

Contents lists available at ScienceDirect

Applied Food Research



journal homepage: www.elsevier.com/locate/afres

Akebia quinata flower is an excellent potential herbal tea: Chemical quality, bioactivity analysis and metabolite profiles of *Akebia quinata* flower with different drying methods

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ARTICLE INFO

Keywords: Akebia quinata flower Herbal tea Drying method Chemical quality Bioactivity Metabolomic analysis

ABSTRACT

Akebia quinata (Houtt.) Decne is a versatile plant with both medicinal and culinary uses. Despite the abundance of *A. quinata* flowers, they remain underutilised and are often wasted. The effects of different drying methods on the chemical quality, bioactivity and metabolic profiles of *A. quinata* flowers were compared. Of the three drying methods, microwave drying (MD) had the highest content of phenolics, flavonoids and anthocyanins, the strongest antioxidant capacity. These indices were 1.4-3 times that of those obtained with hot air drying (HD). MD also improved flavor by increasing the concentration of sweet or umami peptides while reducing astringent and bitter compounds. HD and natural drying (ND) retained stronger α -glucoside inhibitory activity and NO inhibitory activity than MD. In general, *A. quinata* flowers had relatively higher chemical quality and biological activity among the six flower types, highlighting the substantial potential for development as herbal tea. Metabolomic analysis identified 3,902 metabolites, with amino acids, lipids and organic acids being most affected by the drying processes. Considering the quality of the final product, MD was the optimal of the three methods, while ND was suggested to be used in real industrial production due to less energy consumption and economic cost.

1. Introduction

For millennia, people have used plant parts such as roots, stems, leaves, fruits, flowers, seeds, or whole plants to prepare herbal tea (Liu, Ahmed, & Long, 2013). In many cultures, especially in China, drinking herbal tea is considered as a healthy lifestyle practice. Herbal tea contains bioactive compounds such as polyphenols, flavonoids, terpenoids, alkaloids, amino acids, aromatics, organic acids and polysaccharides, which confer numerous health benefits. These include antioxidant, anti-inflammatory, and antibacterial properties, along with hypoglycemic effects, cancer prevention, cardiovascular protection, and weight loss support (Liu et al., 2023; Wu et al., 2023b; Ye, Wang, Duncan, Eigel, & O'Keefe, 2015). Beyond its health benefits, the practice of tea

consumption has evolved into a refined social ritual, promoting relaxation and facilitating meaningful conversation (Pan et al., 2022).

Among the popular herbal teas, flower-based varieties hold a special place due to their attractive appearance as well as their health advantages. Even while there are already several well-liked flower teas available, such as *Chrysanthemum morifolium* Ramat. (Gong et al., 2019; Zhu et al., 2024b), it is still challenging to satisfy the needs and preferences of consumers. So in recent years, researchers have turned their attention to studying the constituents, biological activities, and processing methods of more flowers, leading to the development of novel herbal teas (Venkatesh, Gheena, Ramani, Rajeshkumar, & Ramalingam, 2023; Wu et al., 2023a). However, numerous plant flowers with potential functionalities remain underexplored as candidates for herbal

https://doi.org/10.1016/j.afres.2025.100804

Received 21 February 2025; Accepted 2 March 2025 Available online 3 March 2025 2772-5022/© 2025 The Author(s). Published by Elsevi nc/4.0/).

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teas.

Akebia quinata (Houtt.) Decne, also known as five-leaf akebia, is a versatile plant known for its dual role in both medicine and food. Its dried stem and near-ripe fruit are listed in the "China Pharmacopoeia" (2020) as herbal remedies for conditions such as amenorrhea, dysmenorrhea, edema, and aphtha. Additionally, the ripe fruit is prized in China as a specialty food due to its rich nutrition and distinctive flavor (Li, Yao, Zhong, Chen, & Huang, 2010; Zou at al., 2022). A. quinata flowers, though abundant, are often underutilized, with some plants failing to bear fruit, leading to a waste of floral resources. Although the phytochemical content of A. quinata flowers has not been as thoroughly investigated as that of the stems or fruits, the reports that are now available show that polyphenols are present, which have a variety of beneficial biological effects in humans (Maciag et al., 2021). Furthermore, previous research has shown that A. quinata flowers contain antioxidant capacity (Rim et al., 2006), suggesting their suitability for health food applications such as tea. Therefore, a deeper dive into the composition, biological activity and production technique of A. quinata flowers would provide a data-driven basis for health products.

A key step in the tea production process is drying, which removes moisture, extends shelf life, and affects tea's essential qualities, including flavor, efficacy, fragrance, and color (Qu et al., 2019). The choice of drying method is central to determining the quality and biological activity of the final products, as the same drying technique produces variable efficiencies for different plant materials (Thamkaew, Sjöholm, & Galindo, 2021). In addition, the ever-expanding market for herbal teas requires high quality raw materials and finished products. It is therefore advisable to conduct comparative experiments with the drying methods available to determine the most appropriate drying mode according to the characteristics of the material in order to retain beneficial compounds. The growing field of metabolomics provides new insights into the effects of different drying methods by identifying variations in secondary metabolites (Hua et al., 2024). The most widely used methods are shade drying, sun drying, and hot air drying due to their low-costing. In the meantime, hot air drying is a popular technique since it may dry materials faster than shade drying and sun drying (Miao, Liu, Gao, Lu, & Yang, 2022). Another drying method that is now being used in the herb processing sector is microwave drying, which allows the rapid evaporation of water from materials, resulting in comparatively shorter drying times and lower energy consumption. Comparative studies show that microwave drying preserves or increases the amount of bioactive compounds in high-quality dried products (Thamkaew, Sjöholm, & Galindo, 2021). Unfortunately, nothing has yet been reported about the drying of *A. quinata* flowers.

