

Comprehensive chemical profiling of two *Dendrobium* species and identification of anti-hepatoma active constituents from *Dendrobium chrysotoxum* by Network Pharmacology

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Abstract

Background

D. nobile and *D. chrysotoxum* were important species of the genus *Dendrobium* and has great economic and medicinal value. The material basis of the medicinal effect of *D. nobile* and *D. chrysotoxum* is still unclear, and the biomarkers associated with the anti-cancer are not entirely clear so far. There is no scientific, universal and measurable quality control system, which greatly restricts the development of the *Dendrobium* industry. This study focused on the comprehensive chemical profiling of two *Dendrobium* species and identification of anti-hepatoma active constituents from *Dendrobium chrysotoxum* by Network Pharmacology.

Results

Chemical profiling showed that altogether 65 phytochemicals were identified from *D. nobile* and *D. chrysotoxum*, with major classes as alkaloids, terpenoids, flavonoids, bibenzyls and phenanthrenes. About 18 compounds were identified as the important differential metabolites in *D. nobile* and *D. chrysotoxum*. Furtherly, CCK-8 results showed that the extracts of stems and leaves of *D. nobile* and *D. chrysotoxum* could inhibit the growth of Huh-7 cells, and the anti-hepatoma activity of extracts were dose-dependent. Among the extracts, the extract of *D. chrysotoxum* showed significant anti-hepatoma activity. To find the material basis and mechanisms underlying the anti-hepatoma activity of *D. chrysotoxum*. By constructing and analyzing the compound-target-pathway network, five key compounds and nine key targets were obtained. The five key compounds were chrysotobibenzyl, chrysotoxin, moscatilin, gigantol and chrysotoxene. The nine key targets GAPDH, EGFR, ESR1, HRAS, SRC, CCND1, HIF1A, ERBB2 and MTOR could be considered as the core-targets of the hepatoma activity of *D. chrysotoxum* to hepatoma.

Conclusions

In this study, mass spectrometry-based molecular networking and multivariate statistical analysis was conducted to screen 18 differential metabolites in *D. nobile* and *D. chrysotoxum*. CCK-8 results showed that *D. nobile* and *D. chrysotoxum* extracts could inhibit the growth of Huh-7 cells. The molecular network revealed chrysotobibenzyl, chrysotoxin, moscatilin, gigantol and chrysotoxene were identified as core components of *D. chrysotoxum* on anti-hepatoma. This study compared the chemical composition differences and anti-hepatoma activities between the whole herbs of *D. nobile* and *D. chrysotoxum*, and revealed the anti-hepatoma effects of *D. chrysotoxum* and its potential underlying therapeutic mechanisms in a multi-target and multi-pathway manner.

1. Introduction

Hepatoma is the major cause of cancer associated mortality. Since actual cancer treatments are rarely efficient and often toxic, there is a demand for innocuous and more effective anticancer drugs [1]. Phytochemicals isolated from medicinal plants have been largely neglected in this context, although their pharmacological activities have been well investigated in the past, and they may have considerable potentials for repositioning [2]. In particular, medicinal plant-based can be exploited in the treatment of various ailments including cancer appear as safer and more effective [3]. Various biomolecules present in the plant extract such as alkaloids, terpenoids, polyphenols, polysaccharides, favonoids, tannins, saponins, phenolics, amino acids, and proteins are natural sources of therapeutic drugs [4-8].

Plants of the *Dendrobium* genus, one of the largest in the orchid genera with an upper range of almost 1400 species, manifest a wealth of medicinal effects [9-10]. The stems of *D. nobile* and *D. chrysotoxum* have great economic and medicinal value and are listed in the Chinese Pharmacopoeia (Fig. 1A, B and C) [11]. The chemical constituents of *D. nobile* and *D. chrysotoxum* are mainly alkaloids (Fig. 1D), terpenoids, polyphenols, flavonoids, phenanthrenes, bibenzyls and amino acids [12-14]. Recent pharmacological studies have shown that components of the stems of *Dendrobium*, possess a broad range of activities, encompassing hepatoprotective, antiproliferative activity toward cancer cells, immunomodulatory activity, antidiabetic activity, cataractogenesis-inhibiting activity, neuroprotective activity, anti-inflammatory activity, antiplatelet aggregation activity and hemagglutinating activities and also exert beneficial actions on colonic health and alleviate symptoms of hyperthyroidism [15-17]. Because only the stems of *D. nobile* and *D. chrysotoxum* are permitted for use according to the Chinese Pharmacopoeia, their leaves are largely discarded [18]. However, the leaves of these *Dendrobium* plants possess chemical constituents similar to those found in stems [19-21].

Due to the multicomponent nature of plant extracts and their chemical ingredients, it is difficult to readily understand their mode of action. They act on certain molecular targets due to the synergistic effects of their chemical constituents and the fact that they could interact with many targets simultaneously [3, 22]. In recent years, network pharmacological analysis has been effectively applied for prediction of the protein targets and the related disease pathways of Chinese medicine active compounds. The technique emphasizes on “multi-compounds, multi-target, and multi-disease”, which allows for characterizing the multi-target mechanism of TCM bioactive compounds [23-24]. The core of network pharmacology is to construct a “bioactive compound-target-disease” relationship network, and then predict the mechanism of action of the drug on the organism through a holistic approach network relationship. This approach provides a new perspective to study the relevance between traditional Chinese medicine (TCM) and disease processes, and can reveal possible pharmacological effects of multiple ingredients within chinese herbals [25].

