Available online at www.sciencedirect.com
ScienceDirect
Chinese Journal of Natural Medicines 2019, **17**(6): 0446–0460
Chinese Journal of Natural Medicines

Vitellaria paradoxa nutshells from seven sub-Saharan countries as potential herbal medicines for treating diabetes based on chemical compositions, HPLC fingerprints and bioactivity evaluation

LI Da[1], XIAO Jian-Qi[1], LIU Wen-Yuan[2], ZHANG Chao-Feng[2], AKIHISA Toshihiro[3], ABE Masahiko[3], MASTERS Eliot-T.[4], ZHAI Wei-Wei[1], FENG Feng[1, 5, 6*], ZHANG Jie[5, 6*]

[1] *Jiangsu Food and Pharmaceutical Science College, Huai'an 223003, China;*
[2] *Department of Pharmaceutical Analysis, China Pharmaceutical University, Nanjing 210009, China;*
[3] *Research Institute for Science and Technology, Tokyo University of Science, Chiba 278-8510, Japan;*
[4] *World Agroforestry Centre (ICRAF), Nelson Marlbolough Institute of Technology (NMIT), Nelson 7010, New Zealand;*
[5] *School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing 211198, China;*
[6] *Key Laboratory of Biomedical Functional Materials, China Pharmaceutical University, Nanjing 211198, China*

Available online 20 June, 2019

[ABSTRACT] The aim of the study was to determine the feasibility of the *Vitellaria paradoxa* nutshell as a new medicinal resource for treating diabetes. A total of forty-one compounds were identified by HPLC-DAD-Q-TOF-MS and phytochemical methods in *V. paradoxa* nutshell methanol extract. Based on HPLC fingerprints, four characteristic constituents were quantified and the origin of twenty-eight *V. paradoxa* nutshells from seven sub-Saharan countries was compared, which were classified into three groups with chemometric method. Twenty-eight samples contained high total phenolic content, and exhibited moderate-higher antioxidant activity and strong *α*-glucosidase inhibitory activity. Furthermore, all fractions and isolated compounds were evaluated for their antioxidant and *α*-glucosidase inhibitory activities, and *α*-glucosidase inhibitory action mechanism of four characteristic constituents including protocatechuic acid, 3, 5, 7-trihydroxycoumarin, (2*R*, 3*R*)-(+)-taxifolin and quercetin was investigated via molecular docking method, which were all stabilized by hydrogen bonds with *α*-glucosidase. The study provided an effective approach to waste utilization of *V. paradoxa* nutshell, which would help to resolve waste environmental pollution and provide a basis for developing potential herbal resource for treating diabetes.

[KEY WORDS] *Vitellaria paradoxa*; HPLC fingerprints; Antioxidant activity; *α*-Glucosidase inhibitory activity; Molecular docking

[CLC Number] R917, R96 **[Document code]** A **[Article ID]** 2095-6975(2019)06-0446-15

Introduction

Recently, there has been increasing attention to the natural extracts as a rich source of bioactive compounds with fewer side-effects [1-2], such as ginkgo biloba extract, green tea extract, licorice extract, stevia extract [3-6], which could be used in treatment of cerebral dysfunction disturbance, hyperlipidemia, diabetes, etc [7-10]. These extracts were obtained from the leaves, roots, rhizomes and the other parts of plants [3-6], however, many researchers generally ignored the development of waste parts like nutshell, which tended to cause the resource waste. The *V. paradoxa* nutshell waste separated from shea kernel during industrial production usually brought environmental pollution. Therefore, we made full pre-literature research on *V. paradoxa* nutshell for resolving environmental pollution and resource utilization for treating diabetes.

The shea tree (*Vitellaria paradoxa* C. F. Gaertner) belongs to Sapotaceae family and is indigenous to the savanna

[Received on] 22-Mar.-2019
[Research funding] This work was supported by the General Program Foundation of Jiangsu Food and Pharmaceutical Science College (No. JSFP·2018005-Z), the Natural Science Foundation of Jiangsu Province (No. BK20170742), and the Youth Science Fund Project of National Natural Science Foundation of China (No. 81703383).
[*]**Corresponding authors]** E-mail: fengfeng@cpu.edu.cn (FENG Feng); cpunmc_zj@163.com (ZHANG Jie)
These authors have no conflict of interest to declare.

belt from 16° W to 34° E longitude and 1° N to 15° N altitude [11]. As a medicinal and edible plant, the fruit of *V. paradoxa* is edible and nutritious, and the leave, root and stem bark have been traditionally used in the treatment of inflammation, diarrhea, helminthes, sunburn, dermatitis and rashes for local people [12-13]. Modern pharmacological studies have shown that *V. paradoxa* extract displayed anti-inflammatory, antioxidant, melanogenesis-inhibitory and anthelmintic activities. Phytochemical investigations have discovered that the presence of flavonoids, phenolic acids, terpenoids and glycosides in *V. paradoxa* [12, 14]. Especially, shea butter extracted from shea kernel was widely used in cosmetics industry as product formulations and effective components [14-15].

Diabetes mellitus, a serious metabolic disease, is closely related to oxidative stress characterized by an excessive increase of oxidizing species like reactive oxygen species (ROS), and α-glucsidase is usually used to reduce the postprandial plasma glucose for patients with type 2 diabetes [16]. Moreover, effective *a*-glucosidase inhibitors from herbal plant are gaining more and more attention due to their high effectivities and low toxicities in recent years. Therefore, evaluation of antioxidant activity is an important part of the screening for potential anti-diabetic resources. The pre-experiment was introduced by thin layer chromatography (TLC) method to determine that many phenolic constituents were found in *V. paradoxa* nutshell methanol extracts, which might be used as natural antidiabetic herbal source.

