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Original article

Metabolomics-based comparative analysis of the effects of host and environment on *Viscum coloratum* metabolites and antioxidative activities

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ABSTRACT

Viscum coloratum (Kom.) Nakai is a well-known medicinal hemiparasite widely distributed in Asia. The synthesis and accumulation of its metabolites are affected by both environmental factors and the host plants, while the latter of which is usually overlooked. The purpose of this study was to comprehensively evaluate the effects of host and habitat on the metabolites in *V. coloratum* through multiple chemical and biological approaches. The metabolite profile of *V. coloratum* harvested from three different host plants in two habitats were determined by multiple chemical methods including high-performance liquid chromatography-ultraviolet (HPLC-UV), gas chromatography-flame ionization detector (GC-FID) and ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-QTOF/MS). The differences in antioxidant efficacy of *V. coloratum* were determined based on multiple in vitro models. The multivariate statistical analysis and data fusion strategy were applied to analyze the differences in metabolite profile and antioxidant activity of *V. coloratum*. Results indicated that the metabolite profile obtained by various chemical approaches was simultaneously affected by host and environment factors, and the environment plays a key role. Meanwhile, three main differential metabolites between two environment groups were identified. The results of antioxidant assay indicated that the environment has greater effects on the biological activity of *V. coloratum* than the host. Therefore, we conclude that the integration of various chemical and biological approaches combined with multivariate statistical and data fusion analysis, which can determine the influences of host plant and habitat on the metabolites, is a powerful strategy to control the quality of semi-parasitic herbal medicine.

1. Introduction

Viscum coloratum (Kom.) Nakai (mistletoe) is a well-known medicinal hemiparasite and is widely distributed in Asia. It has been used as a traditional Chinese medicine (TCM) for a long history in China. According to the basic theory of TCM, *V. coloratum* is used to treat ailments such as arthralgia, soreness of the waist and knees, and threatened abortion. In many pharmacological studies, *V. coloratum* has been confirmed to have various pharmacological activities, such as treating cardiovascular system diseases [1–3], anti-aging, anti-oxidation [4], and anti-tumor activities [5–7], and immune regulation [8].

Hemiparasites are capable of photosynthesis. Therefore, like other plants, the synthesis and accumulation of its metabolites are affected by environmental factors. For example, the podophyllotoxin content in *Podophyllum hexandrum*, which grows in different natural habitats, varies greatly [9]. After supplementing UV-B radiation, the total content of flavonoids, kaempferol and quercetin

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glycosides in Brassica napus increased by 150% [10]. *Panax quinquefolius* grown at higher temperatures has higher ginsenoside concentrations than those grown at lower temperatures [11]. Low temperatures stimulate the synthesis of unsaturated fatty acids [12]. The synthesis and accumulation of plant metabolites are also affected by humidity, soil, microbial population and carbon dioxide concentration [13]. Hence, the environment is an important factor influencing the quality of herbal medicine.

On the other hand, hemiparasites support their growth by absorbing water, amino acids, organic acids, mineral nutrients and other substances from host plants [14,15]. For example, the higher water potential and sodium to calcium ratio (Na/Ca>1) allow mistletoe to actively absorb nutrients from the host [16]. The cardiac glycosides could be transferred from *N. indicum* to its parasites [17]. Piwowarczyk et al. [18] found that the metabolites and bioactivities of *Cistanche armena* depend on the species of its host. Vicaş et al. [19] suggested that the phenolic composition of *Viscum album* parasite varies on different trees. Therefore, in addition to environmental factors, host plants also largely affect the metabolite profile in hemiparasites.

At present, *V. coloratum* is purchased and used based on the distinction of origin, while the effect of its host on its metabolites is completely overlooked. This may lead to the uncontrollable quality of *V. coloratum* and affect its effectiveness and safety.

Several previous studies have been conducted to illuminate this problem. For example, Zhao et al. [20] determined ten flavonoids in *V. coloratum* that grow on different hosts and habitats. The common metabolites between *V. coloratum* and its host were analyzed by Long [21]. Vicaş et al. [19] studied the bioactive substances and antioxidant activities of *V. album* from different hosts. However, at present, most of researches focus on either limited numbers of metabolic compounds or merely one of these two influencing factors mentioned above. *V. coloratum* contains various compounds, such as flavonoids, triterpenes, lignins, glucosides, amino acid and volatile oils [22–25]. Thus, a more comprehensive analysis is needed to evaluate the effect of the host and the environment on the metabolic profile of *V. coloratum*, and to determine the most influential factor. In addition, the biological activity is the most direct index to reflect the quality of *V. coloratum*.

To achieve this purpose, in consideration of the characteristics of different compounds, multiple methods were applied to comprehensively analyze the differences in metabolite profile and biological activity of *V. coloratum* that are parasitic on three different host plants harvested from two different habitats (Fig. 1).

First, the metabolite profile of *V. coloratum* was globally determined by multiple methods including high-performance liquid chromatography-ultraviolet (HPLC-UV), gas chromatography-flame ionization detector (GC-FID), and ultra-performance liquid chromatography quadrupole time of flight mass spectrometry (UPLC-QTOF/MS).

Second, the differences in the metabolite profile of *V. coloratum* samples were analyzed by the multivariate statistical analysis. Data fusion is a technology that combines data blocks from different analytical instruments into a single data block, which provides more information than a single technology does [26]. Thus, in addition to separate analyses, a mid-level data fusion strategy was applied in this research.

Third, the antioxidant activities of *V. coloratum* were determined by superoxide radical scavenging and 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging assays.