Screening and identification of active components from TCM is rather challenging due to the diversity and complexity of chemical components. Herein, comprehensive metabolite profiling of the *D. nobile* and *D. chrysotoxum* was conducted by LC-MS using an targeted metabolomics approach. The anti-hepatoma activity of the stems and leaves of *D. Nobile* and *D. chrysotoxum* extracts were compared by CCK-8 assay. Network pharmacology approach was applied to characterize the underlying mechanism of

D. chrysotoxum on anti-hepatoma. Active compounds and potential targets of *D. chrysotoxum*, as well as related genes of hepatoma were obtained from the public databases, the potential targets and signaling pathways were determined by protein-protein interaction (PPI), gene ontology (GO) and pathway enrichment analyses. And the compound-target and target-pathway networks were constructed. This study compared the chemical composition differences and anti-hepatoma activities between the whole herbs of *D. nobile* and *D. chrysotoxum*, and revealed the anti-hepatoma effects of *D. chrysotoxum* and its potential underlying therapeutic mechanisms in a multi-target and multi-pathway manner.

2. Materials And Methods

2.1. Reagents and Materials

Methanol (MeOH), acetonitrile and ethanol (all liquid chromatography–mass spectrometry (LC–MS grade) were obtained from Thermo Fisher Scientific (Waltham, MA, USA). 5-Fluorouracil was bought from Macklin Biochemica Co., Ltd.(Shanghai, China). CCK-8 kit was bought from Beyotime Biotechnology(Shanghai, China); Phosphate Buffered Saline (PBS) was bought from Biological Industries (The State of Israel); DMEM medium; Fetal bovine serum (FBS); Antibiotics; Trypsin were bought from Thermo Fisher Scientific (Massachusetts, US).

D. Nobile and *D. chrysotoxum* were harvested from Wenshan, Yunnan Province, China. The two plants were collected with collection permits and identified by Professor Zongsuo Liang, College of Life Sciences and Medicine, Zhejiang Sci-Tech University. The samples were stored in the Key Laboratory of Plant Secondary Metabolism, Zhejiang Sci-Tech University.

The human hepatoellular carcinomas (Huh7 cell line) was donated by Li Gongchu's research group of Zhejiang Sci-Tech University (Hangzhou, China).

2.2. Extraction procedure

The stems and leaves of the *D. Nobile* and *D. chrysotoxum* were cut into small segments completely dried at 50–60 °C. Then crushed by a pulverizer and put into a sealed bag for storage. 10 g of crushed stems and leaves were extracted with 500 mL of 70% methanol aqueous solution with ultrasonic for 45 min respectively, the filtrate was collected, concentrated by a rotary evaporator, and freeze-dried.

2.3. Liquid chromatography tandem mass spectrometry (LC–MS/MS) analysis for metabolite identification

2.3.1 Sample preparation for metabolomics

The tissue samples were dried at 45°C and ground into a fine powder. Approximately 0.01 g of root was homogenized with 10 mL of MeOH/H₂O (70:30, v/v), centrifuged for 5 min and subjected to ultrasonic treatment for 20 min. The sample was then centrifuged at 10,000 rpm for 10 min, and the resulting

supernatant was passed through a 0.22- μ m pore-size membrane. The filtrate was transferred to sample vials for LC-MS/MS analysis.

2.3.2 UPLC-Q-TOF-MS/MS analysis method

Chromatographic separation was achieved using an Waters ACQUITY UPLC HSS T3 column (1.8 μ m, 2.1 mm $\times$ 100.0 mm; Waters Corporation, Milford, MA, USA). The column temperature and flow rate were set at 40°C and 0.4 mL $\cdot$ min⁻¹, respectively. The injection volume and detection wavelength of the chromatographic column were set at 1 μ L and 254 nm, respectively. The mobile phase was composed of water (A) and acetonitrile (B), with a gradient elution program of 96% A (0–1 min), 96–50% A (1–7 min), 50–30% A (7–8 min), 30–5% A (8–12 min), 5% A (12–15 min), 5–96% A (15–16 min) and 96% A (16–18 min).

Mass spectrometric detection was performed in electrospray mode using a Xevo G2-XS Q-ToF mass spectrometer detector (Waters Corporation, Milford, MA, USA). Argon was used as the desolvation and collision gas. The full-scan data range was 50–1,200 Da, the source temperature was 100°C, the desolvation temperature was 400°C and the scanning frequency was 1.000 s. The locking spray standard was 400 mg $\cdot$ mL⁻¹ with a collision energy of 6.000 V for the low collision energy scan and 30 to 70 V for the high collision energy scan of the mass spectrometer, and the UPLC system was controlled by MassLynx4.1 software (Waters Corporation, Milford, MA, USA).

2.4. Cell culture and CCK-8 assay of the extracts

Human hepatoma cell line Huh-7 cells were maintained routinely in DMEM supplemented with 10% FBS and 1% antibiotics in a humidified atmosphere of 5% CO₂/50% air [26]. The anti-hepatoma activity of the stems and leaves of the *D. Nobile* and *D. chrysotoxum* were extracts were determined by CCK-8 assay. 100 μ L cell suspension were cultured in a 96-well plate for 24 h, then the medium was removed, and the cells were incubated with different concentrations of the extracts, the extracts were dissolved in DMEM medium containing 10% FBS and 1% antibiotics for 48 h. 10 μ L CCK-8 solution was added to each well for 2 h, the absorbance was measured at 450 nm using a microplate reader. The absorbance of blank group without cells was Absblank. 5-Fluorouracil was used as a positive contrast [27]. The cell inhibition rate of extract solution was calculated according to the following formula.