Taking the above into account, high performance liquid chromatography-diode array detector-quadrupole-time of flight (HPLC-DAD-Q-TOF-MS) and phytochemical methods were applied to characterize the main ingredients in *V. paradoxa* nutshell samples. Four characteristic constituents were quantified and chemometric analysis including similarity analysis (SA), hierarchical cluster analysis (HCA) and principal components analysis (PCA) was applied in comparison of the origin on HPLC fingerprints. Twenty-eight *V. paradoxa* nutshells from seven sub-Saharan countries were determined for their yield percentage, total phenolic content (TPC), antioxidant and α-glucosidase inhibitory activities. In addition, all fractions and isolated compounds were evaluated for their antioxidant and α-glucosidase inhibitory activities, and further research was carried out for molecular docking of four characteristic constituents to investigate α-glucosidase inhibitory action mechanism. Our study would help to resolve *V. paradoxa* nutshell waste environmental pollution problem and provide a potential herbal resource for treating diabetes.

Materials and Methods

Chemicals, reagents, and plant materials

Trolox (6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-car- -boxylicacid), 1, 1-diphenyl-2-picrylhydrazul (DPPH), 2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS), 2, 4, 6-tripyridyls-triazine (TPTZ), α-glucosidase powder, *p*-nitrophenyl-α-D-glucopyranoside (*p*NPG) and arbutin were purchased from Yuanye Bio Technology (Shanghai, China). Water was purified by means of a Millipore Milli Q-Plus system (Millipore, Bedford). Folin-Ciocalteu reagent, ferric chloride ($FeCl_3$), acarbose, protocatechuic acid and quercetin (purity ≥ 98%) were obtained from Aladdin (Shanghai, China). 3, 5, 7-Trihydroxycoumarin and (2*R*, 3*R*)-(+)-taxifolin were isolated from *V. paradoxa* nutshell methanol extract in our laboratory and the purity of the standards were over 98% determined by HPLC method. All other reagents and solvents used were of analytical grade, which were purchased from Nanjing Chemical Reagent Corp (Nanjing, China).

The *V. paradoxa* nutshells were obtained from the nut samples collected from twenty-eight sites of seven sub-Saharan countries in Africa, *i.e.*, Côte d'Ivoire (sample no. **S1**), Ghana (**S2**), Nigeria (**S3**–**S13**), Cameroon (**S14**–**S16**), Chad (**S17**–**S24**), Sudan (**S25**–**S26**), and Uganda (**S27**–**S28**) in 2006 (Table 1 and Fig. 1) by one of the authors (E. T. M.) on behalf of the World Agroforestry Centre [11].

Table 1 Longitude, altitude, and elevation of the collection sites of twenty-eight *V. paradoxa* nutshell samples from seven sub-Saharan countries and their yields and total phenolic content of MeOH extracts (Mean ± SD, *n* = 3)

Country	Sample No.	Longitude	Altitude	Elevat-ion (m)	Yields of MeOH extracts (%)	TPC (mg GAE/g DW)
Cote d'Ivoire	**S1**	W 7° 12′ 58″	N 10° 1′ 35″	453	4.42 ± 0.12	155.18 ± 10.11
Ghana	**S2**	W 0° 29′ 27″	N 6° 47′ 11″	145	4.70 ± 0.07	235.38 ± 0.89
Nigeria	**S3**	E 4° 28′ 48″	N 11° 35′ 3″	280	4.99 ± 0.06	248.88 ± 10.68
	S4	E 5° 18′ 7″	N 10° 58′ 9″	337	4.36 ± 0.03	234.15 ± 1.54
	S5	E 6° 13′ 52″	N 10° 10′ 22″	242	5.82 ± 0.14	209.07 ± 0.42
	S6	E 6° 44′ 43″	N 9° 5′ 50″	173	5.46 ± 0.24	130.45 ± 12.83
	S7	E 6° 54′ 45″	N 8° 35′ 4″	683	4.06 ± 0.19	203.35 ± 13.31
	S8	E 6° 56′ 35″	N 10° 38′ 44″	347	6.61 ± 0.27	149.91 ± 12.12
	S9	E 7° 27′ 9″	N 9° 40′ 53″	365	4.27 ± 0.16	147.24 ± 5.93
	S10	E 8° 58′ 54″	N 8° 38′ 34″	160	5.48 ± 0.35	358.78 ± 12.08
	S11	E 11° 34′ 36″	N 9° 9′ 16″	313	3.93 ± 0.18	176.49 ± 6.54
	S12	E 12° 29′ 6″	N 9° 19′ 15″	245	6.92 ± 0.26	223.27 ± 7.72
	S13	E 12° 52′ 13″	N 9° 33′ 36″	267	3.98 ± 0.04	168.11 ± 12.80

