Research Article

Analysis of Bioactive Components in the Fruit, Roots, and Leaves of *Alpinia oxyphylla* by UPLC-MS/MS

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*Alpinia oxyphylla* (*A. oxyphylla*) fruit has long been used in traditional Chinese medicine. In our study, the bioactive components of its roots, fruit, and leaves were investigated, and their potential medical value was predicted. The root, fruit, and leaf samples were analyzed using a UPLC-MS/MS system. The mass spectrometry outcomes were annotated by MULTIAQUANT. The "compound-disease targets" were used to construct a pharmacology network. A total of 293, 277, and 251 components were identified in the roots, fruit, and leaves, respectively. The fruit of *A. oxyphylla* had a higher abundance of flavonols. The roots of *A. oxyphylla* were enriched in flavonols and phenolic acids. The leaves of *A. oxyphylla* exhibited high contents of flavonols, phenolic acids, and tannins. Furthermore, network pharmacology analysis showed that flavonoids are the most important effectors in the fruit of *A. oxyphylla* and phenolic acids are the most important effectors in the roots and leaves. Moreover, the results suggested that the tissues of *A. oxyphylla* might play a role in the regulation of disease-related genes. The whole plant of *A. oxyphylla* is rich in natural drug components, and each tissue has high medicinal value. Therefore, comprehensive utilization of *A. oxyphylla* can greatly improve its economic value.

1. Introduction

*Alpinia oxyphylla* (*A. oxyphylla*) is commonly used in traditional Chinese medicine (TCM). The dried, ripe fruit of *A. oxyphylla* has long been used for treating diarrhea, enuresis, dementia, and other disorders [1]. Modern pharmacological studies have shown that *A. oxyphylla* extracts have antioxidant and anti-inflammatory capacities [2, 3]. In addition, *A. oxyphylla* has been used for the treatment of diabetes [3] and Alzheimer's disease [4].

Numerous chemical constituents, including flavonoids, diarylheptanoids, sesquiterpenes, sterols, and their glycosides, have been isolated from *A. oxyphylla* [1]. The main flavonoids were chrysin, tectochrysin, izalpinin, and kaempferol [5–7]. Yakuchinone A, yakuchinone B, oxyphyllacinol, and neonootkatol were the main diarylheptanoids [6]. The sesquiterpene constituents, including oxyphyllol A–C, nootkatone, and isocyperol, were extracted by aqueous 80% acetone [8]. The nosesquiterpenes, including oxyphylleno diol A, oxyphylleno diol B, oxyphylleneone A, oxyphylleneone B, oxyphyllone E, and oxyphyllone F, have been previously reported [9]. Several steroids have been isolated, such as β-sitosterol, stigmasterol, and β-daucosterol [10]. These results highlight that *A. oxyphylla* fruit has a variety of drug components, and it is still meaningful to comprehensively determine the chemical components of *A. oxyphylla* tissues.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) provides a vital tool to systematically analyze TCM metabolites [11]. Two flavonoids (chrysin and tectochrysin) from *A. oxyphylla* fruit extract were determined by LC-MS/MS with a method exhibiting accuracy ranging from
methanol aqueous solution. (+_he dissolved sample was re-
milligrams of powder was dissolved in 1.0mL of a 70%
with a grinder (mm 400, Retsch, Germany). One hundred
under vacuum and then ground (30Hz, 1.5min) to powder
Fresh samples were freeze-dried
stitute, Haikou, China.
A. oxyphylla
vested in June 2020 from four-year-old cultivated
2.1.PlantMaterials. Fresh A. oxyphylla samples were har-
2. Methods
2.1. Plant Materials. Fresh A. oxyphylla samples were har-\nA. oxyphylla
A. oxyphylla

2.2. Metabolite Extraction. Fresh samples were freeze-dried
under vacuum and then ground (30 Hz, 1.5 min) to powder
with a grinder (mm 400, Retsch, Germany). One hundred
milligrams of powder was dissolved in 1.0 mL of a 70%
methanol aqueous solution. The dissolved sample was re-
frigerated overnight at 4°C three times. The samples were
centrifuged at 10,000xg for 10 min at 4°C, and the super-
natants were collected and then filtered with a microporous
membrane filter (0.22-μm pore size). The prepared extracts
were stored in sampler vials for LC-MS/MS analysis.

