}O$	208.18	0.51
152	34.78	1-Methyl-4-[(2Z)-6-methylhepta-2,5-dien-2-yl]-7-oxabicyclo[4.1.0] heptane	$C_{20}H_{34}O$	290.26	0.45
153	35.06	(2E,6E,10E)-3,7,11,15-Tetramethylhexadeca-2,6,10,14-tetraen-1-ol	$C_{20}H_{34}O$	290.26	0.28
154	35.19	(8E)-2,6,6,10-Tetramethylundeca-8,10-diene-3,7-dione	$C_{15}H_{24}O_2$	236.18	0.37
155	35.66	7,9-Octadecadiynoic acid, DMOX derivative	$C_{22}H_{35}NO$	329.27	0.56
156	35.71	9,11-Octadecadiynoic acid, DMOX derivative	$C_{22}H_{35}NO$	329.27	0.48
157	35.97	4α-Methylcholesta-8,24-dien-3α-ol	$C_{28}H_{46}O$	398.35	1.40
158	36.05	5-Hydroxy-4-nitro-3,4,4a,5,6,7,8,8a-octahydro-2H-naphthalen-1-one	$C_{10}H_{15}NO_4$	213.10	0.46
159	37.91	Bis(6-methylheptyl) benzene-1,2-dicarboxylate	$C_{24}H_{38}O_4$	390.28	2.97
160	38.95	2-Cyclohexyl-4a,7-dimethyl-4,5,6,8a-tetrahydro-3H-1,2-benzoxazine-3-carbonitrile	$C_{17}H_{26}N_2O$	274.20	0.82
161	40.36	[(2E)-3,7-Dimethylocta-2,6-dienyl] hexadecanoate	$C_{26}H_{48}O_2$	392.37	0.95
162	40.70	(Z)-Docos-13-enamide	$C_{22}H_{43}NO$	337.33	0.64
163	42.41	Geranyl oleate	$C_{28}H_{50}O_2$	418.38	0.97
164	42.51	[(2E)-3,7-Dimethylocta-2,6-dienyl] (9Z,12Z,15Z)-octadeca-9,12,15-trienoate	$C_{28}H_{46}O_2$	414.35	0.35
165	45.41	Vitamin E	$C_{29}H_{50}O_2$	430.38	0.86
166	47.16	Ergost-5-en-3α-ol	$C_{28}H_{48}O$	400.37	0.73
167	47.84	Stigmasterol	$C_{29}H_{48}O$	412.37	2.13
168	49.17	γ-Sitosterol	$C_{29}H_{50}O$	414.39	5.99
169	52.53	Stigmast-4-en-3-one	$C_{29}H_{48}O$	412.37	0.42

ASE, accelerated solvent extraction.

both in Caoguo extract and rat plasma samples.

Network pharmacology analysis

According to the screen criteria, twelve active components with 52 target genes in Caoguo were filtered out in the subsequent analysis (*Table 3*).

There are 19 identified target genes focused on bioactive compounds and indigestion including *ESR1*, *PTGS2*, *ADRB1*, *PLAU*, *PGR*, *RXRA*, *PTGS1*, *SLC6A4*, *PPARG*, *MAPK14*, *DPP4*, *SCN5A*, *F10*, *ADRB2*, *NR3C2*, *PIK3CG*, *HTR2A*, *LTA4H*, *OPRD1* by intersection (*Table 4*).

The PPI network is showed in *Figure 3* contained 17 nodes and twenty-six edges, shows the interaction of *PTGS2*, *PPARG*, *MAPK14*, *PGR*, *SLC6A4*, *ADRB2*, *ESR1*, *PLAU*, *PTGS1*, *ADRB1*, *OPRD1*, *LTA4H*, *DPP4*, *HTR2A*, *PIK3CG*, *NR3C2*, *RXRA* ranked according to degree that indicates the importance for treatment of indigestion by Caoguo.

Figure 4 showed the results of GO enrichment analysis of the target genes in biological processes, cell components and molecular functions. The enriched biological processes were dominated by steroid hormone mediated signaling pathway (GO:0043401) and transcription initiation from RNA polymerase II promoter (GO:0006367). Cell components terms mainly contained plasma membrane(GO:0005886). In terms of GO molecular functions, steroid hormone receptor activity (GO:0003707) and enzyme binding (GO:0019899) were mainly enriched.

According to the KEGG enrichment analysis, there were 17 signal pathways enriched and analyzed (*Figure 5*). Based on the results of KEGG, signal pathway of regulation of lipolysis (hsa04923) and serotonergic synapse (hsa04726) played the important role in treatment of indigestion among the enriched pathways which were displayed in *Figures 6* and 7. The component-target-pathway network was constructed with 12 active components, 52 target genes and 17 pathways (*Figure 8*), revealed the probable mechanism of Caoguo in treatment of indigestion which were related to multiple active compounds, target genes and pathways.