Continued

Country	Sample No.	Longitude	Altitude	Elevat-ion (m)	Yields of MeOH extracts (%)	TPC (mg GAE/g DW)
Cameroon	**S14**	E 10° 28′ 49″	N 5° 12′ 55″	1421	4.61 ± 0.15	177.92 ± 0.77
	S15	E 11° 2′ 56″	N 5° 50′ 12″	988	4.36 ± 0.07	156.63 ± 1.50
	S16	E 13° 15′ 42″	N 9° 3′ 56″	418	5.48 ± 0.31	118.53 ± 12.74
Chad	**S17**	E 14° 19′ 34″	N 7° 14′ 57″	974	3.84 ± 0.22	140.62 ± 13.38
	S18	E 15° 29′ 30″	N 9° 36′ 30″	314	5.01 ± 0.08	206.72 ± 7.06
	S19	E 15° 38′ 17″	N 9° 35′ 54″	363	3.14 ± 0.11	181.45 ± 0.71
	S20	E 16° 9′ 17″	N 9° 19′ 41″	400	6.48 ± 0.14	205.88 ± 0.59
	S21	E 16° 21′ 12″	N 8° 31′ 25″	468	5.70 ± 0.04	159.99 ± 1.54
	S22	E 17° 4′ 44″	N 9° 22′ 45″	366	3.10 ± 0.08	111.79 ± 1.74
	S23	E 17° 26′ 45″	N 8° 38′ 16″	390	5.59 ± 0.12	246.54 ± 2.34
	S24	E 18° 2′ 19″	N 9° 1′ 12″	387	5.72± 0.16	149.71 ± 1.96
Sudan	**S25**	E 28° 26′ 55″	N 9° 3′ 56″	418	6.07 ± 0.25	167.22 ± 3.57
	S26	E 30° 31′ 27″	N 7° 17′ 19″	473	5.76 ± 0.19	252.67 ± 3.08
Uganda	**S27**	E 33° 11′ 58″	N 2° 27′ 10″	1207	5.20 ± 0.05	214.48 ± 1.42
	S28	E 33° 40′ 59″	N 2° 21′ 0″	1203	6.20 ± 0.17	191.75 ± 0.61
Mean					5.04	190.08

Fig. 1 Map of elevation in Africa showing the sites (•) of the twenty-eight *V. paradoxa* nutshells which extends from 7°W to 34°E longitude and 2°N to 10°N altitude

Sample preparation

Each accurately weighed powder of *V. paradoxa* nutshell (1.0 g), which had been passed through a 40 mesh screen, was refluxed (60 °C) for 3 h in 10 mL of 100% methanol (MeOH). The extract was then centrifuged at 15 000 r·min^{-1} for 5 min with a HR/T16M centrifuge (Herexi Instrument & Equipment Co., Ltd., Hunan, China). The supernatant solution was evaporated with a rotary vacuum evaporator under reduced pressure at 50 °C and the yield of the extract was determined gravimetrically by dividing the extracted material by the dried weight of *V. paradoxa* nutshell. The sample solution was filtered through 0.45 μm membrane before injected for HPLC analysis.

HPLC-UV and HPLC-DAD-Q-TOF-MS analysis

HPLC analysis was carried out using a L-2000 HPLC instrument (Hitachi, Tokyo, Japan) equipped with a ultraviolet (UV) detector, a quaternary pump, loop injection system, and a column oven. Chromatographic separation was performed on a reversed-phase column (CAPCELL PAK C_{18}, 4.6 mm × 150 mm, 5 μm). To achieve a better chromatographic separation of the chromatograms of twenty-eight *V. paradoxa* nutshell samples, various HPLC parameters were optimized. We compared different mobile phase (MeOH–H_2O and MeCN–H_2O) with different additive (HCOOH and CH_3COOH) in different concentrations (0.02%, 0.05%, 0.1% and 0.2%). Besides, different wavelength (220, 228, 236, 244, 252, 260, 268, 272, 280, 320 and 360 nm), column temperature (30, 35 and 40 °C) and flow rate (0.8, 0.9 and 1.0 mL·min^{-1}) were also compared.

Qualitative analysis in the *V. paradoxa* nutshell extract was performed by means of high-performance liquid chromatography with quadrupole time of flight mass spectrometry (HPLC-Q-TOF-MS) on an Agilent 6520 Q-TOF mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) with a diode array detector (DAD) and electrospray interface (ESI).

Major operating parameters were as follows: negative ion mode, nebulizer pressure of 40 psi, scan spectra from *m/z* 50 to 1000, drying gas temperature of 325 °C, drying gas (N_2) flow rate 8.0 L·min^{-1}, skimmer of 65 V, fragmentor voltage of 100 V, capillary voltage of 3500 V and collision energy 25 eV. Data were processed by Agilent Mass Hunter Workstation Data Acquisition Software Version B.04.00 (Agilent Technologies, Santa Clara, CA).

Validation of methodology

The intraday and interday precisions test were determined by analyzing six injections of the same sample solution on the same day and on three consecutive days, respectively. The repeatability was assessed by analysing six independently prepared sample solutions, respectively. The sample stability test was appraised by examining a single sample solution at room temperature for 0, 2, 4, 8, 12 and 24 h, respectively.

Reference standards were accurately weighed and dissolved separately in MeOH for reservation. And then they were diluted to obtain a series of mixed standard stock solutions with different concentrations. The assay linearity was determined by analyzing six appropriate concentrations of the mixed standard solutions. The standard curves were obtained by plotting peak area against nominal concentration of these four compounds. The limits of detection (LOD) and quantitation (LOQ) of each compound were determined at S/N (signal-noise ratio) = 3 and 10, respectively. The recovery test was conducted to assess the accuracy, spiking known amounts of mixed standard solution (about 50% of the content) with known concentration of *V. paradoxa* nutshell sample, and the mean recoveries were calculated for six replicate determinations. All sample solutions stored at room temperature during this period.

