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Qualitative analysis of chemical components in Lianhua Qingwen capsule by HPLC-Q Exactive-Orbitrap-MS coupled with GC-MS

Shuai Fu [a, **], Rongrong Cheng [a], Zixin Deng [a, b], Tiangang Liu [a, *]

[a] Key Laboratory of Combinatorial Biosynthesis and Drug Discovery, Ministry of Education and School of Pharmaceutical Sciences, Wuhan University, Wuhan, 430071, China
[b] State Key Laboratory of Microbial Metabolism, Joint International Research Laboratory of Metabolic and Developmental Sciences, School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai, 200030, China

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ABSTRACT

The Lianhua Qingwen (LHQW) capsule is a popular traditional Chinese medicine for the treatment of viral respiratory diseases. In particular, it has been recently prescribed to treat infections caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, due to its complex composition, little attention has been directed toward the analysis of chemical constituents present in the LHQW capsule. This study presents a reliable and comprehensive approach to characterizing the chemical constituents present in LHQW by high-performance liquid chromatography-Q Exactive-Orbitrap mass spectrometry (HPLC-Q Exactive-Orbitrap-MS) coupled with gas chromatography-mass spectrometry (GC-MS). An automated library alignment method with a high mass accuracy (within 5 ppm) was used for the rapid identification of compounds. A total of 104 compounds, consisting of alkaloids, flavonoids, phenols, phenolic acids, phenylpropanoids, quinones, terpenoids, and other phytochemicals, were successfully characterized. In addition, the fragmentation pathways and characteristic fragments of some representative compounds were elucidated. GC-MS analysis was conducted to characterize the volatile compounds present in LHQW. In total, 17 compounds were putatively characterized by comparing the acquired data with that from the NIST library. The major constituent was menthol, and all the other compounds were terpenoids. This is the first comprehensive report on the identification of the major chemical constituents present in the LHQW capsule by HPLC-Q Exactive-Orbitrap-MS, coupled with GC-MS, and the results of this study can be used for the quality control and standardization of LHQW capsules.

1. Introduction

Lianhua Qingwen capsule (LHQW), produced by Yiling Pharmaceutical Co., Ltd. (Shijiazhuang, China), is a patented traditional Chinese medicine (TCM). It was developed to treat patients infected with the severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) in China. LHQW is based on two TCM formulas, i.e., Maxing-Shigan-Tang and Yinqiao-San, which were originally mentioned in the classical Chinese books *ShangHanLun* and *WenBingTiaoBian*, respectively [1]. Both Maxing-Shigan-Tang and Yinqiao-San are prescribed to treat fever, inflammation, and seasonal influenza [2,3]. LHQW is composed of 11 herbs [4]: *Forsythiae Fructus*, *Lonicerae Japonicae Flos*, *Ephedrae Herba*, *Armeniacae Semen Amarum*, *Isatidis Radix*, *Dryopteridis Crassirhizomatis Rhizoma*, *Houttuyniae Herba*, *Pogostemonis Herba*, *Rhei Radix Et Rhizoma*, *Rhodiolae Crenulatae Radix Et Rhizoma* and *Glycyrrhizae Radix Et Rhizoma*. In addition, it contains *Gypsum Fibrosum*, a mineral and menthol, extracted from *Menthae Haplocalycis Herba*.

In China, LHQW has been widely used to treat the symptoms of respiratory diseases. Various reports have established the good anti-influenza activity of LHQW against H7N9 [1] and H1N1 [5]

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* Corresponding author.
** Corresponding author.
E-mail addresses: yaqin_29@whu.edu.cn (S. Fu), liutg@whu.edu.cn (T. Liu).

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viruses. In particular, a randomized, double-blind, and positive clinical trial indicated that effectiveness of LHQW against the H1N1 virus is similar to that of Oseltamivir [5]. Another recent study has reported that LHQW is also effective against the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [6], the pathogen that causes the coronavirus disease (COVID-19). Thus, LHQW has been included as a representative TCM prescription in the Guideline for the Diagnosis and Treatment of COVID-19 Pneumonia issued by the National Heath Commission of the People's Republic of China [7]. However, the use of LHQW is limited to China. For LHQW to be globally accepted, its chemical composition and mechanism of action must be well understood. Thus, it is essential to determine the chemical composition of LHQW, which can further enable its quality control and standardization.

Several analytical methods have been developed for the quality control of LHQW. For example, Zhang et al. [8] and Chen et al. [9] developed ultra-performance liquid chromatography (UPLC) fingerprinting methods for the quality control of LHQW. Qiao et al. [10] and Jia et al. [11] developed methods based on gas chromatography with flame-ionization detection and headspace solid-phase microextraction coupled with gas chromatography-mass spectrometry to analyze the volatile constituents and raw materials of an LHQW capsule, respectively. Jia et al. [12] developed a method based on a combination of UPLC with a diode-array detector and quadrupole time-of-flight mass spectrometry to determine the major chemical constituents of an LHQW capsule. Nevertheless, a detailed analysis of the chemical composition of an LHQW capsule has not yet been reported.

Orbitrap mass spectrometry (MS) has become a powerful tool to characterize the chemical components in TCMs because of its high sensitivity, high mass resolution, and high mass accuracy [13,14]. This method also helps analyze MS^n fragments, thus facilitating the structural elucidation of a compound. Gas chromatography-mass spectrometry (GC-MS) has been widely applied for the detection of volatile components in a TCM [15]. In this paper, we present the unprecedented comprehensive analysis of the major chemical components in an LHQW capsule by high-performance liquid chromatography (HPLC)-Q Exactive-Orbitrap-MS coupled with GC-MS. In total, 104 compounds including 6 alkaloids, 33 flavonoids, 7 phenols, 9 phenolic acids, 16 phenylpropanoids, 7 quinones, 12 terpenoids and 14 other phytochemicals, were putatively identified via HPLC-Q Exactive-Orbitrap-MS. In addition, 17 volatile compounds were putatively identified via GC-MS. Thus, a total of 120 compounds were successfully extracted and characterized.