2. Experimental

2.1. Plant materials

V. coloratum on three types of hosts was harvested from Changbai Mountain (CBM, Jilin province, China; temperate continental climate) and Chengde Mountain Resort (CISR, Hebei

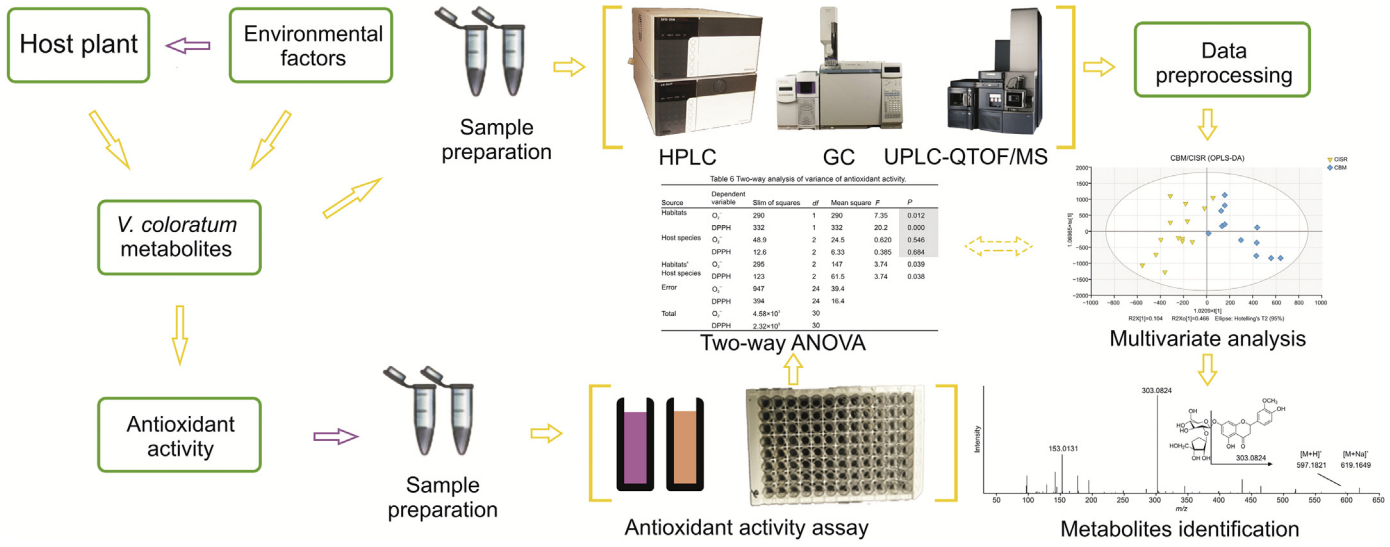

Fig. 1. Integration of multiple chemical and biological approaches for the evaluation of the effects of host and environment on hemiparasite.

Table 1
Viscum coloratum grown on different hosts and environments.

No.	Host species	Origin	No.	Host species	Origin
1–5	*Ulmus pumila* L. (UL)	CBM	16–20	*Ulmus pumila* L. (UL)	CISR
6–10	*Salix babylonica* L. (SA)	CBM	21–25	*Salix babylonica* L. (SA)	CISR
11–15	*Populus ussuriensis* Kom. (PO)	CBM	26–30	*Populus ussuriensis* Kom. (PO)	CISR

CBM: Changbai Mountain, Jilin province, China; CISR: Chengde Mountain Resort, Hebei province, China.

province, China; warm temperate humid monsoon climate). The sample details are shown in Table 1. The collected plants were placed in a ventilated and dark place, and dried at room temperature. The samples with voucher were stored in the State Key Laboratory of Traditional Chinese Medicine (Shenyang Pharmaceutical University, China).

2.2. Chemicals and reagents

The HPLC-grade acetonitrile and HPLC-grade methanol were bought from Fisher Scientific (Fair Lawm, NJ, USA). Tetrahydrofuran normal hexane and methanol were purchased from Tianjin Concord Co., Ltd. (Tianjin, China). Acetic acid and formic acid (HPLC-grade) were purchased from Shenyang Chemical Reagent Factory (Shenyang, China). Tris (hydroxymethyl) methyl aminomethane, pyrogallic acid, pyrogallol and benzaldehyde (HPLC-grade) were purchased from Bodi Chemical Engineering Co., Ltd. (Tianjin, China). Potassium phosphate monobasic and dipotassium phosphate were purchased from Xilong Chemical Co., Ltd. (Shantou, China). Hydrochloric acid was purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). 1,1-Diphenyl-2-picrylhydrazyl free radical was purchased from TCI Chemical Industry Development Co., Ltd. (Shanghai, China). Ultrapure water was used throughout this study.

2.3. Sample preparation

V. coloratum was ground into powder that can pass through the #4 mesh sieve. An accurately weighed 1.0 g *V. coloratum* powder was extracted with 25 mL of 50% methanol in a conical flask with plug under sonication for 30 min. Solvent loss was compensated with 50% methanol when the conical flask was cooled. The extracts were centrifuged at 3,000 r/min for 3 min. The supernatant was then filtered using a 0.22 μm filter for HPLC analysis. The sample solution was diluted twice for UPLC-QTOF/MS.

V. coloratum powder (30 g) was immersed in a 1000 mL flask with 500 mL of distilled water and extracted for 6 h in an essential oil extractor. n-hexane was used as solvent in the essential oil extractor. The solution in the flask was kept slightly boiling during the extraction process. After cooling to ambient temperature, the extract was collected to a 5 mL volumetric flask, and the essential oil extractor was washed twice with n-hexane, which was then added to the extract. n-hexane was added to the volumetric flask until the total volume reached 5 mL. The extract was analyzed by GC after filtering through a 0.22 μm membrane.

2.4. HPLC analysis

A Shimadzu HPLC system, with an LC20ATvp pump coupled with an SPD-20Avp UV-detector (Shimadzu Corp., Kyoto, Japan), was used to analyze the 50% methanol extracts. HPLC analysis was performed according to the previous study [27].

2.5. GC analysis

The assay was performed on an Agilent 6890 N gas chromatograph with a FID (Agilent Corp., Palo Alto, CA, USA). Separation of volatile components was carried out on a PEG-20 M capillary gas chromatographic column (30 m × 0.32 mm × 0.6 μm). The injection volume was 2 μL. The injections were conducted in split mode with a split ratio of 5:1 under the conditions: injector at 250 °C, column oven at 60 °C for 3 min, programmed at a rate of 3 °C/min to 130 °C and then raised to 210 °C at a rate of 2.5 °C/min. Nitrogen served as the carrier gas at 1.2 mL/min.

2.6. UPLC-QTOF/MS analysis

The ACQUITY UHPLC system equipped with the Xevo G2-XS QTOF (Waters Corp., Milford, MA, USA) in ESI-positive ionisation mode was used. The separation was carried out using an ACQUITY UPLC@BEH C_8 column (2.1 mm × 100 mm, 1.7 μm), and the mobile phase included 0.1% formic acid (A) and acetonitrile (B). The elution gradient program was as follows: 0–9 min, 10%–40% B; 9–15 min, 40%–85% B; 15–20 min, 85%–85% B; 20–20.5 min, 85%–10% B; 20.5–22 min, 10%–10% B; flow rate was 0.25 mL/min. The injection volume was 10 μL. The column temperature was kept at 35 °C.