Determination of total phenolic content

The TPC of twenty-eight *V. paradoxa* nutshell samples was determined by the Folin-Ciocalteau method with slight modifications [17]. 100 μL sample solution was put into flask and then 1 mL Foline Ciocalteau's reagent was mixed. After 5 min, 2 mL (29%, *W*/*V*) sodium carbonate was added. After 1 h of incubation, the absorbance was measured (725 nm) at room temperature. The TPC of each sample was expressed as mg of gallic acid equivalents per g dry-extract weight (mg GAEs/g DW).

Measurement of antioxidant capacity

The antioxidant activity of samples was measured using different chemical-based assays including free radicals-scavenging activity (DPPH and ABTS assays) and ferric reducing antioxidant power (FRAP assay). For DPPH assay, result was expressed as IC_{50} values estimated by a nonlinear regression algorithm and for ABTS and FRAP assays, the antioxidant capacity was expressed as mmol of trolox equivalents (TEs) per gram (mmol TEs/g).

DPPH assay

DPPH assay was performed according to previous report with slight modifications [18]. Briefly, a 50 μL aliquot of the samples was added to 3 mL of 60 μmol·L^{-1} DPPH (dissolved in MeOH), and mixed solution was incubated for 30 min in the dark at room temperature and measured at 517 nm.

ABTS assay

ABTS assay was conducted according to previous report with slight modifications [19]. Briefly, ABTS radical cation ($ABTS^+$) solution was prepared by reacting 2.45 mmol·L^{-1} potassium persulfate with ABTS stock solution in the dark at room temperature for 14 h, and then the absorbance of the resulting $ABTS^+$ solution was adjusted to 0.70 ± 0.02 at 734 nm diluted with ethanol. 10 μL of samples or trolox standard solution was added to 200 μL of diluted $ABTS^+$ solution and the absorbance was recorded at 734 nm after 30 min incubation in the dark at room temperature.

FRAP assay

The FRAP assay was performed according to previous report with slight modifications [19]. Briefly, the FRAP working solution was prepared from sodium acetate buffer solution (300 mmol·L^{-1}), TPTZ solution (10 mmol·L^{-1}) and $FeCl_3$ solution (20 mmol·L^{-1}) at a ratio of 10 : 1 : 1, respectively, and kept in the dark at room temperature before use. 5 μL of samples or trolox standard solution was added to 180 μL of FRAP reagent and kept for 30 min in the dark at room temperature and the absorbance was recorded at 593 nm after 30 min incubation in the dark at room temperature.

Determination of α-glucosidase inhibitory activity

The *α*-glucosidase inhibitory activity of samples was determined according to previous report with slight modifications [20]. Briefly, 20 μL of samples, 50 μL of 0.1 mol·L^{-1} phosphate buffer (pH 6.9) and 10 μL the enzyme solution (1 U·mL^{-1}) were plated into 96-well plates and incubated at 37 °C for 20 min. Then, 20 μL *p*NPG solution (5 mmol·L^{-1}) was added. After incubation at 37 °C for 30 min, the absorbance was recorded at 405 nm. Result was expressed as IC_{50} values estimated by a nonlinear regression algorithm.

Molecular docking

To explore the probable binding of four characteristic compounds (protocatechuic acid, 3, 5, 7-trihydroxycoumarin, (2*R*, 3*R*)-(+)-taxifolin and quercetin) with *α*-glucosidase, docking study was carried out. Homology modeling of *α*-glucosidase was conducted by using the crystal structure of *S. cerevisiae* isomaltase (PDB code: 3A4A) as the template [21], which was downloaded from the Protein Data Bank (PDB) website (http: // www.rcsb.org). Result was expressed in terms of free energy (kcal/mol) of ligand-protein binding.

Statistical analysis

All results were expressed as mean ± SD (n = 3). The relative retention time (RRT) and relative peak area (RPA) were used as a normalised data for fingerprint analysis, and the similarity analysis was performed using the software "Similarity Evaluation System for chromatographic fingerprint of TCMs" (Version 2004 A, Chinese Pharmacopoeia Committee). The statistical analyses for hierarchical cluster analysis and principal component analysis were performed

with SPSS software (SPSS 19.0, SPSS, Chicago, IL, USA). Significant differences ($P < 0.05$) between means were determined by a one-way analysis of variance (one-way ANOVA) and correlations between variables were computed using Pearson's correlation values. The molecular docking method was performed using the AutoDock (4.2) tools.

Results and Discussion

Optimization of chromatographic conditions and method validation

In order to achieve satisfactory separations among the chromatographic peaks of test solutions, the optimized HPLC parameters were as follows: the mobile phase consisted of MeCN (A) and H_2O (with 0.2% CH_3COOH) (B) using a gradient elution program (0–10 min, 10%–23% A; 10–17 min, 23%–25% A; 17–30 min, 25%–35% A). In addition, the flow rate was 1.0 mL·min^{-1} and the injection volume was 5 μL. The column temperature was maintained at 30 °C and the detection of peaks was recorded at 252 nm.