$$\text{Inhibition rate (\%)} = 1 - \frac{\text{Abs}_{\text{sample}} - \text{Abs}_{\text{blank}}}{\text{Abs}_{\text{control}} - \text{Abs}_{\text{blank}}} \times 100 \quad (\text{Formula})$$

2.5. Network Pharmacology Analysis

2.5.1 Database Construction and Prediction of Potential Targets

The SIMILES and InChIKey of the chemical components of aerial part of *D. chrysotoxum* were collected from the PubChem database. The GI absorption and bioavailability score of the compounds were calculated by SwissADME, and the compounds with GI absorption were high and bioavailability score > 0.3 were screened. The Swiss Target Prediction database was used to obtain the target corresponding of the components [28]. Use “Liver cancer” as the key words, the liver cancer related genes were screened out by DisGeNET [29], GeneCards [30], OMIM[31]. The obtained targets of the constituents and the disease targets of liver cancer were unified as UniProt ID through the UniProt database [32].

2.5.2 Network Construction

Cytoscape software was used to construct the “drug components-target” network to explain the interactions between the core chemical components of *D. chrysotoxum* and potential targets in liver cancer therapy. In order to better analyze the protein-protein interaction, the PPI network of potential targets was constructed by using the STRING database using the common target of the liver cancer related genes and target corresponding of the components, the species was defined as Homo sapiens [33]. The “drug components-target” network and PPI network was imported into Cytoscape software for topological attribute analysis, and the key nodes and degree values in the network map were analyzed to obtain the core components and targets of *D. chrysotoxum*.

2.5.3 Biological Function and Pathway Analysis

In order to illustrate the role of the core target in the gene function and signal pathway of the active ingredient in the treatment of liver cancer. David database was used to perform gene ontology (GO) function and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis to explore the mechanism of anti-hepatoma activity of *D. chrysotoxum* [34].

2.6. Data analysis

Mass spectrometry data were collected using MassLynx V4.2 software (Waters Corporation, Milford, MA, USA) for automatic peak identification, peak matching, peak alignment, peak extraction, peak integration and normalization. The metabolites and their possible cleavage modes were identified using secondary mass spectrometry data, Unifi (Waters Corporation, Milford, MA, USA), online databases (SciFinder, Chemspider and PubMed) and data from previous studies. Unsupervised principal component analysis (PCA) was performed using SIMCA-P 14.1 (Umetrics Corporation, Umea, Sweden) software to obtain overall metabolic differences between sample regions and determine the magnitude of variation between samples within groups. Supervised orthogonal partial least squares discriminant analysis (OPLS-DA) was performed to obtain information about related metabolites that were significantly different between subgroups. The median inhibitory concentration (IC₅₀) of different samples was obtained by processing the efficacy data with graphpad software. All the experiments were performed in triplicates and the results are given as mean ± standard deviation (SD).

3. Results And Discussion

3.1. Overview of the metabolomes of different tissues

Comprehensive metabolite profiling of the *D. nobile* and *D. chrysotoxum* was conducted by LC-MS by using an untargeted metabolomics approach. By looking at the trend of ion peaks visible in BPI between samples and the metabolomes from samples were grouped into the same cluster, indicating high similarity between different tissues of the same species (Fig. 2A). To analyze different samples tissue-specific accumulated metabolites, metabolite types that have been identified are localized to different samples. In *D. nobile*, the major metabolite types were alkaloids (21 metabolites), terpenoids (8 metabolites), flavonoids (3 metabolites) and phenanthrenes (3 metabolites); in *D. chrysotoxum*, the largest number of metabolite types belonged to bibenzyls (4 metabolites), phenanthrenes (3 metabolites) and phenolic acids (2 metabolites) (Fig. 2B). Notably, As for the metabolites specifically enriched in *D. nobile*, alkaloids were abundant in stems but also were observed in leaves (Fig. 2C). In both plants, the predominant differential metabolites were alkaloids and bibenzyls.

3.2. Metabolomic multivariate statistical analysis of *D. nobile* and *D. chrysotoxum*

3.2.1. Metabolic profiling of *D. nobile* and *D. chrysotoxum*

Analysis using our in-house and public databases, tentatively identified 65 metabolites for both the negative and positive-ion modes to give a comprehensive metabolome for the stems and leaf of *D. nobile* and *D. chrysotoxum* (Tables S1, S2, S3 and S4). The majority of metabolite types were alkaloids, terpenoids, bibenzyls, phenanthrenes, phenolic acids, flavonoids, carboxylic acids. Figure 3A presents two heat-maps corresponding to the relative content of different classes of metabolites among the different plant stems and leaf. The alkaloids clearly had greater metabolite diversity, especially in *D. nobile* stem and leaf, such as dendrobine, N-isopentenyl-dendrobinium, N-isopentenyl-dendroxinium, N-methyldendrobinium, homocrepidine B and dendroxine. As shown in Fig. 3A, phenanthrenes were detected in all the plant stems as the major metabolites, however, different contents and types exist among these two plant parts. Chemometric analysis was applied to quantify metabolite abundance among the *D. nobile* and *D. chrysotoxum*. PCA and OPLS-DA analysis could clearly distinguish the plant parts (Fig. 3B-C). The VIP value ≥ 1.0 derived from the OPLS-DA analysis revealed differences in metabolite profiles between the *D. nobile* and *D. chrysotoxum* (Fig. 4). Notably, 18 compounds were identified as the important differential metabolites in *D. nobile* and *D. chrysotoxum*, including alkaloids, phenanthrenes and terpenoids.

