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DRYING CHARACTERISTICS, CHEMICAL CONSTITUENTS, VOLATILES PROFILES OF DIFFERENT ROOT SYSTEMS FROM HAIRY FIG (*FICUS HIRTA VAHL.*) AND ANTIOXIDANT ACTIVITY

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ABSTRACT

Hairy fig (*Ficus hirta* Vahl.) root is a traditional medicinal and food homologous plant in China. Due to the lignified roots are extremely difficult to cut and package, postharvest treatment is very difficult. Therefore, when the roots of the hairy fig are used as a soup ingredient, the root cortex is simply peeled off and used.In order to promote the development and utilization of hairy fig root, chemical composition, volatile profiles and antioxidant activities of entire root (ER), root cortex (RC) and root internal tissue (RIT) of hairy fig were investigated. Hairy fig root was rich in fat, protein, soluble sugar, polyphenols, flavones and other nutrients, which had significant difference in ER, RC and RIT ($P<0.05$). Chemical components and nutrients were highest in RC, and lowest in RIT, as well as psoralen and bergapten. In ER, RC and RIT, content of psoralen was 0.70, 0.77, 0.32 mg/g, and 0.27, 0.42, 0.14 mg/g for bergapten. Phosphorus, potassium, magnesium, calcium were the main minerals, and arginine was the main free amino acid. There were 47 volatile components detected in ER, RC and RIT, where aldehydes and heterocyclic compounds were the dominant components. The relative level of aldehydes was 16.98~34.09%, and 6.71~45.23% for heterocyclic compounds. In addition, total relative amount of volatile components was 75.68% in RC, but 57.94% in RIT. From chemical components, nutritional components and volatile components content, the quality of RC was better than ER and RIT. The results might be related to chemicals accumulation position and the physiological structure of different parts of plants. According to antioxidant activity, ER, RC and RIT have scavenging activity of 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH) and hydroxyl radicals. The half inhibitory concentration (IC50) of RC on ABTS, DPPH and hydroxyl radical were 3.51, 0.08 and 1360.40 mg/mL, respectively, which were lower than ER and RIT. Based on the drying process of the samples, the RC and RIT exhibited the best drying efficiency. All in all, from the point of view of saving energy and quality, RC not only had better quality but need less energy to dry. These results provided a reference for the development and utilization of hairy fig root cortex as a raw material in medicine, functional food or dietary additives.

1.Introduction

With more than 800 species of trees, shrubs, hemiepiphytes, climbers, and creepers in the tropics and subtropics worldwide, Ficus is one of the largest genera of angiosperms. There are 23 species of lithophytes and hemiepiphytes in the ficus family that form aerial and creeping root systems (Ronsted, et al., 2008). Among these, hairy fig (*Ficus hirta* Vahl.) is one of the important member in the family of Moraceae (Shu, et al., 1975). It widely distributed in southern China and it's known as Wuzhimaotao in Chinese language (Dai, et al., 2018). It is a popular herbal medicine and a food ingredient among Hakka people in China. Hairy fig is a wild edible plant in Assam and Meghalaya,India. It is popular as a folk medicine in Vietnam for the treatment of hepatitis, nephritis, mastitis and rheumatism (Yi, et al., 2013) . Hairy fig root has a pleasant fragrance and medicinal potential for the treatment of hepatitis, anticancer, antioxidation and improving fatigue resistance in China (Au, et al., 2008; Zeng, et al.,2012; Liang, et al., 2021).

In recent years, many studies have been done on the hairy fig root for understanding its chemical compositions. From the stem and root extracts of hairy fig, hundreds of various therapeutically important phyto-compounds such as phenylpropanoids, flavonoids, terpenoids, sterols and volatile oils have been identified (Wang and Chen, 2013; Cheng, et al., 2017; Ye, et al., 2019). Hairy fig root is rich with polyphenols (197.16 mg/g) and flavonoid (9.10 mg/g) (Gui, et al., 2018; Chen, et al., 2021). The content of volatile oils in root cortex and root internal tissue of hairy fig was 0.3 and 0.25 mL/Kg, respectively. The popular linear furanocoumarins such as psoralen and bergapten were considered as the most valuable quality evaluation indexes component (Liu, et al., 2004; Wei, et al., 2005). The content of psoralen and bergapten in hairy fig root was 0.0008-1.187mg/g and 0.0039-0.034mg/g, respectively (Cai, et al., 2019; Chen, et al., 2022).

According to various reports, different plants have a distinct distribution of metabolites in their roots. Paeoniflorin and its derivatives are primarily found in the cortex and phloem of Paeonia suffruticosa root, although they are also present in Paeonia lactiflora's xylem rays (Li, et al., 2021). As well as in Tripterygium root, the quinone methides compound, celastrol is observed only in periderm, but the intense signals of sesquiterpene pyridine alkaloids are detected throughout the root cortex (Lang, et al., 2016). The antibacterial activity of root cortex extract in Berberis heteropoda Schrenk is superior than root internal tissue against Pseudomonas aeruginosa and Enterococcus faecalis, whereas their antibacterial activity against Escherichia coli and Staphylococcus aureus is comparable (Zhu and Li, 2018). Periplocin and 4-methoxysalicylaldehyde are crucial index component in Periploca sepium Bunge, but they are found in greater concentrations in the root cortex than the root internal tissue of the plant (Zhang, et al., 2012). It is indicated that the accumulation of plant metabolites are varying in different parts of herbal root.

Various studies have been done on the hairy fig root and revealed the presence of numerous bioactive phyto molecules. Nevertheless, the studies were a general approach to understand the contents in the entire root. But based on the various studies (Zhang, et al., 2012; Lang, et al., 2016; Zhu and Li, 2018; Li, et al., 2021), it is understood that each root parts possess different level of bioactive molecules. So the current study has been designed to analyse the two common forms (entire root (ER) and root cortex (RC)) and the new root part, root internal tissue (RIT). Chinese Pharmacopoeia (1977 edition) mention that hairy fig root with thick cortex and nice fragrance can be an improved product, but no studies have shown that whether root cortex has higher content of active ingredients or antioxidant activity. Although, Liu et al. (2004) find that the content of volatile oil and psoralen in root cortex is higher than that of root internal tissue. Another study report that the contents of psoralen, polyphenols and flavonoids in the thin root are higher than thick root (Gui, et al., 2018). But there is no systematic study about the

difference of chemical component and antioxidant activity in different part of hairy fig root.

In this paper, the drying curves of ER, RC and RIT of hairy fig root were analyzed under the hot air drying condition at 80℃, and the energy required to dry the different parts was calculated. Then, the differences of chemical components, volatile flavor components and antioxidant activities in different parts of hairy fig root were detected, and the relationship between chemical components and microscopic structure were analyzed. The results from this study will be useful not only at the scientific level to find out the effective parts accurately but also to encourage farmers, large-scale breeders and the pharmaceutical industry to explore the development and utilization of hairy figs.

2.Materials and methods

2.1.Plant Materials

Fresh hairy fig (HF) roots were collected from the experimental farm of the Chinese Academy of Tropical Agricultural Sciences (CATAS; 19°32′55″N, 109°28′30″E) in Danzhou City. The entire root (ER)of HF was separated to root cortex (RC) and root internal tissue (RIT), and the three samples were dried in a hot air drying oven (GZX-9240MBE, China) at 80 ℃ to constant weight. Hairy fig plant, entire root, root cortex and root internal tissue of hairy fig were shown in Figure 1. Then the dried samples were ground into powder and sieved through 100 mesh sieves. The hairy fig root powder was collected and stored at -20℃ prior to further use.

