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ORIGINAL ARTICLE

Geographical Origin Discrimination of *Amomi fructus* using an Ultra-Performance Liquid Chromatography-Quadrupole Time-of-Flight Mass Spectrometry-Based Metabolomics Approach Combined with Antioxidant Activity Analysis

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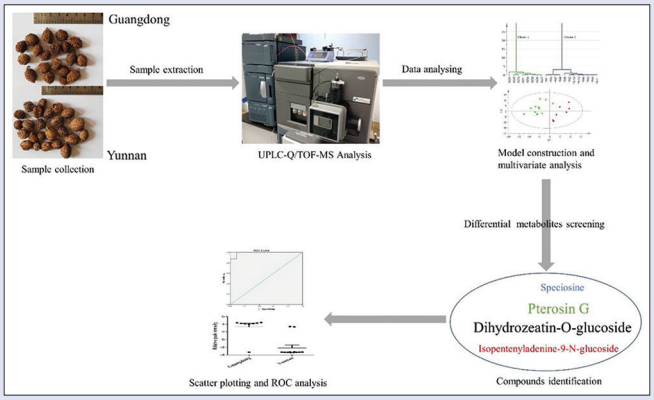
ABSTRACT

Background: *Amomi fructus* as edible and medicinal food can be used as a flavoring in the daily cooking and herbal tea, the consumption of *A. fructus* is gradually aggregate in recent years. The quality of *A. fructus* from Yangchun city of Guangdong province in China is familiar as the best and the value is higher than other areas, the flavor and pharmacological activities is mostly affected by its quality. However, geographical origin confusion often occurs in the market, so it is obligatory to develop technology to differentiate the geographical origin of *A. fructus*. **Objectives:** The objective of this study is to assess the antioxidant activity of *A. fructus* and discriminate the geographical origin of *A. fructus* using nontarget metabolomics technology. **Materials and Methods:** Ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry was used for acquired data in positive ionization modes, principal component analysis, and orthogonal partial least-squares discrimination analysis. The online analysis website Metabo Analyst 4.0 (https://www.metaboanalyst.ca/) was used for the ensuing analysis. A reducing power test, chelating power of ferrous ions test, 1,1-diphenyl-2-picryl-hydrazyl-hydrate assay, and hydroxyl radicals scavenging ability assays were used to assess the differences in antioxidant activity between the samples from the two geographical locations. **Results:** Eleven differential metabolites were attained, among which eight were established as marker compounds with the greatest contribution to the discernment between the two production areas. The antioxidant activity happens obvious differences due to dissimilar geographical origin. **Conclusion:** Untargeted metabolomics combined with multivariate statistical analysis is a commanding strategy to extricate the samples from the same species and to comprehend the quality differences from the viewpoint of overall metabolic profile.

Key words: *Amomi fructus,* antioxidant activity, metabolic profiling, metabolomics, ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry

SUMMARY

- Twenty batches of *Amomum villosum* Lour. from Yunnan and Guangdong province in China were assessed by an ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry-based metabolomics and antioxidant activity. Principal component analysis and orthogonal partial least-squares discrimination analysis model exposed differences between the samples from two geographical locations, 11 differential compounds were recognized, and eight of them were documented as the chemical markers metabolites in charge of the geographical origins discrimination.



Abbreviations used: UPLC-Q/TOF-MS: Ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry; PCA: principal component analysis; OPLS-DA: orthogonal partial least squares discrimination analysis; PLS-DA: partial least squares discrimination analysis; DPPH: 1,1-diphenyl-2-picryl-hydrazyl-hydrate; ESI: electrospray ionization; SD: standard deviation, VIP: variable importance in projection; HRSA: hydroxyl radicals scavenging ability; ROC: Receiver operating characteristics.