3.3. Evaluations of in vitro anti-hepatoma activity

CCK-8 results showed that all four extracts could inhibit the growth of Huh-7 cells, and the anti-hepatoma activity of extracts were dose-dependent. According to the Table 1, among the extracts, the extract of leaves of *D. chrysotoxum* showed most significant anti-hepatoma activity, the stem extract of *D. nobile* showed the weakest anti-hepatoma activity.

Table 1

Anti-hepatoma activity of four extracts determined by CCK-8.

Sample	Anti-hepatoma activity <i>IC</i> ₅₀ (μg/mL)
5-Fluorouracil	30.04 ± 1.16 ^a
<i>D. Nobile</i> -Stem	1315.67 ± 37.50 ^e
<i>D. Nobile</i> -Leaf	815.00 ± 70.73 ^c
<i>D. chrysotoxum</i> -Stem	1051.67 ± 48.09 ^d
<i>D. chrysotoxum</i> -Leaf	493.50 ± 28.56 ^b

3.4. Network pharmacology analysis

3.4.1. PPI network analysis

Because the stems and leaf of *D. chrysotoxum* have excellent anti-hepatoma effect, it was selected to perform a network pharmacological analysis. About 1526 liver cancer related targets were obtained by DisGeNET, GeneCards and OMIM. Finally, 112 potential targets for anti-hepatoma activity of *D. chrysotoxum* were obtained, the PPI network of the 112 potential targets were constructed by using the STRING database (Fig. 5A). The required interaction score was set to 0.400. The PPI network contained 112 nodes, 1192 edges, an average node degree of 21.3. Each node in the figure represents a protein, and the connections between the nodes represent the interaction between the proteins. The common target may be related to the anti-hepatoma activity of *D. chrysotoxum*. According to the topological attribute analysis of PPI network, GAPDH, EGFR, ESR1, HRAS, SRC, CCND1, HIF1A, ERBB2 and MTOR were the nodes with high degree and could be considered as the core-targets of the hepatoma activity of *D. chrysotoxum* to liver cancer.

3.4.2. Pathway enrichment analysis

Biological function analysis of anti-hepatoma activity of chemical components were performed by David database. The GO enrichment results for the first 12 terms of biological process, cellular component and molecular function are shown in Fig. 5B. According to *p* value analysis, the results of the first 20 KEGG

pathways were shown in Fig. 5C, including prostate cancer, pathways in cancer, endocrine resistance, EGFR tyrosine kinase inhibitor resistance, central carbon metabolism in cancer, FoxO signaling pathway, etc.

3.4.3. Herbs-chemicals-targets-pathways-therapeutic effects network analysis

22 components were identified by UPLC-Q-TOF-MS, 6 non-target protein were eliminated and 454 targets were obtained by Pubchem, SwissADME and Swiss target prediction. Chrysotobibenzyl, chrysotoxin, moscatilin, gigantol and chrysotoxene were identified as core components by topological attribute analysis of “drug components-target”.

Discussion

Quality oriented UPLC-Q-TOF-MS Metabolite fingerprint

Plants are unique in the richness and diversity of their secondary metabolism, the literature suggests that the number of metabolites produced in the plant kingdom exceeds 200,000 [35]. Although there are hundreds of millions of phytochemicals, only a small number have been isolated and identified from plants [36, 37]. The advancement of metabolomics in terms of techniques for measuring small molecule composition has enabled the rapid assay and quantification of numerous the endogenous metabolites of an organism [38]. With the optimization of the analytics platform, such as gas or liquid chromatography (GC-MS and LC-MS, respectively) and nuclear magnetic resonance spectroscopy (NMR), have enabled simultaneous characterizing the dynamic overall level of metabolites [39–41]. Based on this, UPLC-Q-TOF-MS was used to identify *D. nobile* and *D. chrysotoxum* comprehensive metabolome in different tissues. Research shows that the levels of compounds from different *Dendrobium* varied greatly. Among them, *D. nobile* stem is abundant and diversified in alkaloids, such as dendrobine, N-isopentenyl-dendrobinium and N-methyldendrobinium, in *D. chrysotoxum*, bibenzyls and phenanthrenes secondary metabolites were significantly accumulated, such as chrysotobibenzyl, chrysotoxin, moscatilin, and chrysotoxene, which was consistent with previous studies [42–44]. In addition, we also found that there was no significant difference in the chemical composition of stems and leaves of *Dendrobium* species, indicating that leaves of *Dendrobium* species are also essential sources of natural lead compounds [45, 46]. Chemometric analysis of the metabolites differences between the two species revealed that they are quite different in evolution [46, 47]. However, due to the wide variation in the types and contents of secondary metabolites of different *Dendrobium* species, the differences in their active ingredients may lead to the differences in their pharmacological activities.