According to the operation method, precision, repeatability and stability of *V. paradoxa* nutshell samples were evaluated by calculating the RSD of retention time (t_R) and of the common peak areas (PAs). The RSD of t_R and PAs of precisions varied from 0.07% to 0.87% and 0.64% to 2.09%, respectively, whereas RSD of the repeatability test was within the range 0.07%–0.78% (t_R) and 0.60%–2.48% (PAs), respectively. What's more, stability test yielded RSD between 0.10% and 0.66% (t_R) and 0.58% and 2.42% (PAs), respectively. All results suggested that the HPLC method for fingerprint analysis of *V. paradoxa* nutshell was valid and applicable.

The proposed HPLC method was also fully validated of the four characteristic compounds in this study (Table 1 in Supplementary material). The RSD values of intra- and inter-day precision, repeatability and stability of the four compounds were all lower than 3%, and the calibration curves of these four compounds demonstrated good linearity ($r^2 \geq 0.999$ 5) within the test ranges. The LODs and LOQs of were in the range of 0.06–1.56 and 0.24–3.91 μg·mL^{-1}, respectively, which indicated the high detection sensitivity of the analysis method. All the mean recoveries were between 96.22% and 102.75% with RSD less than 3%. These results revealed that the developed method was validated and satisfactory for quantitative analysis of *V. paradoxa* nutshell samples.

Identification of chemical constituents

Identification of constituents by HPLC-DAD-Q-TOF-MS

The negative and positive ion modes were both adopted for MS analysis. Our results indicated that chemical constituents of *V. paradoxa* nutshell showed more obvious response in the negative mode than positive mode. Therefore, the negative ion mode was finally adopted, and the typical total ion chromatogram (TIC) was illustrated in Fig. 2A.

A total of twenty-eight constituents were identified by comparing t_R, $[M - H]^-$ ions and fragmentations with literature [22-36]. These compounds included fourteen flavonoids (**10**, **13**, **15**–**19**, **22**, **23**, **25**, **26**, **28**, **29**, **33**), nine phenolic acids (**4**–**9**, **11**, **12**, **21**), two lignins (**27**, **30**) and three organic acids (**1**–**3**), among which, twenty-seven compounds (**1**–**3**, **5**–**13**, **14**–**19**, **21**–**23**, **25**–**28**, **30**, **33**) were reported for the first time in *V. paradoxa* extract. Compounds were numbered in their elution order (Table 2).

Identification of constituents by phytochemical method

V. paradoxa nutshell (**S15**) was extracted with 100% MeOH to get the extract, which was suspended in H_2O and then partitioned with PE, CH_2Cl_2, EtOAc and *n*-BuOH, respectively. Separation of CH_2Cl_2 and EtOAc fractions by repeated column chromatography on silica gel, ODS and RP-HPLC resulted in the isolation of twenty-one compounds (**VP-1**–**VP-21**), which were identified as (+)-pinoresinol (**VP-1**), (+)-medioresinol (**VP-2**), (+)-syringaresinol (**VP-3**), 5, 7, 3', 5'-tetrahy-droxyflavanone (**VP-4**), (2*R*, 3*R*)-(+)-taxifolin (**VP-5**), quercetin (**VP-6**), kaempferol (**VP-7**), luteolin (**VP-8**), 5, 7-dihydroxyl coumarin (**VP-9**), 3, 5, 7-trihydroxyl coumarin (**VP-10**), *p*-hydroxybenzoic acid (**VP-11**), protocatechuic acid (**VP-12**), salicylic acid (**VP-13**), *p*-hydroxybenzaldehyde (**VP-14**), protocatechuic acid methyl ester (**VP-15**), *p*-methoxybenzoic acid (**VP-16**), *p*-methoxyphenylacetic acid (**VP-17**), vanillic acid (**VP-18**), 2-hydroxy-5-(2-metho-xyethyl) benzoic acid (**VP-19**) coniferaldehyde (**VP-20**) and ferulic acid (**VP-21**) by comparing their MS and NMR spectroscopic data and optical rotation with literature. Among which, nineteen compounds were reported for the first time in *V. paradoxa* extract except compounds **VP-6** and **VP-12**. Their extraction, separation, structures, spectral data and reference literature were shown in the Supplementary material.

Quantitative application

The established quantification method was subsequently applied to measure amounts of four characteristic compounds, including protocatechuic acid (**6**), 3, 5, 7-trihydroxycoumarin (**14**), (2*R*, 3*R*)-(+)-taxifolin (**16**) and quercetin (**29**) (Fig. 2B and 2C) in twenty-eight samples. The contents of four characteristic compounds differed between twenty-eight samples (Table 3), and the total concentration of the four compounds was from 29.29 (**S19**) to 139.31 mg·g^{-1} (**S10**). Compound **16** was the most predominant component and showed high concentrations (27.64–132.04 mg·g^{-1}, mean 67.16 mg·g^{-1}) in all MeOH extracts. Compound **29** was the second most abundant in all *V. paradoxa* nutshells and its content was within 0.49–11.56 mg·g^{-1} (mean 2.08 mg·g^{-1}), while compounds **6** and **14** were found the lower contents in all samples (mean 0.46 and 0.80 mg·g^{-1}, respectively). Meanwhile, there was significant difference ($P < 0.01$) in contents of four characteristic compounds between twenty-eight samples, which might cause their difference in bioactivities. Therefore, we would discuss the correlation between them in the following section.