Figure 1. Hairy fig plant (A) and entire root (B) , root cortex (C) and root internal tissue (D) of hairy fig

2.2.Chemicals and reagents

Acetonitrile (HPLC grade) were purchased from Merck (Darmstadt, Germany). Psoralen and bergapten were purchased from Aladdin (Shanghai, China). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) were purchased from Sigma Chemical Co.LTD,

(St. Louis, MO, USA). Gallic acid, rutin (analytical grade) was purchased from sinopharm chemical reagent Co., LTD. (Shanghai, China). Folin-Ciocalteu was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). Methanol, ethanol was purchased from Xilong Scientific Co., Ltd. (Guangdong, China). Ultrapure water (~18.2 MΩ, 25 ℃) obtained from a Master-S plus UVF ultra-pure water system (Shanghai Fushite environmental protection technology Co., Ltd, China). Glucose (Glu) and phenol and other chemicals and reagents used were analytical grade.

2.3.Drying curve

The moisture contents of fresh and dried samples were analyzed by an oven drying method at 105 °C (AOAC, 2005). The samples were weighed every 30 min until the weight of sample became constant. The initial and final moisture contents of the samples were recorded. The drying rate is expressed as the amount of evaporated moisture over time. It is calculated as

$$\textbf{Drying rate} = \frac{MC_1 - MC_2}{t_1 - t_2} \quad (1)$$

where MC_1 and MC_2 are moisture contents of sample (g water/g dry matter) at time t_1 and t_2 (h), respectively (Akpinar and Toraman, 2016).

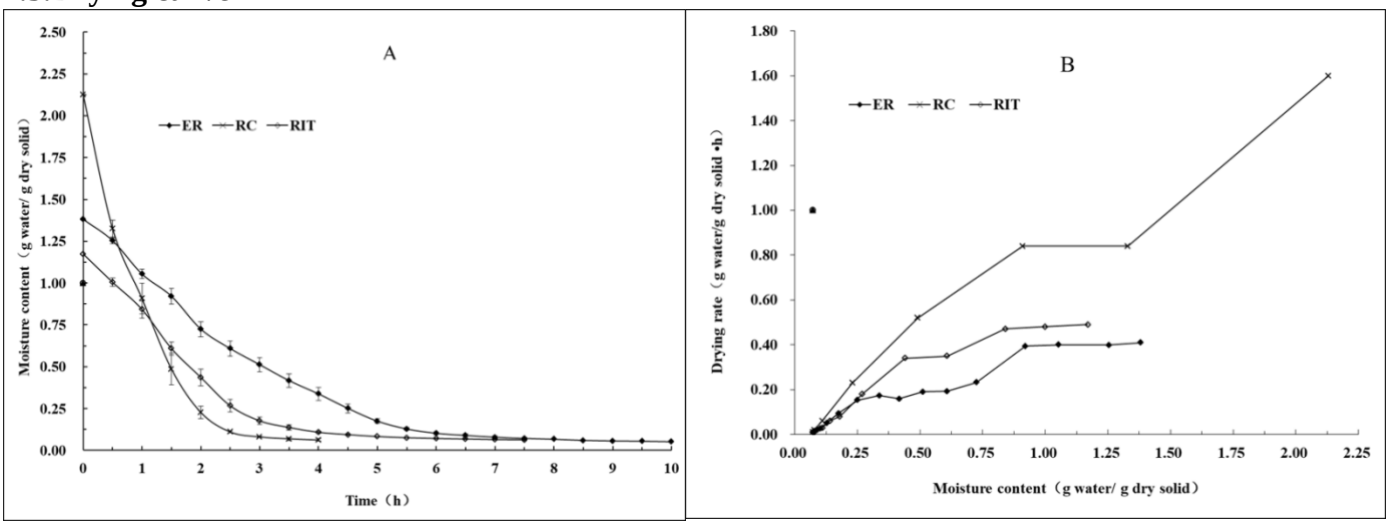


Figure 2. Drying curves (A) and drying rate (B) of ER, RC and RIT of hairy fig root dried by hot air at 80 ℃. ER, entire root; RC, root cortex; RIT, root internal tissue.

2.4.Chemical composition analysis

Chemical compositions including ash (AOAC 938.08), protein (AOAC 2001.11) and fat (AOAC 920.39) were determined according to the methods recommended by AOAC (2005). The soluble sugar was determined by phenol-sulfuric acid method with glucose as a standard (Rover, et al., 2014).

2.5.Extraction for phenolic, flavonoid, psoralen, bergapten analysis

2 g hairy fig powder was extracted three times by 60% ethanol (*V/V*) with ultrasonic at 40 KHz (SB5200DTS, Ningbo Scientz Biotechnology Co., Ltd., Zhejiang, China) for 0.5 h. The extraction solutions were combined to be filtered and concentrated to 100 mL and stored at -80 ℃ for phenolic, flavonoid, psoralen, bergapten analysis.

2.6.Determination of total phenolic and total flavonoid

The total phenolic concentration (TPC) and total flavonoid concentration (TFC) were determined according to the method reported by Chumroenphat et al. (2021). Briefly, the 60% ethanol extract of hairy fig (0.1 mL) was added Folin–Ciocalteu reagent (0.5 mL) and 10%

Na_2CO_3 (1.5 mL). The TPC of the sample was quantified by constructing standard curves with different concentrations of gallic acid at 760 nm (UV-2600, Shimadzu Co., Kyoto, Japan). TFC determination was performed by adding 5% $NaNO_2$ (0.15 mL) and 10% $AlCl_3$ (0.15 mL) solutions to the 60% ethanol extract (1 mL), and then adding 1 M NaOH (1 mL) for color development after avoiding light for 5 min. TFC in the sample was quantified by making standard curves with different concentrations of quercetin at 506 nm (UV-2600, Shimadzu Co., Tokyo, Japan). psoralen, bergapten analysis. TPCs and TFCs were expressed as equivalents of gallic acid and rutin per kilogram of dry matter, expressed as mg GAE/g and mg RE/g, respectively.

2.7. Determination of psoralen and bergapten

Psoralen and bergapten content were determined by high performance liquid chromatography (HPLC), discribed by Yang et al. (2010) with small modification, an Agilent 1200 liquid chromatography system (Agilent, USA) equipped with a quaternary solvent delivery system, an autosampler and a column compartment was used for all analysis. The chromatographic separation was performed on an Agilent Zorbax Eclipse XDB-C18 column (250 mm × 4.6 mm, 5 μm), and the column temperature was kept at 35℃. The mobile phase consisted of ultrapure water and acetonitrile (65:35,V/V) with a flow rate of 1.0 mL/min, Psoralen and bergapten were monitored at 245 nm.

The appropriate amounts of psoralen and bergapten were separately weighed and dissolved in methanol to make the stock solution containing 0.80 mg/ml of psoralen and bergapten. A series of working solutions of these analytes were freshly prepared by diluting mixed standard solution with methanol at appropriate ratios to yield concentrations of 3.20, 16.00, 80.00 and 400.00 ug/mL. Hairy fig extract was filltered with 0.45 μm millipore filter. The psoralen and bergapten composition were calculated according to the standard curve.

2.8. Determination of mineral elements

Mineral elements were determined according to the method described by Zhang et al. (2022). Briefly, 0.25 g sample powder was mixed with 6.0 mL nitric acid. Then the mixture was predigested for 1 h. After that, 2 mL hydrogen peroxide were added to digest thoroughly in the Anton Paar multiwave pro microwave digestion instrument (Graz, Austria). Then mineral elements were determined by Perkin Elmer Nexion 300X Inductively coupled plasma mass spectrometry (ICP-MS) (Waltham, MA, USA). The content of each element was calculated with reference of standard curve.