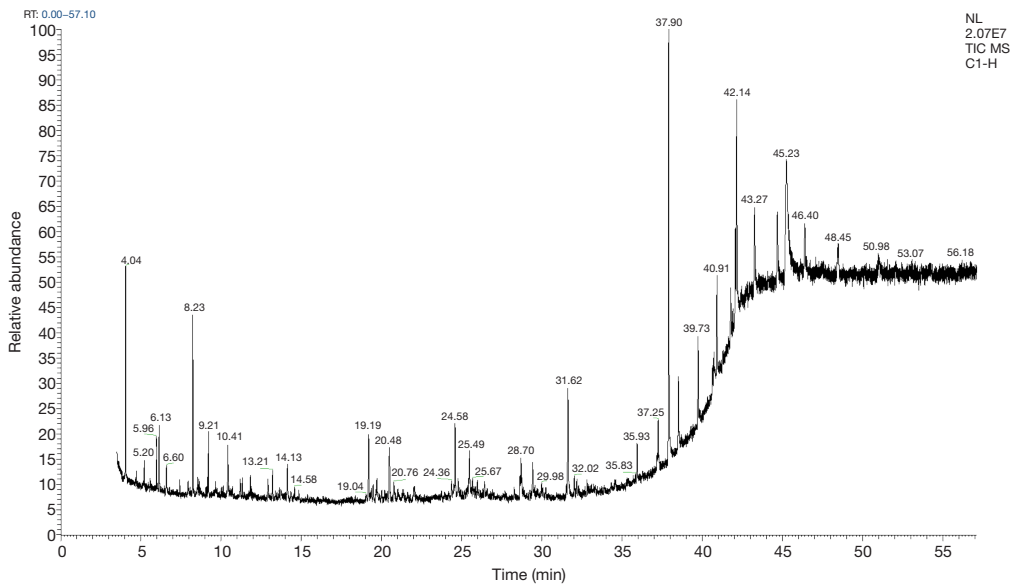

Figure 2 The total ion chromatograms of Caoguo processed by ASE in rats plasma. ASE, accelerated solvent extraction.

Table 2 The identified compounds of Caoguo processed by ASE in rats plasma

No.	Retention time (min)	Compound name	Molecular formula	Molecular weight	Content (%)
1	4.04	Cyclohepta-1,3,5-triene	C_7H_8	92.06	3.25
2	6.13	1,3-Xylene	C_8H_{10}	106.08	2.22
3	6.46	(Z)-Octadec-9-enal	$C_{18}H_{34}O$	266.26	3.08
4	7.42	(5Z,8Z,11Z,14Z,17Z)-Icosa-5,8,11,14,17-pentaenoic acid	$C_{20}H_{30}O_2$	302.22	0.10
5	8.23	β-Pinene	$C_{10}H_{16}$	136.13	2.78
6	9.21	Eucalyptol	$C_{10}H_{18}O$	154.14	1.43

Table 2 (*continued*)

Table 2 (*continued*)

No.	Retention time (min)	Compound name	Molecular formula	Molecular weight	Content (%)
7	9.74	2,4,6-Trimethyldecane	$C_{13}H_{28}$	184.22	0.29
8	10.41	Nonanal	$C_9H_{18}O$	142.14	0.87
9	10.63	3-Methyl-2,3-dihydro-benzofuran	$C_9H_{10}O$	134.07	0.22
10	11.81	(4Z,6Z,9Z)-Nonadeca-4,6,9-triene	$C_{19}H_{34}$	262.27	0.43
11	13.21	6-Methyloctadecane	$C_{19}H_{40}$	268.31	0.22
12	20.19	3,7,11-Trimethyldodecan-1-ol	$C_{15}H_{32}O$	228.25	0.21
13	21.62	[(2E)-3,7-Dimethylocta-2,6-dienyl] 3-methylbutanoate	$C_{15}H_{26}O_2$	238.19	0.73
14	21.93	Hexadec-9-enoic acid	$C_{16}H_{30}O_2$	254.22	0.60
15	22.23	(22E)-Ergosta-5,22-dien-3-yl acetate	$C_{30}H_{48}O_2$	440.37	0.74
16	24.58	2,6,10,15-Tetramethylheptadecane	$C_{21}H_{44}$	296.34	2.39
17	24.76	2,6,10-Trimethyltetradecane	$C_{17}H_{36}$	240.28	0.39
18	25.49	2-Methylicosane	$C_{21}H_{44}$	296.34	1.60
19	25.59	Icosan-2-ylcyclohexane	$C_{26}H_{52}$	364.41	0.91
20	31.53	(8Z,11Z,14Z)-Icosa-8,11,14-trienoic acid	$C_{20}H_{34}O_2$	306.26	0.25
21	31.62	Methyl (E)-octadec-6-enoate	$C_{19}H_{36}O_2$	296.27	3.85
22	32.35	2-Pentadecyl-1,3-dioxocane	$C_{21}H_{42}O_2$	326.32	0.02
23	32.80	5,8-Diethyldodecane	$C_{16}H_{34}$	226.27	0.14
24	33.24	Oleic Acid	$C_{18}H_{34}O_2$	282.26	0.09
25	34.31	(2-Phenyl-1,3-dioxolan-4-yl)methyl (Z)-octadec-9-enoate	$C_{28}H_{44}O_4$	444.32	2.07
26	34.53	1,25-Dihydroxyvitamin D3,	$C_{30}H_{52}O_3Si$	488.37	0.06
27	35.93	Hexadecan-2-ol	$C_{16}H_{34}O$	242.26	1.06
28	37.90	Bis(6-methylheptyl) benzene-1,2-dicarboxylate	$C_{24}H_{38}O_4$	390.28	13.72
29	38.06	(2-Phenyl-1,3-dioxolan-4-yl)methyl (Z)-octadec-9-enoate	$C_{28}H_{44}O_4$	444.32	0.07
30	38.51	2-Methyloctadecane	$C_{19}H_{40}$	268.31	2.39
31	38.68	16-Hydroxy-5',7,9,13-tetramethylspiro[5-oxapentacyclo[10.8.0.02,9.04,8.013,18]icos-1(12)-ene-6,2'-oxane]-11-one	$C_{27}H_{40}O_4$	428.29	0.08
32	38.77	Ethyl iso-allocholate	$C_{26}H_{44}O_5$	436.32	1.02
33	39.73	7-Hexylicosane	$C_{26}H_{54}$	366.42	3.00
34	40.65	1,1,3,3,5,5,7,7,9,9,11,11-Dodecamethylhexasiloxane	$C_{12}H_{38}O_5Si_6$	430.13	0.86
35	40.73	(Z)-Icos-11-enamide	$C_{20}H_{39}NO$	309.30	2.17
36	40.91	11-Pentan-3-ylhenicosane	$C_{26}H_{54}$	366.42	4.88
37	42.14	Cholesta-3,5-diene	$C_{27}H_{44}$	368.34	8.61
38	43.27	11-Decyltetracosane	$C_{34}H_{70}$	478.55	5.12
39	44.69	3-Ethyl-5-(2-ethylbutyl)octadecane	$C_{26}H_{54}$	366.42	3.70
40	45.23	Cholesterol	$C_{27}H_{46}O$	386.35	3.81
41	46.40	9-Hexylheptadecane	$C_{23}H_{48}$	324.38	3.10
42	47.07	2-(3-Acetoxy-4,4,14-trimethylandrost-8-en-17-yl)-Propanoic acid	$C_{27}H_{42}O_4$	430.31	0.41
43	48.45	1-Chloroheptacosane	$C_{27}H_{55}CL$	414.40	3.03