2.3. Untargeted Metabolomics Analysis. All samples were
analyzed using an ultraperformance liquid chromatography
(UPLC, Shim-pack UFLC SHIMADZU CBM30A)-tandem
mass spectrometry (MS/MS, Applied Biosystems 4500
QTRAP) system. First, separation was achieved on a Waters
ACQUITY UPLC HSS T3 C18 column (2.1 mm × 100 mm,
1.8-μm particle size) using the UPLC system (Waters, Herts,
UK). The column oven was maintained at 40°C, and the flow
rate was set at 0.4 mL/min. The mobile phase was composed
of solvent A (water with 0.04% acetic acid) and solvent B
(acetonitrile with 0.04% acetic acid). Gradient elution
conditions were set as follows: 0 min, 95 : 5 V/V (A:B);
11.0 min, 5 : 95 V/V; 12.0 min, 5 : 95 V/V; 12.1 min, 95 : 5 V/
V; and 15.0 min, 95 : 5 V/V.

High-resolution MS/MS was used to detect metabolites
euluted from the column. The electrospray ionization tem-
perature was set at 550°C, and the MS voltage was set at
5500 V. The curtain gas was set at 25 psi. The collision-ac-
tivated dissociation was set at high.

To compare the differences in the metabolites, the mass
spectral peaks of each metabolite detected in different
samples were corrected to ensure the accuracy of qualitative
and quantitative analyses. Figure S1 shows the integral
correction results of the quantitative analysis of randomly
selected metabolites in different samples. The abscissa is
the retention time (min) of the metabolite, and the ordinate is
the ion current intensity of metabolite ion detection. The
metabolites were quantified by the multiple reaction mon-
itoring (MRM) mode of triple-quadrupole mass spec-
trometry [14]. Quality control samples were prepared by
mixing sample extracts and analyzing the repeatability of
samples by the same treatment methods. In the process of
instrumental analysis, a quality control sample was analyzed
every ten samples to monitor the repeatability of the UPLC-
MS/MS system over the entire detection process.

2.4. Bioinformatics Dataset of Untargeted Metabolism (for
TCM). Raw UPLC-MS/MS data were processed using the
following procedures. For each sample, a matrix of mo-
lecular features, such as the retention time and mass-to-
charge ratio (m/z), was generated using Analyst 1.6.3
software with default parameters. The structures of me-
tabolites were analyzed with reference to MASSBANK
(http://www.massbank.jp/), KNAPSAcK (http://kanaya.
naist.jp/KNAPSAcK/), HMDB (http://www.hmdb.ca/)
[15], MoToDB (http://www.ab wur.nl/moto/), and METLIN
(http://metlin.scripps.edu/index.php) [16].

After obtaining the mass spectrometric data of metab-
olites from different samples, the peak area of all mass
spectral peaks was integrated, and the peaks of the same
metabolite in different samples were integrated and cor-
corrected [14].

The mass spectrometry file of each sample was opened
with MULTIAQUANT software, and the integration and
correction of chromatographic peaks were conducted. The
peak area of each chromatographic peak represents the
relative levels of the corresponding substances.

2.5. Target Identification and Network Construction. The
target compounds were searched against the SWISSADME
(http://www.swissadme.ch/) [17] and TargetNet (http://
targetnet.scbdd.com/calcnet/index/) databases [18], which
are designed to identify potential target compounds via
various prediction algorithms. Homo sapiens origin targets
were used in the following analysis. Only targets with 95%
possibility were included for the disease-related targets.

To compile the disease targets for susceptibility to ath-
erosclerosis, Alzheimer’s disease, liver disease, diabetes
mellitus, allergies, Parkinson’s disease, and depression, we
searched the GeneCards database [19]. For each disease,
duplicated targets were removed. The intersection between
the drug and disease targets was determined to screen key
targets.

The “compound-disease targets” were the intersection of
A. oxyphylla compound targets and disease targets. The
network was constructed and analyzed with the Cytoscape
platform [20].
2.6. Systematic Correlativeity Analysis and Statistical Analysis. Pearson’s correlation, one-way analysis of variance (ANOVA), and hierarchical (average linkage) clustering were conducted for the untargeted metabolism analyses. P-values of the ANOVA were adjusted for the false discovery rate. Principal component analysis (PCA) and partial least squares discrimination analysis (PLS-DA) of the metabolites were performed using SIMCA v14.0 (Umetrics, Umea, Sweden).