Variation on anti-cancer effects of *D. nobile* and *D. chrysotoxum*

Modern pharmacological studies have shown that *D. nobile* and *D. chrysotoxum* has had a strong anti-cancer effect [15, 48]. For example, *D. chrysotoxum* natural products caused moderate growth delay in

xenografted human hepatoma Bel7402 and melanoma A375 and induced significant vascular shutdown within 4 h of administering 100 mg/kg of the drug [49]. In addition, *D. nobile* extracts down regulated the expression level of decoy receptor-3 and synergized with Fas ligand to bring about apoptotic cell death in pancreatic adenocarcinoma cells [50]. Our study found that *D. nobile* and *D. chrysotoxum* significantly inhibited the proliferation rates of Huh7 cells, this result is consistent with the results of other studies and may contribute to the development of *D. nobile* and *D. chrysotoxum* as pharmacological therapies for hepatoblastoma or hepatocellular carcinoma. However, the inhibition rates of the two dendrobium species on hepatocellular carcinoma cells were different. The study showed that the anti-hepatoma effect of *D. chrysotoxum* was significantly better than that of *D. nobile*, and it was worth noting that *D. chrysotoxum* leaves had the best anti-hepatoma effect. In traditional processing of *Dendrobiums*, leaves were removed after thoroughly washing. Meanwhile, the leaves on the *Dendrobiums* stem are sometimes peeled sometimes for aesthetic reasons. According to our results, removal of leaves of *D. nobile* and *D. chrysotoxum* may leads to loss of bioactive constituents.

Conclusion

In this study, mass spectrometry-based molecular networking and multivariate statistical analysis was conducted to screen the differential metabolites in *D. nobile* and *D. chrysotoxum*. We screened 18 differential ions ($[M + H]^+$ and $[M - H]^-$) in the positive-ion and negative-ion model of UPLC-Q-TOF-MS/MS using OPLS-DA and PCA. CCK-8 results showed that *D. nobile* and *D. chrysotoxum* extracts could inhibit the growth of Huh-7 cells, and the anti-hepatoma activity of extracts were dose-dependent. The molecular network revealed chrysotobibenzyl, chrysotoxin, moscatilin, gigantol and chrysotoxene were identified as core components of *D. chrysotoxum* on anti-hepatoma. Our research provides a effective method for rapid screening and identification of the differential metabolites in different *Dendrobium* species, and provides candidate chemical markers for quality control of *D. nobile* and *D. chrysotoxum*.

Declarations

Ethics approval and consent to participate

The authors declared that experimental research works on the plants described in this paper comply with institutional, national and international guidelines. Collections of both *D. nobile* and *D. chrysotoxum*. Species have been permitted or licensed and comply with the relevant regulations for scientific research. Field studies were conducted in accordance with local legislation and get permissions from provincial department of forest and grass of Yunnan province. The specimens were then stored in the Key Laboratory of Plant Secondary Metabolism, Zhejiang Sci-Tech University.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on request.

Competing interests

We declare that we have no competing interests.

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Authors' contributions

JX and JNY were responsible for the experimental study, data analysis and manuscript writing, TGZ, FJH and YF for the sample collection, LGG, ZYX and YY for the composition testing, corresponding authors XDZ, ZSL were responsible for the overall design, planning of the project and revision of the manuscript.