For mass spectrometry detections, data were acquired using sensitivity mode with resolution >22,000 FWHM under positive electrospray ionisation. Acquisition range was 50–1000 *m/z*. Capillary voltage was 3 kV. Cone voltage was 30 V. The temperatures of source and desolvation gas were maintained at 150 °C and 450 °C, respectively, cone gas flow at 50 L/h and desolvation gas flow at 800 L/h. An alternation of low-energy (collision cell energy of 6 V) and elevated energy (collision cell energy ramped from 10 to 30 V) acquisition was used to obtain the precursor ion (MS) and the fragmentation ion. The QC solution was obtained by mixing equal amounts of each sample solution and was injected once every 5 samples throughout the analytical run.

2.7. Data pre-processing and statistical analyses

The original HPLC-UV data and GC-FID data were respectively normalized, which produced a data matrix including the sample name (ID), retention time (t_R), and relative peak intensity.

The original multiple variables were transformed into a few comprehensive indicators (i.e., principal components (PCs)) by principal component analysis (PCA), thereby achieving feature extraction and visualization for large-scale complex data. Then, the processed data were imported in SIMCA 14.0 (Umetrics, Umea, Sweden) for further analysis through PCA with Pareto scaling mode, partial least squares discrimination analysis (PLS-DA), and orthogonal partial least squares discriminant analysis (OPLS-DA).

The UPLC-QTOF/MS raw data were processed on Progenesis QI 2.3 (Waters, Milford, MA, USA). The main procedures included chromatographic peak alignment, peak picking, normalisation, deconvolution, and compound identification. The 36 runs were aligned on the basis of an automatically selected QC. The selected adduct ions, including $[M\text{-}H_2O+H]^+$, $[M+H]^+$, $[M+2H]^+$, $[M+NH_4]^+$, $[M+Na]^+$, $[M+K]^+$ and $[M+ACN+H]^+$, were used to deconvolute the data. Subsequently, a matrix was generated, which contained aligned peaks with normalized peak intensities and sample numbers.

Data fusion was carried out on Matlab R2016a. The mid-level data fusion concatenates latent variables extracted from each data source into a single data set. This data set was then used for further multivariate classification. As the purpose of this study was to investigate the factors with greater influences (better classification), the unsupervised analysis (PCA) was adopted. At first, every raw data matrix was normalized using z-scores, and PCA was then applied for latent variable extraction. The scores of PCs from each data source were concatenated to obtain the fusion data, based on which PCA was performed subsequently [28].

2.8. Identification of characteristic components

Characteristic components were selected by five criteria: variable influence in projection (VIP) >1, |p|>0.1, |p (corr)|>0.5 (correlation coefficient), ANOVA $P \leq 0.05$, and max fold change ≥2. The first three parameters were calculated by OPLS-DA model, and the last two parameters were obtained from Progenesis QI.

On the basis of the abovementioned five criteria, the characteristic components were identified using a self-established *V. coloratum* compounds information database, which was established through references and querying databases such as SciFinder and PubMed. This database contains information regarding compound structure, precise molecular weight, and compound mass spectrum fragment information.

2.9. Evaluation of the antioxidant activity

A total of 0.15 g *V. coloratum* powder was vortexed for 2 min with 4 mL of 0.1 mol/L phosphate buffer (pH 7.2). After kept at room temperature for 1 h, the mixture was sonicated for 1 min. The mixture was then centrifuged at 13,000 r/min for 5 min. The supernatant was used for superoxide radical scavenging assay.

Superoxide radical scavenging activity was determined according to pyrogallol autoxidation method as previously reported [29]. In short, 0.1 mL of the test solution and 0.4 mL of pyrogallol (2.5 mmol/L) were added into 4.5 mL of Tris-HCl buffer (0.05 mL/L, pH 8.2). Then, the mixture was incubated for 4 min at 25 °C followed by recording the absorbance at 299 nm. The free radical scavenging potential (FRSP) was calculated according to the previously reported method.

DPPH free radical scavenging assay was performed according to the method previously reported [30]. In brief, a series of the *V. coloratum* sample solutions (1 mL) were dispensed into a 96-well plate with 2 mL of DPPH methanolic solution (0.2 mM). The absorbance at 517 nm was recorded up to 45 min in the dark. The dose-effect curve was plotted with the clearance rate as the ordinate and the concentration of the sample solution as the abscissa. The concentration of the sample at a clearance rate of 50% (IC_{50}, μmol/L) value was calculated based on the dose-response curve.

3. Results and discussion

3.1. HPLC-UV analysis

Typical chromatogram obtained from HPLC is shown in Fig. 2. The original HPLC-UV data were normalized in Excel, which generated a 51 × 30 data matrix including the sample number (ID), t_R, and relative peak intensity.

The 30 samples could be divided into two groups (CBM and CISR) according to their origin and divided into three groups (PO, SA, and UL) according to their host plant. PCA was performed to analyze the differences in metabolic profile of *V. coloratum*. The score plot is shown in Fig. 3.

The result of PCA showed that the samples from CBM and CISR groups were scattered into different regions while those from PO, SA and UL groups were located together, indicating that the metabolic profile of *V. coloratum* is affected by more the environment than the host.

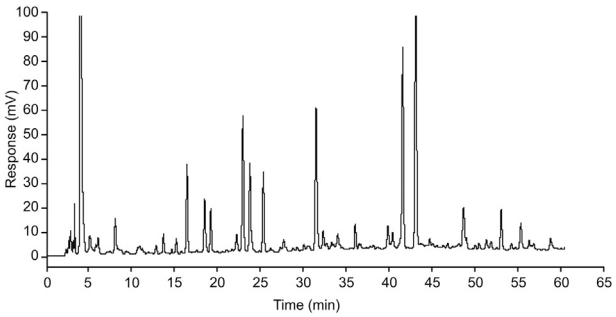

Fig. 2. Typical HPLC-UV fingerprint of *Viscum coloratum*.