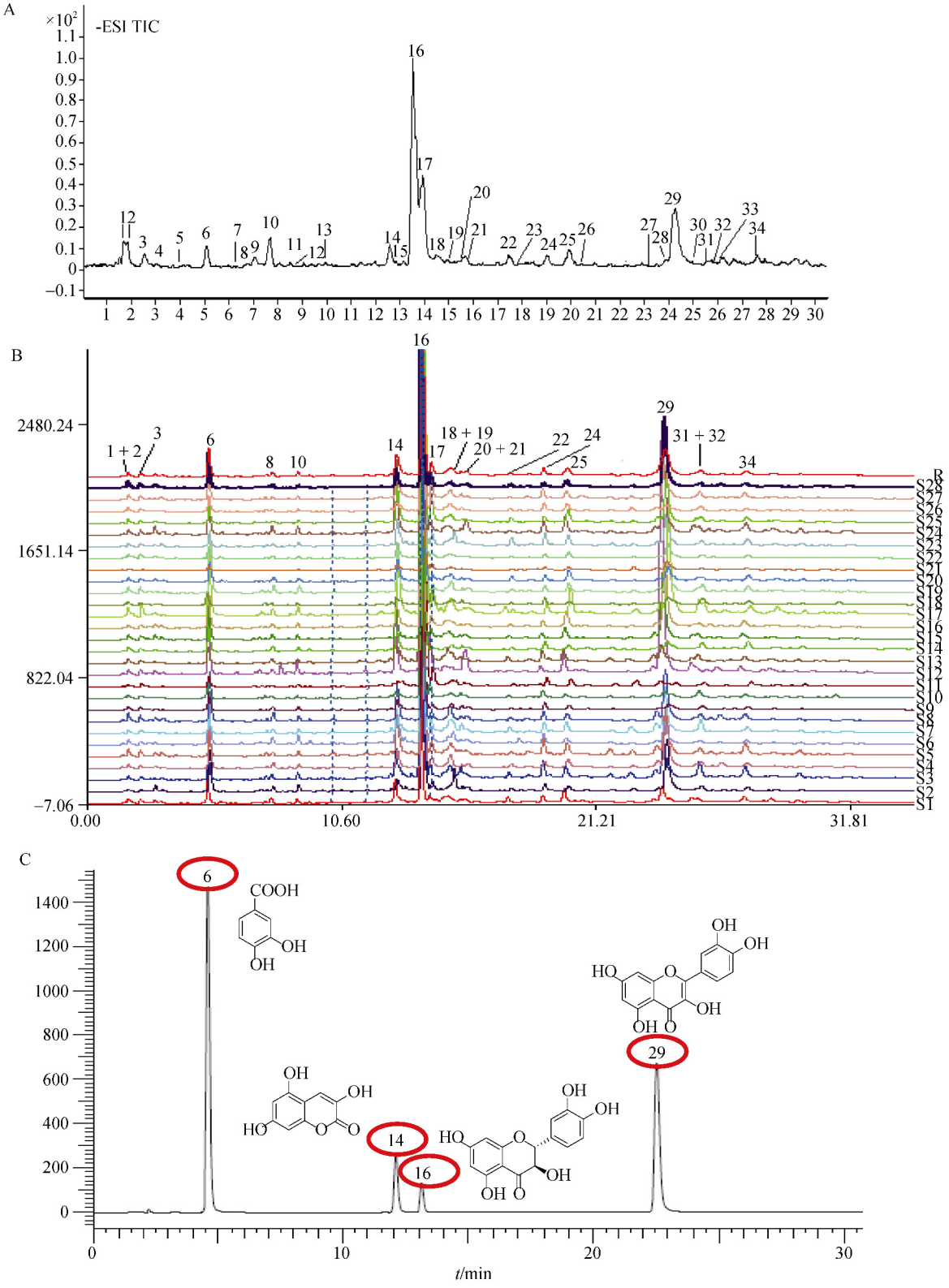


Fig. 2 Total ion chromatogram in negative mode (A), HPLC chromatograms of chromatographic fingerprints (B) and mixed reference compounds (C) for *V. paradoxa* nutshells