2. Materials and methods

2.1. Chemicals and materials

LHQW capsules (Lot No. B2001158) were obtained from Yiling Pharmaceutical Co., Ltd. (Shijiazhuang, China). Acetonitrile (ACN, HPLC grade) and methanol (HPLC grade) were obtained from Merck KGaA (Darmstadt, Germany). Watsons-distilled water was purchased from Jingdong Mall (Beijing, China). Formic acid (FA), acetone (HPLC grade), and hexane (HPLC grade) were purchased from Thermo Fisher Scientific (Waltham, MA, USA). Ethyl acetate was purchased from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Sample preparation

For the HPLC-Q Exactive-Orbitrap-MS analysis, the raw material (brown powder; 0.4 g) of the LHQW capsule was accurately weighed, dissolved in 60% methanol (*V/V*; 20 mL), and sonicated for 30 min. The solution was centrifuged at 12,000 r/min and the supernatant was filtered through a 0.22 μm membrane.

For GC-MS analysis, three different extraction solvents were used: hexane, acetone, and ethyl acetate. The raw material (1 g) was accurately weighed, dissolved in the extracted solvent (10 mL), and sonicated for 20 min. The resulting extracts were centrifuged at 12,000 r/min and the supernatants were analyzed.

2.3. HPLC-Q Exactive-Orbitrap-MS analysis

An HPLC-high resolution mass spectrometer (HPLC-HRMS; Ultimate 3000 HPLC system, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a reversed-phase Hypersil Gold aQ C_{18} column (2.1 mm × 150 mm, 3 μm; Thermo Fisher Scientific, Waltham, MA, USA) was used to determine the chemical constituents present in the LHQW sample. The column temperature was maintained at 40 °C. The mobile phases included 0.1% FA in ACN (A) and 0.1% FA in H_2O (B). A constant flow rate was maintained (0.2 mL/min). The elution gradient was maintained at 5% A for 1 min, increased to 95% A over 41 min, and maintained at this phase for 4.9 min. The column was then re-equilibrated at 5% A for 3 min. The samples were maintained at room temperature and the injection volume was 5 μL.

The Q Exactive mass spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) equipped with a heated electrospray ionization interface was operated under both electrospray ionization (ESI) negative and ESI positive modes. The instrument was calibrated with the calibration solutions provided by the manufacturer. Data were acquired using Xcalibur 4.1 software (Thermo Fisher Scientific, Waltham, MA, USA), and all obtained data were processed by the Compound Discoverer (CD) 3.0 (Thermo Fisher Scientific, Waltham, MA, USA) and Xcalibur 4.1 software packages. The source parameters were optimized with a spray voltage of 3.5 kV (+)/3.2 kV (−). The other parameters were set as follows: capillary temperature, 320 °C; auxiliary gas heater temperature, 350 °C; sheath gas pressure, 40 arb; auxiliary gas pressure, 15 arb; sweep gas pressure, 0 arb; S-lens RF level, 50 V.

The Q Exactive detector was operated in full scan and data-dependent MS^2 (dd MS^2) modes. In full scan mode, the resolution was set at 70,000. The automatic gain control (AGC) target and maximum injection time (IT) were 1×10^6 ions capacity and 100 ms, respectively. For the dd MS^2 mode, the acquisition parameters were: resolution, 17,500; AGC target, 2×10^5 ions capacity; maximum IT, 50 ms; scan range, 100–1500 *m/z*; loop count, 3; normalized collision energy (stepped), 20%, 40%, 60%; isolation window, 1.2 Da; apex trigger, 5–15 s; dynamic exclusion, 5 s. The Top N (N = number of top abundant ions for fragmentation) was set to 5.

2.4. GC-MS analysis

The GC-MS analysis was conducted on a Trace GC Ultra system equipped with an AS 3000 auto-sampler, a split/splitless injector, and TSQ Quantum XLS MS detector with triple quadrupole (Thermo Fisher Scientific, Waltham, MA, USA). The TR-5 MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness) was purchased from Thermo Fisher Scientific (Waltham, MA, USA).

The injection temperature and ion source temperature were 250 °C. The oven temperature program was maintained at 50 °C for 1 min, increased to 200 °C (rate: 8 °C/min), maintained at 200 °C for 5 min, increased to 280 °C (rate: 10 °C/min), and maintained for 5 min. Helium was used as the carrier gas (flow rate: 1 mL/min). Sample injection volume and split ratio were 1 μL and 50:1, respectively. The ionizing energy was 70 eV. The chromatograms were obtained by collecting the total ion currents in the scan range of *m/z* 50–550. Data were acquired using the Xcalibur 2.2 software.

2.5. Data processing and compound identification

The raw data acquired from the HPLC-Q Exactive-Orbitrap-MS analysis were processed by the CD 3.0 software. The data matrices of the molecular masses, retention time, fragments, and peak areas from both ESI positive and negative modes were extracted and aligned to the mzVault library, which was integrated in the CD software. The mzVault spectral library (Thermo Fisher Scientific, Waltham, MA, USA) contained the retention time, precise mass ions, and MS^2 fragments of 1200 commercial reference standards, which were analyzed by Q Exactive-Orbitrap-MS. The CD software equipped with the mzVault library identified peaks with a high mass accuracy (< 5 ppm) and an isotope pattern variation within 85%. The molecular compositions adhered to the hydrogen to carbon ratio rules, and were matched to potential compounds using rings and double-bonds equivalents. The MS^2 spectra were compared with the reference spectra from the mzVault library. Compound identification was accepted only when the matching score was greater than 85 (total score = 100). In addition, the accuracy of the compound identification was improved by comparing the obtained data and possible fragmentation patterns with those reported in the literature.