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INTRODUCTION

Amomi fructus, the ripened fruit of *Amomum villosum* Lour, belongs to the perennial herb family *Zingiberaceae*. It is a well-known traditional Chinese medicine extensively used to treat the gastrointestinal diseases. As edible and medicinal food of *A. fructus* has been permitted by the Chinese Ministry of Health.[1] It is used as a flavoring in the day-to-day cooking and herbal tea and is also used widely to make soup for a long time in China. *A. fructus* also has a long history of the medicinal use in China and possesses various pharmacological activities, such as reducing intestinal inflammation, warming the spleen, antidiarrheal effects, removing dampness, and preventing miscarriage.[2,3] Earlier studies have specified that volatile oils, flavonoids, phenolic acid, and polysaccharides are the main bioactive compounds in *A. fructus*, which are closely related to quality and are largely prejudiced by processing methods, storage conditions, geographical position of cultivation, and harvesting time.[4] *A. fructus* is mainly dispersed in Guangdong, Yunnan, and Guangxi provinces in China. *A. fructus* produced from Yangchun City in Guangdong province, has a good quality and is usually protected as a Geographical Indication Product.

With the growing demand for high quality food materials, food products with a clear geographical source cause augmented consumer trust, such that consumers are more willing to pay a higher price for the product. Therefore, classifying the geographical origin of food products has involved augmented research attention. The identification and quality evaluation of food products are chiefly important because they are directly related to food safety and taste. In fact, the quality of *A. fructus* in the market has been variable, with huge differences in the quality and price of *A. fructus* from different production areas. However, it is difficult to extricate *A. fructus* from different geographical origins in terms of its appearance and texture, especially for the samples from the same species. Unfortunately, to attain more economic benefits, some unscrupulous resellers misidentify the geographical origin of *A. fructus* and this behavior extremely damages the interests of consumers and legitimate producers. Morphological and physicochemical identification typically rely on abundant experience; nevertheless, analysis and determination strategies based on one or several constituents cannot differentiate samples of the same species from different sources well. Tiny differences affected by the geographical cultivation environment are hard to classify using traditional finding methods. Therefore, to reinforce market supervision, it is needed to develop technology to separate the geographical origin of *A. fructus.*

Metabolites are the end products of cell life activities, and their levels are measured to be the decisive response of organisms to external stimulation.[5] Compared with other omics techniques, metabolomics has probable benefits in terms of the close affinity of metabolites to diseases and plant phenotypes.[6,7] Metabolomics delivers a new way to resolve the problem of geographical source identification. This technology can provide plentiful evidence on the metabolic response of an organism under various stimulations and the levels of endogenous metabolites.[8] To date, metabolomics has been applied widely in areas such as disease diagnosis, cancer biomarker discovery, drug discovery, personalized medicine, molecular epidemiology, and to uncover new drug targets.[9,10] Moreover, in recent years, metabolomics technology has started to be applied to the analysis of food and Chinese herbal medicine, such as wine,[11] orange,[12] and *Siraitia grosvenorii*.[13] In the present study, we intended to employ a ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF)-MS-based metabolomics method to decide the geographical origin of *A. fructus* samples.

Overproduction of free radicals can cause oxidative damage, abolish cellular homeostasis and damage biomolecules, comprising nucleic acids, proteins, and lipids. To evade the toxic damage of free radicals, plants have established two antioxidant defense systems, comprising enzymatic and non-enzymatic systems.[14] Low-molecular-weight antioxidants and high-molecular-weight secondary metabolites establish the non-enzymatic antioxidant system of plants. Metabolites with antioxidant functions in plants have specific pharmacological activities.[15] The antioxidant activity of plants is frequently related to metabolites such as phenolics, flavonoids, and alkaloids.[16,17] The ecological factors in different geographical locations often lead to the changes in metabolite levels.[18] Therefore, difference among metabolites with antioxidant properties could be considered indirectly by assessing the total antioxidant capacity.

In the present study, we collected 20 batches of *A. fructus* samples from Yunnan (YN) and Guangdong (GD) provinces in China and determined the differences in their metabolite profiles using an untargeted metabolomics method. In addition, the antioxidant ability of the samples was evaluated using reducing power tests, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assays, and hydroxyl radicals scavenging ability assays. Eleven differentially abundant metabolites were acquired after the analysis of the metabolomics data and eight of them were established as markers with the greatest influence to the discrimination of the samples from the two production areas. The present study is expected to form the basis for a new research strategy to discourse the quality control (QC) and to classify the geographic origin among *A. fructus* samples. As far as we know, this research is the leading report on the identification of the geographic origin of *A. fructus* by nontargeted metabolomics.