ASE, accelerated solvent extraction.

Table 3 Twelve active compounds in Caoguo were retrieved from databases and previous experiments

MOL ID	Molecule name	Target name	Target
MOL000073	ent-Epicatechin	Prostaglandin G/H synthase 1	PTGS1
		Estrogen receptor	ESR1
		Prostaglandin G/H synthase 2	PTGS2
		Heat shock protein HSP 90	HSP90
		Beta-lactamase	#N/A
		mRNA of PKA Catalytic Subunit C-alpha	#N/A
MOL000074	(4E,6E)-1,7-bis(4-hydroxyphenyl)hepta-4,6-dien-3-one	Estrogen receptor	ESR1
		Peroxisome proliferator activated receptor gamma	PPARG
		Prostaglandin G/H synthase 2	PTGS2
		Beta-2 adrenergic receptor	ADRB2
		Mitogen-activated protein kinase 14	MAPK14
		Leukotriene A-4 hydrolase	LTA4H
		mRNA of PKA Catalytic Subunit C-alpha	#N/A
MOL000085	beta-daucosterol_qt	Progesterone receptor	PGR
MOL000096	(-)-catechin	Prostaglandin G/H synthase 1	PTGS1
		Estrogen receptor	ESR1
		Prostaglandin G/H synthase 2	PTGS2
		Heat shock protein HSP 90	HSP90
		Beta-lactamase	#N/A
		mRNA of PKA Catalytic Subunit C-alpha	#N/A
		Nuclear receptor coactivator 2	NCOA2
		Calmodulin	CALM
		Fatty acid synthase	FASN
		Peroxisome proliferator-activated receptor gamma	PPARG
		Krueppel-like factor 7	KLF7
MOL000098	quercetin	Prostaglandin G/H synthase 1	PTGS1
		Androgen receptor	AR
		Peroxisome proliferator activated receptor gamma	PPARG
		Prostaglandin G/H synthase 2	PTGS2
		Heat shock protein HSP 90	HSP90
		Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, gamma isoform	PIK3CG
		Nuclear receptor coactivator 2	NCOA2
		Dipeptidyl peptidase IV	DPP4

Table 3 (*continued*)

Table 3 (*continued*)

MOL ID	Molecule name	Target name	Target
		Aldose reductase	AR
		Trypsin-1	PRSS1
		DNA topoisomerase II	TOP2
		Thrombin	#N/A
		Potassium voltage-gated channel subfamily H member 2	KCNH2
		Sodium channel protein type 5 subunit alpha	SCN5A
		Coagulation factor Xa	F10
MOL005970	Eucalyptol	Prostaglandin G/H synthase 1	PTGS1
		Dopamine D1 receptor	DRD1/DRD5
		Muscarinic acetylcholine receptor M3	CHRM3
		Thrombin	#N/A
		Muscarinic acetylcholine receptor M1	CHRM1
		Sodium channel protein type 5 subunit alpha	SCN5A
		Muscarinic acetylcholine receptor M5	CHRM5
		Prostaglandin G/H synthase 2	PTGS2
		Carbonic anhydrase II	CA2
		Muscarinic acetylcholine receptor M4	CHRM4
		Retinoic acid receptor RXR-alpha	RXRA
		Delta-type opioid receptor	OPRD1
		Acetylcholinesterase	ACHE
		5-hydroxytryptamine 2A receptor	HTR2A
		Alpha-1A adrenergic receptor	ADRA1A
		Muscarinic acetylcholine receptor M2	CHRM2
		Beta-2 adrenergic receptor	ADRB2
		Alpha-1D adrenergic receptor	ADRA1D
		Sodium-dependent serotonin transporter	SLC6A4
		Mu-type opioid receptor	OPRM1
		Heat shock protein HSP 90	HSP90
		Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, gamma isoform	PIK3CG
		Neuronal acetylcholine receptor protein, alpha-7 chain	CHRNA7
		Ig gamma-1 chain C region	IGHG1
		Nuclear receptor coactivator 2	NCOA2
		Nuclear receptor coactivator 1	NCOA1