3. Results and discussion

3.1. HPLC-Q Exactive-Orbitrap-MS analysis

The base peak chromatograms of the LHQW capsule in positive and negative ion modes are depicted in Fig. 1. In total, 104 compounds, including alkaloids, flavonoids, phenols, phenolic acids, phenylpropanoids, quinones, terpenoids, and other phytochemicals, were putatively identified (as listed in Table 1 [16–44]) using the library alignment method. According to previous studies [16–44], the identified compounds are present in the herbs used in the LHQW formulation. No phytochemical was identified from *Dryopteridis Crassirhizomatis Rhizoma*. The compound identification of several specific compounds is presented below.

3.1.1. Flavonoids

The molecular formula of compound **71** was $C_{16}H_{14}O_5$, as established by the $[M-H]^-$ peak at *m/z* 285.0760. The MS^2 spectrum was similar to that previously reported [31] for licochalcone B. The MS^2 spectrum and the possible fragmentation pathway are shown in Fig. S1A. The peak at *m/z* 270.0530 indicates the formation of $[M-H-CH_3]^-$. The base peak at *m/z* 150.0316 is attributed to the fragment generated by the cleavage of groups around the carbonyl carbon, followed by loss of CH_3 unit. The fragment responsible for the peak at *m/z* 177.0189 is shown in Fig. S1A. The mzVault library alignment results (Fig. S1B; score: 89.9) revealed that the compound was licochalcone B, which is a phytochemical present in *Glycyrrhizae Radix Et Rhizoma*.

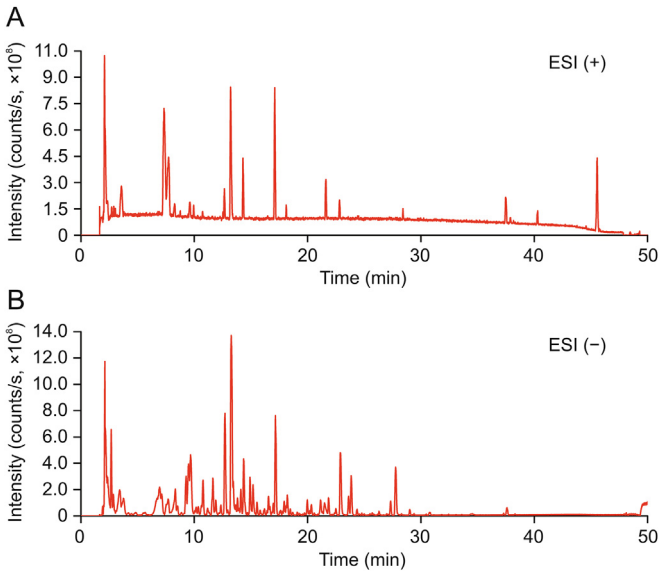

Fig. 1. Base peak chromatogram of the Lianhua Qingwen (LHQW) capsule analyzed using HPLC-Q Exactive-Orbitrap-MS analysis in (A) electrospray ionization (ESI) positive mode and (B) ESI negative mode.

3.1.2. Terpenoids

The molecular formula of compound **26** was $C_{16}H_{22}O_9$, as indicated by the molecular ion peak ($[M+H]^+$) at *m/z* 359.1335. Fragmentation occurred via the loss of a neutral glucose (Glc) molecule (*m/z* 197.0810; $[M+H-Glc]^+$), and the resulting ion was further fragmented to generate $[M+H-Glc-H_2O]^+$ (*m/z* 179.0704) and $[M+H-Glc-H_2O-CO]^+$ (*m/z* 151.0755). The base peak at *m/z* 127.0392 is attributed to the fragment that was generated by the retro-Diels-Alder fragmentation, as shown in Fig. S2A. The library alignment (Fig. S2B; score: 96.2) and comparison with literature data [24] revealed that compound **26** was sweroside, a phytochemical present in *Lonicerae Japonicae Flos*.

3.1.3. Quinones

The molecular formula of compound **97** was $C_{15}H_{10}O_5$, as established by the $[M-H]^-$ peak at *m/z* 269.0454. Peaks at *m/z* 241.0502, 225.0553, and 197.0603 can be attributed to $[M-H-CO]^-$, $[M-H-CO-O]^-$, and $[M-H-CO-O-CO]^-$ fragments, respectively (Fig. S3A). Library alignment (Fig. S3B; score: 96.8) and comparison with literature data [42] revealed that the compound was emodin, a constituent of *Rhei Radix Et Rhizoma*.

3.1.4. Phenolic acids

Compound **31** had a molecular formula of $C_9H_{10}O_5$, as evaluated from the molecular ion peak at *m/z* 197.0451 ($[M-H]^-$) in the MS^2 spectrum (Fig. S4A). The characteristic fragments were $[M-H-C_2H_4]^-$ (*m/z* 169.0138) and $[M-H-C_2H_4-CO_2]^-$ (*m/z* 125.0239). Comparison with literature data [25] and library alignment (Fig. S4B; score: 94) revealed that compound **31** was ethyl gallate, a component of *Rhodiolae Crenulatae Radix Et Rhizoma*.