MATERIALS AND METHODS

Plant materials

In our research, 20 batches of dried fruit of *A. fructus* were collected from Guangdong and Yunnan province in China, respectively. The identification of those samples as *A. villosum* Lour. was performed at Sun Yat-Sen University. All the samples were powdered and passed through a 50-mesh sieve. The sampled were filled self-sealing bags and stored at the room temperature (10°C–30°C) in a desiccator. Voucher specimen was placed in the Laboratory of Pharmacognosy, Sun Yat-Sen University.

Sample treatment

The dried mature fruit of *A. villosum* Lour was pulverized using a high-speed disintegrator and about 0.5 g of the powder (dry weight) was extracted using ultrasound in 10 mL of a 50% methanol in water solution (v/v) for 30 min. The supernatants were collected by centrifugation at 12000 rpm for 10 min. An aliquot of this supernatant was used for UPLC-Q/TOF-MS analysis. The QC sample was prepared by mixing a 100 μL aliquot of all samples.

Ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry analysis

Chromatograms were attained from the Waters ACQUITY UPLC I-class system (Waters Corp., Milford, MA, USA) associated to a SYNAPT G2-Si high definition MS (Waters). UPLC separation was performed using an ACQUITY UPLC HSS T3 column (2.1 mm × 100 mm, 1.8 μm; Waters, Dublin, Ireland). The mobile phase contained of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). The gradient elution procedures were performed as follows: 2%–30% B for 0–8 min, 30%–98% B for 8–16 min, and 98% of B for 16–19 min. The column was equilibrated with 2% of mobile phase B for 3 min before each sample run. The flow rate of the mobile phase was 0.4 mL/min, the column temperature was

35°C, and the injection volume was 0.5 µL. The positive-ion (electrospray ionization+) modes were applied to obtain the MS data; the mass range was set at m/z 50–1200 in full scan resolution mode.

Data processing

The raw data files were developed in positive ionization modes and then pre-processed (peak detection, filtering, and alignment) using the Progenesis QI software (Waters). The raw data were tabularized as a data matrix arranged by retention time, m/z and peak intensities of each sample after processing. Then, MetaboAnalyst 4.0 and SIMCA 14.1 (Umetrics, Umea, Sweden) software was applied for the additional analysis of the data.

Many missing values could affect the downstream analysis; therefore, the default method substitutes all the missing values with small values. We obvious to eliminate the features with >50% missing values. The method of "replace by a small value" was used to assess the remaining missing values, which was delivered by the online website MetaboAnalyst 4.0.

Data filtering is crucial for modeling the data, this is a key process used for untargeted metabolomic dataset analysis. The interquartile range was used as a strong estimate for data filtering. In addition, data normalization, data transformation, and data scaling were performed in the normalization process. Data transformation and scaling are two diverse ways to make discrete features more comparable. Thus, that the data were regulated by their sum, logarithmic transformation, and pareto scaling.

Statistical analysis and metabolite identification

The experimental results were articulated as the mean ± standard deviation (SD) values. The antioxidant activity of the samples from the two geographical origins was analyzed using the one-way analysis of variance (ANOVA) with Graph Pad Prism 7.0.(Graph Pad Software Inc., La Jolla, CA, USA). $P < 0.05$ was measured statistically significant.

The data were managed as defined above and then imported into SIMCA 14.1 software (Umetrics AB, Umea, Sweden) for multivariate analysis, such as principal component analysis (PCA) and orthogonal partial least-squares discrimination analysis (OPLS-DA). The model and class were designated separately; PCA is an unsupervised model that was employed to visualize any possible clustering of samples on the basis of two geographical origins.

For metabolites measured to play an important role in distinguishing *A. fructus* samples from two different locations, their variable importance in projection (VIP) values should be >1 and the fold change describes difference between two locations groups should be >2, with a $P < 0.001$. Metabolites that met above three criteria were considered to be differentially abundant metabolites, which contribute most suggestively to the classification. Online databases (Human Metabolome Database, PubMed, ChemSpider and MassBank) and relevant available literature were used for the tentative identification of the compounds.