2.9.Determination of free amino acid

The free amino acid were profiled using LC-MS with slight modification of Zhang et al. (2022). Briefly, 0.2 g sample powder was ground with liquid nitrogen and 3 mL 50% acetonitrile contained 0.1% hydrochloric acid (V/V), and stored at 4 ℃ for 30 min after vortexed. Then the extracts were centrifuged at 13800×g for 10 min at 4 ℃. The supernatant was extracted 3 times and filtered through a 0.22 μm filter before analysis. The LC-MS system consisted of an high-performance liquid chromatography (Ultimate3000, Thermo Fisher, USA) system with a high resolution mass spectrometer (Exactive Plus, Thermo Fisher, USA). Chromatographic separation was achieved using an Poroshell SB-Aq column (3×150mm, 2.7 μm; Agilent), kept at 30 ℃ with 0.3 mL/min flow rate. The mobile solutions were water with 0.1% formic acid (A) and acetonitrile (B). The following gradient was employed: 0-2 min, 0-2% B; 2-5 min, 2-8% B; 5-5.5 min, 8-80% B; 5.5-8 min, 80% B, the initial conditions were maintained for 2 min to equilibrate the column. The injection volume was 2 μL. MS detection was performed in the positive mode with Full MS/AIF mode. The ion spray voltage was set to 3,500 V, and the source temperature was set to 320 ℃. The nebulizer and heater gases were maintained at 55 psi. The content of each amino acid per gram of sample in dry weight was calculated by establishing a standard curve of the standard substance.

2.10. Analysis of volatile components by headspace solid-phase microextraction–gas chromatography–mass spectrometry (HS-SPME-GC-MS)

1.0 g of hairy fig powder was taken into an extraction bottle and added 10 mL distilled water. The sample vials were preheated using the solid-phase microextraction unit at 100 ℃ for 20 min, and then solid-phase microextraction (SPME) fibers (50/30 μm PDMS/CAR/DVB; Supelco Co., PA, USA) were inserted into the headspace of the sample and extracted for 30 min. Volatile components were qualitatively determined using a 7890B-5977 GC–MS system equipped with a HP-5MS column (0.25μm, 30.0 m × 250 μm; Agilent Co., CA, USA). The initial column temperature was 40 ℃ (maintained for 3 min), followed by an increase up to 120 ℃ at a rate of 5℃/min, and then again hiked up to 280 ℃ at 10 ℃/min, then it maintained for 5 min). The injector temperature was 250 ℃, and the carrier gas was He with a flow rate of 1.0 mL/min. The mass spectrometer was set to electron ionization (EI) mode, with an ion source temperature of 230 ℃, quadrupole temperature of 250 ℃, and scanning quality range of 45-400 m/z. The volatile compounds were identified by mass spectra from NIST 17.0 database (Liu, et al., 2022).

2.11.In vitro antioxidant assays

Radical scavenging activity (ABTS assay)

The radical scavenging activity of ABTS radical of the 60% ethanol extracts of ER, RC and RIT was assessed as described by Euch et al. (2015). The radical solution consisted of a mixture of equal volumes of 2.45 mM of potassium persulphate and 7 mM ABTS, kept in the dark for 12-16 h at room temperature and further diluted with ethanol in a ratio 1:70 to obtain a 0.700 ± 0.003 absorbance at 734 nm.

A mixture of 0.1 mL of diluted sample extracts and 900 mL of ABTS solution was incubated in the dark for 6 min. The absorbance was then measured at 734 nm, and the ascorbic acid (Vc) as a positive control. The ABTS radical scavenging activity of the extract was expressed as the inhibition concentration (IC_{50}) i.e., the concentration of the extract required to decrease the initial ABTS radical concentration by 50% (IC_{50}) under the specified experimental condition. The ability to scavenge the ABTS radical was evaluated according to the following formula:

$$\text{ABTS radical scavenging effect (\%)} = \left[\mathbf{1} - \frac{\boldsymbol{A}}{\boldsymbol{A_0}}\right] \times 100 \quad (2)$$

Where: $\boldsymbol{A_0}$: control absorbance; A: sample absorbance.

2.11.Radical scavenging activity (DPPH assay)

DPPH assay was conducted according to Oke-Altuntas et al. (2017). Serial dilutions of each 60% ethanol extracts of ER, RC and RIT were prepared. A diluted sample (100 mL) and 900 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in ethanol solution (5×10^(-5)M) were mixed. After 30 min of incubation in the dark, the absorbance at 520 nm was measured, Vc as a positive control. The antiradical activity was expressed as (IC_{50}). The ability to scavenge the DPPH radical was calculated as follows:

$$\text{Radical scavenging effect (\%)} = \left[\mathbf{1} - \frac{\boldsymbol{A}}{\boldsymbol{A_0}}\right] \times 100 \quad (3)$$

Where: A_0: control absorbance; A: sample absorbance.

2.12.Hydroxyl radical scavenging activity assay

The hydroxyl radical scavenging activity of 60% ethanol extracts of ER, RC and RIT was assayed according to zhang et al. (2012), and Vc is used as a positive control. The reaction mixture, containing 1 mL of 60% ethanolic extracts, was incubated with 0.3 mL of $FeSO_4$ (8 mM), 1 mL of salicylic acid (3 mM) and 0.25 mL of H_2O_2 (20 mM) at 37 ℃ for 30 min. The reaction was cooled to room temperature by keeping in the water. Then 0.45 mL distilled water was added into the mixture to make the end volume 3.0 mL, then centrifuged at 3000×g

for 10 min. The absorbance of the supernatant was measured at 510 nm, and 1 mL of solvent solution was used instead of 60% ethanol extracts solution as a control. The extract activity against hydroxyl radicals was expressed as (IC_{50}), the concentration of the compound required to decrease the initial hydroxyl radical concentration by 50%. Hydroxyl radical scavenging activity was calculated as follows：

Hydroxyl radical scavenging effect (%) = $\left[\mathbf{1}-\frac{\boldsymbol{A}}{\boldsymbol{A_0}}\right]$*100. (4)

Where: A_0: Control absorbance; A: sample absorbance

2.13.Fourier-transform infrared (FT-IR) spectroscopy

Dried samples powder was mixed with KBr powder evenly and pressed into a pellet and measured on FT-IR spectroscopy (IS50, Thermo Fisher Scientific, MA, USA) in the wave number range from 400 cm-1 to 4000 cm^{-1}.

2.14.Statistical analysis

All obtained data were expressed as mean ± standard deviation and analyzed by one-way analysis of variance (ANOVA). Microsoft Office 2007 and SPSS 17.0 software were used for drawing and data calculation. Principal component analysis and partial least squares-discriminate analysis (PLS-DA) using Omics share 6.4.5 online analysis (Genedenovo Co.ltd, Guangzhou, China). Differences at $P<0.05$ were taken as statistically significant.

3.Results and Disscusion

3.1.Drying characteristics

The entire root were separated into root cortex and root internal tissue (Figure 1). The initial moisture content of ER, RC and RIT was 56.22%, 66.02% and 51.10% w.b., respectively, while the final moisture content of the dried products was in the range of 6.21-7.64 % w.b are presented in Table 1. Drying curves and drying rate of ER, RC and RIT are presented in Figure 2. ER, RC and RIT were needed 10, 4, and 7.5 hours, respectively, to reach a certain moisture content (Table 1). From Figure 1B, RC had higher drying rate, followed by RIT and ER. The falling drying rate period occurred through the whole drying process except for at the beginning 0.5 h of ER and RIT and 0.5-1.0 h of RC, in which the constant rate period was also observed when moisture content between 1.00-1.50 g water/g dry solid. The results indi cated that in hairy fig root drying process, the surface water was evaporated faster than the diffusion of water inside the sample to the surface and there was no saturated water surrounding the sample surface. Therefore, diffusion is the mechanism describing the removal of water in this process (Akpinar and Toraman, 2016).