Given these observations, this study aims to compare *A. quinata* flowers processed using three drying methods—natural air drying in the shade (ND), hot air drying (HD), and microwave drying (MD)—to five popular herbal teas in the Chinese market, focusing on their chemical qualities and bioactivities. Additionally, the study investigates changes in the metabolic composition of *A. quinata* flowers using non-targeted metabolomics, providing a theoretical foundation for their use in herbal tea and insights into the optimal drying methods for their preparation.

2. Materials and methods

2.1. Plant materials

The whole inflorescences of *A. quinata* at full-blossom were collected from Jiujiang City, Jiangxi Province, China on March 2024 (Fig. 1A). Since sun drying tends to cause a degradation of aroma and color, ultimately resulting in low-quality products, we chose three drying methods that are widely used in industry. The *A. quinata* flowers were dried to a constant weight to ensure uniformity. Natural air drying (ND):



Fig. 1. Quality analysis of *A. quinate* flowers dried using three methods. (A) The image of *A. quinate* flowers. (B) The heatmap analysis of chemical composition contents and biological activities from different flowers. (C) The IC₅₀ values against α -glucosidase of water extracts from different flowers. Effect of water extracts from different flowers on cell viability (D) and NO concentration (E) of LPS-induced RAW264.7 cells. ND: natural air drying; HD: hot air drying; MD: microwave drying. Different letters mean significant difference from each other (*P* < 0.05).

A. quinata flowers were left to dry in the shade naturally at 25 °C about 2 days; Hot air drying (HD): *A. quinata* flowers were dried in hot air drying oven (DHG-9245A, Yiheng, Shanghai, China) at 65 °C (HD) about 12 h; Microwave drying (MD): *A. quinata* flowers were placed into microwave oven (P70D20TL-D4, Galanz, Guangdong, China) at 560 W about 15 min. All dried samples were stored in ziplock bags at -20 °C refrigerator. Commercial herbal tea including peach blossom (*Prunus persica* 'Dan Fen'), rose (*Rose rugosa* cv. Plena), jasmine (single-petal *Jasminum. sambac* var. *unifoliatum*), honeysuckle (*Lonicera japonica* Thumb.) and foetus chrysanthemum (*Chrysanthemum morifolium* Ramat.) flowers were purchased from Fumingyuan Tea Co., Ltd., Fujian, China.

2.2. Chemical reagants

The standards rutin (purity \geq 98 %) and gallic acid (purity \geq 98 %) were purchased from national institutes for food and drug control, Beijing, China. Trolox was bought from GLPBIO Co., Ltd., USA. The α -glucosidase (enzyme activity \geq 50 units/mg protein), acarbose (purity \geq 98 %), p-nitrobenzene- α -glucoside (PNPG), and parthenolide were purchased from Yuanye Biotechnology Co., Ltd., Shanghai, China. TPTZ (2, 4, 6-tri (2-pyridyl)–1, 3, 5-triazine and DPPH (1,1-diphenyl-2-pic-rylhydrazyl) were purchased from TCI Co., Ltd., Japan. ABTS (2, 2'-azino-bis-(3-ethyl-benzothiozoline-6-sulfonic acid)) was purchased from Solarbio Biotechnology Co., Ltd., Beijing, China. Other commonly used chemical reagents, such as Folin-Ciocalteu, ferric chloride, sodium acetate, sodium carbonate, sodium hydroxide, etc. are bought from Sinopharm Reagent Co., Ltd.

2.3. Extraction method

Every dried or purchased flower (1.0 g) was soaked in 100 mL boiling deionized water. After 10 min, the water extract was obtained through filtration. Then the water extract (10.0 g/L) was cooled to room temperature and stored in the refrigerator at 4 °C. Triplicate water extracts were prepared for each flower.

2.4. Quantification of chemical composition

2.4.1. Total phenolic content (TPC)

TPC of water extracts was determined by the Folin-Ciocalteu method with a slight change (Lawag, Nolden, Schaper, Lim, & Locher, 2023). Firstly, 0.2 mL water extract or gallic acid standard solution with different concentrations was mixed with 2 mL 10 % Folin-Ciocalteu reagent. After 5 min, 2 mL 7.5 % (w/v) Na₂CO₃ was added. Then the resulting mixture was left in the dark at room temperature for 30 min, followed by reading of the absorbance at 765 nm using a spectrophotometer (BioSpectrometer, Eppendorf, Germany). TPC was expressed as gallic acid equivalents (GAE) per gram of dried flowers (mg GAE/g DF).

2.4.2. Total flavonoid content (TFC)

TFC of water extracts were determined by aluminum chloride assay with a slight change (Alide, Wangila, & Kiprop, 2020). Firstly, 1 mL sample water extract or rutin standard solution with different concentrations was mixed with 0.5 mL 5 % (w/v) NaNO₂ solution. After 5 min, 0.5 mL 10 % (w/v) AlCl₃ solution was added and kept for 5 min. Then 3 mL NaOH solution (1 mol/L) was added and kept for 15 min at room temperature. Finally, the absorbance of the mixture at 510 nm was read using a spectrophotometer (BioSpectrometer, Eppendorf, Germany). TFC was expressed as rutin equivalents (RE) per gram of dried flowers (mg RE/g DF).

2.4.3. Total anthocyanin content (TAC)

TAC of dried flowers was measured with a spectrophotometer (Bio-Spectrometer, Eppendorf, Germany) as described in the literature (Wei et al., 2016). Briefly, 0.1 g dried flower was extracted with 10 mL of 0.1 mol/L HCl in ethanol at 60 °C for 30 min with intermittent shaking. The extract was centrifuged at 2290 g for 10 min and then absorbance of the supernatant was read at 530, 620 and 650 nm, respectively.

$$\Delta A = (A530 - A620) - 0.1(A650 - A620)$$

TAC $(\mu \text{mol} / \text{g DF}) = (\Delta A \times V) / (\varepsilon \times M) \times 10^6 = \Delta A \times 10 / 4.62.$

 ΔA was the anthocyanin absorbance; ϵ was the molar extinction coefficient of anthocyanin, the value was 4.62×10^4 ; M was the quality of the dried flower (0.1 g); V was the volume of the extract (0.01 L).