3.1.5. Phenylpropanoids

The peak at *m/z* 579.2079 in the MS spectrum of compound **75** is attributed to the $[M+HCOO]^-$ adduct, while the peak at *m/z* 533.2019 corresponds to $[M-H]^-$. Characteristic peaks were *m/z* 371.1490, 356.1250, and 121.0288 in the MS^2 spectrum. Library alignment (Fig. S5B) and a comparison with literature data [29] revealed that compound **75** was forsythin, a phytochemical present in *Forsythiae Fructus*. The possible fragmentation pathways and the characteristic fragments are shown in Fig. S5A.

3.1.6. Phenols

The peak at *m/z* 299.1133 in the MS^2 spectrum of compound **17** is attributed to the $[M-H]^-$ ion. Some characteristic peaks and the corresponding fragments were at *m/z* 137.0605 ($[M-H-Glc]^-$) and *m/z* 119.0496 ($[M-H-Glc-H_2O]^-$), and the data are in accordance with that of previous studies [25]. The possible fragmentation pathway is shown in Fig. S6A. Library alignment (Fig. S6B; score: 96.6) identified the compound as salidroside, a compound derived from *Rhodiolae Crenulatae Radix Et Rhizoma*.

3.1.7. Alkaloids

The molecular formula of compound **7** was $C_5H_5N_5$. There were only two characteristic peaks in the MS^2 spectrum (Fig. S7A): *m/z*

Table 1
Identification of the chemical constituents in the LHQW capsule using HPLC-Q Exactive-Orbitrap-MS.

No.	t_R (min)	Error (ppm)	Formula	$[M+H]^+$		$[M-H]^-$		MS^2		Alignment score	Potential compounds	Herb	Refs.
				Theo. *m/z*	Exp. *m/z*	Theo. *m/z*	Exp. *m/z*	ESI (+)	ESI (−)				
1	2.07	1.7	$C_6H_{14}O_6$			181.0717	181.0714		101.0239, 71.0134[b], 59.0135	86.5	Mannitol	*IR*	[16]
2	2.09	1.5	$C_{12}H_{22}O_{11}$			341.1089	341.1084		101.0238, 71.0134, 59.0135[b]	95.6	Sucrose	*IR*	[17]
3	2.12	1.6	$C_7H_{12}O_6$			191.0561	191.0558		191.0556[b], 127.0394, 85.0289	94.8	Quinic acid	*LJ*	[18]
4	2.13	−2.6	$C_5H_9NO_2$	116.0706	116.0709			70.0659[b]		86.4	Proline	*HH*	[19]
5	2.7	−2.4	$C_6H_5NO_2$	124.0393	124.0396			124.0396[b], 96.0449, 81.0534		89.3	Nicotinic acid	*IR*	[17]
6	2.7	1.6	$C_9H_{12}N_2O_6$			243.0622	243.0618		110.0242[b], 82.0294	93.1	Uridine	*IR*	[20]
7	2.71	−1.5	$C_5H_5N_5$	136.0617	136.0619			136.0619[b], 119.0356		89.8	Adenine	*IR*	[21]
8	2.71	2.1	$C_6H_8O_7$			191.0197	191.0193		111.0082[b], 87.0082	93.9	Citric acid	*RR*	[17]
9	2.9	−0.7	$C_{10}H_{13}N_5O_4$	268.1040	268.1042			163.0619[b]		92.6	Adenosine	*IR*	[20]
10	2.97	−0.4	$C_{10}H_{13}N_5O_5$	284.0989	284.0990			152.0569[b]		87.7	Guanosine	*IR*	[20]
11	3.5	3.0	$C_7H_6O_5$			169.0142	169.0137		125.0239[b]	92.3	Gallic acid	*RR*	[22]
12	5.57	3.3	$C_8H_{10}O_3$			153.0557	153.0552		123.0447, 109.0289[b]	87.3	3,4-Dihydroxyphenylethanol	*FF*	[17]
13	5.6	2.6	$C_7H_6O_4$			153.0193	153.0189		109.0289[b]	88.6	Protocatechuic acid	*LJ*	[18]
14	6	−1.5	C_5H_7NOS	130.0321	130.0323			70.0659[b]		86.5	Epigoitrin	*IR*	[23]
15[a]	6.8	0.8	$C_{16}H_{24}O_{10}$			375.1296	375.1293		213.0765, 151.0758, 125.0602[b]	89.4	Loganic acid	*LJ*	[24]
16	7.7	3.6	$C_7H_6O_3$			137.0244	137.0239		137.0238[b]	92.1	Protocatechualdehyde		