Table 2 Compounds identified in the MeOH extract of *V. paradoxa* nutshells

Peak No.	t_R (min)	UV λmax (nm)	$[M-H]^-$ (*m/z*)	Error (ppm)	Error (mDa)	MS/MS fragments (*m/z*)	Formula	Identification	Literature
1[a]	1.711	220	195.050 0	–5.25	1.03		$C_6H_{12}O_7$	Gluconic acid	22
2[a]	1.745	220	191.055 7	2.37	0.46	109.027 7, 93.035 9, 85.029 0	$C_7H_{12}O_6$	Quinic acid	22, 23
3[a]	2.461	225	133.014 3	–0.41	–0.05	114.614 8, 89.011 7, 72.992 2	$C_4H_6O_5$	Malic acid	22
4	3.014	280	169.014 3	–0.56	–0.09	125.018 4	$C_7H_6O_5$	Gallic acid	24
5[a]	4.114	290	167.035 1	–0.43	–0.07		$C_8H_8O_4$	Homogentisic acid	25
6[a]	5.040	260, 295	153.019 2	0.69	0.11	109.029 3, 91.018 2	$C_7H_6O_4$	Protocatechuic acid	22
7[a]	6.009	—	465.103 9	0.07	0.03	303.048 1	$C_{21}H_{22}O_{12}$	Delphinidin-3-glucoside	26
8[a]	6.771	225, 285	181.050 5	0.68	0.12	137.023 0, 107.011 7	$C_9H_{10}O_4$	Homovanillic acid	27
9[a]	6.979	230, 280, 310	137.024 6	–1.12	–0.15	93.033 2	$C_7H_6O_3$	*p*-Hydroxybenzoic acid	27
10[a]	7.602	290	303.050 9	0.41	0.13	607.109 5, 199.045 6, 177.011 7, 175.038 4, 151.000 9, 125.024 7, 123.040 4	$C_{15}H_{12}O_7$	Taxifolin isomer	28
11[a]	8.502	220, 260, 290	167.034 6	2.04	0.34	108.020 9, 91.018 3	$C_8H_8O_4$	Vanillic acid	23
12[a]	8.710	220, 280	197.045 4	0.93	0.16	166.999 3, 123.007 0	$C_9H_{10}O_5$	Syringic acid	29
13[a]	9.956	230, 290	317.029 6	2.06	0.65	151.003 7	$C_{15}H_{10}O_8$	Myricetin	30
14[a, b]	12.553	240, 300, 330	193.014 1	0.79	0.15		$C_9H_{10}O_9$	3,5,7-Trihydroxycoumarin	
15[a]	13.349	230, 290	285.040 8	–1.21	–0.35	217.316 8, 213.022 3, 185.853 0, 151.002 0	$C_{15}H_{10}O_6$	Luteolin	31
16[a, b]	13.487	235, 280, 305	303.051 5	–1.55	–0.47	607.110 2, 153.019 2, 125.024 3	$C_{15}H_{12}O_7$	(2*R*, 3*R*)-(+)-Taxifolin	28
17[a]	13.937	230, 290	303.051 1	–0.3	–0.09	607.109 1, 217.050 2, 199.039 9, 175.039 5, 125.024 1	$C_{15}H_{12}O_7$	Taxifolin isomer	28
18[a]	14.526	230, 290	303.051 4	–1.23	–0.36	607.102 5, 217.049 5, 199.039 7, 175.037 2, 125.024 4	$C_{15}H_{12}O_7$	Taxifolin isomer	28
19	14.837	230, 285	447.092 4	1.92	0.86	301.036 3	$C_{21}H_{20}O_{11}$	Quercitrin	22
20	15.564	230, 295	207.066 6	–1.54	–0.32	177.018 6, 149.023 4, 121.028 8, 105.033 6, 93.035 0, 77.040 2	$C_{11}H_{12}O_4$	Unknown	
21[a]	15.634	235, 295, 340	177.055 7	–0.14	–0.03		$C_{10}H_{10}O_{13}$	Methyl ester coumaric acid	32
22[a]	17.503	230, 285	287.056 9	–2.59	–0.75	151.002 5, 135.044 8	$C_{15}H_{12}O_6$	Eriodictyol	33
23[a]	17.849	235, 290	317.066 8	–0.36	–0.11	298.000 3	$C_{16}H_{14}O_7$	Quercetin methyl eter	34
24	18.957	235, 285	329.139 7	–0.71	–0.23	299.092 7, 269.080 3, 147.045 3, 121.027 9	$C_{19}H_{22}O_5$	Unknown	
25[a]	19.857	230, 290	283.024 6	0.91	0.26	255.033 9, 211.041 7, 195.044 2, 184.043 1	$C_{16}H_{12}O_5$	Calycosin	28
26[a]	20.376	235, 285	285.040 3	0.56	0.16	267.017 1, 257.043 4, 211.033 9	$C_{15}H_{10}O_6$	Scutellarein	23
27[a]	23.181	235, 280	417.154 8	1.57	0.66		$C_{22}H_{26}O_8$	Syringaresinol	35
28[a]	23.804	230, 285	285.040 5	–0.22	–0.06	199.030 2, 119.045 0	$C_{15}H_{10}O_6$	Kaempferol	22
29	24.219	255, 370	301.035 0	1.07	0.32	178.997 7, 151.003 4, 121.029 3, 107.013 5	$C_{15}H_{10}O_7$	Quercetin	24, 28
30	24.912	—	357.1344	4.91	1.76		$C_{20}H_{22}O_6$	Pinoresinol	35
31	25.569	235, 265, 295	409.0571	–1.37	–0.56	257.007 4, 231.027 7, 229.012 6, 201.018 7	$C_{21}H_{14}O_9$	Unknown	
32	25.708	235, 290	337.1071	3.08	1.04	307.057 8, 279.064 1, 178.995 3, 151.004 6	$C_{20}H_{18}O_5$	Unknown	
33[a]	26.500	235, 265, 290	299.0204	–2.43	–0.73		$C_{16}H_{12}O_6$	Diosmetin	36
34	27.577	230, 285	299.0187	3.41	1.02	277.022 5, 227.030 1, 199.038 4, 151.004 4	$C_{15}H_8O_7$	Unknown	

[a] Reported for the first time in *V. paradoxa*; [b] Identified by comparing with reference standard separated by phytochemical method

Table 3 The contents of four marker compounds of twenty-eight *V. paradoxa* nutshells samples and similarity of their chromatographic fingerprints