Table 3 (*continued*)

Table 3 (*continued*)

MOL ID	Molecule name	Target name	Target
MOL005030	gondoic acid	Prostaglandin G/H synthase 1	PTGS1
		Nuclear receptor coactivator 2	NCOA2
MOL006202	LAX	Prostaglandin G/H synthase 1	PTGS1
		Prostaglandin G/H synthase 2	PTGS2
MOL002879	Diop	Sodium channel protein type 5 subunit alpha	SCN5A
		Beta-2 adrenergic receptor	ADRB2
		Muscarinic acetylcholine receptor M3	CHRM3
MOL000449	Stigmasterol	Progesterone receptor	PGR
		Mineralocorticoid receptor	NR3C2
		Nuclear receptor coactivator 2	NCOA2
		Alcohol dehydrogenase 1C	ADH1C
		Ig gamma-1 chain C region	IGHG1
		Retinoic acid receptor RXR-alpha	RXRA
		Nuclear receptor coactivator 1	NCOA1
		Prostaglandin G/H synthase 1	PTGS1
		Prostaglandin G/H synthase 2	PTGS2
		Alpha-2A adrenergic receptor	ADRA2A
		Sodium-dependent noradrenaline transporter	SLC6A2
		Sodium-dependent dopamine transporter	SLC6A3
		Beta-2 adrenergic receptor	ADRB2
		Aldose reductase	AR
		Urokinase-type plasminogen activator	PLAU
		Leukotriene A-4 hydrolase	LTA4H
		Amine oxidase [flavin-containing] B	MAOB
		Amine oxidase [flavin-containing] A	MAOA
		mRNA of PKA Catalytic Subunit C-alpha	#N/A
		Chymotrypsinogen B	CTRB1
		Muscarinic acetylcholine receptor M3	CHRM3
		Muscarinic acetylcholine receptor M1	CHRM1
		Beta-1 adrenergic receptor	ADRB1
		Sodium channel protein type 5 subunit alpha	SCN5A
		5-hydroxytryptamine 2A receptor	HTR2A
		Alpha-1A adrenergic receptor	ADRA1A
		Gamma-aminobutyric-acid receptor alpha-3 subunit	GABRA3

Table 3 (*continued*)

Table 3 (*continued*)

MOL ID	Molecule name	Target name	Target
		Muscarinic acetylcholine receptor M2	CHRM2
		Alpha-1B adrenergic receptor	ADRA1B
		Gamma-aminobutyric acid receptor subunit alpha-1	GABRA1
		Neuronal acetylcholine receptor protein, alpha-7 chain	CHRNA7
MOL002320	γ-sitosterol	Progesterone receptor	PGR
		Nuclear receptor coactivator 2	NCOA2
MOL001507	stigmast-4-en-3-one	Progesterone receptor	PGR

Table 4 Nineteen potential targets of Caoguo related to indigestion

Target	Protein name
ADRB1	Beta-1 adrenergic receptor
ADRB2	Beta-2 adrenergic receptor
DPP4	Dipeptidyl peptidase IV
ESR1	Estrogen receptor
F10	Coagulation factor Xa
HTR2A	5-hydroxytryptamine 2A receptor
LTA4H	Leukotriene A-4 hydrolase
MAPK14	Mitogen-activated protein kinase 14
NR3C2	Mineralocorticoid receptor
OPRD1	Delta-type opioid receptor
PGR	Progesterone receptor
PIK3CG	Phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit, gamma isoform
PLAU	Urokinase-type plasminogen activator
PPARG	Peroxisome proliferator activated receptor gamma
PTGS1	Prostaglandin G/H synthase 1
PTGS2	Prostaglandin G/H synthase 2
RXRA	Retinoic acid receptor RXR-alpha
SCN5A	Sodium channel protein type 5 subunit alpha
SLC6A4	Sodium-dependent serotonin transporter