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Figures

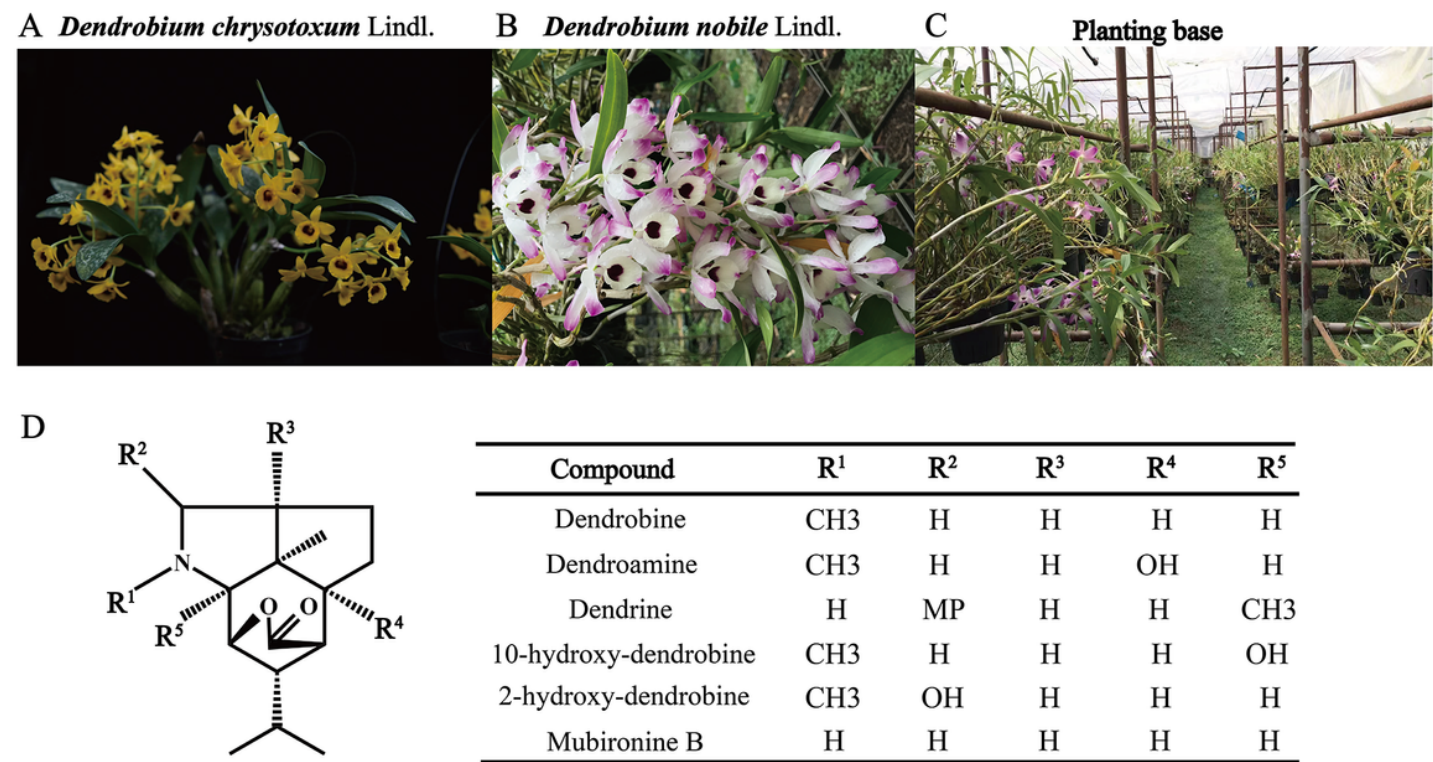


Figure 1

Plants and metabolites. (A, B, C) *Dendrobium* species planting base in Kunming, Yunnan; (D) Main alkaloids of *Dendrobium*.

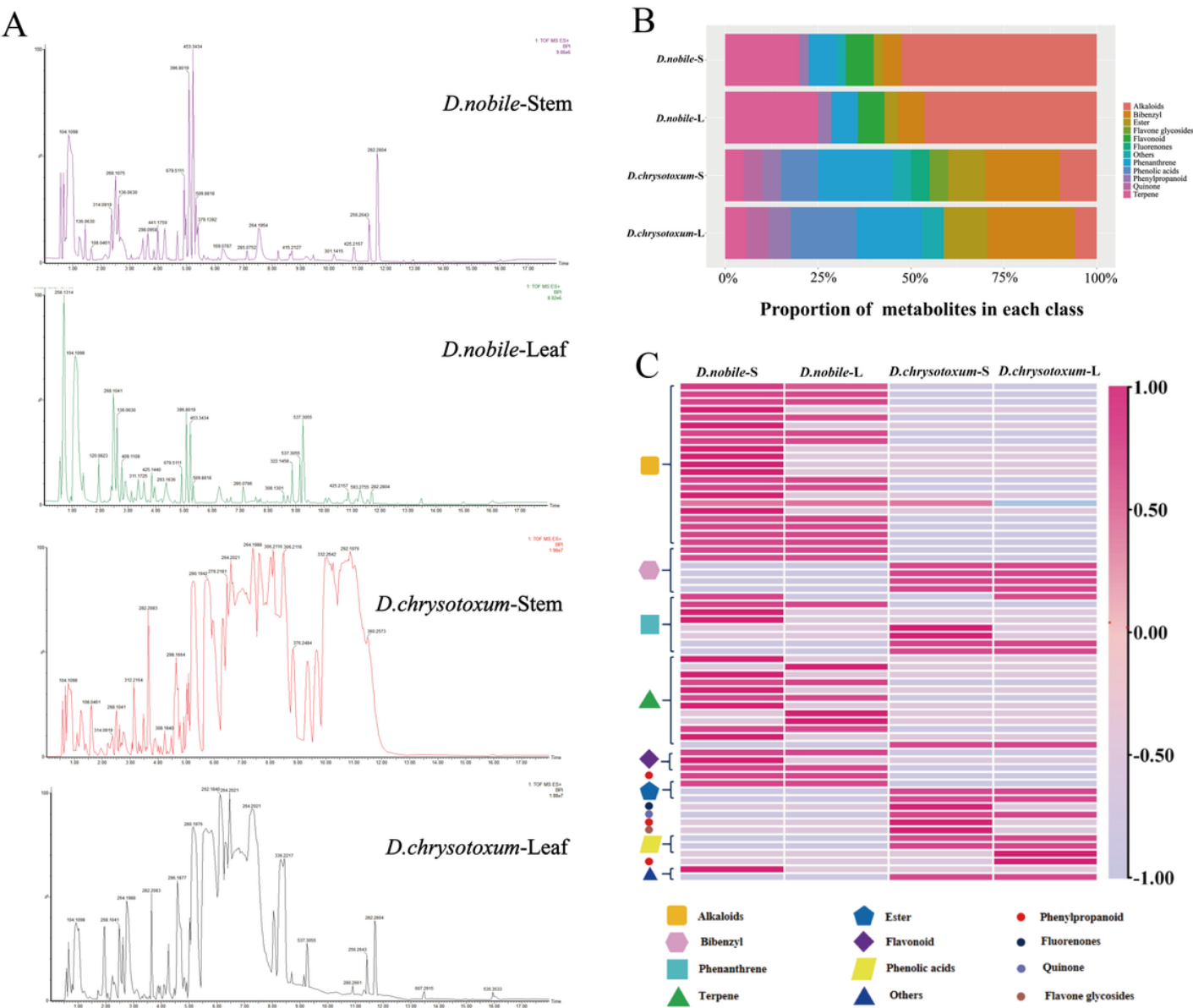


Figure 2

Differentially accumulated metabolites among the four tissues. (A) The base peak ion chromatogram (BPI) of different samples; (B) Proportions of metabolites in each samples; (C) A heatmap of the abundance of metabolites in the four tissues.