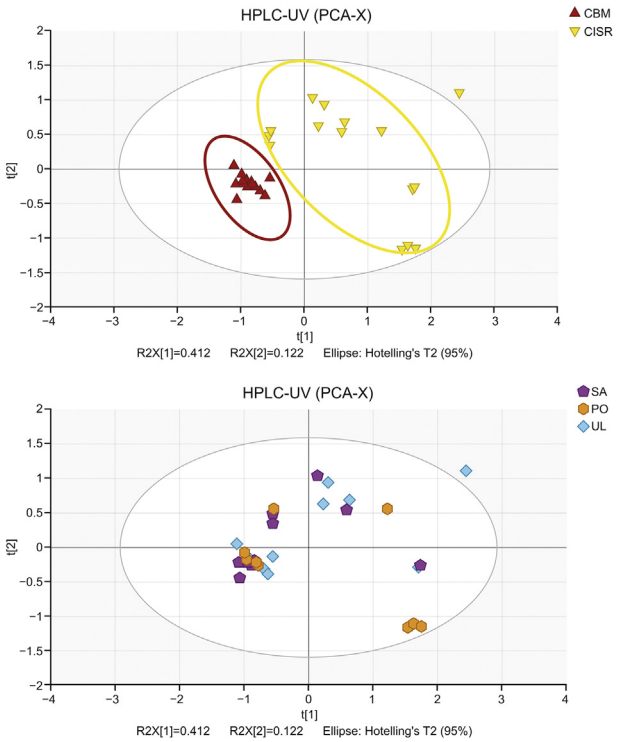

Fig. 3. Score plot of principal component analysis (PCA) for metabolic profile of *Viscum coloratum* obtained from HPLC-UV.

Samples in the CISR group were relatively scattered. This may be possibly attributed to the fierce competition for survival. The forests in Chengde Imperial Summer Resort are denser, and the fierce competition for survival and complex conditions of light and water resources may lead to a large difference in the growth of *V. coloratum*.

3.2. GC analysis

The typical chromatogram of GC is shown in Fig. 4. The original GC data were normalized in Excel, which generated a 51 × 30 data matrix including the sample name (ID), t_R and relative peak intensity. PCA was performed to analyze the data in the matrix. The score plot of PCA for GC metabolic profile is shown in Fig. 5.

As shown in Fig. 5, neither environment nor the host could clearly distinguish *V. coloratum* samples, but a separation trend was observed in both score plots. Therefore, the OPLS-DA model of each pair of comparative host groups and corresponding origin groups

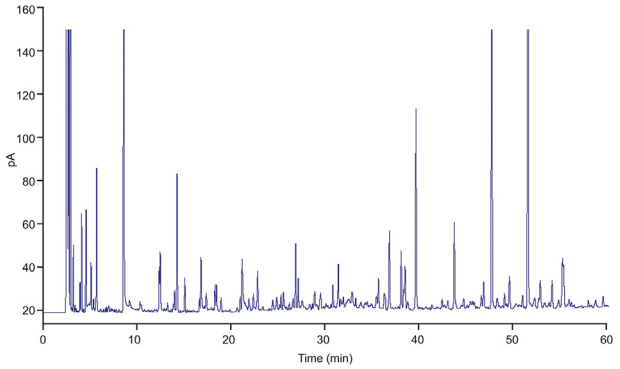

Fig. 4. Typical GC fingerprint of *Viscum coloratum*.

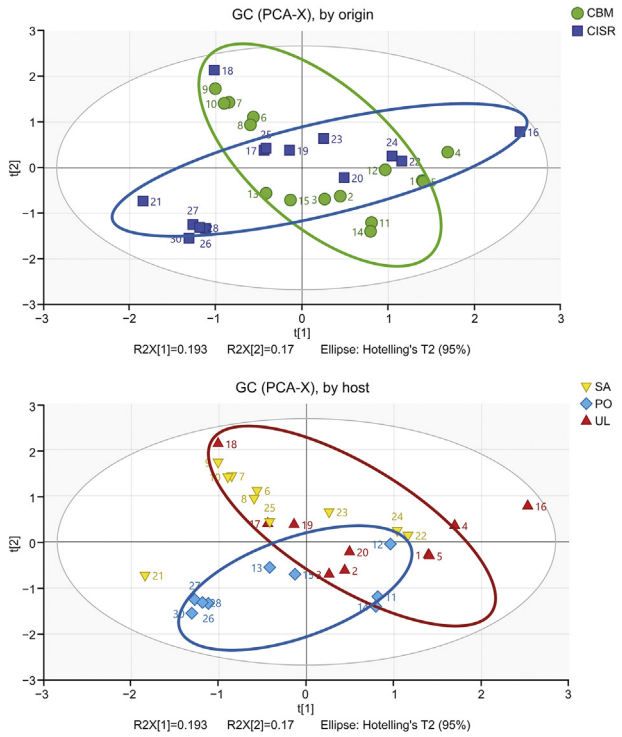

Fig. 5. Score plot of PCA for metabolic profile of *Viscum coloratum* obtained by GC.

was constructed. The score plots of six comparative groups (UL/SA, SA/PO, UL/PO and CBM/CISR in UL/SA, SA/PO and UL/PO) from OPLS-DA are shown in Fig. 6.

The effects of environment and host plants on metabolic profile were evaluated based on the quality of models, which was represented by R2X or R2Y and Q2 terms (Table 2).

In Table 2, Q2 represents the predictability of the model. R2X or R2Y represents the explanation proportion of variance. For all parameters, the closer to 1, the better the model.

An obvious separation between the CBM and CISR groups was observed in the score plots as shown in Figs. 6A–C, but no distinct separation could be seen between the UL and SA groups (Fig. 6D) or between the UL and PO groups (Fig. 6E). Although the samples from SA and PO groups were separated according to principal component 1 (t[1]) (Fig. 6F), they were separated better based on the sample origin (Fig. 6C).

The parameters (Table 2) of every CBM/CISR model were greater than those of the model constructed between host groups. Overall, the comparison results indicate that both environment and host influence the metabolic profile of *V. coloratum*, while different environments largely contribute to the changes of metabolic profile.

The volatile components, most of which are unstable and evaporable, were examined by GC-FID. Due to the fact that the current result can be affected by sample acquisition and storage time, the effect of the host plants and environment on the volatile components of *V. coloratum* requires further study.