Evaluation of antioxidant capacity of samples from two geographical origins

Hydroxyl radicals scavenging ability

The HRSA assay was performed according to the literature with some changes.[19] Briefly, the reaction mixture contained 20 µL of ferrous sulfate (9 mM), 20 µL of salicylic acid-ethanol (9 mM), 100 µL distilled water, and 10 µL of sample methanol extract. The reaction was started by the addition of 20 µL of hydrogen peroxide (0.1% v/v). After incubating for 0.5 h at a constant temperature (37°C), the absorbance of the samples was measured at 510 nm. The HRSA was calculated as follows:

Inhibition rate (%) = (A1–[A2–A3])/A1 × 100

Where A1 is the absorbance of the blank (containing all the reagents except the sample), A2 is the absorbance of samples (the sample with all reagents) and A3 is the absorbance of the sample blank (the sample with all the reagents except H_2O_2).

Evaluation of reducing power

The reducing power was determined according to a previous method with some modifications.[20] The sample extract solutions from two geographical regions were mixed with 200 µL of 0.2 M phosphate buffer (pH 6.6) and 200 µL of potassium ferricyanide (1%) and then incubated at 50°C for 20 min. Thereafter, 200 µL of 10% trichloroacetic acid was added, 200 µL of the mixture was transferred into a new micro-centrifuge tube and distilled water (200 µL) and 0.1% ferric chloride (40 µL) were added immediately. The sample was mixed thoroughly on a vortex machine and centrifuged at 12000 rpm for 5 min. Then, 100 µL of the supernatant were added to the 96-well plate and measured at 700 nm using a micro plate reader. Each sample was measured three times.

Chelating power of ferrous ions

The chelating effect of ferrous ions was measured using the colorimetric method according to an earlier report, with some changes.[21] An 10 µL aliquot of the sample solution was placed in a micro-centrifuge tube and then 25 µL of a 2 mM $FeCl_2$ solution and 225 µL of methanol were added. The mixture was vortexed and incubated for 5 min and 200 µL of ferrozine (5 mM in water) was added. The reaction was leftward standing at the room temperature for 10 min and then centrifuged (12000 rpm) for 5 min. The supernatant was shifted into the wells of a 96 well microplate, and the absorbance was attained at 562 nm. The control sample confined 1 mL of extract solvent. The results were calculated using a Na_2EDTA standard curve and expressed as Na_2EDTA equivalents (mmol/LNa_2EDTA).

1,1-diphenyl-2-picryl-hydrazyl-hydrate assay

Antioxidant activity was determined using DPPH as defined earlier, with slight alterations.[22] Reaction mixtures contained 0.4 mM DPPH dissolved in ethanol and the crude extract in 50% methanol. The mixture was stirred entirely and incubated at the room temperature for 0.5 h in the dark. The absorbance at 517 nm was then measured. The radical scavenging activity was calculated as follows:

$$\text{Scavenging activity (\%)} = \frac{\text{A1 - A2}}{\text{A1}} \times 100$$

A1 and A2 represent the absorbance at 517 nm of the control and sample, respectively. All investigates were performed in triplicate. The results were expressed as the mean and SD of three repeats.

Statistical analysis

The statistical analysis was accomplished using IBM SPSS Statistics software (version 22.0, IBM Corp., Armonk, NY, USA). The analyses were conducted in triplicate, and the data are accessible as mean and SDs. The antioxidant activity differences were calculated using the one-way ANOVA between the samples. Statistical significance was set at $P < 0.05$.

RESULTS

Metabolic profiles of *Amomi fructus* from Yunnan and Guangdong Provinces in China

PCA is an unsupervised model that imitates altering trends in the dataset according to the resemblances or differences of complete metabolites and is often used to examine and decrease the dimensionality of datasets, particularly for multivariate analysis. Figure 1a shows the findings of

unsupervised PCA analysis. According to the PCA score plot, all of the GD and YN samples were detached completely. The Ward method was used for hierarchical cluster analysis based on above PCA model and the relationships among samples was revealed in the form of a dendrogram [Figure 1b]. All GD samples were classified into Cluster-1 and all YN samples were categorized into Cluster-2.