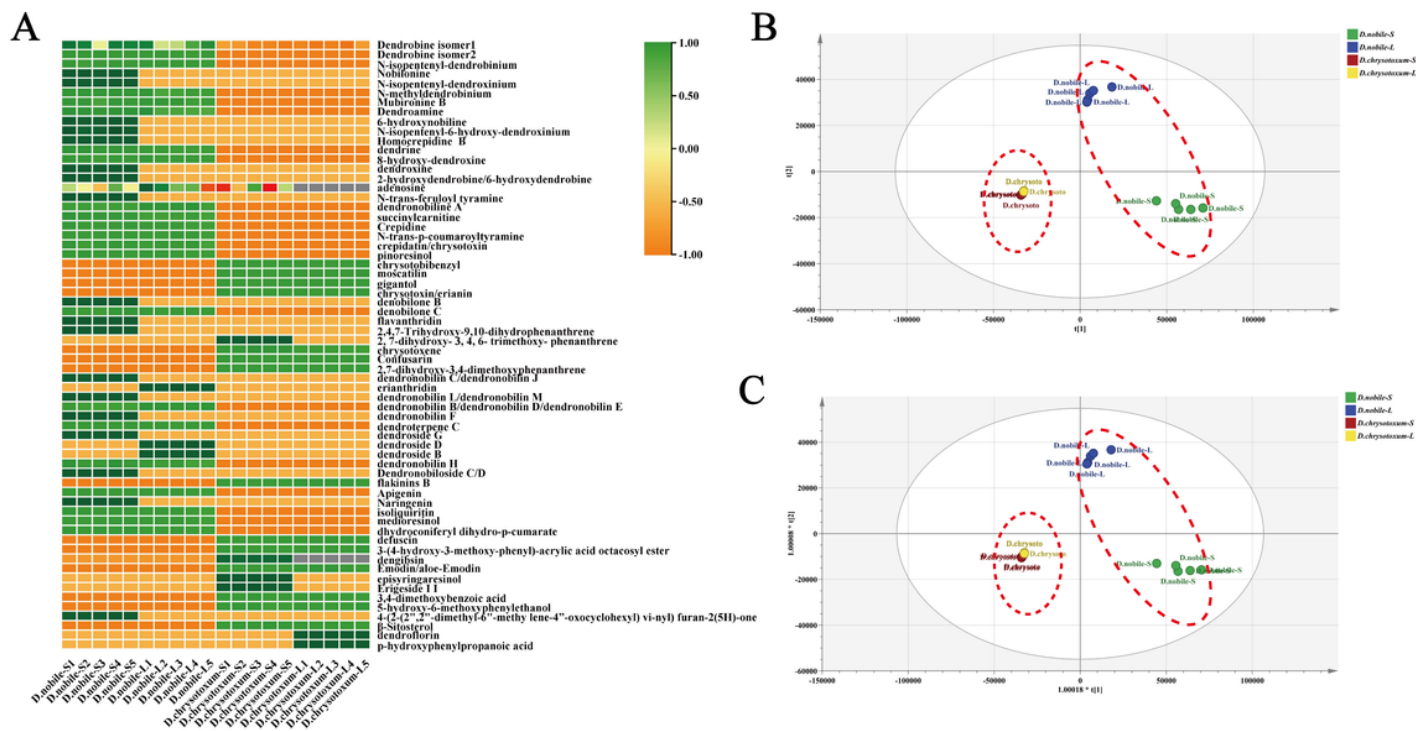


Figure 3

The results of non-targeted metabolomics and chemometric analysis of *D. nobile* and *D. chrysotoxum*. (A) Heat map of metabolomics and chemometrics clustering of *D. nobile* and *D. chrysotoxum*; (B) Principal component analysis of *D. nobile* and *D. chrysotoxum*; (C) OPLS-DA analysis of *D. nobile* and *D. chrysotoxum*.