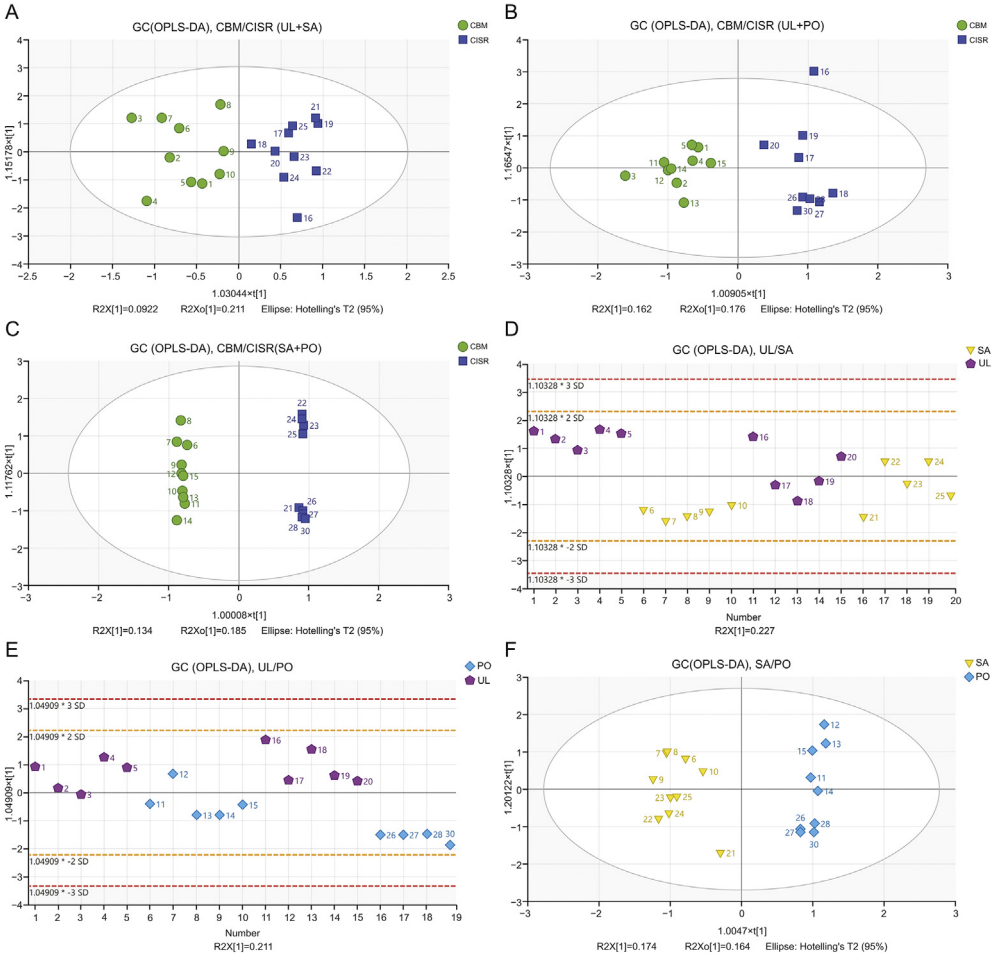

Fig. 6. Score plots of OPLS-DA for six comparative groups. (A) Changbai Mountain, Jilin province, China (CBM)/Chengde Mountain Resort, Hebei province, China (CISR) comparative group in UL and SA groups. (B) CBM/CISR comparative group in PO and UL groups. (C) CBM/CISR comparative group in PO and SA groups. (D) UL/SA comparative group. (E) UL/PO comparative group. (F) SA/PO comparative group.

Table 2
Parameters of six models.

No.	Type	N	R2X (cum)	R2Y (cum)	Q2 (cum)	Model
1	OPLS-DA	20	0.227	0.477	0.129	UL/SA
2	OPLS-DA	20	0.304	0.816	0.375	CBM/CISR in (UL + SA)
3	OPLS-DA	19	0.457	0.952	0.829	SA/PO
4	OPLS-DA	19	0.859	0.998	0.856	CBM/CISR in (SA + PO)
5	OPLS-DA	19	0.211	0.627	0.358	UL/PO
6	OPLS-DA	19	0.337	0.905	0.72	CBM/CISR in (UL/PO)

OPLS-DA: orthogonal partial least squares discriminant analysis.

3.3. UPLC-QTOF/MS analysis

3.3.1. Multivariate statistical analysis

A typical UPLC-QTOF/MS chromatogram is shown in Fig. 7. The raw data were imported in Progenesis QI 2.3 (Waters, Milford, MA,

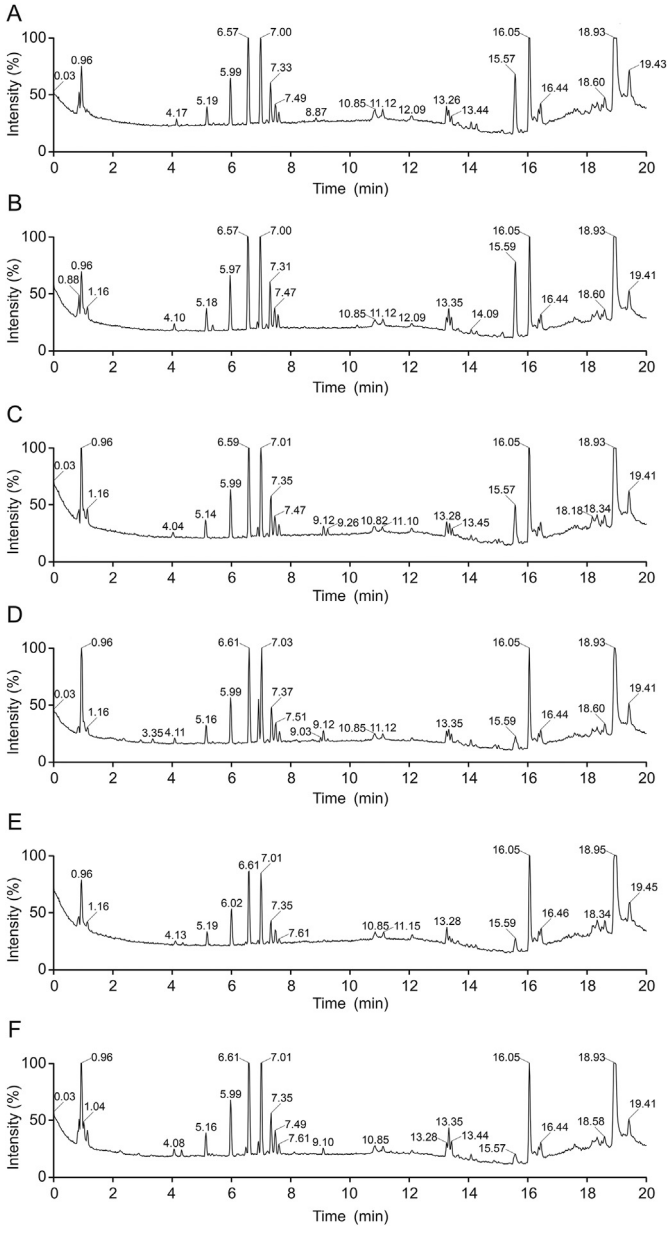

Fig. 7. Typical positive TIC chromatograms of *Viscum coloratum*. (A) Host on PO from CISR, (B) host on UL from CISR, (C) host on SA from CISR, (D) host on UL from CBM, (E) host on PO from CBM, and (F) host on SA from CBM.