2.4.4. Total protein content

The total protein content of water extracts was measured by BCA kit (Yamei, Shanghai, China). The diluted water extracts (20 μ L) were added to a 96-well plate, then 200 μ L working solution were added. After mixing, the mixture was incubated at 37 °C for 30 min, then the absorbance was read at 562 nm after cooling to room temperature. The total protein in water extract was expressed as a percentage of dry weight of *A. quinata* flowers.

2.5. Bioactivity analysis

2.5.1. Antioxidant capacity

DPPH assay: the DPPH assay was performed by colorimetric method with some modifications (Nurcholis, Putri, Husnawati, Aisyah, & Priosoeryanto, 2021). Sample water extract or Trolox standard solution (0.1 mL) was mixed with 2 mL 0.2 mol/L DPPH anhydrous ethanol solution. After 30 min in the dark, the absorbance at 517 nm of reaction solution was read. The value of Trolox equivalents per gram of dried flowers (µmol TE/g DF) was used to express DPPH free radical scavenging activity.

ABTS assay: the ABTS assay was carried out as described in Rumpf et al. with some modifications (Rumpf, Burger, & Schulze, 2023). ABTS stock solution containing 25 mL of 7 mmol/L ABTS radical cation solution and 0.44 mL 140 mmol/L K₂S₂O₈ was prepared and incubated in the dark at 25 °C for 20 h. Then, the stock solution was diluted with distilled water to achieve an absorbance at 734 nm of 0.70 \pm 0.02 as working solution. Sample water extract or Trolox standard solution of 0.1 mL was added into 5 mL ABTS working solution and incubated in the dark for 30 min at 25 °C. Then, distilled water was zeroed as a blank and the absorbance at 734 nm of reaction solution was read. ABTS free radical scavenging capacity was expressed as Trolox equivalents (TE) per gram of dried flowers (µmol TE/g DF).

FRAP assay: the total antioxidant capacity (T-AOC) of the water extract was measured by Ferric-reducing antioxidant power (FRAP) assay (Parit, Dawkar, Tanpure, Pai, & Chougale, 2018). The FRAP working solution was formed before measurement by mixing 300 mmol/L acetate buffer (pH 3.6), 20 mmol/L FeCl₃ solution and 10 mmol/L TPTZ in HCl (40 mmol/L) in a ratio of 10:1:1 (v/v/v). FRAP working solution was preheated at 37 °C for 10 min before use. Then determined T-AOC as following steps: added 180 µL FRAP working solution to the 96-well plate, and 5 µL Trolox standard solution or water extract was added the to sample wells, while 5 µL deionized water was added to the blank well. Next, the 96-well plate was incubated at 37 °C for 30 min without light. The absorbance of the reaction solution at 595 nm was subsequently read. The results of the T-AOC were calculated from the standard curve and expressed as the concentration of Trolox standard solution (mmol/L).

2.5.2. α -glucosidase inhibitory activity

α-glucosidase inhibitory activity experiment was conducted as described in Miao et al. with some modifications (Miao et al., 2018). At first, 50 μL water extract (0.05 g/L-10 g/L), 50 μL α-glucosidase (1.0 U/mL) were added to wells of a 96-well plate and incubated at 37 °C for 10 min. Acarbose was used as positive control. 100 μL PNPG (10

mmol/L) was added subsequently. After 30 min at 37 °C, 1 mL Na₂CO₃ (1 mol/L) was added to end the reaction. Finally, the 96-well plate was placed on a spectramax (cMAX PLUS, Molecular devices, USA) to read the absorbance at 405 nm. The inhibitory rate of α -glucosidase was calculated as following:

Inhibition rate (%) = $1 - \left(\left(OD_A - OD_a \right) / OD_b \right) \times 100$

 OD_A represented absorbance of water extract with α -glucosidase; OD_a represented absorbance of water extract with PBS buffer; OD_b represented absorbance of distilled water with α -glucosidase. The half-maximal inhibitory concentration (IC_{50}) was obtained from the % inhibition, and calculated by GraphPad Prism. IC_{50} was used as an expression of α -glucosidase inhibitory activity.

2.5.3. Cell viability assay

Cell culture: RAW 264.7 cells, a mouse macrophage cell line originally derived from tumor tissue induced by Abelson murine leukemia virus mouse cells were cultured at 37 °C in incubator with 5 % CO₂. The cell culture medium was Dulbecco's Modified Eagle's Medium (DMEM) containing 80 % fetal bovine serum (FBS) and 1 % penicillin/streptomycin antibiotics.

Cell viability assay: The cells $(5 \times 10^3 \text{ cells in } 100 \,\mu\text{L}$ culture medium) were cultured in 96-well plate. After 24 h, cells were treated with water extract of flowers at 2 g/L. Cells treated with the same volume culture medium were used as control group (ck). Continue to incubate for another 24 h, 10 μ L cell counting kit-8 (CCK-8) solution (GIPBIO, USA) was added followed by an incubation at 37 °C for 1.5 h. Then, the OD₄₅₀ was measured (cMAX PLUS, Molecular devices, USA).