17	7.72	1.0	$C_{14}H_{20}O_7$			299.1136	299.1133		137.0605, 119.0496, 89.0239, 71.0134, 59.0135[b]	96.6	Salidroside	*RC*	[25]
18[a]	8.06	0.5	$C_{16}H_{22}O_{10}$			373.1140	373.1138		149.0602, 123.0446, 59.0135[b]	91.1	Geniposidic acid		
19	8.3	0	$C_{20}H_{30}O_{12}$			461.1664	461.1664		205.0709, 135.0446[b]	95.7	Forsythoside E	*FF*	[26]
20	8.59	2.2	$C_8H_8O_5$			183.0298	183.0294		183.0930[b], 168.0066	88.5	Methylgallate		
21	9.23	−0.5	$C_{10}H_8O_4$	193.0495	193.0496			119.0495, 95.0497[b], 91.0549		88.5	4-Methyl-6,7-dihydroxycoumarin		
22	9.55	0.4	$C_{20}H_{27}NO_{11}$			456.1511	456.1509		323.0979, 161.0450, 113.0239, 101.0239, 71.0134, 59.0135[b]	89.4	Amygdalin	*AS*	[27,28]
23	9.64	0.6	$C_{16}H_{18}O_9$			353.0878	353.0876		191.0556, 173.0449, 136.0446[b]	97	Cryptochlorogenic acid	*LJ*	[29]
24	9.8	2.4	$C_9H_6O_4$			177.0193	177.0189		177.0288[b], 133.0289, 105.0341	90.5	Esculetin		
25	9.96	3.3	$C_7H_6O_2$			121.0295	121.0291		121.0290[b]	89.8	*p*-Hydroxybenzaldehyde	*IR*	[17]
26	10.75	−0.3	$C_{16}H_{22}O_9$	359.1336	359.1335			197.0810, 127.0392[b], 179.0704, 151.0755		96.2	Sweroside	*LJ*	[24]
27	11.18	0	$C_{18}H_{19}NO_4$	314.1386	314.1386			265.0860[b], 237.0911, 222.0678		88.1	Norisoboldine		
28	11.2	0.5	$C_{27}H_{30}O_{15}$			593.1511	593.1508		473.1089, 383.0769, 353.0664[b]	91.7	Vicenin II	*GR*	[17]
29	11.5	0	$C_8H_8O_3$	153.0546	153.0546			125.0600, 111.0445[b]		85.2	2-Hydroxy-4-methoxybenzaldehyde		
30	11.63	1.0	$C_{17}H_{24}O_{11}$			403.1246	403.1242		121.0289[b]	93.4	Secoxyloganin	*LJ*	[24]
31	11.7	2.0	$C_9H_{10}O_5$			197.0455	197.0451		169.0138, 140.0111, 125.0239[b]	94	Ethyl gallate	*RC*	[25]
32	11.7	0.9	$C_{32}H_{42}O_{16}$			681.2400	681.2394		357.1340, 151.0395[b], 136.0161	89.7	Pinoresinol diglucoside	*FF*	[30]
33[a]	11.8	0	$C_{19}H_{21}NO_4$	328.1543	328.1543			297.1124[b], 282.0889, 265.0862, 237.0912, 220.0677		85.9	Boldine		
34	11.8	1.6	$C_{26}H_{28}O_{14}$			563.1406	563.1397	383.0769, 353.0662[b], 297.0763		94.1	Isoschaftoside	*GR*	[31]
35	12.2	2.5	$C_9H_8O_3$			163.04	163.0396		119.0496[b]	92.9	*p*-Hydroxy-cinnamic acid		
36	12.4	−0.4	$C_{21}H_{20}O_{11}$			447.0932	447.0934		357.0611, 327.0506[b], 133.0289	90.7	Homoorientin		
37	12.4	−0.5	$C_9H_{10}O_4$	183.0651	183.0652			155.0704, 140.0470, 123.0444, 95.0497[b]		87.4	Syringaldehydes	*EH*	[17]
38[a]	13.2	0.8	$C_{15}H_{12}O_4$	257.0808	257.0806			147.0442, 137.0235[b], 119.0495		95.3	Isoliquiritigenin	*GR*	[31]
39[a]	13.2	1.1	$C_9H_8O_4$	181.0495	181.0493			163.0391[b], 135.0443		85.9	Caffeic acid	*LJ*	[29]
40[a]	13.2	1.0	$C_{29}H_{36}O_{15}$			623.1981	623.1975		161.0238[b], 133.0289	96.3	Forsythoside I	*FF*	[26]
41	13.3	0	$C_{27}H_{32}O_{15}$			595.1668	595.1668		459.1141, 151.0031[b], 135.0447	88.9	Eriocitrin	*MH*	[32]
42	13.3	−0.2	$C_{27}H_{30}O_{14}$	579.1708	579.1709			433.1133 313.0708 283.0603[b]		87.5	Vitexin-2-O-rhamnoside		
43[a]	13.34	1.3	$C_{26}H_{30}O_{13}$			549.1617	549.1610		255.0659[b], 153.0878, 135.0083, 119.0497	93.9	Liguiritigenin-7-O-β-D-apiosyl-4′-O-β-D-glucoside		
44[a]	13.35	0.7	$C_{21}H_{22}O_9$			417.1191	417.1188		135.0082, 119.0496[b], 91.0184	93.6	Liquiritin	*GR*	[31]