3. Results

3.1. Untargeted Metabolite Profiling of the Metabolites in Different Tissues. A total of 312 secondary metabolites were found by untargeted metabolomics analysis (Table S1), including phenolic acids, flavonoids, tannins, lignans, coumarins, terpenoids, alkaloids, and quinones. PCA data showed three distinct sample groups, indicating that there was separation among the three tissues (Figure 1(a)). As shown in Figure 1(b), the roots, fruit, and leaves contained 293, 277, and 251 metabolites, respectively. In total, the abundance of metabolites in the roots and fruit was not significantly different, while the abundance of metabolites in the leaves was approximately 51.41% of that in the fruit. All annotated metabolites were classified to identify the differentially accumulated metabolites between tissues (Figure 1(b)). In the roots, 111 flavonoids accounted for 47.40% of the total abundance, 97 phenolic acids accounted for 17.51%, and 15 terpenoids accounted for 13.70% (Figures 1(b) and 1(c)). Among the fruits, flavonoids were the most abundant, with 113 species in total, accounting for 58.86% of the total abundance. The abundance of phenolic acids ranked second, with 91 species, accounting for 14.09%. The abundance of tannins was the third highest, with 13 species, accounting for 5.00% (Figures 1(b) and 1(c)). In the leaves, the three compounds with the highest contents were flavonoids (90 species, accounting for 33.50%), phenolic acids (82 species, accounting for 20.08%), and tannic acids (12 species, accounting for 13.91%) (Figures 1(b) and 1(c)). Moreover, 116 metabolites predominantly accumulated in the roots, 120 metabolites were present at relatively high abundance in the fruits, and 76 metabolites were more highly distributed in the leaves (Figure 1(d)). Therefore, the characteristics of metabolites in the fruit, roots, and leaves of *A. oxyphylla* were significantly different.

3.2. Variations in the Abundance Levels of Flavonoids among Tissues. As shown in Figure 2, 115 flavonoids were identified in *A. oxyphylla* tissues. Heatmap clustering analysis found that more flavonoids accumulated in the fruit than in the roots and leaves (Figure 2(a)). The phenolic acids with the highest abundance in the fruit were prunetin, rhamnetin, and luteolin-7-O-glucuronide-5-O-rhamnoside. The 3 most abundant phenolic acids in the roots were delphinidin-3-O-(6''-O-p-coumaroyl) glucoside, hyperin, and quercetin-7-O-(6''-malonyl) glucoside. The phenolic acids with the highest abundance in the leaves were pinostrobin, epicatechin glucoside, and catechin-catechin-catechin.

Furthermore, the metabolites were assigned to multiple synthetic pathways of flavonoids (Figure 2(b)). Naringenin, dihydrokaempferol, quercetin, methylnaringenin, two quercetin derivatives, four kaempferol derivatives, and six luteolin derivatives were highly accumulated in the fruit. Kaempferol, kaempferol derivatives, six quercetin derivatives, and seven kaempferol derivatives were highly accumulated in the roots. Therefore, the fruit, roots, and leaves of *A. oxyphylla* might adopt different pathways to synthesize flavonoids, resulting in different dominant flavonoids in these tissues.

3.3. Variations in the Abundance Levels of Phenolic Acids among Tissues. As shown in Figure 3, a large number of phenolic acids accumulated in the roots, fruit, and leaves (Figure 3(a)). Moreover, the dominant phenolic acids in the fruit, roots, and leaves of *A. oxyphylla* were quite different. 3,4,5-Trimethoxyphenyl-1-O-glucoside, 4-O-glucosyl-sinapate, and dibutyl phthalate were the 3 most abundant phenolic acids in the fruit. 3,4,5-Trimethoxyphenyl-1-O-glucoside, 1,7-bis (4-hydroxy-3-methoxyphenyl) hepta-4,6-dien-3-one, and feruloylmalic acid were the most abundant phenolic acids in the roots. 1,7-Bis(4-hydroxy-3-methoxyphenyl) hepta-4,6-dien-3-one, dibutyl phthalate, and vanillin were the 3 most abundant phenolic acids in the leaves.

Then, phenolic acids were enriched in known synthetic pathways (Figure 3(b)). Cinnamic acid, caffeic acid, vanillic acid, four coumaroyl derivatives, and three feruloyl derivatives were highly accumulated in the roots. p-Coumaric acid, p-coumaroyl, quinic acid, chlorogenic acid, coniferol, four coumaroyl derivatives, two feruloyl derivatives, and three sinapoyl derivatives were highly accumulated in the fruit. Ferulic acid, coniferol, sinapate, and sinapaldehyde were highly accumulated in the leaves. Therefore, different phenolic acid synthetic strategies were employed in the fruit, roots, and leaves of *A. oxyphylla*.