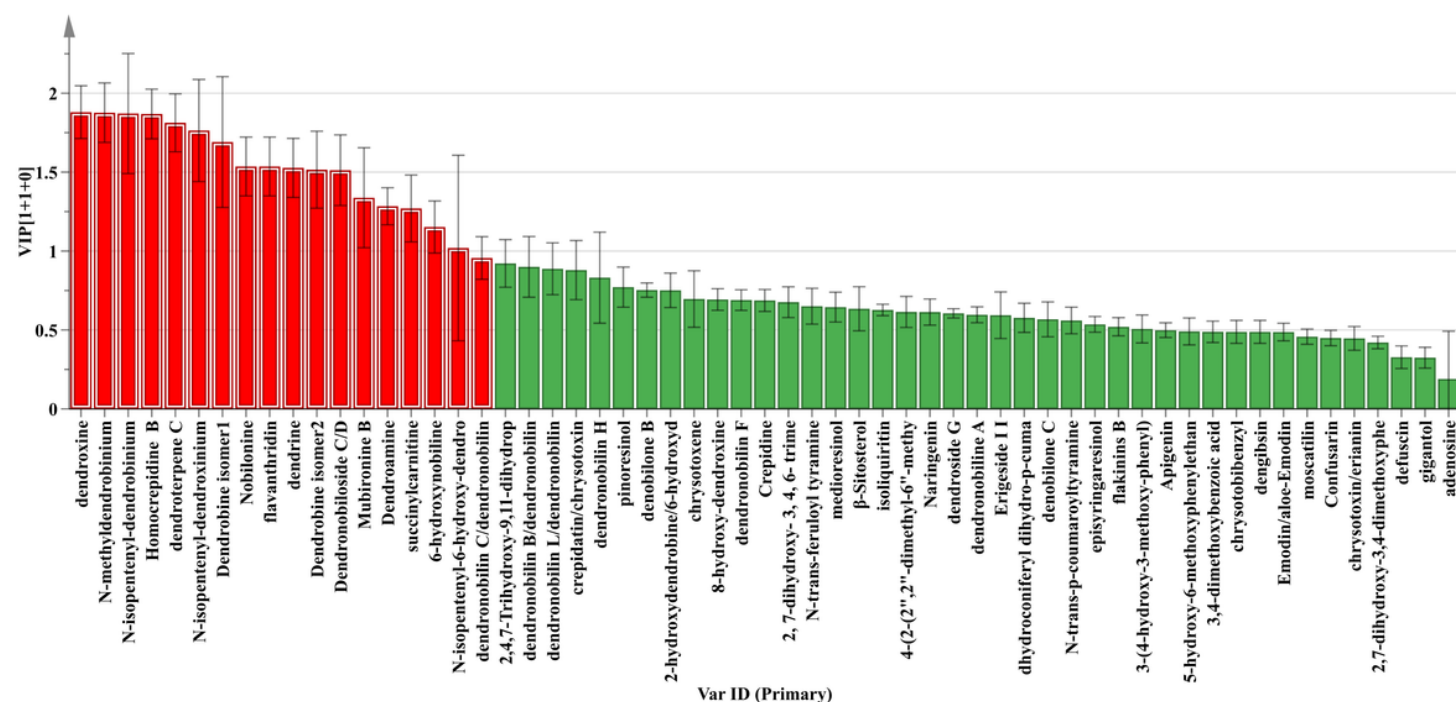


Figure 4

The values of VIP of the *D. nobile* and *D. chrysotoxum*.

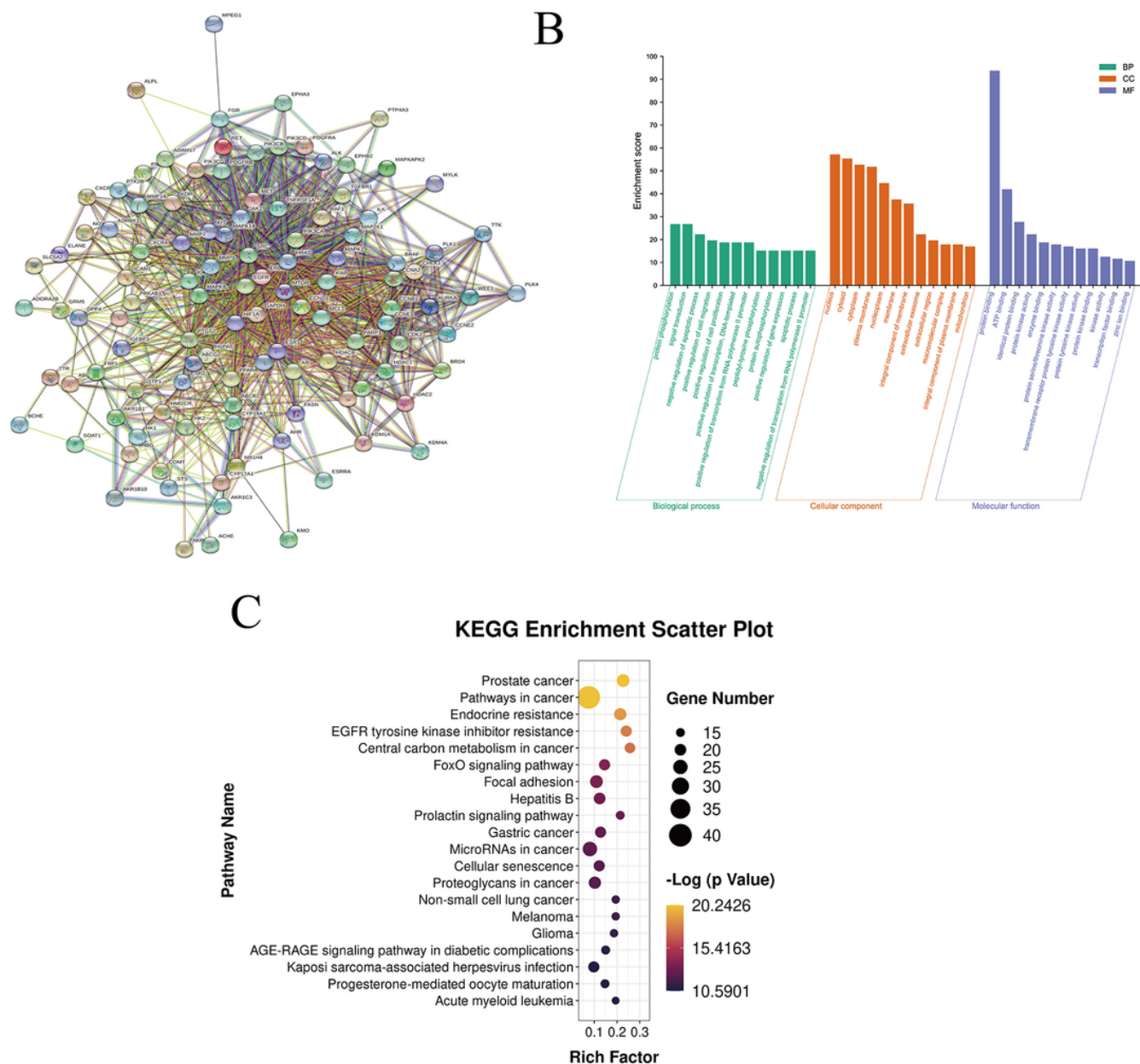


Figure 5

The integrated pharmacology network of “herbs-chemicals-targets-pathways-therapeutic effects” of *D. chrysotoxum* in the treatment of anti-hepatoma. (A) The PPI network of the potential targets. (B) The top 12 items in the GO biological process, cellular component and molecular function; (C) The top 20 items in Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathway.

Supplementary Files

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