45	13.4	0.3	$C_{27}H_{30}O_{16}$			609.1461	609.1459		300.0271[b],271.0246, 255.0296, 243.0295, 151.0032	94.2	Rutin	*LJ* [29]
46[a]	13.5	0.9	$C_{21}H_{20}O_{10}$			431.0983	431.0979		311.0557, 283.0606, 269.0451[b]	86	Vitexin	*EH* [33]
47[a]	13.6	0	$C_{21}H_{20}O_{12}$			463.0881	463.0881		271.0246[b], 255.0296, 151.0032	96.4	Hyperoside	*LJ* *HH* [18,34]
48	13.6	0.7	$C_{14}H_6O_8$			300.9989	300.9987		300.9987[b], 283.9956, 229.1361	94.1	Ellagic acid	
49[a]	13.8	0.3	$C_{27}H_{30}O_{15}$			593.1512	593.1510		285.0401[b]	95.1	Lonicerin	*LJ* [24]
50	13.8	0.2	$C_{21}H_{20}O_{11}$			447.0932	447.0931		285.0401[b]	94	Cynaroside	*LJ* [18]
51	13.8	0	$C_{21}H_{18}O_{12}$			461.0725	461.0725		285.0402[b], 133.0289	90.2	Luteolin 7-glucuronide	*MH* [35]
52[a]	13.8	0.6	$C_{23}H_{26}O_{11}$			477.1402	477.1399		161.0239[b], 133.0289	95.7	Calceolarioside B	
53	14.07	0.6	$C_{16}H_{18}O_9$	355.1023	355.1021			163.0392[b], 135.0443		85.4	Chlorogenic acid	*LJ* [24]
54	14.09	1.2	$C_{25}H_{24}O_{12}$			515.1195	515.1189		353.0875, 119.0556, 179.0344, 173.0449, 135.0446[b]	96.3	Isochlorogenic acid B	*LJ* [24]
55	14.18	3.6	$C_7H_6O_3$			137.0244	137.0239		93.0340[b]	87.6	4-Hydroxybenzoic acid	*LJ* [17]
56	14.3	0.8	$C_{26}H_{32}O_{11}$			519.1871	519.1867		151.0395[b], 136.0159	90.9	Pinoresinol-4-O-glucoside	*FF* [30]
57	14.4	1.0	$C_{25}H_{24}O_{12}$			515.1195	515.1190		353.0873, 191.0556[b], 135.0446	96.3	3,5-Dicaffeoylquinic acid	*LJ* [24]
58	14.7	0	$C_{20}H_{23}NO_4$	342.1699	342.1699			296.1046, 280.1095[b], 265.0861, 237.0912		85.2	Corydine	
59[a]	14.8	0.7	$C_{15}H_{10}O_7$	303.0499	303.0497			303.0497[b], 229.0497, 153.0183		94.2	Quercetin	*LJ* [24]
60	14.89	0.4	$C_{21}H_{20}O_{11}$			447.0932	447.0930		271.0244[b], 255.0295, 151.0031	95.6	Quercitrin	*HH* [34]
61	14.9	0	$C_{27}H_{30}O_{14}$	579.1708	579.1708			271.0603[b], 153.0833		91.9	Rhoifolin	*LJ* [18]
62	14.9	2.2	$C_9H_{10}O_4$			181.0506	181.0502		153.0188, 109.0289[b]	93.3	Ethyl protocatechuate	
63	14.9	2.1	$C_9H_{16}O_4$			187.0975	187.0971		125.0966[b], 97.0653	95.2	Azelaic acid	
64	15.15	1.0	$C_{25}H_{24}O_{12}$			515.1195	515.1190		191.0555, 173.0449, 135.0446[b]	95.7	Isochlorogenic acid C	*LJ* [24]
65	15.27	0	$C_{28}H_{32}O_{15}$	609.1813	609.1813			463.1235, 301.0709[b], 286.0474		91.2	Diosmin	*MH* [32]
66	15.4	−0.2	$C_{22}H_{22}O_{11}$	463.1234	463.1235			301.0708[b], 286.0473, 258.0523		86.4	Diosmetin-7-O-β-D-glucopyranoside	
67[a]	16.13	0	$C_{15}H_{10}O_6$	287.0550	287.0550			287.0552[b], 153.0184		91	Kaempferol	*KJ* [18]
68	16.3	0	$C_{22}H_{22}O_9$	431.1337	431.1337			269.0809[b]		95.2	Ononin	*GR* [36]
69[a]	16.5	0.2	$C_{21}H_{22}O_9$			417.1191	417.1190		255.0659, 148.0161[b], 119.0497	93.5	Isoliquiritin	*GR* [31]
70[a]	16.89	1.1	$C_{15}H_{20}O_4$	265.1434	265.1431			219.1356, 204.1150[b]		87.7	Abscisic acid	*IR* [37]
71	16.9	2.8	$C_{16}H_{14}O_5$			285.0768	285.0760		270.0530, 177.0189, 285.0760, 150.0316[b]	89.9	Licochalcone B	*GR* [31]
72[a]	16.9	0.8	$C_{15}H_{12}O_4$			255.0663	255.0661		135.0082, 119.0497[b], 91.0184	92.3	Liquiritigenin	*GR* [31]
73	16.9	0.7	$C_{15}H_{12}O_6$			287.0561	287.0559		151.0032, 135.0447[b], 107.0132	93.4	Eriodictyol	*EH* *MH* [17]
74[a]	17	1.3	$C_{27}H_{30}O_{15}$	595.1659	595.1651			287.0552[b]		90.7	Kaempferol-3-O-rutinoside	*LJ* [18]
75	17.12	−0.4	$C_{27}H_{34}O_{11}$	535.2173	535.2175			337.1434, 201.0912, 189.0913, 175.0756, 151.0756, 137.0599[b]		91.8	Forsythin	*FF* [29]
76	17.55	0.8	$C_{28}H_{32}O_{14}$	593.1864	593.1859			447.1286, 285.0752[b]		89.9	Linarin	*MH* [38]
77	17.8	1.8	$C_{16}H_{12}O_5$	285.0757	285.0752			285.0759[b], 270.0525, 134.0364		85.6	Calycosin	*GR* [31]
78	17.9	0.7	$C_{15}H_{10}O_6$			285.0404	285.0402		285.0402[b], 133.0289	95.3	Luteolin	*LJ* [31]
79	17.9	1.4	$C_{11}H_{12}O_4$			207.0662	207.0659		179.0344, 161.0238, 135.0446[b]	94.4	Ethyl caffeate	*LJ* [39]
80[a]	18.1	0.6	$C_{20}H_{22}O_6$	359.1489	359.1487			187.0756, 137.0599[b]		87.7	(+)-Pinoresinol	*FF* [26]
81[a]	18.4	0.2	$C_{21}H_{20}O_9$			415.1034	415.1033		253.0504[b], 225.0553	94.9	Chrysophanol-8-O-β-D-glucoside	
82[a]	18.4	0.8	$C_{15}H_{10}O_4$			253.0506	253.0504		253.0503, 225.0553[b]	88.1	Rubiadin	
83[a]	19.02	0.3	$C_{15}H_{12}O_5$			271.06119	271.0611		151.0031, 119.0496[b], 107.0133	94.6	Naringenin	*GR* [31]
84	19.6	0.4	$C_{16}H_{14}O_4$	271.0964	271.0963			229.0861, 177.0547, 121.0288[b], 111.0443		86.2	Retrochalcone	*GR* [31]
85	19.9	−0.4	$C_{30}H_{46}O_3$	455.3519	455.3521			409.3471, 189.1642, 95.0861[b]		85.7	Oleanonic acid	*PH* [40]
86	20.04	0.4	$C_{16}H_{12}O_5$			283.0611	283.0610		240.0425[b]	86.7	Physcion	*RR* [17]
87[a]	21.6	0	$C_{16}H_{12}O_4$	269.0808	269.0808			269.0809[b], 254.0575		94	Formononetin	*GR* [31]
88[a]	22.826	0	$C_{30}H_{46}O_4$	471.3468	471.3468			471.3476[b], 453.3369, 189.1639		89.2	Glycyrrhetic acid	*GR* [41]
89	22.9	0	$C_{16}H_{14}O_4$	271.0964	271.0964			137.0599[b], 109.0653		88.5	Medicarpin	*GR* [36]
90	23.15	−0.4	$C_{15}H_{24}O_2$	237.1849	237.1850			237.1853[b], 219.1744, 135.0807		88.1	Curdione	
91	23.422	0.4	$C_{15}H_{10}O_5$			269.0455	269.0454		269.0453[b], 240.0424	91.8	Aloeemodin	*RR* [22]
92	23.57	1.0	$C_{42}H_{62}O_{16}$			821.3965	821.3957		351.0575, 193.0350, 113.0350[b]	93.1	Glycyrrhizic acid	*GR* [31]