To screen the metabolites that are meaningfully affected by the different geographical origins, the OPLS-DA modeling was adopted. The samples were evidently separated depending on the holistic metabolites difference in the OPLS-DA score plot [Figure 1c], the values of R^2Y and Q^2 were 0.966 and 0.923, respectively, which showed a good fitness and predictive ability of the OPLS-DA model. The reliability of the OPLS-DA model was authenticated using a chance permutation test (n = 200). The results showed that the OPLS-DA model was dependable and steady (R^2 = [0.0, 0.454], Q^2 = [0.0, −0.201]) [Figure 1d]. The detailed parameters of the model are revealed in supplementary Table S1.

Identification of potential marker compounds associated with production area

Based on the criteria fold change >2, P < 0.001 and VIP >1, 11 of metabolites were identified as differentially abundant, representing that they make the utmost contribution to the discernment of the samples from the two production areas. Eight of them were recognized, as shown in supplementary Table S2. A heat map was employed to imagine the regional differences of differentially abundant metabolites in the samples [Figure 2]. Eight metabolites with a VIP >1.5 were designated from the mentioned eleven metabolites as possible production area markers. Binary logistic regression and receiver operating characteristics analysis were useful to assess the predictive specificities and sensitivities [Figure 3], which indicated that the eight marker compounds had high predictive sensitivities and specificities. The areas under the curve for the eight metabolites features ranged from 0.854–0.990, which showed that the eight metabolites keep high accuracy to distinguish the geographical origin. The relative peak intensities of the potential production area markers are obtainable in supplementary Figure S1.

Evaluation of the antioxidant capacity of *Amomi fructus* from two geographical areas

Hydroxyl radicals scavenging ability

The methanol extracts of *A. fructus* were demonstrated to have hydroxyl radical scavenging activity. The scavenging ability in samples varied suggestively with the two different geographical origins. All test results are accessible in Table 1. Sample YN9 had the highest HRSA and among the all tested samples, the average scavenging activity of the samples from Guangdong was higher than that of the samples from Yunnan.

Table 1: Scavenger effect of *Amomi fructus* samples from different geographical origins on hydroxyl radicals scavenging

Samples	Hydroxyl radical scavenger activity (percentage inhibition)
GD1	55.67±1.22
GD2	69.12±0.64
GD3	83.87±1.25
GD4	79.82±0.64
GD5	83.71±1.29
GD6	81.68±1.72
GD7	85.01±1.84
GD8	83.79±1.10
YN1	67.59±2.53
YN2	78.08±2.18
YN3	60.31±0.55
YN4	66.87±0.64
YN5	73.20±0.91
YN6	61.67±0.69
YN7	74.64±0.37
YN8	80.48±0.60
YN9	87.23±0.24
YN10	75.57±0.36
YN11	82.35±0.14
YN12	71.87±0.82