3.4. Network Pharmacology Analysis Based on Major Components in Tissues. In fruits, the 20 most abundant metabolites accounted for 52.90% of the total, of which 13 flavonoids accounted for 36.77% (Table 1). These compounds were accepted as candidates to predict the targets. The GeneCards database was used to predict the disease (cancer, osteoporosis, allergic disease, dementia, Parkinson’s disease, kidney disease, diabetes mellitus, cardiovascular disease, and depression) targets. Three hundred fourteen overlapping genes were selected as potential targets for integrative network analysis. Thirteen flavonoids had 184 target genes, while phenolic acids had 44 targets. Network pharmacology analysis showed that flavonoids are the main effectors, which could interfere not only with cancer, cardiovascular disease, kidney diseases, and diabetes mellitus but also with depression (Figure 4).

In the roots, among the 20 most abundant metabolites, there were 9 flavonoids (27.5%), 4 phenolic acids (9.52%), and 4 terpenes (8.46%) (Table 2). In addition, a total of 379 genes were obtained for integration network analysis.
Among them, phenolic acids may be involved in the regulation of 190 genes, flavonoids are associated with 126 genes, and terpenoids may target 34 genes. Network pharmacology analysis showed that phenolic acid is the main effective component of the roots, and it has the potential to interfere with diseases such as cancer, cardiovascular disease, kidney disease, diabetes, depression, dementia, and Parkinson’s disease (Figure 4).

In leaves, the top 20 metabolites accounted for 65.79%, and the highest contents were observed for flavonoids (6 species, accounting for 23.99%), tannins (5 species, accounting for 11.53%), and phenolic acid compounds (3 types, accounting for 7.86%) (Table 3). Furthermore, 418 target genes were found, among which phenolic acids target 305 genes and flavonoids may affect the expression of 137 genes. Therefore, phenolic acids may also be the main active...
Figure 2: Accumulation of flavonoids in the three tissues. (a) The heatmap scale ranges from −1 to +1 after data homogenization. (b) The biosynthetic pathway of flavonoids. The green color text indicates that the relative concentration was higher in the roots than in the other tissues, the purple color text indicates that the relative concentration was higher in the fruit, and the yellow color text indicates that the relative concentration was higher in the leaves.

Figure 3: Accumulation of phenolic acids in the three tissues. (a) The heatmap scale ranges from −1 to +1 after data homogenization. (b) The biosynthetic pathway of phenolic acid. The green color text indicates that the relative concentration was higher in the roots than in the other tissues, the purple color text indicates that the relative concentration was higher in the fruit, and the yellow color text indicates that the relative concentration was higher in the leaves.
components in leaves (Figure 4). Therefore, the roots, leaves, and fruit of *A. oxyphylla* can be used as a candidate component source to intervene in multiple diseases.

### 4. Discussion

Multiple bioactive components have been separated from *A. oxyphylla* fruit [8]. In the present study, we analyzed the chemical components in the roots, leaves, and fruit of *A. oxyphylla* through UPLC-MS/MS. Based on the analysis results, the intervention effects of different tissues of *A. oxyphylla* on multiple diseases were predicted.

In this study, PCA showed that the components in fruits, roots, and leaves were quite different. The metabolome data were further analyzed by orthogonal partial least squares discriminant analysis (OPLS-DA), which further demonstrated the differences among the fruit, roots, and leaves [21]. Permutation verification of OPLS-DA (*n* = 200, 200 permutation experiments) showed that the R2′ and Q2′ were both smaller than the R2 and Q2 of the original model, and this model was meaningful (Figure S2).

Previous studies have separated hundreds of essential oil components and 128 other types of components from the fruit of *A. oxyphylla*, including 81 terpenes, six diaryleptanoids, seven flavonoids, and five steroids [1]. These studies used different methods to extract the fruit of *A. oxyphylla* and obtained a variety of components. In addition, due to the different production areas of *A. oxyphylla*, the components were also affected [22]. In this study, 70% methanol was used for extraction, and a total of 277 secondary metabolites were obtained in the fruit of *A. oxyphylla*. Some components have been reported in previous studies, such as kaempferol, while a number of components were highlighted here for the first time. Moreover, the relative abundance of each component was also quantified, which provided a basis for the functional prediction of *A. oxyphylla* fruit.