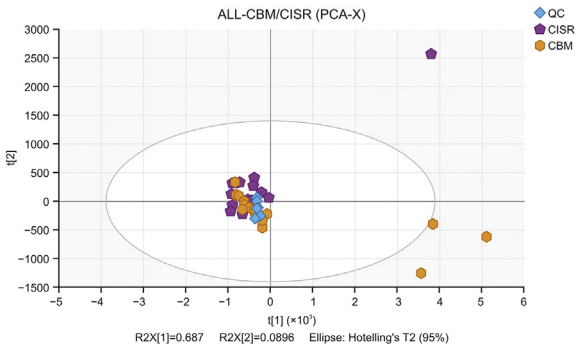

Fig. 8. Score plot of PCA for metabolic profile of *Viscum coloratum* obtained from UPLC-QTOF/MS.

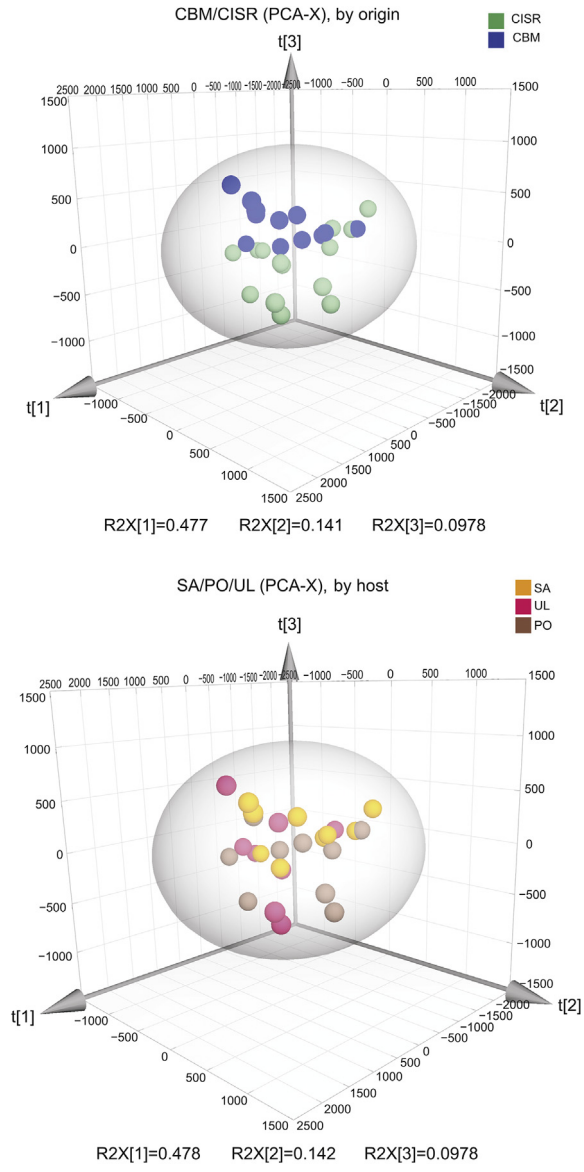

Fig. 9. 3D score plot of PCA for metabolic profile of *Viscum coloratum* analyzed by UPLC-QTOF/MS.

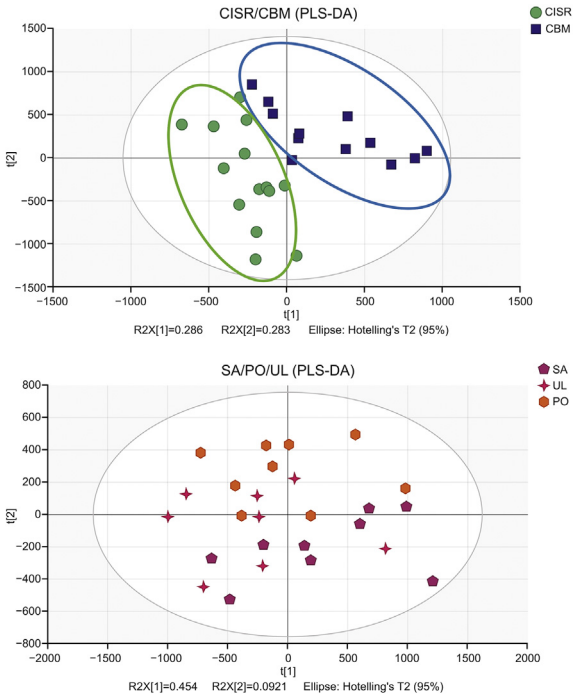

Fig. 10. Score plot of partial least squares discrimination analysis (PLS-DA) for metabolic profile of *Viscum coloratum* obtained from UPLC-QTOF/MS.

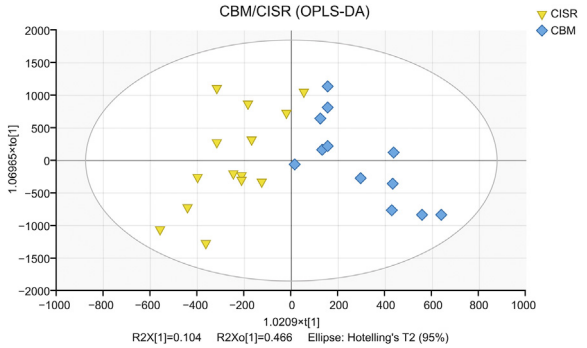

Fig. 11. Score plot of OPLS-DA for metabolic profile of *Viscum coloratum* obtained from UPLC-QTOF/MS.