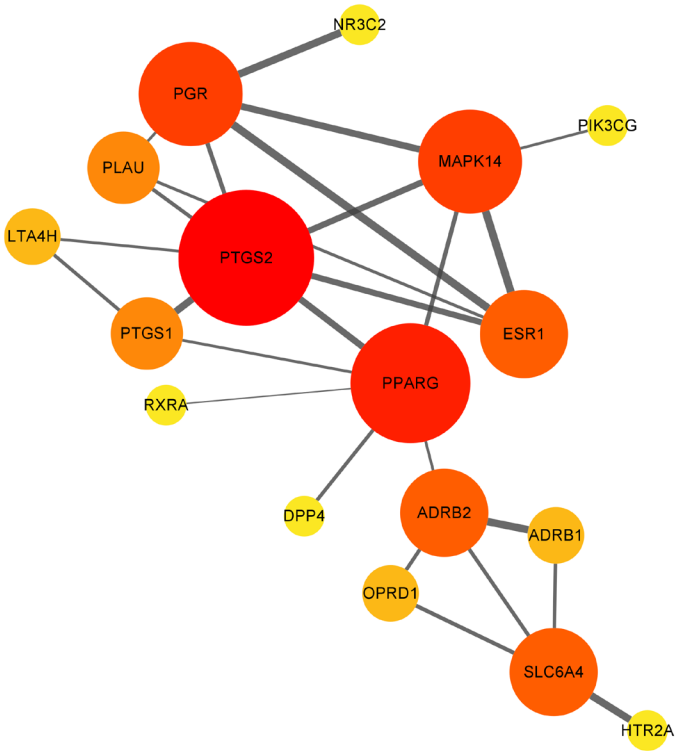

Figure 3 A PPI network (the size and colour indicate the importance of the target genes proportional to their degree and edges represent associations between protein and protein). PPI, protein-protein interaction.

Discussion

Accelerated solvent extraction (ASE) is a new extraction method which uses organic solvents at high pressure and temperature above boiling point. The ASE procedure was introduced by Dionex (Sunnyvale, CA, USA) in recent years. It uses organic solvents to extract solid and semi-solid samples at higher temperatures (50–200 ℃) and pressure (10.3–20.6 MPa) than those used in traditional solvent extraction procedures (22). High temperature can increase the solubility of the target components in the solvent and reduce solvent viscosity, which is beneficial to the diffusion of solvent molecules into the matrix and increases the dissolution rate of them (23). The high pressure can increase the boiling point of the solvent to above normal boiling point and keep the solvent in a liquid state at this elevated temperature (24). Compared with the traditional extraction method, ASE can significantly improve the extraction rate, shorten analysis time, and reduce environmental pollution. Additionally, as one of the best methods for sample preparation, ASE can reduce or even eliminate errors caused by individual differences in manual operation and increase the sensitivity, accuracy, and reproducibility of analytical tests. In this study, Caoguo was first extracted by ASE which was able to extract a plenitude of potential chemical constituents than other methods reported before (16,25).

Gas chromatography-mass spectroscopy (GC-MS) is one of the most versatile and widely applied technology in modern volatile constituents identification (26) due to its inherent advantages of high resolution (27), rapid separation, low cost, and easy linkage with sensitive and selective detectors (28). Mass spectrometry is a better technique which affords rather complete structural information (29), and it is suitable for the analysis of complex mixtures (30). Some methods based on GC or GC-MS have been reported to detect volatile components in Caoguo (31), but there is still no published data on the *in vitro-in vivo* components analysis. This is the first time using the GC-MS to identify the components of Caoguo *in vivo*.