Studies have shown that multiple flavonoids and phenolic acids were isolated from *A. oxyphylla* fruit [7, 23–25]. The isolated flavonoids included tectochrysin, izalpinin, kaempferide, kaempferol-7,4-dimethyl ether, chrysin, rhamnocitrin, and pinocembrin [7, 23, 24]. The isolated phenolic acids included protocatechuic acid, vanillic acid, 3,5-dihydroxy-4-methoxybenzoic acid, and isovanillin [25]. In the present study, the analysis results showed that the

### Table 1: Top 20 abundant components in fruits.

| Rank | Formula | Compounds                                           | Class I      | Class II     | Mean abundance |
|------|---------|-----------------------------------------------------|--------------|--------------|----------------|
| 1    | C16H12O5 | Prunetin (5,4′-dihydroxy-7-methoxyisoflavone)       | Flavonoids   | Isoflavones  | 47867500       |
| 2    | C16H12O7 | Rhamnetin (7-O-methyl quercetin)                     | Flavonoids   | Flavonols    | 36629167       |
| 3    | C22H30O16 | Luteolin (7-O-glucuronide-5-O-rhamnoside)           | Flavonoids   | Flavonol     | 25471383       |
| 4    | C20H28O6 | 5-Hydroxy-1,7-bis (4-hydroxy-3-methoxyphenyl) heptan-3-one | Others       | Others       | 25408500       |
| 5    | C20H26O7 | 3,5-Dihydroxy-meodah                                | Others       | Others       | 21946233       |
| 6    | C16H14O4 | Pinostrobin                                         | Flavonoids   | Dihydroflavone | 19776650     |
| 7    | C22H30O12 | Quercetin-3-O-galactoside (hyperin)                  | Flavonoids   | Flavonols    | 16930800       |
| 8    | C21H28O7 | Luteolin-7-O-glucoside (cyanoside)                  | Flavonoids   | Flavonoid    | 14381533       |
| 9    | C22H30O7 | 5-Hydroxy-1-(4-hydroxy-3,5-dimethoxyphenyl)-7-(4-hydroxy-3-methoxyphenyl) heptan-3-one | Others       | Others       | 12284683       |
| 10   | C21H28O12 | Quercetin-3-O-glucoside (isoquercitrin)             | Flavonoids   | Flavonols    | 12108717       |
| 11   | C18H20O3 | Oxyphyllol D                                       | Terpenoids   | Sesquiterpenoids | 11446750   |
| 12   | C18H21O4 | Pinocembrin (dihydrochrysin)                         | Flavonoids   | Dihydroflavone | 11027850     |
| 13   | C18H21O18 | Catechin-catechin-catechin                           | Flavonoids   | Flavonol     | 10100900       |
| 14   | C22H24O11 | Hesperetin-5-O-glucoside                            | Flavonoids   | Dihydroflavanol | 10053150   |
| 15   | C22H26O6 | 2-Hydroxy-3-carboxy-4-linylidihydrx                  | Others       | Others       | 9552900        |
| 16   | C22H30O16 | Quercetin-3-O-robinobioside                         | Flavonoids   | Flavonols    | 9571433        |
| 17   | C22H28O5 | Gingerenone A                                     | Others       | Others       | 9503050        |
| 18   | C22H28O15 | Quercetin-7-O-(6''-malonyl) glucoside               | Flavonoids   | Flavonols    | 9290433        |
| 19   | C22H29O15 | Quercetin-3-O-(6''-malonyl) galactoside             | Flavonoids   | Flavonols    | 9010300        |
| 20   | C18H20O8 | 3,4,5-Trimethoxyphenyl-1-O-glucoside               | Phenolic acids | Phenolic acids | 8675383       |

![Figure 4: Comprehensive representation of the built network of bioactive constituents in *Alpinia oxyphylla* and diseases.](image)
flavonoids and phenolic acids in the fruit were the main components, accounting for 72.95% of the total abundance. Among flavonoids, prunetin, rhamnetin, pinostrobin, oxyphyllol D, pinocembrin, gingerenone A, luteolin derivatives, and quercetin derivatives were the dominant flavonoids. A variety of kaempferol derivatives have also been found. Among phenolic acids, the abundance of sinapoyl derivatives, coumaroyl derivatives, and $p$-coumaric acid was