% of cell viability = Abs of test/Abs of ck×100

2.5.4. Nitric oxide (NO) assay

Cell culture was as described in 2.5.3. Lipopolysaccharide (LPS) was used to cause inflammation in the RAW 264.7 cells. Firstly, 100 μ L RAW 264.7 cells (2 \times 10⁴ cells/well) were cultured in 96-well plates for 24 h. The supernatant was discarded and cells were treated with water extract (2 g/L). Parthenolide solution (5 μ mol/L) acted as the positive control. After incubation for 1 h at 37 °C in incubator with 5 % CO₂, 10 μ L 1 μ g/mL LPS was added into the wells. The cells untreated with LPS served as control group (ck). The incubation was continued for 24 h. Then, 50 μ L supernatant or NaNO₂ standard solution was mixed with 50 μ L Griess reagent A and Griess reagent B, and absorbance at 540 nm was read after 5 min. By comparing the NO produced in cells treated with LPS alone to cells treated with LPS and extracts, the percentage inhibition of extracts on NO generation in the RAW 264.7 cell line was determined.

2.6. Non-targeted metabolomics analysis

2.6.1. Sampe preparation

An appropriate amount of the water extract of *A. quinata* flower dried by three methods was freeze-dried and added with a certain amount of 70 % methanol. Then, the mixture was vortexed, sonicated in ice-water bath and centrifuged at 4 °C. Finally, the supernatant was collected and stored in the sample vial for LC-MS/MS detection.

2.6.2. LC-MS/MS analysis conditions

HPLC conditions: all samples were separated by chromatography using the Shimadzu LC-30A (1.8 μ m, 2.1 mm \times 100 mm) at a flow rate of 0.40 mL/min at 40 °C. The injection volume was 4 μ L. The solvent system contained water (0.1 % formic acid) (A) and acetonitrile (0.1 % formic acid) (B). Sample measurements were performed with a gradient program: 0–5 min, 95 % A; 5–6 min, 35 % to 1 % A; 6–7.5 min, 1 % A; 7.5–7.6 min, 1 % A to 95 % A; 7.6–10 min, 95 % A.

MS conditions: the detection was operated using the AB TripleTOF 6600 mass spectrometry with positive and negative ion modes, respectively. The ion source temperature was 550 $^{\circ}$ C. IonSpray voltages were

5000 V (positive ion mode) and -4000 V (negative ion mode). The gases were set as follows: ion source gas1 (GAS1), 50 psi; ion source gas 2 (GAS2), 60 psi; curtain gas (CUR), 35 psi. Collision energies were 30 or -30 V in positive or negative modes, respectively. The maximum number of candidate ions to monitor per cycle was 12.

2.6.3. Data processing

ProteoWizard software was used to convert the original LC-MS/MS data file into mzXML format. The peaks were then extracted, aligned and retention times corrected using the XCMS program. Support vector regression (SVR) was used to correct the peak area. In each group of samples, peaks with a detection rate of <50 % were eliminated. Metabolite identification information was obtained by searching the laboratory's database (MetMare, China), the integrated public database, the AI database and the proprietary database.

2.7. Statistical analysis

The results were presented as the mean \pm standard deviation (SD) of each sample's triplicate measurements. With SPSS 26, one-way analysis of variance (ANOVA) and Duncan's test were carried out. The Pearson method of correlation analysis was used, along with Origin software. Principal component analysis (PCA) was performed using the statistics function prcomp inside the R (base package), while orthogonal partial least squares-discriminant analysis (OPLS-DA) was generated using the R package MetaboAnalystR. R package was used to create heat maps of hierarchical cluster analysis (ComplexHeatmap).

3. Results and discussion

3.1. Chemical qualities of A. quinata flower

Flavonoids, important phenolic compounds, are key quality indicators in teas due to their health-promoting bioactivities, which include antioxidant, antimicrobial, and anti-hypertensive properties (Hu et al., 2020; Lorenzo & Munekata, 2016). Anthocyanins, a subclass of flavonoids, also contribute to the red, blue, and purple pigmentation in plants and are known for their health benefits, including antioxidant activity. The *A. quinata* flower is recognized for its rich phenolic, flavonoid, and anthocyanin content (Maciag et al., 2021). To evaluate the chemical composition of *A. quinata* and compare it with other popular herbal teas, we first measured its total phenolic content (TPC), total flavonoid content (TFC), and total anthocyanin content (TAC).

As shown in the heat map of Fig. 1B and Table S1, A. quinata flower exhibited significantly higher TPC and TFC than peach blossom, rose, jasmine, and chrysanthemum flowers, and significantly lower levels than honeysuckle flowers alone. Among the three methods, the highest TFC was recorded for MD at 68.57 mg RE/g DF, followed by ND at 63.00 mg RE/g DF, and HD at 40.31 mg RE/g DF. Similarly, MD preserved the highest TPC (36.11 mg GAE/g DF), followed by ND and HD. Compared with peach blossom, rose, jasmine and chrysanthemum flowers, the TPC and TFC of MD were about 0.6-3.8 times and 1-22 times higher, respectively, than those of them. Even HD, which had the lowest TPC and TFC of the three drying methods, had much higher values than these. Rose (Rose rugosa cv. Plena) is known for its high anthocyanin content and is an excellent plant source for food and cosmetic additives (Feng et al., 2023). Here, we found that the TAC of A. quinata flowers, in particular MD and ND, was comparable to that of rose (Fig. 1B and Table S1).

Therefore, *A. quinata* flowers have a good metabolic composition with comparatively higher TPC and TFC of peach blossom, rose, jasmine, chrysanthemum and high levels of anthocyanins comparable to roses. The drying method significantly influenced the chemical composition of *A.* quinata flower. MD yielded higher TPC, TFC, and TAC, likely due to electromagnetic waves breaking chemical bonds and enhancing the release of phenolic compounds (Belwal et al., 2022). In

contrast, ND allowed oxidation by polyphenol oxidase, reducing TPC and TFC, while HD caused the breakdown of thermolabile compounds, resulting in a substantial decrease in chemical quality (Belwal et al., 2022).