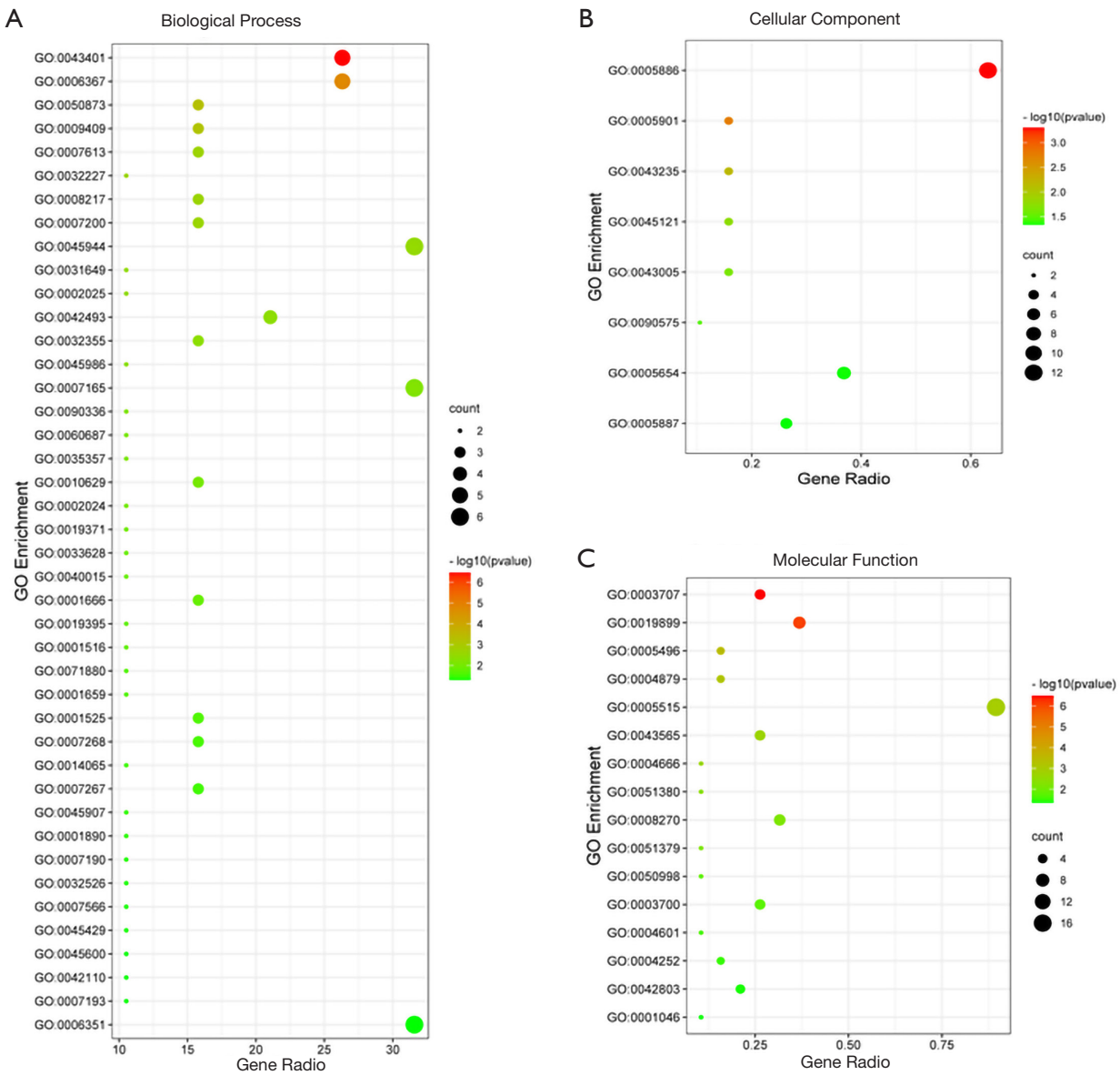

Figure 4 GO enrichment. (A) Biological processes; (B) cell components; (C) molecular functions. GO, Gene Ontology.

Recent years, with the popularization of network pharmacology, it has been used in traditional Chinese medicine research, which could reveal the relationships between disease and active components of traditional Chinese medicine by linking the chemical constituents, targets and pathways to establish a compound-target-pathway network to explore the possible mechanisms in disease treatment. Niu *et al.* used network pharmacology to predict the active components and targets of *Pterocypsela elata* for treatment of cerebral ischemia (32). The anti-Alzheimer's effects of Bushen Tiansui formula was studied by Zhang *et al.* using network pharmacology approach (33). In a review, Luo *et al.* summarized the methodology, application and prospective of network pharmacology technology providing us a new research strategy in the area of Chinese medicine (34).

In this study, 12 identified active components of Caoguo were considered to have potential key roles in the treatment

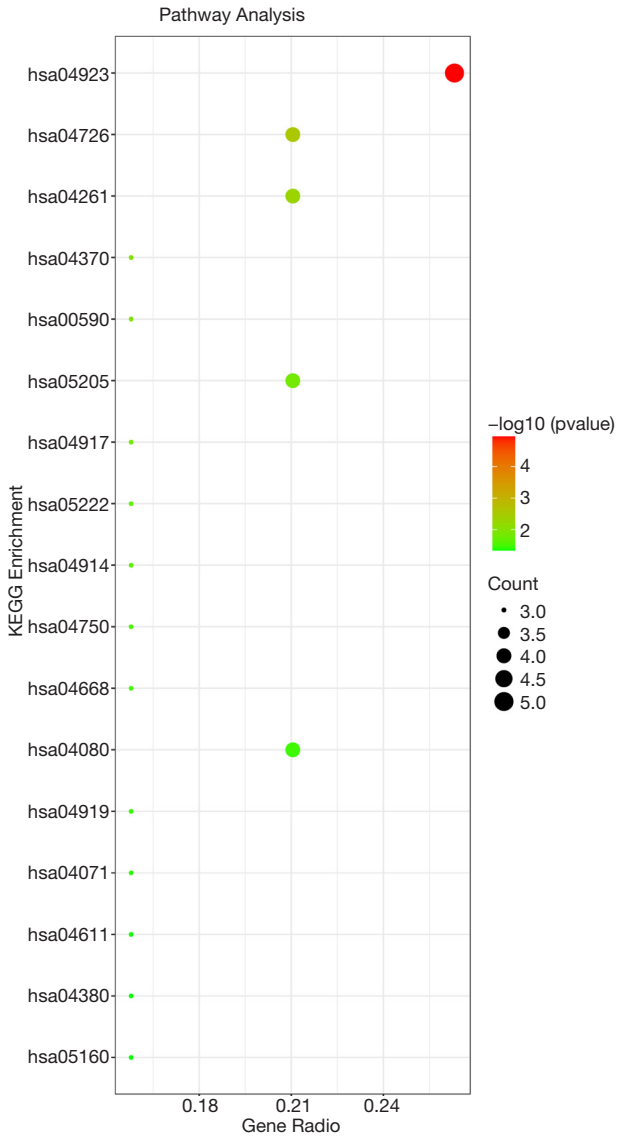

Figure 5 KEGG enrichment analysis of 17 signal pathways.

of indigestion by acting on 19 targets. Based on KEGG enrichment analysis, 17 signal pathways were enriched and analyzed, 2 of which had strong significance and relation. A total of 7 key targets were involved in the top two enriched pathways including regulation of lipolysis in adipocytes (hsa04923) (related to the key target *ADRB1*, *ADRB2*, *PTGS2*, *PIK3CG*, *PTGS1*) and serotonergic synapse (hsa04726) (related to the key target *HTR2A*, *PTGS2*, *SLC6A4*, *PTGS1*).