Table 1. Drying time, moisture content of the fresh and dried Hairy fig root

Samples	Drying time (h)	Moisture content (% w.b.)	
		Fresh	Dried
ER	10	56.22 ± 0.69^{b}	7.64 ± 0.07^{c}
RC	4	66.02 ± 0.25^{c}	6.21 ± 0.06^{a}
RIT	7.5	51.10 ± 0.14^{a}	7.27 ± 0.01^{b}

* ER, entire root; RC, root cortex; RIT, root internal tissue; w.b., wet base.

3.2.Chemical composition

Chemical contents such as fat, protein, ash, total polyphenols (TPC) and total flavonoids (TFC) in RC was higher than in RIT and ER, yet, the total soluble sugar was higher in RIT (4.48%). As results shown in Table 2, ER, RC and RIT of hairy fig root have significant differences ($p<0.05$) in fat, protein, ash, total polyphenols and total flavonoids content. The content of fat, protein, ash, TPC and TFC in RC

was 8.48%, 9.27%, 8.39%, 3.42 mg/g and 3.16 mg/g, respectively, and it was 8.43%, 4.70%, 1.63%, 1.56 mg/g and 0.78 mg/g in RIT. The lowest content of fat and total soluble sugar in ER might be due to the physical or chemical reactions which occurred as a result of the long drying time.

Table 2. Chemical composition in hairy fig root

Parameter	**ER**	**RC**	**RIT**
Fat（%）	5.73±0.21^{a}	8.48±0.39^{c}	8.43±0.11^{b}
Protein (%)	5.88±0.13^{b}	9.27±0.22^{c}	4.70±0.08^{a}
total soluble sugar(mg/g)	3.98±0.13^{a}	4.12±0.12^{b}	4.48±0.15^{c}
Ash (%)	3.55±0.05^{b}	8.39±0.11^{c}	1.63±0.04^{a}
TPC (mg GAE/g)	2.61±0.20^{b}	3.42±0.10^{c}	1.56±0.03^{a}
TFC（mg RE/g）	1.89±0.01^{b}	3.16±0.02^{c}	0.78±0.01^{a}
Psoralen	0.70±0.00^{b}	0.77±0.00^{c}	0.32±0.00^{a}
Bergapten	0.27±0.00^{b}	0.42±0.00^{c}	0.14±0.00^{a}

*ER, entire root; RC, root cortex; RIT, root internal tissue. Different letters in the right superscript of the same line showed significant differences (P< 0.05).

Table 3. Mineral elements in hairy fig root

Mineral Elements	**Content (mg/kg)**		
	ER	**RC**	**RIT**
Macro-minerals			
Phosphorus	1325.82±82.22^{b}	2490.23±164.99^{c}	1050.91±71.31^{a}
Sodium	24.18±1.51^{a}	46.88±3.73^{b}	23.16±2.27^{a}
Potassium	4303.08±120.97^{b}	8179.86±157.58^{c}	3127.37±192.88^{a}
Magnesium	1139.88±69.39^{b}	2403.03±211.14^{c}	714.46±8.71^{a}
Calcium	5099.40±83.60^{b}	11456.80±346.95^{c}	2910.42±117.61^{a}
Micro-minerals			
Copper	2.63±0.04^{b}	4.74±0.14^{c}	1.89±0.06^{a}
Zinc	81.46±1.44^{c}	76.57±1.62^{b}	64.63±3.02^{a}
Manganese	478.67±4.69^{b}	1063.67±69.50^{c}	255.66±30.49^{a}
Iron	206.98±8.09^{b}	655.60±18.51^{c}	50.07±2.02^{a}
Other-minerals			
Nickel	2.26±0.03^{c}	1.94±0.06^{a}	2.16±0.02^{b}
Aluminum	1570.60±60.92^{c}	563.68±10.41^{b}	701.86±31.04^{a}
Rubidium	26.02±1.08^{b}	42.61±0.89^{c}	17.42±1.17^{a}
Tin	0.02±0.00^{b}	0.04±0.00^{c}	0.02±0.00^{a}
Boron	6.05±0.21^{b}	12.47±0.85^{c}	3.43±0.22^{a}

* Different letters in right superscript of the same line showed significant differences (P<0.05). ER, entire root; RC, root cortex; RIT, root internal tissue.

The content of other chemical composition were protein, ash, TPC and TFC between RC and RIT. The results showed that primary and secondary metabolites were accumulated more in RC, which was similar to the results reported by Aielloa et al. (2015).

Psoralen and bergapten were common plant secondary metabolites in Lemon juice (Jungen,

et al., 2023), celery (Beier, et al., 1983) and *Psoralea corylifolia* L (Guo, et al., 2005). They were regarded as marker compounds in hairy fig (Liu, et al., 2004; Wei,et al., 2005). Content of psoralen in ER, RC and RIT was 0.70, 0.77 and 0.32 mg/g, respectively, and content of bergapten was 0.27, 0.42, 0.14 mg/g. This result was consistent with Aielloa et al. (2015) whose conclusion that the root cortex as the main accumulation site for marker compounds. As we known, psoralen and bergapten were belong to furanocoumarins. It was reported that coumarins in primary root of A. *dahurica* were concentrated in the periderm, cortex, and phloem, whereas they were concentrated in the phloem in lateral roots (Gao and Li, 2023), which provided guidance for further analysis of the accumulation of psoralen, bergapten and other active ingredients in hairy fig root.

3.3.Analysis of Mineral Elements

Fourteen mineral elements were detected in ER, RC and RIT (Table 3). All minerals had significant difference between ER, RC and RIT ($P<0.05$), except for sodium in ER and RIT ($p>0.05$). Calcium and potassium were two main macro-minerals in hairy fig root. Calcium was the highest mineral in ER and RC, whose content was 5099.40 and 11456.80 mg/kg. In RIT potassium content was the highest for 3127.37 mg/kg. Manganese was the highest micro-minerals which was 478.67, 1063.67 and 255.66 mg/kg in ER, RC and RIT, followed by iron, zinc and copper. As other-minerals, Aluminum was the highest mineral, the next was rubidium, boron, nickel and tin. Compared to the amount of elements in the roots of sweet potato, the content of calcium, magnesium, phosphorus were higher in hairy fig root (Md Mokter, et al., 2022). Content of potassium in RIT was similar to sweet potato root reported by Senthilkumar et al. (2020) and Sanoussi et al. (2016). From Table 3, the results showed that hairy fig root had abundant mineral elements, especially in RC. Moreover, this could explain why ash content in RC was the highest.