**Table 2: Top 20 abundant components in roots.**

| Rank | Formula | Compounds | Class I | Class II | Mean abundance |
|------|---------|-----------|---------|----------|---------------|
| 1    | C$_{30}$H$_{27}$O$_{14}$ | Delphinidin-3-O-(6''-O-p-coumaroyl)glucoside | Flavonoids | Anthocyanins | 40513500 |
| 2    | C$_{21}$H$_{26}$O$_{11}$ | Quercetin-3-O-galactoside (Hyperin) | Flavonoids | Flavonols | 23952000 |
| 3    | C$_{24}$H$_{20}$O$_{15}$ | Quercetin-7-O-(6''-malonyl) glucoside | Flavonoids | Flavonols | 22008667 |
| 4    | C$_{14}$H$_{22}$O$_{3}$ | Oxyphyllol D | Oxyphyllol | Oxyphyllol | 21040167 |
| 5    | C$_{23}$H$_{20}$O$_{4}$ | 1-(4-Hydroxy-3-methoxyphenyl)-7-phenyl-3,5-diheptanone | Others | Others | 19360450 |
| 6    | C$_{23}$H$_{20}$O$_{15}$ | Quercetin-3-O-(6''-malonyl) galactoside | Flavonoids | Flavonols | 17750167 |
| 7    | C$_{24}$H$_{20}$O$_{12}$ | Quercetin-3-O-glucoside (Isoquercitrin) | Flavonoids | Flavonols | 17015000 |
| 8    | C$_{12}$H$_{22}$O$_{8}$ | 3,4,5-Trimethoxyphenyl-1-O-glucoside | Phenolic acids | Phenolic acids | 16549150 |
| 9    | C$_{21}$H$_{26}$O | Oxyphyllol A | Terpenoids | Sesquiterpenoids | 14884910 |
| 10   | C$_{30}$H$_{27}$O$_{12}$ | 5-Hydroxy-1,7-bis (4-hydroxy-3-methoxyphenyl) heptan-3-one | Others | Others | 14610700 |
| 11   | C$_{22}$H$_{22}$O$_{11}$ | Quercetin-3-O-(6''-malonyl) galactoside | Flavonoids | Flavonols | 17750167 |
| 12   | C$_{21}$H$_{20}$O$_{12}$ | Quercetin-3-O-glucoside (Isoquercitrin) | Flavonoids | Flavonols | 17015000 |
| 13   | C$_{15}$H$_{22}$O$_{8}$ | 3,4,5-Trimethoxyphenyl-1-O-glucoside | Phenolic acids | Phenolic acids | 16549150 |
| 14   | C$_{15}$H$_{24}$O$_{3}$ | Oxyphyllol A | Terpenoids | Sesquiterpenoids | 14884910 |
| 15   | C$_{21}$H$_{20}$O$_{12}$ | Oxyphyllol A | Terpenoids | Sesquiterpenoids | 14884910 |
| 16   | C$_{12}$H$_{18}$O$_{3}$ | Oxyphyllene B | Terpenoids | Sesquiterpenoids | 12010683 |
| 17   | C$_{16}$H$_{22}$O$_{5}$ | Rhamnetin (7-O-methyl quercetin) | Phenolic acids | Phenolic acids | 11396750 |
| 18   | C$_{20}$H$_{24}$O$_{5}$ | 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl) heptan-3-one | Others | Others | 11270433 |
| 19   | C$_{16}$H$_{20}$O$_{10}$ | 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl) heptan-3-one | Others | Others | 11270433 |
| 20   | C$_{14}$H$_{14}$O$_{4}$ | Pinostrobin* | Flavonoids | Dihydroflavone | 28276667 |