3.2. Bioactivity analysis of A. quinata flower

Oxidative stress plays a role in the development of numerous diseases, such as diabetes mellitus, atherosclerosis, cancer, and hypertension. Phenolic compounds are known to counteract oxidative stress by donating electrons or hydrogen atoms to neutralize free radicals (Albuquerque, Heleno, Oliveira, Barros, & Ferreira, 2021). Given the importance of antioxidant capacity in tea, we evaluated DPPH scavenging capacity, ABTS scavenging capacity and T-AOC of the flowers (Fig. 1B and Table S1).

The antioxidant capacity for different flowers also showed signicantly differences. A. quinata flower displayed a strong antioxidant capacity, aligning with its TPC and TFC levels, second only to honeysuckle flower. For DPPH scavenging capacity, ABTS scavenging capacity and T-AOC, MD-treated samples held the maximum values among the three drying methods with 199.28 µmol TE/g DF, 177.82 µmol TE/g DF, and 3.16 mmol/L, respectively, indicating that MD had the stronger comprehensive antioxidant capacity compared with HD and ND. Similar outcomes were observed in microwave-dried herbal tea from strobilanthes crispus leaves, which showed higher ferric reducing antioxidant power and DPPH scavenging capacity in comparison with leaves dried in a hot air oven (Lasano, Rahmat, Ramli, & Bakar, 2018). However, for spearmint, the results were completely different from ours, as convection oven and microwave drying showed the lowest antioxidant capacity including FRAP and DPPH scavenging capacity comparing to freeze-drying, sun-drying, and shade-drying (Orphanides, Goulas, & Gekas, 2013). These differences may be due to the unique metabolite composition of the plant materials, making each material differently adaptable to the same drying method. In contrast, jasmine presented the most weak antioxidant capacity across all the flowers, containg merely 18.66 µmol TE/g DF of DPPH scavenging capacity, 30.68 µmol TE/g DF of ABTS scavenging capacity, and 0.4 mmol/L of T-AOC, accounting for only 9.3 %, 17.2 %, and 12.7 % of those in MD, respectively. When HD and ND were compared, they were dominant in various assays, specifically the DPPH and ABTS scavenging capacities of ND were superior to HD, while the T-AOC of HD was stronger than ND.

Furthermore, the inhibitory activity of *A. quinata* flower against α -glucosidase, an enzyme involved in glucose digestion, was examined due to its relevance in managing type 2 diabetes ((Kumar et al., 2020; Papoutsis et al., 2021). Various degrees of α -glucosidase inhibition were observed in all flowers except jasmine (Fig. 1C). With an IC₅₀ value of 0.09 \pm 0.03 g/L, rose flowers notably showed the strongest inhibition. This is consistent with the findings of Simin et al. (2024), who found that roses expressed extremely high inhibitory to α -glucosidase, suggesting that roses could be used as plant sources to inhibit α -glucosidase activity. Moreover, The IC₅₀ values of ND and MD had no significant differences, but were significantly lower than HD, indicating stronger inhibition to α -glucosidase activity.

An important part of the anti-inflammatory process is played by macrophages. LPS and other stimuli cause macrophages to become activated, which in turn produces pro-inflammatory cytokines and inflammatory mediators likeNO. An essential inflammatory marker is the amount of NO present (Hu et al., 2023). Therefore, anti-inflammatory activity of flowers was assessed by measuring NO production in LPS-stimulated macrophages. All water extracts produced high cell viability of more than 90 % without appreciable cytotoxicity as compared to the control group (ck group in Fig. 1D). It is noteworthy that the cell viability of peach, rose, jasmine, honeysuckle, chrysanthemum, and *A. quinata* flower under MD was greatly increased. This might be because specific protein kinases required for cellular development were triggered or additional nutritions were supplied by

complex compounds (Peng, Li, Pi, & Yue, 2024). Additionlly, all flower extracts inhibited NO production to varying degrees (Fig. 1E), with chrysanthemum and honeysuckle flowers showing the most potent inhibition (above 95 % and 85 %, respectively), which can be supported by previous studies (Le Thi, Phan, Nguyen, & Do Hong, 2020; Li et al., 2019; Nikzad-Langerodi et al., 2017). A. quinata flowers exhibited moderate NO inhibition (50-60 %), with ND and HD treatments may providing better anti-inflammatory effects than MD. This result was inconsistent with the effect of drying methods on antioxidant capacity, possibly because non-phenolic compounds played an anti-inflammatory role, as the akebiasaponin from A. quinata stems has been reported to have anti-inflammatory effects (Maciag et al., 2021). As far as we are aware, this is the first time the A. quinata flower's anti-inflammatory properties have been discovered. Futher search focus on the separation and purification of extract from A. quinata flower, identification of anti-inflammatory substances and analysis of specific anti-inflammatory pathways.

Overall, these findings demonstrated that drying methods significantly affected bioactive compound retention and influenced the overall bioactivity of the flowers. Meanwhile, our analysis suggested that *A. quinata* flower had a variety of biological activities, including NO inhibition, α -glucosidase inhibition activity, and superior antioxidant capacity, making it a potential functional food application such as herbal tea.

3.3. Correlation between TPC, TFC, TAC and bioactivities

Correlation analysis revealed a extremely significant positive correlation between TPC, TFC, and antioxidant capacity. TPC and DPPH scavenging capacity, ABTS scavenging capacity, and T-AOC had correlation coefficients of 0.81, 0.85, and 0.82 (P < 0.01), respectively, whereas TFC had correlation coefficients of 0.81, 0.86, and 0.81 (P < 0.01) with DPPH scavenging capacity, ABTS scavenging capacity, and T-AOC, respectively (Fig. S1), highlighting the role of phenolic compounds in oxidative stress protection (Alara, Abdurahman, & Ukaegbu, 2021). However, no significant correlation was found between TAC and antioxidant capacity, suggesting that the antioxidant capacity of *A. quinata* flower is driven more by non-anthocyanin flavonoids. Interestingly, the α -glucosidase inhibitory activity and NO inhibition did not correlate significantly with TPC, TFC, or TAC, implying the involvement of non-phenolic compounds, such as saponins and terpenes (Papoutsis et al., 2021).