3.4.Analysis of amino acid composition

Different levels of ten free amino acids were detected in the test-parts of hairy fig root (Table 4). Total free amino acids in ER, RC and RIT were 2219.48, 2656.81 and 2052.72 mg/Kg, respectively, with significant differences ($P<0.05$). Only five essential amino acids (histidine, threonine, valine, methionine and phenylalanine) and five nonessential amino acids (aspartic acid, glutamic acid, arginine, glycine and serine) were detected. The content of methionine was 0.27 and 5.05 mg/Kg in ER and RC, respectively, but it was not detected in RIT. The content of arginine was 2013.51(ER), 2405.76 (RC) and 1853.13 mg/Kg (RIT), which was the highest among all detected free amino acids. Besides arginine, the main free amino acids in ER were aspartic acid (110.59 mg/Kg) and phenylalanine (24.27 mg/Kg). Aspartic acid (81.60 mg/Kg), valine (50.44 mg/Kg), and phenylalanine (44.55 mg/Kg) were three main amino acids in RC. The content of aspartic acid (122.17 mg/Kg) and valine (19.35 mg/Kg) in RIT was also noticeable. So, from Table 4, we could see that amino acid content was lower in hairy fig root, and fewer amino acid varieties were detected. Yang et al. (2019) reported that sixteen amino acids were detected in Codonopsis pilosula root, the lowest content was 0.01% for methionine and the highest content was 1.19 % for arginine, which were lower in hairy fig root. But it had similar results that arginine was highest and methionine was lowest in root.

3.5.Analysis of volatile profiles

Table 5 showed the presence of a total of 47 volatile compounds in hairy fig root, including twelve aldehydes, seven alcohols, four ketones, one acids, two esters, thirteen hydrocarbons, and three other compounds. Total relative amount of volatile compounds in entire root, root cortex and root internal tissue were 65.08%, 75.68% and 58.16%. Hydrocarbons were the most abundant volatile compounds, accounting for 32.5% of all volatile compounds. However, the highest relative content of volatile compounds in ER and RIT were aldehydes, which was 34.09%

and 27.69%, respectively, while heterocyclic compounds were the highest relative content of 45.23% in RC. The second largest group of volatiles were heterocyclic compounds for ER (16.20%), aldehydes for RC (16.98%), and alcohols for RIT (13.56%). Moreover, the relative content of six compounds in three samples were consisted of hexanal, nonanal, methyl salicylate, decanal, (E)-2-decenal and ficus in with a significant difference.

Table 4. Amino acid compositions in hairy fig root

Amino Acid	Content (mg/Kg)		
	ER	**RC**	**RIT**
Histidine	16.99±0.01^{b}	13.91±0.01^{a}	18.52±0.00^{c}
Threonine	13.14±0.01^{b}	19.88±0.01^{c}	9.67±0.00^{a}
Valine	13.05±0.05^{a}	50.44±0.03^{c}	19.35±0.06^{b}
Methionine	0.27±0.02^{a}	5.05±0.05^{b}	-
Phenylalanine	24.27±0.28^{b}	44.55±0.02^{c}	12.43±0.00^{a}
Aspartic acid	110.59±0.06^{b}	81.60±0.03^{a}	122.17±0.08^{c}
Glutamic acid	4.42±0.00^{b}	7.45±0.01^{c}	0.45±0.01^{a}
Arginine	2013.51±0.24^{b}	2405.76±6.31^{c}	1853.13±27.60^{a}
Glycine	4.27±0.02^{b}	5.78±0.01^{c}	3.13±0.01^{a}
Serine	19.04±0.01^{b}	22.39±0.01^{c}	13.87±0.01^{a}
Total	2219.48±0.07	2656.81±0.65	2052.72±3.09

* "-" represented not detected, different letters in right superscript of the same line showed significant differences (P< 0.05).ER, entire root; RC, root cortex; RIT, root internal tissue.

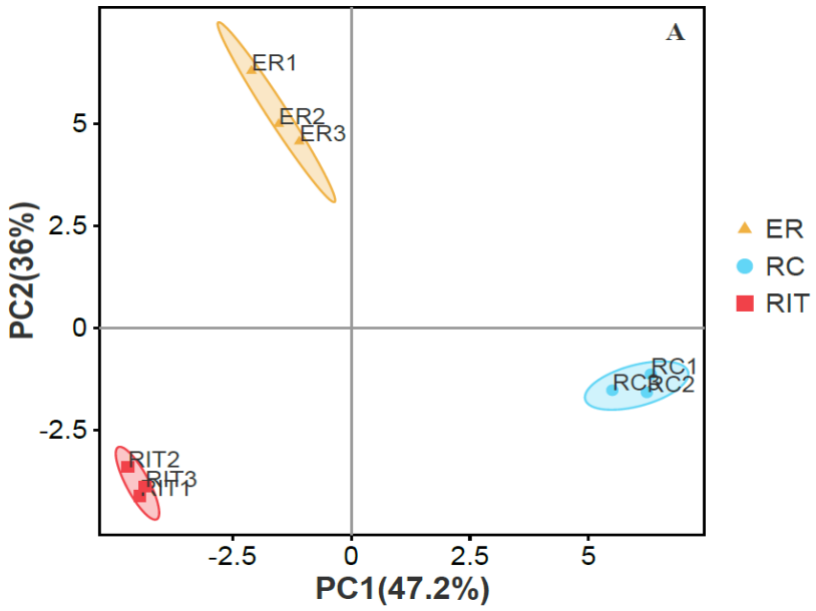
Table 5. Volatile compounds identified from the hairy fig root in GC-MS

NO.	Retention Time(min)	Compound Name	CAS#	Match	Relative Amount (%)		
					ER	**RC**	**RIT**
1	5.88	Hexanal	66-25-1	91	12.27±1.75^{c}	2.81±1.30^{a}	6.46±1.08^{b}
2	8.33	1-Hexanol	111-27-3	83	-	-	10.81±1.52
3	9.26	2-Heptanone	110-43-0	80	-	0.55±0.11	-
4	9.63	Heptanal	111-71-7	86	0.57±0.13	-	-
5	9.91	Oxime-, methoxy-phenyl-	222-86-6	86	2.43±0.56^{b}	2.02±0.33^{b}	1.41±0.21^{a}
6	10.36	Phenol, 2-ethyl-	90-00-6	80	-	-	0.30±0.03
7	12.06	Benzaldehyde	100-52-7	95	5.20±0.67^{a}	5.74±0.67^{a}	2.93±0.05^{a}
8	12.72	1-Heptanol	111-70-6	83	-	-	1.03±0.23
9	13.67	Furan, 2-pentyl-	3777-69-3	91	1.84±0.48^{a}	2.15±0.45^{a}	1.92±0.32^{a}
10	14.56	2,4-Heptadienal, (E,E)-	4313-03-5	87	1.85±0.15^{a}	2.34±0.08^{b}	1.61±0.26^{a}
11	15.47	1,3-Hexadiene, 3-ethyl-2-methyl-	61142-36-7	91	1.04±0.19^{a}	0.89±0.25^{a}	-
12	15.64	Benzyl alcohol	100-51-6	97	-	0.34±0.03	-