(*continued on next page*)

Table 1 (*continued*)

No.	t_R (min)	Error (ppm)	Formula	$[M+H]^+$		$[M-H]^-$		MS^2		Alignment score	Potential compounds	Herb	Refs.
				Theo. m/z	Exp. m/z	Theo. m/z	Exp. m/z	ESI (+)	ESI (−)				
93	23.8	0.7	$C_{15}H_8O_6$			283.0248	283.0246		239.0346, 229.0499, 183.0446[b]	92.8	Rhein	*RR*	[22]
94	23.831	1.3	$C_{14}H_8O_4$			239.0349	239.0346		239.0346[b], 211.0396, 183.0446	88.2	Antrapurol		
95	26	0	$C_{15}H_{24}O_2$	237.1849	237.1849			121.1016[b]		87.4	Dihydroartemisinic acid		
96	26.69	−0.6	$C_{21}H_{22}O_4$	339.159	339.1592			121.0288[b]		89.4	Licochalcone A	*GR*	[31]
97	27.75	0.4	$C_{15}H_{10}O_5$			269.0455	269.0454		269.0454[b], 241.0502, 225.0553, 197.0603	96.8	Emodin	*RR*	[42]
98	28.49	−0.4	$C_{12}H_{16}O_4$	225.1121	225.1122			207.1019, 139.0392, 81.0706[b]		96	Pogostone	*PH*	[43]
99	28.5	−0.5	$C_{15}H_{22}O$	219.1743	219.1744			219.1745[b], 95.0861		88.7	(+)-Nootkatone		
100	29.6	−0.5	$C_{25}H_{28}O_4$	393.206	393.2062			337.1431, 167.0342[b], 149.0236		88.4	Kanzonol C	GR	[44]
101	30.5	−0.5	$C_{15}H_{22}O$	219.1743	219.1744			219.1746[b], 137.0964		92.8	Germacrone		
102	32.39	−0.8	$C_{30}H_{48}O_4$	473.3625	473.3629			409.3465, 205.1592[b], 189.1639		91	Maslinic acid	*EH*	[17]
103	36.3	−0.4	$C_{18}H_{30}O_2$	279.2318	279.2319			109.1016, 95.0861, 81.0706, 67.0551[b]		92.4	α-Linolenic acid	*IR*	[17]
												AS	
104[a]	39.8	−0.5	$C_{30}H_{48}O$	425.3777	425.3779			137.1327, 95.0862[b]		87.6	Lupenone	*IR*	[17]

t_R: retention time.

[a] Representative retention time of this compound. More than one peak was identified as this compound.

[b] Base fragment ion. Theo. *m/z*: theoretical mass to charge ratio (*m/z*) of the compound. Exp. *m/z*: experimental *m/z* of the compound. *FF: Forsythiae Fructus; LJ: Lonicerae Japonicae Flos; EH: Ephedrae Herba; AS: Armeniacae Semen Amarum; IR: Isatidis Radix; DC: Dryopteridis Crassirhizomatis Rhizoma; HH: Houttuyniae Herba; PH: Pogostemonis Herba; RR: Rhei Radix Et Rhizoma; RC: Rhodiolae Crenulatae Radix Et Rhizoma; GR: Glycyrrhizae Radix Et Rhizoma; MH: Menthae Haplocalycis Herba.*

136.0619 ($[M+H]^+$) and *m/z* 119.0356 ($[M+H-NH_3]^+$). Comparison with literature data [21] and the library alignment results (Fig. S7B) revealed that compound **7** was adenine, an alkaloid present in *Isatidis Radix*.