3.4. Metabolite profiling of A. quinata flower dried by three methods

To better understand the effect of drying on A. quinata flower, nontargeted metabolomics based on LC-MS/MS was employed to profile the metabolites. A total of 3902 metabolites were identified, including amino acids, organic acids, alkaloids, phenolic acids, and flavonoids, among others (Fig. 2A, Table S2). Amino acids and their derivatives (1250) were the most abundant, accounting for 32.03 % of the total metabolites, followed by 446 organic acids, 370 benzene and substituted derivatives, 235 alkaloids, 155 phenolic acids and 146 flavonoids, 139 nucleotides and derivatives, 125 heterocyclic compounds, 125 alcohol and amines, 105 lipids, 84 terpenoids, 73 glycerophospholipids, 54 fatty acyls, 45 lignans and coumarins, 26 steroids, 22 glycerolipids, 13 quinones, 9 sphingolipids, 7 tannins, 2 tryptamines, cholines and pigments and 471 others (Fig. 2A and Table S2). Total positive and negative ion current of quality control (QC) samples and correlation analysis indicated that the detection process was stable and the data obtained was reliable (Fig. S2).

Principal component analysis (PCA) revealed clear separation between the different drying treatments, indicating significant metabolic variation (Fig. 2B). PC1 and PC2 explained 43.54 % and 23.18 % of the variability, respectively. The close clustering of the ND, HD, and MD samples suggested strong analytical repeatability and experiment



Fig. 2. Metabolome analysis of *A. quinate* flowers. (A) Ring plot of total metabolites in *A. quinata* flower. GL: Glycerolipid; SL: Sphingolipid; GP: Glycerophospholipids; FA: Fatty acyls. (B) PCA analysis for three drying samples groups of *A. quinate* flowers. (C) Heat map of hierarchical cluster analysis for total metabolites in *A. quinata* flowers. (D) OPLS-DA score plot of *A. quinata* flowers under three drying methods. ND: natural air drying; HD: hot air drying; MD: microwave drying.

stability.

The hierarchical cluster analysis heat map showed variations in the metabolite accumulation patterns. The relative concentrations of the majority of metabolites varied across the groups (Fig. 2C). All of the metabolites could be easily separated into three clusters: the compounds in cluster 1, cluster 2, and cluster 3 were mainly accumulated in HD-treated, MD-treated, and ND-treated samples, respectively. In summary, the data above indicated that the types and quantities of the

chemicals were significantly influenced by the drying methods.

3.5. Differential metabolites screening

To investigate the effects of the drying process on the chemical quality and biological activity of *A. quinata* flowers, differential metabolite screening was performed. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA), a multivariate statistical method with

supervised pattern recognition, was employed to screen for significant differentially accumulated metabolites (DAMs) while eliminating irrelevant variations. As depicted in Fig. 2D, clear separations between groups along component 1 indicated that the drying process had a substantial impact on *A. quinata* flowers. However, no significant separation was observed among biological replicates within each group along component 2. The model's R^2X , R^2Y , and Q^2 values were 0.671, 0.995, and 0.909, respectively, demonstrating high explained variance and predictive capability (Fig. S3).

The variable importance in projection (VIP) values based on OPLS - DA model were used to determine the impact of specific metabolites on group separation. Metabolites with VIP > 1, a *t*-test p value < 0.05 and a fold change ≥ 2 or ≤ 0.5 were considered as significantally differential. A total of 1902 metabolites met the criteria, representing approximately half of all the detected metabolites.

Further analysis revealed notable differences between groups. As illustrated in Fig. 3A, 824 DAMs were identified in the HD vs ND group (434 up-accumulated and 390 down-accumulated), 1189 DAMs in the MD vs ND group (364 up-accumulated and 825 down-accumulated), and 1137 differential metabolites in the HD vs MD group (807 up-accumulated and 330 down-accumulated) Interestingly, the number of down-accumulated metabolites was greater in the MD vs ND comparison, whereas the HD vs MD comparison exhibited a higher number of

up-accumulated metabolites. Overall, metabolite levels were generally higher in HD-treated *A. quinata* flowers.

The differential metabolites were further categorized into 21 groups (Fig. 3B). Amino acids and their derivatives were the most affected, with 435, 302, and 420 DAMs in the MD vs ND, HD vs ND, and HD vs MD comparisons, respectively. HD treatment particularly increased the upaccumulation of amino acids and their derivatives, indicating that pathways related to amino acid biosynthesis and protein hydrolysis might have been activated.

Additionally, certain compound classes responded differently to the drying processes. ND treatment led to more up-accumulated alkaloids, heterocyclic compounds, nucleotides and derivatives, alcohols and amines, fatty acids, and phenolic acids, whereas HD treatment significantly up-accumulated benzene derivatives, flavonoids, lipids, and organic acids. This suggests that the drying process either activated or inhibited specific metabolic pathways, leading to variation in the expression levels of these compounds.

A Venn diagram (Fig. 3C) showed common and unique differential metabolites across the three group comparisons. There were 279, 260, and 266 distinct DAMs in the MD vs ND, HD vs ND, and HD vs MD groups, respectively. Importantly, 151 metabolites were common across all comparisons and were considered as key DAMs. Among these, amino acids and their derivatives accounted for the largest proportion (38.4



Fig. 3. Differential metabolite analysis of *A. quinate* flowers. (A) Volcano plot of HD vs ND, MD vs ND and HD vs MD, respectively. Red spots present the upaccumulated DAMs, green spots present the down-accumulated DAMs. (B) The number of up-accumulated and down-accumulated DAMs in each group by category. GL: Glycerolipid; SL: Sphingolipid; GP: Glycerophospholipids; FA: Fatty acyls. (C) Veen diagram of DAMs of HD vs ND, MD vs ND and HD vs MD. (D) Ring plot displaying the classification of the 151 key DAMs, FA: Fatty acyls. ND: natural air drying; HD: hot air drying; MD: microwave drying.