13	15.79	Phenylethyl Alcohol	100-51-6	94	-	1.08±0.09^{b}	0.29±0.07^{a}
14	15.97	3-Octen-2-one	1669-44-9	93	1.36±0.54^{a}	-	1.08±0.06^{a}
15	16.82	2-Octenal, (E)-	2548-87-0	91	7.30±1.60^{b}	2.62±0.36^{a}	4.17±0.09^{a}
16	17.51	3,5-Octadien-2-one	38284-27-4	93	-	0.78±0.04^{a}	2.29±0.80^{b}
17	17.67	(5-Ethylcyclopent-1-enyl)methanol	36431-59-1	94	-	-	0.35±0.07
18	19.10	Nonanal	124-19-6	93	2.29±0.13^{c}	1.50±0.24^{a}	1.89±0.36^{b}
19	20.82	trans-3-Nonen-2-one	18402-83-0	81	-	-	0.18±0.03
20	21.16	Benzene, 1,2-dimethoxy-	91-16-7	93	0.43±0.06^{a}	-	0.54±0.05^{b}
21	21.72	2-Nonenal, (E)-	18829-56-6	86	-	-	1.07±0.30
22	22.32	1-Nonanol	143-08-8	91	0.52±0.06^{a}	-	1.08±0.29^{b}
23	22.56	Naphthalene	91-20-3	95	0.76±0.24^{a}	1.60±0.15^{a}	-
24	23.56	Methyl salicylate	119-36-8	97	1.77±0.21^{b}	4.04±0.20^{c}	1.02±0.09^{a}
25	23.91	Decanal	112-31-2	91	0.65±0.04^{c}	0.43±0.02^{a}	0.54±0.05^{b}
26	24.22	2,4-Nonadienal, (E,E)-	5910-87-2	95	1.68±0.16^{b}	1.06±0.08^{a}	1.70±0.17^{b}
27	26.45	2-Decenal, (E)-	3913-81-3	80	1.13±0.18^{b}	0.48±0.07^{a}	1.42±0.07^{c}
28	27.63	Naphthalene, 1-methyl-	90-12-0	91	0.34±0.04^{a}	0.43±0.00^{b}	0.38±0.05^{ab}
29	28.87	2,4-Decadienal, (E,E)-	25152-84-5	81	-	-	5.90±1.07
30	30.82	2(3H)-Furanone, dihydro-5-pentyl-	104-61-0	90	0.69±0.01^{a}	3.46±0.31^{b}	-
31	30.9	2-Dodecenal	4826-62-4	83	1.15±0.45	-	-
32	32.29	Tetradecane	629-59-4	97	0.70±0.08^{a}	0.69±0.04^{a}	0.54±0.12^{a}
33	33.97	Seychellene	20085-93-2	97	0.36±0.05^{a}	0.45±0.08^{b}	-
34	34.36	Pentadecane, 2,6,10-trimethyl-	3892-00-0	81	0.28±0.10	-	-
35	35.08	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethenyl)-, [4aR-(4a.alpha.,7.alpha.,8a.beta.)]-	17066-67-0	99	0.37±0.10	-	-
36	35.4	Heneicosane	629-94-7	86	0.71±0.10^{b}	0.46±0.11^{a}	0.35±0.02^{a}
37	35.52	Pentadecane	629-62-9	95	0.34±0.09^{a}	0.35±0.00^{a}	0.25±0.02^{a}
38	35.89	2,4-Di-tert-butylphenol	96-76-4	97	1.42±0.57^{a}	0.65±0.19^{a}	0.92±0.06^{a}
39	36.69	Hentriacontane	630-04-6	86	0.34±0.16^{a}	-	0.38±0.07^{a}
40	38.26	Hexadecane	544-76-3	97	0.83±0.30^{a}	0.81±0.15^{a}	0.71±0.11^{a}
41	39.68	Aciphyllene	87745-31-1	97	0.41±0.20	-	-

42	40.7	Heptadecane	629-78-7	96	0.27±0.07[a]	-	0.20±0.01[ab]
43	42.19	1-Naphthalenemethanol, 1,4,4a,5,6,7,8,8a-octahydro-2,5,5,8a-tetramethyl-	19078-37-6	83	0.28±0.02	-	-
44	43.48	Ficusin	66-97-7	98	10.55±2.14[b]	35.02±2.54[c]	2.99±0.77[a]
45	44.43	Phthalic acid, isobutyl nonyl ester	1000309-04-4	90	1.63±0.70[a]	-	0.84±0.20[a]
46	46.18	n-Hexadecanoic acid	57-10-3	98	0.15±0.02[a]	0.35±0.08[b]	-
47	47.83	7H-Furo[3,2-g][1]benzopyran-7-one, 4-methoxy-	484-20-8	98	0.69±0.19[a]	2.58±0.20[b]	0.39±0.18[a]
Aldehydes (12)					34.09±1.46	16.98±0.83	27.69±2.61
Alcohols (7)					0.80±0.02	1.42±0.0.08	13.56±1.00
Ketones (4)					1.36±0.08	1.33±0.07	3.55±0.13
Acids (1)					0.15±0.02	0.35±0.08	-
Esters (2)					3.40±0.54	4.04±0.46	1.86±0.22
Hydrocarbons (13)					6.75±0.09	5.68±0.06	2.81±0.08
Heterocyclic compounds (5)					16.20±3.46	45.23±5.23	6.71±1.05
Others (3)					1.85±0.01	0.65±0.19	1.76±0.06
Total					64.60±5.85	75.68±8.12	57.94±3.73

* "-" is not detected, CAS# refer to the number assigned to each chemical substance by the Chemical Abstracts Service (CAS), an organization under the American Chemical Society. The numbers in brackets after aldehydes, esters, acids, ketones, esters and pyrazines indicated the quantity of substances. Different letters in the right superscript of the same line showed significant differences (P< 0.05).