USA) for data processing, in which a 10,383 × 36 data block including the sample number (ID), t_R, and relative peak intensity was generated.

PCA was used to analyze the data. The score plot from PCA (Fig. 8) shows that the QC samples were clustered closely together located at the centre, indicating that the system stability was excellent. Moreover, four samples (Nos. 1, 5, 15, and 24) were outside the range of the ellipse, indicating that these samples were outliers. Therefore, the remaining samples excluding Nos. 1, 5, 15 and 24 were used in further analyses.

The score plot of PCA excluding outliers (Fig. 9) showed a good separation pattern between the CISR and CBM groups, while no obvious separation was observed among the UL, SA and PO groups, indicating that the metabolites are more affected by the environment than by the host.

PLS-DA was used to differentiate the metabolic profiles between the CBM and CISR groups and among the PO, SA and UL groups. As shown in Fig. 10, samples were divided into two groups according to habitats, while the samples were clustered when grouped by hosts. All parameters of these models are summarised in Table 3. Obviously, the model parameters of CBM/CISR had significantly higher values, whereas the model of SA/PO/UL had lower values of goodness of fit, with Q2 (cum) < 0 and R2Y = 0.336. This result indicated that the metabolites in *V. coloratum* were more affected by the environment than the host plant. Therefore, differential metabolites were screened between CBM and CISR.

Table 3
Parameters of five models.

No.	Type	N	R2X (cum)	R2Y (cum)	Q2 (cum)	Title
1	PCA-X	36	0.777		0.619	ALL
2	PCA-X	26	0.618		0.574	/
3	PLS-DA	26	0.569	0.709	0.603	CBM/CISR
4	PLS-DA	26	0.547	0.336	−0.00074	SA/PO/UL
5	0PLS-DA	26	0.569	0.709	0.572	CBM/CISR

PCA: principal component analysis; PLS-DA: partial least squares discrimination analysis.

3.3.2. Identification of characteristic components

Different metabolites between CBM and CISR groups were analyzed using OPLS-DA. Score plots are presented in Fig. 11.

Characteristic components were screened based on the criteria described in Section 2.8. Ten differential metabolites were screened according to the five standards, and three compounds were identified using a self-established *V. coloratum* compounds information database (Table 4 and Fig. 12).

3.3.3. Data fusion

The latent variables (PCs) were obtained by PCA analysis on three data blocks from HPLC-UV, GC-FID, and UPLC-QTOF/MS. As a result, PC1 and PC2 in HPLC-UV, GC-FID, and UPLC-QTOF/MS explained 56.19%, 52.24%, and 69.64% variances, respectively.

Mid-level data fusion was carried out according to the combination of PCs scores from each data block. Subsequently, PCA was performed on the fused data. The model generated similar results compared with those of the separate analysis. Most samples were clearly classified according to origin (Fig. 13), while they were fused together in accordance with their hosts (Fig. 14). The results of the comprehensive analysis of the data from three platforms indicated that origin accounts for the main influential source, which is consistent with the results from the separate analysis.

3.4. Antioxidant activity

The results from the antioxidant assay of the 30 samples are listed in Table 5. The effects of environment and host plants on the antioxidant activity of *V. coloratum* were determined by two-way ANOVA using IBM SPSS Statistics (IBM, Armonk, NY, USA). The results (Table 6) suggest that the environment exerts a significant impact on the antioxidant activity ($P<0.05$). This finding is consistent with the results from metabolic analysis, which indicated that the quality of *V. coloratum* in the CBM group might be better.

Türe et al. [31] studied the nutritional relationships between mistletoes and its hosts in different habitats, and found that the nutrient absorption of mistletoes from the hosts is mainly influenced by the type of habitat. Scalon et al. [32] found that temperature and humidity significantly affect nitrogen concentration and carbon isotopic composition of leaves in mistletoes and host plants. Moreover, they also suggested that nutrient absorption by mistletoes is closely related to the type of habitat.

Aside from the nutritional characteristics, the numbers of *V. coloratum* on hosts reveal important information for evaluating

Table 4
UHPLC-QTOF/MS data of the characteristic components in *Viscum coloratum* between CBM and CISR groups.

No.	Compounds	Retention time (min)	$[M+H]^+$ and fragments (*m/z*)			Elemental composition
			Calculated	Observed	Error (ppm)	
1	Homoeriodictyol-7-O-β-D-apiosiyl-(1→ 2)-O-β-D- glucoside	6.89	597.1819	597.1830	1.84	$C_{27}H_{33}O_{15}$
			303.0869	303.0844	−8.25	$C_{16}H_{15}O_6$
2	Homoeriodictyol	6.89	303.0869	303.0856	−4.29	$C_{16}H_{15}O_6$
			153.0188	153.0171	−11.11	$C_7H_5O_4$
3	Homoeriodictyol-7-O-β-D-glucoside	6.89	465.1397	465.1379	−3.87	$C_{22}H_{25}O_{11}$
			303.0869	303.0854	−4.95	$C_{16}H_{15}O_6$

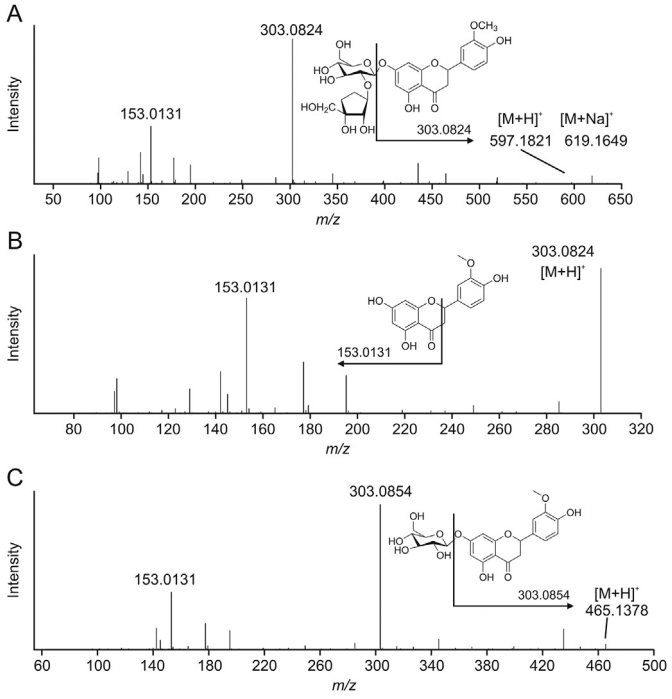

Fig. 12. Fragmentation trace for (A) compounds 6.89_596.1757n, (B) 6.89_302.0625n, and (C) 6.89_464.1294n.