In the pathway of regulation of lipolysis in adipocytes, Caoguo plays a pharmacological role by promoting the effect of epinephrine and norepinephrine on lipid metabolism and inhibiting the inhibitory effect of insulin and prostaglandin on lipid metabolism. Adrenoceptor beta 1 (*ADRB1*) and adrenoceptor beta 2 (*ADRB2*) are responsible to activate the regulation of lipolysis in adipocytes. The assembly of G proteins produces a large number of second messengers cAMP which are dispersed into the cells and then activate PKA which enable lipase activation to participate in lipid metabolism and transfer triglycerides into glycerol and fatty acids to the liver. The protein *PIK3CG* is responsible for the PI3K-Akt insulin signal pathway. Activation of PI3K/Akt/mTOR pathways may be involved in pathogenesis of the gastrointestinal tract (35). The PI3K/Akt signaling pathways is involved in soluble uric acid and the gut excretion in human intestinal cells (36). The proteins *PTGS1* and *PTGS2* are responsible for arachidonic acid metabolism, which then affects the release of PGE2, causing the direct activation of longitudinal smooth muscle contraction of the colon in rats (37).

In the regulation of serotonergic synapse pathway, Caoguo plays a pharmacological role by inhibiting 5-HT reuptake and promoting the combination of 5-HT to 5-hydroxytryptamine receptor. Solute carrier family 6 member 4 (*SLC6A4*) is responsible for the reuptake process by SERT. Up-regulation of SERT levels in the midbrain and thalamus may relate to pathogenesis of the gut (38). SERT reuptakes excessive 5-HT and participates in the regulation of gastrointestinal motility, thus intestinal dysregulation may be due to up-regulation of SERT expression, leading to the development of gastrointestinal diseases (39). Additionally, HTR2A is responsible for the activation of the 5-HT receptor 2A which leads to release of calcium ion and play an important role in controlling gastric emptying (40).

Conclusions

In this article, a systematic study of the essential volatile chemical components in Caoguo extract and in rats plasma were conducted by application of ASE technique and GC-MS method, which played an important role in the development, modernization, and quality control of Caoguo formulations. Network pharmacology was also introduced to reveal the possible mechanism of Caoguo in treatment of indigestion. This study provided a new strategy using ASE combined with GC-MS for Caoguo extraction and analysis, while enriched our knowledge about its volatile components and the therapeutic material basis. In next work, the prediction signaling pathways need be confirmed

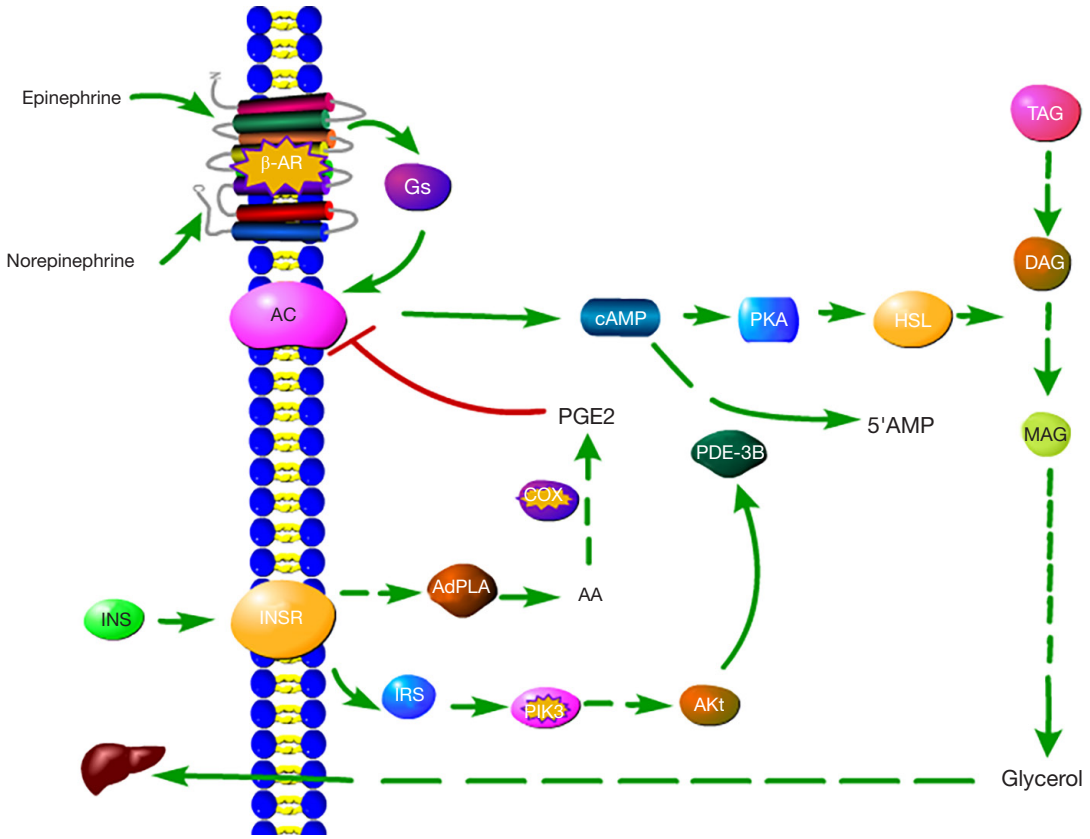

Figure 6 Regulation of lipolysis in adipocytes signal pathway.