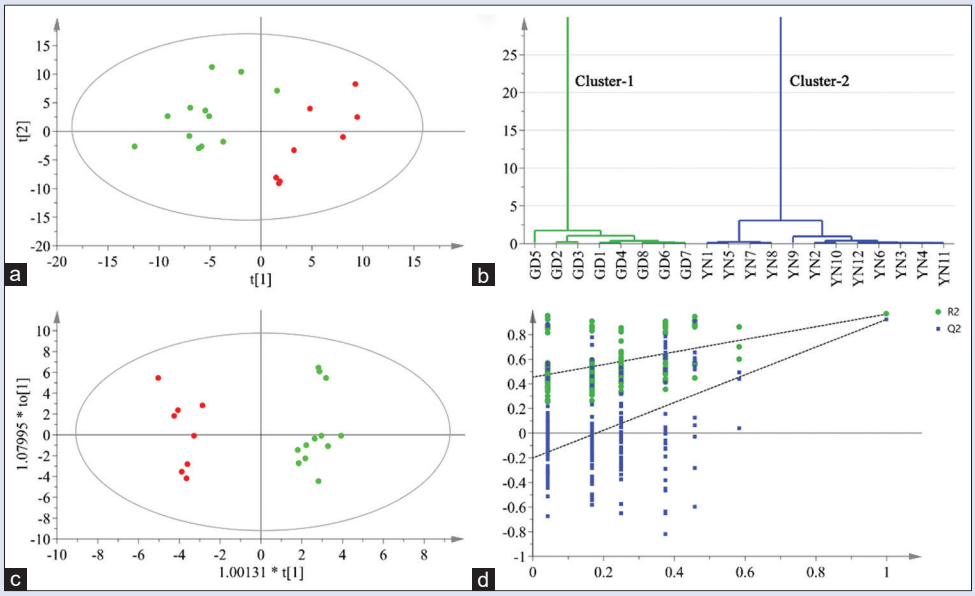

Figure 1: Discrimination of samples from Guangdong and Yunnan. (a) principal component analysis score plot of *Amomi fructus* samples from Guangdong and Yunnan. (b) Dendrograms of the hierarchical-cluster-analysis result. (c) Orthogonal partial least-squares discrimination analysis score plot showing the discrimination the metabolome of *Amomi fructus* from Guangdong and Yunnan (R^2 = [0.0, 0.454], Q^2 = [0.0–0.201]). (d) The discrimination between the samples from two locations by chance permutation test at 200 times

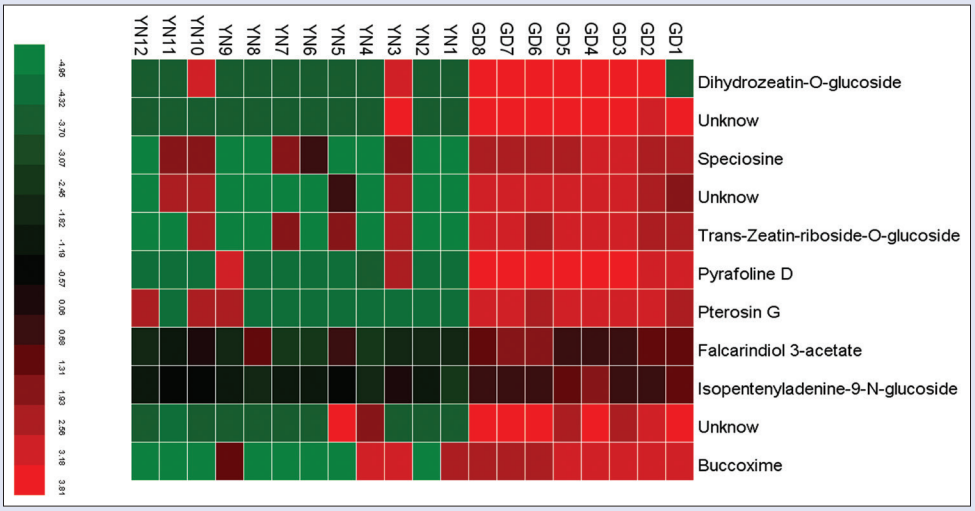

Figure 2: Heat map presentation of the differential metabolites found between the samples from Guangdong and Yunnan. YN1 to YN 12 obtained from Yunnan province, GD1 to GD8 obtained from Guangdong province