**Table 3: Top 20 abundant components in leaves.**

| Rank | Formula | Compounds | Class I | Class II | Mean abundance |
|------|---------|-----------|---------|----------|---------------|
| 1    | C$_{14}$H$_{14}$O | Pinostrobin* | Flavonoids | Dihydroflavone | 28276667 |
| 2    | C$_{21}$H$_{26}$O$_{11}$ | Epicatechin glucoside | Flavonoids | Flavanols | 12130550 |
| 3    | C$_{21}$H$_{26}$O$_{10}$ | 5'-Hydroxyhexahydrocurcumin | Others | Others | 11798517 |
| 4    | C$_{22}$H$_{30}$O$_{7}$ | 3,5-Dihydroxy-meadah | Others | Others | 11779017 |
| 5    | C$_{21}$H$_{36}$O$_{18}$ | Catechin-catechin-catechin* | Flavonoids | Flavanols | 11665500 |
| 6    | C$_{16}$H$_{22}$O$_{5}$ | Rhamnetin (7-O-methyl quercetin) | Flavonoids | Flavanols | 10399500 |
| 7    | C$_{21}$H$_{26}$O$_{15}$ | 1,7-Bis(4-hydroxy-3-methoxyphenyl) hepta-4,6-dien-3-one | Others | Others | 9825767 |
| 8    | C$_{21}$H$_{26}$O$_{5}$ | 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl) heptan-3-one | Others | Others | 11270433 |
| 9    | C$_{21}$H$_{26}$O$_{5}$ | 1,7-Bis(4-hydroxy-3-methoxyphenyl) hepta-4,6-dien-3-one | Others | Others | 11270433 |
| 10   | C$_{16}$H$_{20}$O$_{4}$ | Gingerenone A | Terpenoids | Sesquiterpenoids | 9651967 |
| 11   | C$_{21}$H$_{26}$O$_{5}$ | 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl) heptan-3-one | Others | Others | 11270433 |
| 12   | C$_{21}$H$_{26}$O$_{5}$ | 1,7-Bis(4-hydroxy-3-methoxyphenyl) hepta-4,6-dien-3-one | Others | Others | 11270433 |
| 13   | C$_{16}$H$_{20}$O$_{4}$ | Gingerenone A | Terpenoids | Sesquiterpenoids | 9651967 |
| 14   | C$_{21}$H$_{26}$O$_{5}$ | 5-Hydroxy-1-(4-hydroxy-3-methoxyphenyl)-7-(4-hydroxyphenyl) heptan-3-one | Others | Others | 11270433 |
| 15   | C$_{16}$H$_{20}$O | Oxyphyllene B | Terpenoids | Sesquiterpenoids | 12010683 |
| 16   | C$_{16}$H$_{20}$O | Oxyphyllene B | Terpenoids | Sesquiterpenoids | 12010683 |
| 17   | C$_{16}$H$_{20}$O | Oxyphyllene B | Terpenoids | Sesquiterpenoids | 12010683 |
| 18   | C$_{16}$H$_{20}$O | Oxyphyllene B | Terpenoids | Sesquiterpenoids | 12010683 |
| 19   | C$_{16}$H$_{20}$O | Oxyphyllene B | Terpenoids | Sesquiterpenoids | 12010683 |
| 20   | C$_{16}$H$_{20}$O | Oxyphyllene B | Terpenoids | Sesquiterpenoids | 12010683 |
relatively high. Therefore, flavonoids and phenolic acids are the characteristic components of A. oxyphylla fruit and can reflect its medicinal value.

Recent studies have shown that A. oxyphylla fruit can delay heart aging [26], provide neuroprotection in Alzheimer’s disease [4], enhance kidney function [27], and induce cancer cell apoptosis [28]. Moreover, prunetin could induce cell death in gastric cancer cells, relax aortic rings, and promote bone regeneration [29–31]. Rhamnetin could play a role in inducing cancer cell apoptosis, inhibiting cell proliferation, and preventing cancer formation [32–34]. Rhamnetin has the potential to treat oxidative myocardiomyopathy disease [35]. Pinostrobin may serve as a novel agent for lipid management, cancer treatment, and Parkinson’s disease neuroprotection [36–38]. Pinocembrin is effective in treating ischemic stroke, and it also shows excellent neuroprotective potential [39]. Gingerenone A may be used as a potential therapeutic candidate for the treatment of obesity and diabetes [40, 41]. Studies have shown that kaempferol has multiple bioactivities, such as antioxidant, neuroprotective, anticancer, anti-inflammatory, anti-diabetic, and antiosteoporotic activities [42, 43]. Phenolic acids have many unique functions, such as memory improvement, antioxidant, anti-diabetic, anti-inflammatory, and antiaging functions [44–46]. In the present study, the 20 most abundant components in fruit could dock to 314 genes, which were categorized into various pathways, such as respiratory electron transport, LPA receptor-mediated events, and the HIF-1-alpha transcription factor network (Table S2). Network pharmacology predictions showed that the components and targets were associated with cancer, cardiovascular disease, kidney diseases, diabetes mellitus, and depression. These results match those observed in previous studies showing that A. oxyphylla fruit can play a role in the treatment of a variety of diseases.

The roots of some Alpinia species, such as A. officinarum, are used for medicine [47]. The main components in the roots of A. officinarum were kaempferol, quercetin, diphenylheptane, and volatile oils [47]. A. officinarum is used to treat digestive disorders, stomachache, flatulence, and the common cold [47]. However, the medicinal value of A. oxyphylla roots is still mostly unknown. In the present study, a large number of secondary metabolites were detected in the roots. Flavonoids and phenolic acids accounted for 64.91% of the total abundance, and terpenoids accounted for 13.70%. Therefore, flavonoids, phenolic acids, and terpenoids are the representative components of A. oxyphylla roots.