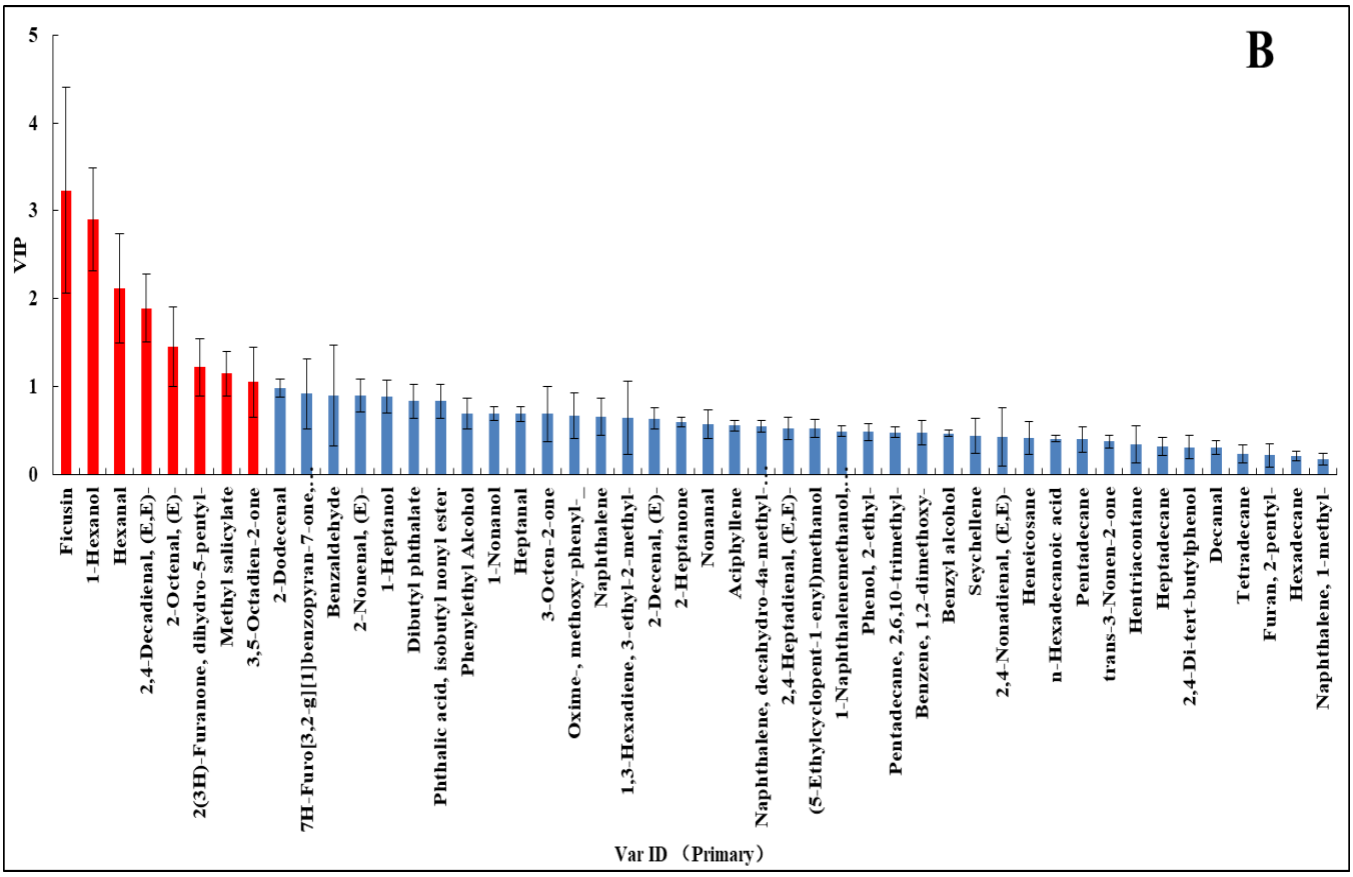


Figure 3. PCA of volatile flavour compounds in different part of hairy fig root(A) and VIP based on PLS-DA (B). The yellow,blue and red ellipse represented the 95% confidence interval in Figure 3A.

The principal component analysis (PCA) of all identified substances showed that the cumulative variance contribution rates of the first two principal components were 47.20% and 36.00%, respectively (Figure 3A). The first and second principal components expressed almost all information of the volatile components in the three samples, they could be used for the comparative analysis of volatile components between different samples. Moreover, the close relative distance between the same group of samples, indicated that the detection repetition of samples was good. The obvious spacing between different groups showed obvious difference in volatile substances among them (Figure 3A). These results pointed that GC-MS could distinguish entire root, root cortex and root internal tissue well by the difference in volatile substances. Figure 3B showed that 8 potential differential metabolites that were screened (VIP≥1, $p< 0.05$) by PLS-DA (Figure 3B), including ficusin, 1-hexanol, hexanal, (E,E)-2,4-Decadienal, (E)-2-Octenal, dihydro-5-pentyl-2(3H)-Furanone, methyl salicylate and 3,5-Octadien-2-one. Hairy fig root has special flavor, it may be related to ficusin, dihydro-5-pentyl-2(3H)-furanone and methyl salicylate, which had high relative amount. The result was similar to Liu et al. (2004) .

3.6.Infrared spectrum analysis

The FT-IR spectra of ER, RC and RIT were shown in Figure 4. The three samples showed 8 characteristic absorption peaks from 4000-400 cm-1. The strong absorption peak at 3372.43 cm-1 was assigned to the O-H vibration of cellulose and hemicellulose (Zhang, et al., 2020). The absorption peak at 2924.77 cm-1 was related to the vibration of C-H from some methylene groups of polysaccharides (Sanoussi,

et al., 2016). The absorption peak around 1736.41 cm-1 and 1630.84 cm^{-1} were indicated to C=O stretching vibration of protein and uronic acid, and the absorption peak at 1383.84 and 1244.28 represented the variable angle vibration of C-H and stretching vibration of C-O, respectively (Cao, et al., 2018). The absorption peak observed at 1026.96 cm^{-1} and 535.69 cm-1 of fingerprint area were associated with the β-glycosidic linkage of polysaccharides (Chen, et al., 2019). On the whole, the FT-IR spectra of hairy fig root were basically the same, which indicated that the organic functional groups of ER, RC and RIT were similar.

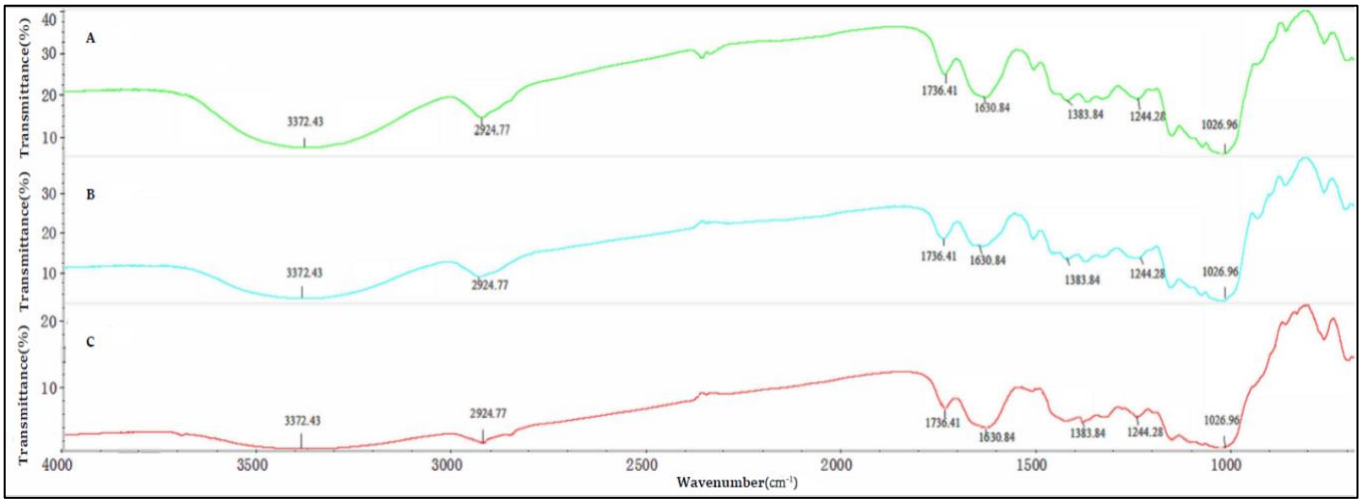


Figure 4. FT-IR spectroscopy of hairy fig root. Entire root (ER), root internal tissue (RIT) and root cortex (RC) were represented byA, B and C.

3.7.In vitro antioxidant analysis

ABTS radical scavenging activity

The radical scavenging ability measured by ABTS assay is given in Table 6 and expressed as IC_{50}. In this experiment, a lower IC_{50} value denotes a higher level of free radical scavenging action. The ABTS radical scavenging rate of ER, RC and RIT were 98.03%, 97.31% and 75.69%, respectively, at 20 mg/mL (Figure 5A). And the corresponding IC_{50} values were 4.25 mg/mL, 3.51 mg/mL and 8.77 mg/mL (Table 6). RC exhibited significantly higher free radical activities than ER and RIT.

Table 6. IC_{50} value of ethanol extract of hairy fig root on ABTS, DPPH and Hydroxyl radical

Parameters		**ER**	**RC**	**RIT**	**Vc***
IC_{50} value	ABTS	4.25 ± 0.07^{b}	3.51 ± 0.06^{a}	8.77 ± 0.35^{c}	12.03±0.01
	DPPH	0.58 ± 0.04^{b}	0.08 ± 0.02^{a}	2.29 ± 0.06^{c}	3.23±0.03
	Hydroxyl	238.47 ± 38.20^{b}	40.80 ± 2.09^{a}	1360.40 ± 380.04^{c}	141.82±1.32

The units of all results was mg/mL except for the data marked with * indicated the unit was μg/mL. Different letters in right superscript of the same row showed significant differences (P<0.05).

3.8.DPPH radical scavenging activity

In DPPH radical scavenging ability, the scavenging rate of ER, RC and RIT at the concentration of 5 mg/mL could reach to 89.57%, 91.82% and 72.60%, respectively (Figure 5B). IC_{50} values of scavenging DPPH

radicals for the ER, RC and RIT were 0.58 mg/mL, 0.08 mg/mL and 2.29 mg/mL, respectively (Table 6) and the free radical scavenging ability were in the following: RC > ER > RIT.