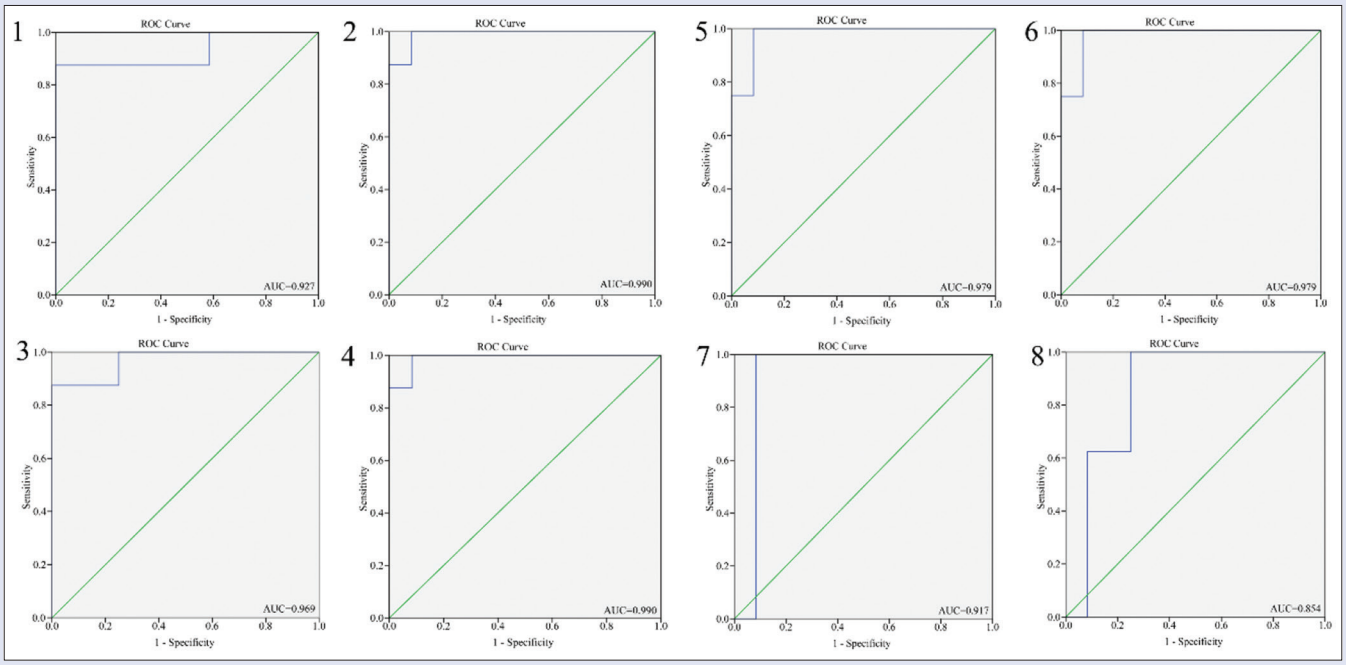

Figure 3: Receiver operating characteristics analysis and binary logistic regression used for evaluate the predictive sensitivities and specificities of the eight marker compounds. (1) Dihydrozeatin-O-glucoside (2) Unknow (3) Unknow (4) Trans-Zeatin-riboside-O-glucoside (5) Pyrafoline D (6) Pterosin G (7) Unknow (8) Buccoxime

Reducing power

Reducing capacity (reproducing the electron donation capacity) can indicate antioxidant activity to a certain range.[23] Antioxidants bind with free radical by giving up their own electrons, which can decrease the Fe^{3+} of potassium ferricyanide to Fe^{2+}. Thereafter, Prussian blue, with a maximum absorbance at 700 nm, is further formed by the reaction of Fe^{2+} and $FeCl_3$. Thus, the absorbance recorded at 700 nm can circuitously reproduce the reduction capacity of antioxidants. The greater the absorbance, the stronger the reduction capacity, the reducing power is entirely correlated with the absorbance. In our test, the absorbance at 700 nm showed a noticeable change among samples with different geographical origins [Table 2]. The strongest reducing ability (in sample YN11) was 29.5 times higher than of the lowest reducing ability (in sample YN3). Table 2 also shows obvious differences in the reduction capacity of samples from the same production area.

Chelating power of ferrous ions

The ability of the methanol extract to chelate iron was assayed, which was expressed as mmol/L Na_2EDTA. As revealed in Table 3, the chelating power of ferrous ions differed decidedly among the samples with different geographical origins. The highest and lowest values were documented for the samples YN3 (4.19 mmol/L) and GD1 (2.86 mmol/L), respectively. In general, the chelating capacity of the Yunnan samples was slightly

Table 2: Evaluation reducing power of *Amomi fructus* samples from different geographical origins

Samples	OD_{700}
GD1	0.56±0.01
GD2	0.08±0.00
GD3	0.80±0.02
GD4	0.07±0.00
GD5	0.74±0.01
GD6	0.78±0.02
GD7	0.81±0.03
GD8	0.87±0.05
YN1	1.17±0.01
YN2	1.01±0.01
YN3	0.04±0.01
YN4	1.17±0.02
YN5	0.29±0.01
YN6	0.35±0.00
YN7	1.11±0.03
YN8	1.12±0.04
YN9	1.15±0.02
YN10	1.16±0.02
YN11	1.18±0.02
YN12	1.12±0.01