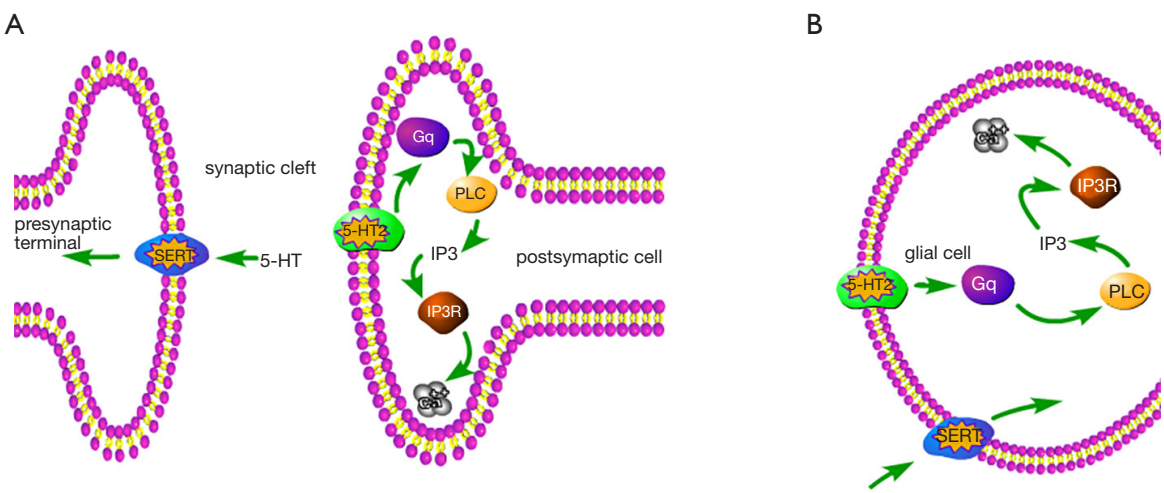

Figure 7 Serotonergic synapse signal pathway.

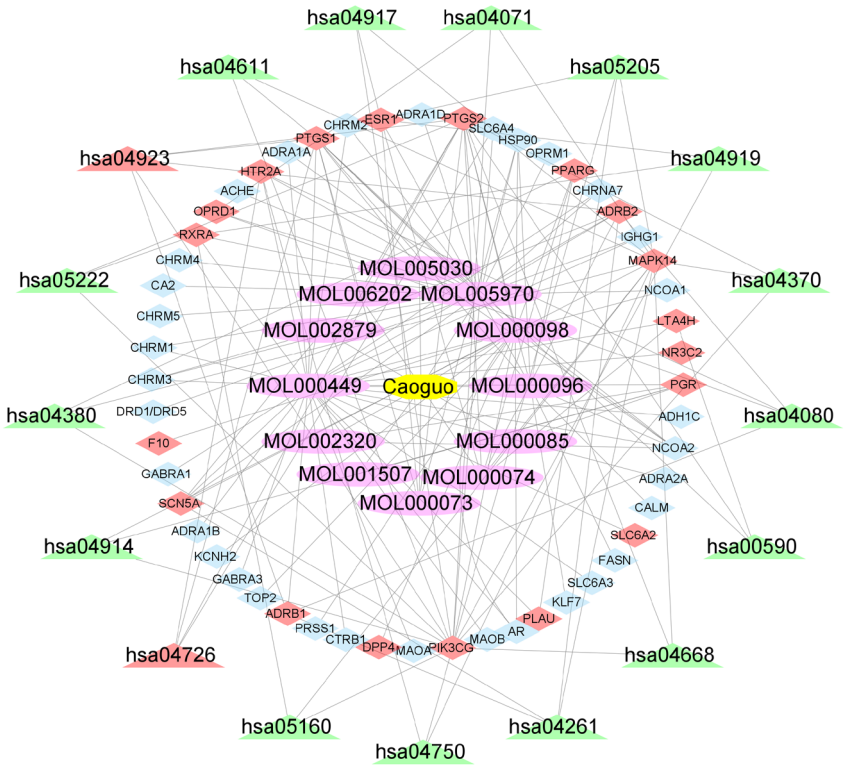

Figure 8 The component-target-pathway network (the yellow node represents the herbal medicine; the 12 purple nodes represent the active components in Caoguo; the 52 rhombuses represent the target genes of the active components in Caoguo, in which 19 red nodes represent the intersecting targets; the 17 triangles represent the pathways and two of them marked in red considered as important).

and verified by further study.

Acknowledgments

Funding: This work was financially supported by Liaoning Province Natural Science Foundation of China (2020-BS-106).

Footnote

Reporting Checklist: The authors have completed the ARRIVE reporting checklist. Available at https://dx.doi.org/10.21037/atm-21-3245

Data Sharing Statement: Available at https://dx.doi.org/10.21037/atm-21-3245

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://dx.doi.org/10.21037/atm-21-3245). Dr. Chu reports funding support from Liaoning Province Natural Science Foundation of China (2020-BS-106). The other authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Experiments were performed under a project license (No.: SYPU-IACUC-C2018-12-26-103) granted by institutional ethics board of Shenyang Pharmaceutical University, in compliance with Shenyang Pharmaceutical University institutional guidelines for the care and use of animals.

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(English Language Editor: J. Jones)

Cite this article as: Shi S, Luo Y, Ma Y, Chu Y, Wang Y, Chen X, Chu Y. Identification of *in vitro-in vivo* components of Caoguo using accelerated solvent extraction combined with gas chromatography-mass spectrometry integrated with network pharmacology on indigestion. Ann Transl Med 2021;9(15):1247. doi: 10.21037/atm-21-3245