It has been reported that the physiological activities of quercetin include anticancer, hypoglycemic, and antiobesity activities [42, 43, 48]. Delphinidin has a variety of pharmacological activities, including anticancer, cardiovascular protection, neuroprotection, anti-diabetes, and antiobesity activities [49]. Ferulic acid could offer beneficial effects, such as anticancer, anti-diabetes, and antineurodegenerative effects [50]. Nootkatol could prevent UV-induced photoaging [51]. In the present study, delphinidin, kaempferol derivatives, and quercetin derivatives were the dominant flavonoid components in A. oxyphylla roots, while feruloylmalic acid and feruloyl derivatives were the dominant phenolic acid components. Among terpenoids, oxyphyllol D, oxyphyllol A, oxyphyllolene B, and nootkatol were present at higher levels. Moreover, the top 20 abundant components in the roots docked with 378 genes, which were related to 220 pathways, including lipid metabolism, inflammatory response, and neurotransmitter metabolism (Table S2). The target genes might be involved in multiple diseases, including cancer, cardiovascular disease, kidney diseases, diabetes mellitus, depression, dementia, and Parkinson’s disease. These analysis results indicate that A. oxyphylla roots also have high medicinal value.

Few studies have analyzed the chemical constituents of volatile oil and organic acids from the leaves of A. oxyphylla [52]. Systematically analyzed chemical components of A. oxyphylla leaves have not been reported. The present study demonstrated that the total abundance of metabolites in leaves was approximately 51.40% of that in the fruit, and the dominant components were flavonoids (33.50%), phenolic acids (20.08%), and tannins (13.91%). Pinostrobin, epicatechin, rhamnetin, pinocembrin, and prunetin were the most abundant flavonoids. Among the tannins, procyanidin C1, procyanidin B2, procyanidin B3, procyanidin B4, and procyanidin C2 had a high abundance. Therefore, this study systematically analyzed the drug components of A. oxyphylla leaves and clarified the main chemical components of A. oxyphylla leaves.

Recent studies have shown that epicatechin plays a role in improving cardiovascular and cerebrovascular diseases and exerts anti-inflammatory, anti-diabetic, and neuroprotective effects [53]. Procyanidin is considered to be involved in lipid regulation and cancer treatment [54, 55]. In the present study, the top 20 abundant components in the leaves of A. oxyphylla docked with 416 genes, which were related to multiple pathways, including respiratory electron transport, IL1-mediated signaling events, and the TNF receptor signaling pathway (Table S2). Network pharmacology predictions showed that components in the leaves were also associated with a variety of diseases. Even its target genes had a higher relationship degree with the analyzed diseases than those of roots and fruits. A. oxyphylla leaves are readily available. Thus, the use of leaves as medicine can significantly increase the economic value of A. oxyphylla.

In summary, metabolic profiles revealed that the levels of metabolite accumulation might vary significantly among the fruit, roots, and leaves of A. oxyphylla. The representative components of A. oxyphylla fruit were flavonoids and phenolic acids. Flavonoids, phenolic acids, and terpenoids were the main components in A. oxyphylla roots. Flavonoids, phenolic acids, and tannins were the dominant components in A. oxyphylla leaves. Furthermore, the network pharmacology predictions suggest that the fruit, roots, and leaves of A. oxyphylla were associated with cancer, cardiovascular disease, kidney diseases, and diabetes mellitus. In addition, different tissues of A. oxyphylla could be used to treat more different diseases. Therefore, further studies on the drug components and functions of tissues of A. oxyphylla will help to improve the medicinal value and economic value of A. oxyphylla.
Abbreviations

TCM: Traditional Chinese medicine
LC-MS/MS: Liquid chromatography-tandem mass spectrometry
UPLC: Ultraperformance liquid chromatography
MS: Mass spectrometry
ANOVA: Analysis of variance
PCA: Principal component analysis
PLS-DA: Partial least squares discrimination analysis.

Data Availability

All the datasets generated and analyzed during the current study were uploaded with the manuscript as additional files.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors’ Contributions

Guankui Du contributed to conceptualization, review and editing of the paper, project administration, and funding acquisition; Deli Wang contributed to methodology; Li Ying performed to data curation and original draft preparation. All the authors have read and agreed to the published version of the manuscript.

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Supplementary Materials

Figure S1: MRM metabolite detection. The multiplex diagram shows the substances that were detected in the sample, and each mass spectral peak with different colors represents one detected metabolite. Figure S2: OPLS-DA analysis model verification diagram. Table S1: 312 identified metabolites. Table S2: the target genes were enriched in multiple pathways. (Supplementary Materials)

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