%), followed by organic acids (8.6 %) and lipids (7.3 %) (Fig. 3D).

In summary, the drying process significantly altered the metabolite profiles of *A. quinata* flowers, with HD generally increasing metabolite levels, particularly in amino acids and their derivatives. This suggests that drying methods can materially influence both the chemical quality and potential biological activity of the flowers through alterations in specific metabolic pathways.

3.6. KEGG pathway enrichment analysis

KEGG pathway enrichment analysis was conducted to explore the biological mechanisms underlying the alterations in metabolites of *A. quinata* flowers. The majority of DAMs were associated with metabolic pathways and the biosynthesis of secondary metabolites. In the HD vs ND, MD vs ND, and HD vs MD comparisons, the DAMs were enriched and mapped to 67, 83, and 75 pathways, respectively (Fig. S4). Across these comparisons, 57 metabolic pathways were common, highlighting that the drying process significantly affected most metabolic pathways (Table S3).

Fig. 4 presents the top 20 enriched pathways for each pairwise comparison. Among these pathways, alpha-linolenic acid metabolism, amino sugar and nucleotide sugar metabolism, neomycin, kanamycin,

and gentamicin biosynthesis, carbapenem biosynthesis, biotin metabolism, isoquinoline alkaloid biosynthesis, and tryptophan metabolism were notably enriched, with p-values < 0.05. These pathways primarily involved compounds such as amino acids and alkaloids.

Consistent with the identification of key DAMs, pathways related to amino acid metabolism and biosynthesis—including tryptophan metabolism, lysine metabolism, beta-alanine metabolism, and cysteine and methionine metabolism—were particularly significant. These results suggest that the drying process plays a critical role in regulating pathways related to amino acids and alkaloids, further influencing the chemical composition and biological activity of *A. quinata* flowers.

3.7. Alterations in metabolites during drying process

3.7.1. Amino acids and derivatives

During the drying process, proteins undergo degradation into amino acids and small peptides due to proteolytic enzymes and microbial action (Lu et al., 2024; Xue et al., 2022). Amino acids and peptides are key contributors to flavor, including bitterness, umami, sweetness, astringency, and kokumi (Zhao, Schieber, & Gänzle, 2016). In addition, active peptides with antioxidant, antihypertensive, hypolipidemic, and hypoglycemic properties have also been reported (Yating et al., 2021; Ye



Fig. 4. Metabolomic enrichment pathway analysis. ND: natural air drying; HD: hot air drying; MD: microwave drying.

The water extract analysis revealed high protein content in *A. quinata* flowers, ranging from 28.4 % to 50.4 % of dry weight (Fig. S5). With HD promoting the up-accumulating amino acids and derivatives, this suggested significant protein degradation. Among the 151 key DAMs, 58 were amino acids and derivatives, primarily small peptides, and their changes were visualized using a heat map (Fig. 5A). The 58 compounds were categorized into three classes: 23 in Class I, 15 in Class II, and 20 in Class III, with higher levels of amino acid derivatives and small peptides accumulated in MD and ND treatments.

Bitterness in peptides often arises from the presence of proline and

other amino acids such as Gly, Ala, Val, Leu, Tyr, and Phe (Zhao et al., 2016). In this study, the identified peptides, including Gly-Leu, Gly-Pro-Phe, Tyr-Ser-Leu, and Pro-Pro-Gly, predominantly imparted bitterness, particularly in the HD group. Glu-Val, however, contributed to a sweet or umami taste and was more abundant in MD (Maehashi & Huang, 2009; Wieser & Belitz, 1976). Thus, to reduce bitterness and enhance flavor, MD appears to be a preferable drying method for *A. quinata* flowers.

Although bitter substances give us a bad feeling in taste, but often have some unique bioactivities. It has been demonstrated that the bitter tripeptide Tyr-Ser-Leu (Tyroserleutide) inhibits human



Fig. 5. Heat maps of hierarchical cluster analysis for key DAMs. (A) amino acids and derivatives, (B) organic acids, (C) lipids, (D) phenolic acids and (E) flavonoids in *A. quinata* flowers. ND: natural air drying; HD: hot air drying; MD: microwave drying.

hepatocarcinoma, presumably via blocking the PI3K/AKT pathway's ability to transmit signals related to tumor cell proliferation (Fu et al., 2014). This suggests that the bioactivities of *A. quinata* flowers could provide opportunities for further exploration.

3.7.2. Organic acids

Organic acids, key intermediates in plant metabolic processes, were significantly affected during the drying process. Eight aromatic acids and five aliphatic acids showed substantial differences in abundance. Overall, key organic acids were most abundant in MD and ND, and the lowest in HD (Fig. 5B). Some of these organic acids are classified as derivatives or metabolites of useful chemicals, whereas others have distinct biological activity.

For example, 3-methoxybenzenepropanoic acid, which inhibits α -amylase, shows potential as a type 2 diabetes inhibitor (Galarce-Bustos et al., 2023). Indicaxanthin, a natural orange-yellow pigment, exhibits antioxidant properties (Butera et al., 2002), while hippuric acid, a metabolite of chlorogenic acid, undergoes microbial transformation and is a regular component of urine (Ogawa, 2015). Chlorogenic acid was identified among the metabolites, and its relative concentration was lowest in MD, suggesting its conversion to hippuric acid during MD (Fig. S6). Hippuric acid's diuretic properties make it a potential biomarker for the diuretic effects of polyphenols (Angappan et al., 2018). Additionally, pyridoxamine phosphate (vitamin B6), DL-pantothenic acid (vitamin B5), and (6R)–5-methyltetrahydrofolic acid (a form of vitamin B9) were identified, linking these acids to vitamin-related metabolic pathways.