Table 3: The results achieved by the chelating power of ferrous ions assays

Samples	Fe (II) (mmol/L Na_2EDTA)
GD1	2.86±0.06
GD2	3.40±0.16
GD3	3.04±0.12
GD4	3.71±0.04
GD5	3.05±0.02
GD6	2.96±0.09
GD7	2.99±0.03
GD8	3.13±0.11
YN1	3.15±0.04
YN2	3.16±0.04
YN3	4.19±0.02
YN4	3.55±0.06
YN5	3.73±0.02
YN6	2.88±0.04
YN7	3.20±0.03
YN8	3.61±0.01
YN9	3.37±0.02
YN10	3.01±0.02
YN11	3.37±0.01
YN12	3.54±0.02

EDTA: Ethylenediaminetetraacetic acid

stronger than that of the Guangdong samples; however, there were also alterations among the samples from each province.

1,1-diphenyl-2-picryl-hydrazyl-hydrate free radical scavenging activity

In the present study, the methanol extract of *A. fructus* was demonstrated to scavenge DPPH radicals. Supplementary Table S3 shows that the percent inhibition of free radicals varied significantly variation among all the samples. YN3 and YN9 had the lowest and highest DPPH radical scavenging activity (11.97% and 92.88%), respectively.

DISCUSSION

Metabolomics technology affords a good chance to widely analyze the metabolic variations among samples attained from *A. fructus* grown in two different geographical environments. The accumulation of plant metabolites is a complex process that mainly affected by genotype differences and geographical environment factors.[24] In the present study, the metabolites accountable for the discrimination among *A. fructus* samples were found. To evaluate the impact of geographical factors, the metabolic profiles of all samples were visualized using unsupervised PCA. In Figure 1a, samples from two different locations were detached completely. Ward's minimum-variance method was used to display the relationships between two geographical origin samples in the form of a dendrogram.

For the data analysis, OPLS-DA was implemented as a supervised model, which can be used to filter system noise,[25] and orthogonal signal correction filters are presented to deal with orderly changes related to, or uncorrelated with, the Y variables, respectively. The OPLS-DA method can be used as the discriminatory components and Y-orthogonal components separately, resulting in a model that is easier to understand than the standard PLS-DA model.[26] Thus, the OPLS-DA model has improved differentiation efficacy than the PCA model, particularly for complex experimental samples.

Differences in antioxidant activity were detected among the different samples. Certain secondary metabolites in plants exert antioxidant activity, such as phenolic and flavonoid compounds, and their content and species are closely related to a plant's antioxidant capacity.[27] The antioxidant capacity of the samples from two locations can indirectly reflect the differences of their secondary metabolites. Earlier studies have found that the impact of the soil type on the essential oil chemical composition,[28] the light quality, sunlight exposure and elevation also affect growth, and secondary metabolites accumulation in plants.[29,30] Therefore, we speculated that differences in the antioxidant activity were caused by multiple factors, for example, climate, soil type, temperature, rainfall, and sunlight time, which lead to the changes in secondary metabolites accumulation. A slight change in metabolite accumulation formed the basis for the discrimination of geographical origins, metabolomics platform permissible us to identify these slight changes in overall metabolite levels and combined with multivariate statistical analysis, made it likely to better differentiate between samples with different geographical origins. A limitation of the study was that the intra-specific and inter-specific variability among the samples was not evaluated. In addition, samples from more geographical regions need to be examined in upcoming investigation.

CONCLUSION

The untargeted UPLC-QTOF-MS-based metabolomics approach showed a reliable and sensitive way to differentiate *A. fructus* samples with different geographical origins. The notable differentially components between two geographical origin samples were recognized. Through a comprehensive analysis of the samples, the metabolomics technology provided abundant metabolic profiling evidence that could be used to recognize the geographical origin of a sample. The metabolomics-guided classification approach could be contributing to discrimination of the geographical origin of food and herbal medicine.

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Conflicts of interest

There are no conflicts of interest.

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