3.9.Hydroxyl radical scavenging activity

In hydroxyl radical scavenging test, the scavenging rate of ER, RC and RIT were the lowest among the three assays. At 50 mg/mL of ER, RC and RIT, the scavenging rate was 28.82%, 64.49 %and 20.47 %, respectively (Figure 5C). And the IC_{50} values of ER, RC and RIT were 238.47 mg/mL, 40.80 mg/mL and 1360.40 mg/mL (Table 6). The radical scavenging activities of different part of hairy fig root followed the order: RC>ER>RIT. This order was in accordance with those of TPC, TFC and bergapten, and the results was in in accordance with previous reports, which was reported that the scavenging capacity was increased by the TPC content (Zhao, et al., 2014; Dong, et al., 2015). Li et al. (2022) found that the DPPH and ABTS radical scavenging capacities were positively correlated with TPC and TFC.

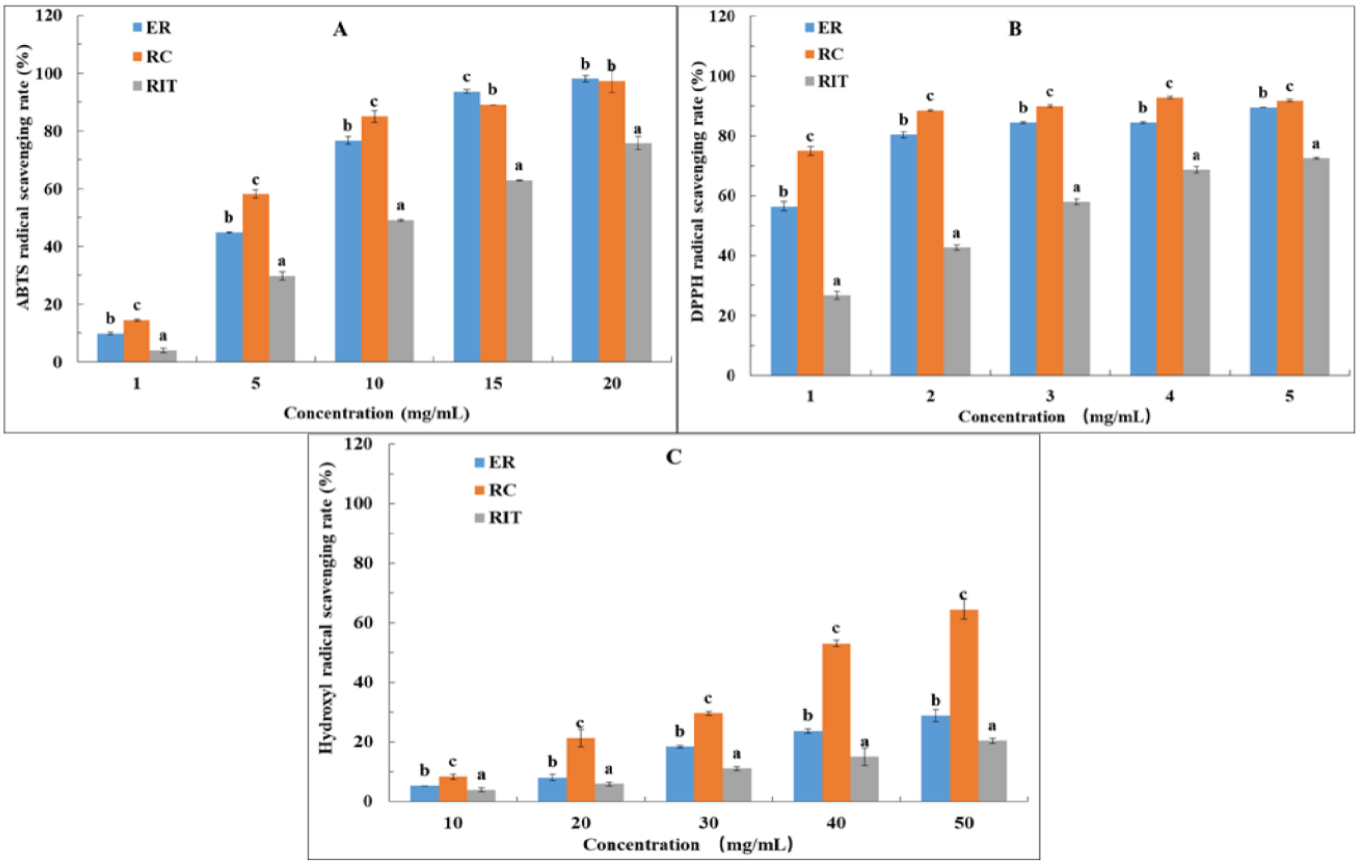


Figure 5. ABTS and DPPH, hydroxyl radical scavenging activities of ER, RC and RIT (A, B, C). The error bar are standard deviations. Different letters on the same concentration of ER, RC and RIT showed significant difference ($P<0.05$), and the significant difference was not analysis among different concentrations of each samples.

Another study emphasize that IC_{50} values of bergapten (23.98 ug/mL) was examined in DPPH assay (S ü zgeç-Selçuk and Dikpınar, 2021), which was indicated that bergapten was contributed to antioxidant activity of hairy fig root. Although, RC had high content of psoralen, but Guo et al. (2005) reported that psoralen had no antioxidant activities at 0.02% and 0.04%

levels. It was indicated that psoralen didn't make contributions to antioxidant activity of hairy fig root. As we known, hydroxyl radical model was suitable to evaluate hydrophilic antioxidants (Süzgeç-Selçuk and Dikpınar, 2021), the results might be explained that there were more hydrophilic substance in RC. So, the results might indicated that TPC, TFC and bergapten were the main components contributed to antioxidant activity.

4.Conclusions

RC and RIT are two important constituent part of hairy fig root. In this article, dry characteristics, chemical components, nutritional compositions, antioxidant activity and structure of ER, RC and RIT were analyzed. The results showed that the time of ER, RC and RIT dried to constant weight was 10, 4, 7.5 h at 80 ℃, respectively. In the whole drying process, decreasing rate drying was the main drying process with a short time constant rate drying. It was indicated that diffusion is the mechanism describing the removal of water in this process, and when hairy fig root was separate into RC and RIT, it was conducive to water diffusion, improve drying efficiency, and save energy and resources. In this study, the results demonstrated that there were more chemical composition, nutritional composition and flavor composition accumulated in RC, also had better antioxidant activity. There were significant differences among ER, RC and RIT of all components and antioxidant activity ($P<0.05$). However, the result of FT-IR was shown that there were no differences among these three samples, which was indicated no new functional group substances appeared in ER, RC and RIT, namely, the types of substances were similar, but the content was different. Results of this study were similar to that the root cortex was the main accumulation site for secondary metabolites which was reported by Aielloa et al. (2015). So, it can be concluded that RC had better quality and biological activity than RIT.

The results from this study proved that RC was the effective part of hairy fig root. It provided a basis for the study of the metabolism and accumulation mechanism of active ingredients in hairy fig root and also providing a reference for the development and utilization of hairy fig root cortex as a raw material in medicine, functional food or dietary additives. Moreover, it could address to scientific researcher, farmers, whole-salers and phyto-pharmaceutical industries that hairy fig root with a thicker cortex was needed to be plant or for sale.

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Conflicts of Interest:

The authors declare that they have no known competing financial interest orpersonal relationship that could have appeared to influence the work reported in this paper.