3.7.3. Lipids

Free fatty acids, an important class of lipids, were significantly affected by the drying process. Among the 11 key lipid metabolites, 10 were more abundant in ND, and only one in MD (Fig. 5C). Pinellic acid, 13(S)-HpOTrE(gamma), 9,12,13-Trihydroxy-10,15-octadecadienoic acid, 9(S)-HpOTrE, 1-Linoleoyl-sn-glycerol-diglucoside and 9-OxoODE are metabolites of linolenic acid or oxidized linolenic acid (Kumar et al., 2016; Yuan et al., 2013). The accumulation of the above linoleic acid metabolites, except 1-linoleoyl-sn-glycerol-diglucoside, was significantly increased under ND treatment. Meanwhile, similar patterns were observed for 11-HEDE, palmitoyl serinol, octadecapentaenoic acid, 5S-hydroperoxy-18R-HEPE and 18-hydroxyoleic acid. KEGG analysis hinted that ND activated the metabolic pathway of fatty acids, especially linolenic acid as the high temperature produced by the MD and HD processes was not conducive to the activity of enzymes, such as oxidases (Fig. S3).

Uncontrolled inflammation is an underlying etiology for multiple diseases and macrophages orchestrate inflammation largely through the production of oxidized fatty acids known as oxylipids (Mattmiller et al., 2014). A. quinata flower, as well as *Bupleurum falcatum, Pinellia ternate*, and other plants contain pinellic acid, a trihydroxyoctadecenoic acid that functions as an anti-inflammatory by preventing NO generation in LPS-activated murine microglial cells (Kim et al., 2014; Nagai et al., 2002). Because 9-oxoODE reduces TNF- α production, it can lessen the oxidant-induced macrophage inflammatory response (Mattmiller et al., 2014). Fatty acids also contribute to the flavor of tea through interactions with amino acids, carbonyl compounds, and lipid oxidation products (Huang et al., 2024).

3.7.4. Phenolic acids and flavonoids

Although phenolic acids and flavonoids comprised a smaller fraction of the 151 key DAMs, their biological activities and influence on flower quality made them of particular interest. Phenolic acids generally exist as amides, esters, or glycosides, with only a few occurring in free form (Mattila & Hellström, 2007). Five key phenolic acids were identified, including three free phenolic acids and two phenolic acid esters (Fig. 5D). Dodecyl gallate and ethyl gallate, both esters of gallic acid, are known for their antioxidant and antibacterial activities (Gabe et al., 2019; Masek, Chrzescijanska, Zaborski, & Piotrowska, 2014) and were more abundant in HD and MD, respectively.

The key flavonoids identified included flavokawain A, kaempferol 3-O-beta-D-glucopyranosyl-7-O-alpha-L-rhamnopyranoside and 5,7,3',4'-Tetrahydroxy-6,8-dimethoxyflavone (Fig. 5E). Flavokawain A, a chalcone family member, has demonstrated anti-tumor and antiinflammatory properties by inhibiting the production of iNOS and COX-2 in RAW 264.7 cells through the blockage of NF-kB and AP-1 activation (Kwon, Ju, Youn, Choi, & Park, 2013; Liu et al., 2022). While, Kaempferol derivatives contribute to the astringency of tea infusions (Zhu et al., 2024a). ND treatment increased the levels of kaempferol derivatives, potentially leading to a stronger astringent taste compared to HD and MD.

4. Conclusions

In this study, we explored the potential of *A. quinata* flowers as a herbal tea. *A. quinate* flowers demonstrated relatively superior chemical quality across six kinds of flowers, exhibiting higher TPC, TFC compared to peach blossom, rose, jasmine, and chrysanthemum flowers and higher TAC compared to peach blossom, jasmine, honeysuckle, and chrysanthemum flowers. Furthermore, the *A. quinate* flower showed significant antioxidant capacity, a certain degree of α -glucosidase inhibitory activity, and NO inhibition at the same time, indicating multiple potential in the areas of antioxidants, treatment of type 2 diabetes and anti-inflammatory, and its comprehensive biological activity was unmatched by other herbal teas.

Among the three drying methods tested, MD emerged as the most effective in enhancing the chemical quality and antioxidant capacity as well as improved the flavor by increasing the concentration of sweet or umami peptides while reducing astringent and bitter compounds. However, ND and HD had stronger preserved stronger α -glucosidase inhibitory activity, and NO inhibition. Metabolomics analysis, applied for the first time to A. quinata flowers, identified 3902 metabolites, providing systemic insights into the choice of drying method and the metabolite composition. Multivariate analysis revealed significant alterations in key metabolites, with amino acids, lipids, and organic acids being the most affected by the drying process. Taking into account the flavor, chemical quality and antioxidant capacity, MD was the optimal of the three methods, while ND was suggested to be the advantageous technology in actual industrial production in terms of energy consumption and economic cost. Given its superior chemical profile and bioactivity. A. quinata flower holds substantial potential for development as a functional herbal tea. Future directions could include expanding the metabolomics approach to identify more bioactive compounds and conducting sensory evaluations to better understand consumer preferences as well as exploring additional health benefits of this plant and optimize its preparation methods for commercial use.

Ethical statement-Studies in humans and animals

The work involved no studies in humans and animals.

Funding

The work was supported by the National Natural Science Foundation of China (32460101), the Natural Science Foundation of Jiangxi Province (20232BAB216121) and Jiujiang City Talent Project (JJXC2023134).

CRediT authorship contribution statement

Tianjiao Jia: Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft. Mian Faisal Nazir: Writing – review & editing. Tao Zhang: Formal analysis. Qiuping Zhu: Data curation. Jie Xu: Formal analysis. Longyu Dai: Investigation. **Yafang Zhao:** Data curation. **Shuaiyu Zou:** Conceptualization, Resources, Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2025.100804.

Data availability

Data will be made available on request.

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