3.1.8. Miscellaneous

Compound **22** had a molecular formula of $C_{20}H_{27}NO_{11}$, as indicated by the peaks at *m/z* 458.1675 ($[M+H]^+$) and *m/z* 475.1922 ($[M+NH_4]^+$). The peak at *m/z* 296.1129 is attributed to $[M+NH_4-Glc]^+$, and the other characteristic fragments, along with the possible fragmentation pathway are shown in Fig. S8A. Comparison of the possible fragmentation pathway with reference data [27,28], along with the library alignment (Fig. S8B) of the retention time in ESI(−) mode indicated that compound **22** was amygdalin, a chemical constituent present in *Armeniacae Semen Amarum*.

3.2. GC-MS analysis

The volatile constituents in the LHQW capsule were extracted in three different solvents, i.e., hexane, acetone, and ethyl acetate, and then detected using GC-MS. The total ion chromatograms (TICs; Fig. S9) of all three extracts were similar. The TIC of the LHQW capsule extracted in ethyl acetate is shown in Fig. 2. In total, 17 volatile compounds (Table 2) [43, 45–48] were putatively identified by comparing the obtained data with that from the NIST mass spectra library and previously reported literature [11]. The results showed that the major constituent was levomenthol with a relative percentage of 97.89%, as calculated by the peak area normalization method. The other most abundant components were patchouli alcohol (0.47%), β-pathchoulene (0.33%), α-guaiene (0.22%), and α-bulnesene (0.20%). Patchouli alcohol has anti-virus and anti-inflammatory activities reported by Kiyohara et al. [49]. Most of the components were terpenoids derived from *Menthae Haplocalycis Herba* and *Pogostemonis Herba*.

4. Conclusion

In this study, HPLC-Q Exactive-Orbitrap-MS coupled with GC-MS method was used for the first time to identify the chemical

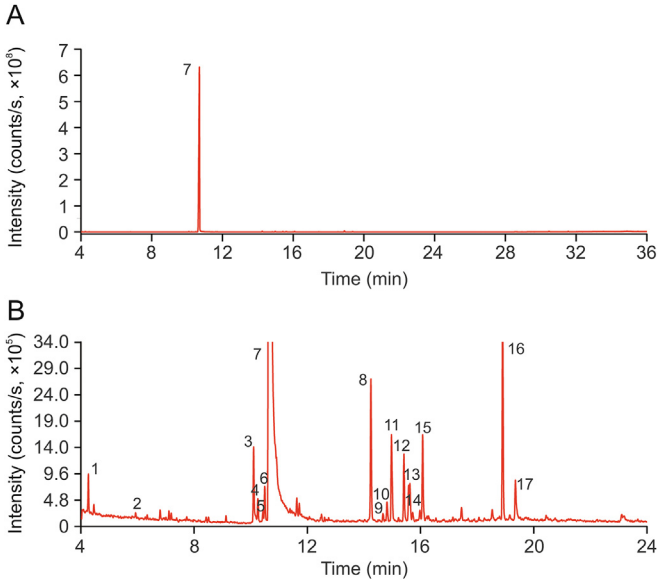

Fig. 2. (A) Total ion chromatogram (TIC) of the LHQW capsule (extracted in ethyl acetate) analyzed by GC-MS. (B) Enlarged image of the TIC from 4 to 24 min.

Table 2
Identification of the volatile components in the LHQW capsule (extracted in ethyl acetate) using GC-MS.

No.	t_R (min)	Potential component	Formula	Relative amount (%)	Herb	Refs.
1	4.25	2,4-dimethylhept-1-ene	C_9H_{18}	0.07		
2	6.8	α-phellandrene	$C_{10}H_{16}$	0.01		
3	10.11	neoisopulegol	$C_{10}H_{18}O$	0.15		
4	10.25	L-menthone	$C_{10}H_{18}O$	0.05	*MH*	[45]
5	10.43	isomenthone	$C_{10}H_{18}O$	0.02		
6	10.5	d-menthol	$C_{10}H_{20}O$	0.07	*MH*	[46]
7	10.7	levomenthol	$C_{10}H_{20}O$	97.89	*MH*	[46]
8	14.25	β-patchoulene	$C_{15}H_{24}$	0.33	*PH*	[47]
9	14.68	ë-selinene	$C_{15}H_{24}$	0.02	*PH*	[47]
10	14.82	isocaryophillene	$C_{15}H_{24}$	0.05		
11	14.98	α-guaiene	$C_{15}H_{24}$	0.22	*PH*	[48]
12	15.42	seychellene	$C_{15}H_{24}$	0.15	*PH*	[48]
13	15.59	α-patchoulene	$C_{15}H_{24}$	0.07	*PH*	[47]
14	15.63	10s,11s-Himachala-3(12),4-diene	$C_{15}H_{24}$	0.09		
15	16.08	α-bulnesene	$C_{15}H_{24}$	0.20	*PH*	[47]
16	18.91	patchouli alcohol	$C_{15}H_{26}O$	0.47	*PH*	[48]
17[a]	19.36	pogostone	$C_{12}H_{16}O_4$	0.14	*PH*	[43]

[a] Compound was also detected by HPLC-Q Exactive-Orbitrap-MS. t_R: retention time. *MH: Menthae Haplocalycis Herba; PH: Pogostemonis Herba.*

constituents in the LHQW capsule, a popular TCM prescribed to treat the symptoms of SARS-CoV-2 infections. In addition, the MS and MS^2 spectrum library alignment method was found to be a convenient approach to rapidly identifying the components of a complex mixture. A total of 120 constituents, including alkaloids, flavonoids, phenols, phenolic acids, phenylpropanoids, quinones, terpenoids, and other phytochemicals, were successfully detected and putatively identified in this study. The findings of our study can contribute to future investigations on the active chemical constituents and the mechanism of action of the LHQW capsule.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jpha.2021.01.004.

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