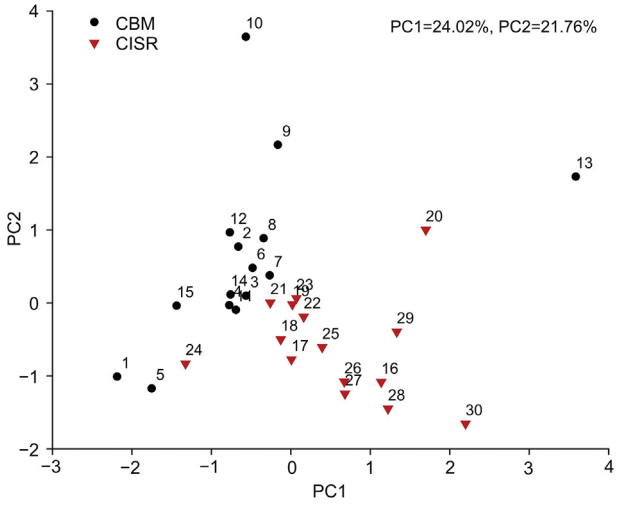

Fig. 13. Scores plot from PCA group by origin for mid-level data fusion.

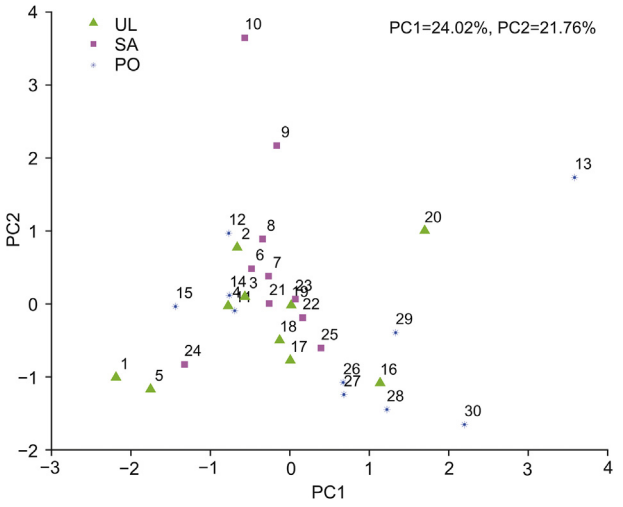

Fig. 14. Scores plot from PCA group by host for mid-level data fusion.

Table 5
Antioxidant activity of *Viscum coloratum* grown on different hosts and origins.

No.	$O_2^{-\cdot}$ FRSP (%)	DPPH IC_{50} (μg/mL)	No.	$O_2^{-\cdot}$ FRSP (%)	DPPH IC_{50} (μg/mL)
1	54.26	4.746	16	28.81	11.893
2	34.34	4.480	17	35.22	11.728
3	29.70	0.996	18	32.07	1.106
4	51.96	11.321	19	36.80	4.325
5	50.79	3.200	20	34.19	7.286
6	31.38	1.166	21	34.29	6.789
7	33.25	1.193	22	34.00	22.962
8	36.31	1.194	23	33.45	8.609
9	37.35	1.686	24	35.13	13.225
10	38.28	2.952	25	52.94	16.905
11	45.62	6.837	26	35.22	11.321
12	38.97	4.301	27	32.91	10.506
13	50.00	8.580	28	37.84	13.115
14	45.17	1.105	29	33.50	6.144
15	44.89	1.002	30	32.61	8.657

FRSP: free radical scavenging potential.

environmental factors. Improvements in *V. coloratum* greatly depends on the ability to absorb water and minerals from their host plants [33]. Hosts may make an attempt to make up for the increased nutritional requirements of *V. coloratum* by acquiring more nutrients from the environment [34]. Generally, nutrients are easily absorbed by host plants in areas with abundant water resources; therefore, the nutrition potential of *V. coloratum* from the hosts is mainly affected by the type of habitat. CBM is covered with original dense forests and rich water resources, whereas CISR is with a semi-arid habitat. Considering the findings from this study, we believe that host plants

Table 6
Two-way analysis of variance of antioxidant activity.

Source	Dependent variable	Sum of squares	*df*	Mean square	*F*	*P*
Habitats	$O_2^{-\cdot}$	290	1	290	7.35	0.012
	DPPH	332	1	332	20.2	0.000
Host species	$O_2^{-\cdot}$	48.9	2	24.5	0.620	0.546
	DPPH	12.6	2	6.33	0.385	0.684
Habitats × Host species	$O_2^{-\cdot}$	295	2	147	3.74	0.039
	DPPH	123	2	61.5	3.74	0.038
Error	$O_2^{-\cdot}$	947	24	39.4		
	DPPH	394	24	16.4		
Total	$O_2^{-\cdot}$	4.58×10^3	30			
	DPPH	2.32×10^3	30			

and environment can have influence on the secondary metabolism and antioxidant activity of *V. coloratum*, while the influence of environment is greater than that of host plants.

4. Conclusions

The metabolite profile of *V. coloratum* is simultaneously affected by host plant and environment, and the environment factors play a key role in the synthesis and accumulation of the metabolites. Meanwhile, three main differential metabolites between two environment groups are identified. The results of antioxidant activity assay indicated that the biological activity of *V. coloratum* is more affected by the environment factors as well, and the quality of *V. coloratum* in CBM may be better.

Overall, various chemical analysis methods can provide multi-dimensional metabolite profile information for the integral quality evaluation. This study may provide new ideas and references for the quality control of semi-parasitic herbal medicine.

CRediT author statement

Rui-Zhen Zhang: Conceptualization, Methodology, Software, Formal analysis, Writing - Original draft preparation; **Jing-Tao Zhao:** Investigation, Formal analysis; **Wei-Qing Wang:** Investigation, Methodology; **Rong-Hua Fan:** Formal analysis; **Rong Rong:** Methodology; **Zhi-Guo Yu:** Resources, Supervision; **Yun-Li Zhao:** Writing - Reviewing and Editing, Supervision, Funding acquisition, Project administration.

Declaration of competing interest

The authors declare that there are no conflicts of interest.

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