Sample No.	Content (mg·g^{-1})				Total	Similarity
	3, 4-Dihydroxybenzoic acid	3, 5, 7-Trihydroxy-coumarin	(2*R*, 3*R*)-(+)-Taxifolin	Quercetin		
S1	0.76 ± 0.04	1.09 ± 0.05	49.32 ± 0.81	2.36 ± 0.09	53.53	0.945
S2	0.16 ± 0.01	0.16 ± 0.01	102.94 ± 1.25	0.53 ± 0.15	103.80	0.978
S3	0.33 ± 0.01	1.40 ± 0.05	80.09 ± 0.73	2.23 ± 0.01	84.04	0.987
S4	0.38 ± 0.02	0.99 ± 0.04	76.71 ± 1.12	1.69 ± 0.11	79.77	0.988
S5	0.25 ± 0.02	1.09 ± 0.02	84.79 ± 0.26	1.99 ± 0.02	88.12	0.987
S6	0.22 ± 0.01	0.37 ± 0.03	31.57 ± 0.68	0.64 ± 0.03	32.80	0.980
S7	0.22 ± 0.02	0.31 ± 0.02	74.84 ± 0.89	0.87 ± 0.07	76.24	0.991
S8	0.50 ± 0.03	0.60 ± 0.04	50.68 ± 0.64	3.07 ± 0.18	54.85	0.947
S9	0.53 ± 0.04	0.39 ± 0.02	40.41 ± 1.72	1.22 ± 0.08	42.55	0.963
S10	1.11 ± 0.07	1.58 ± 0.05	132.04 ± 0.95	4.58 ± 0.05	139.31	0.818
S11	0.15 ± 0.01	0.81 ± 0.06	63.38 ± 0.78	1.65 ± 0.08	65.99	0.988
S12	0.65 ± 0.03	0.88 ± 0.03	75.16 ± 0.95	3.07 ± 0.02	79.76	0.984
S13	0.32 ± 0.02	0.38 ± 0.01	84.32 ± 1.22	0.72 ± 0.02	85.74	0.989
S14	0.81 ± 0.06	0.33 ± 0.01	65.15 ± 0.55	1.34 ± 0.11	67.63	0.989
S15	0.21 ± 0.03	0.28 ± 0.04	59.03 ± 0.83	2.65 ± 0.05	59.78	0.951
S16	0.08 ± 0.01	0.24 ± 0.01	39.23 ± 0.76	0.69 ± 0.02	40.24	0.970
S17	0.36 ± 0.02	0.69 ± 0.03	27.64 ± 0.46	0.60 ± 0.04	29.29	0.978
S18	1.14 ± 0.06	2.22 ± 0.14	63.39 ± 1.21	11.56 ± 0.28	78.33	0.586
S19	0.24 ± 0.01	0.21 ± 0.01	69.39 ± 0.83	0.60 ± 0.04	70.44	0.992
S20	0.21 ± 0.01	0.30 ± 0.01	75.53 ± 1.27	0.49 ± 0.02	76.54	0.992
S21	0.94 ± 0.04	1.22 ± 0.08	47.57 ± 0.42	1.52 ± 0.08	51.24	0.950
S22	0.24 ± 0.01	0.16 ± 0.01	50.78 ± 0.56	0.51 ± 0.02	51.68	0.961
S23	0.24 ±0.01	0.77 ± 0.05	106.23 ± 1.03	2.06 ± 0.12	109.30	0.978
S24	0.30 ± 0.02	0.77 ± 0.03	32.00 ± 0.98	1.09 ± 0.05	34.17	0.955
S25	0.65 ± 0.05	0.41 ± 0.01	68.70 ± 0.77	2.26 ± 0.03	72.02	0.989
S26	0.34 ± 0.01	1.11 ± 0.07	88.07 ± 0.65	1.99 ± 0.13	91.51	0.984
S27	0.57 ± 0.05	1.01 ± 0.05	91.47 ± 0.74	2.98 ± 0.05	96.02	0.986
S28	0.54 ± 0.04	2.26 ± 0.12	53.09 ± 0.42	3.50 ± 0.07	59.39	0.934
Mean	0.46	0.80	67.16	2.08		

Chemometric analysis

Chromatographic fingerprint analysis is an effective and comprehensive technique for quality assessment of herbal medicines. As a statistical tool, chemometrics could overview complex data from the chromatographic profiles [37]. Therefore, HPLC fingerprints combined with powerful chemometrics would be an important tool for systemic quality assessment of herbal medicine.

Similarity analysis

The chromatographic fingerprints for *V. paradoxa* nutshells (**S1**–**S28**) were estalished and reference (R) was produced by the median of twenty-eight chromatograms. Twenty peaks (**1** + **2**, **3**, **6**, **8**, **10**, **14**, **16**, **17**, **18** + **19, 20** + **21**, **22**, **24**, **25**, **29**, **31** + **32**, **34**) existing in all chromatograms of twenty-eight *V. paradoxa* nutshell samples were assigned as "common peaks". The correlation coefficients of all sample fingerprints were calculated and shown in Table 3. The results showed that *V. paradoxa* nutshell samples from Chad (**S18**) and Nigeriahad (**S10**) relatively low similarities (0.586 and 0.818) and the others were all more than 0.9, which indicated that quality of the samples **S10** and **S18** was different from others from other origins in the contents of common peaks.

Hierarchical cluster analysis

For HCA analysis, calculation of degree of association is expressed as distance and the smallest distance between two groups indicates how highest the degree of association was [37]. The sample clusters were shown in a dendrogram (Fig. 3A) using the relative areas of common eight characteristic peaks as clustering variable. The results demonstrated that the twenty- eight samples could be categorized into three main clusters (Cluster I, Cluster II and Cluster III). The cluster I consisted of **S18**, cluster II consisted of **S10**, and the other samples, **S1**–**S9**, **S11**–**S17**, and **S19**–**S28** were belonging to cluster III, which was in accordance with the result of HPLC fingerprints similarity above.

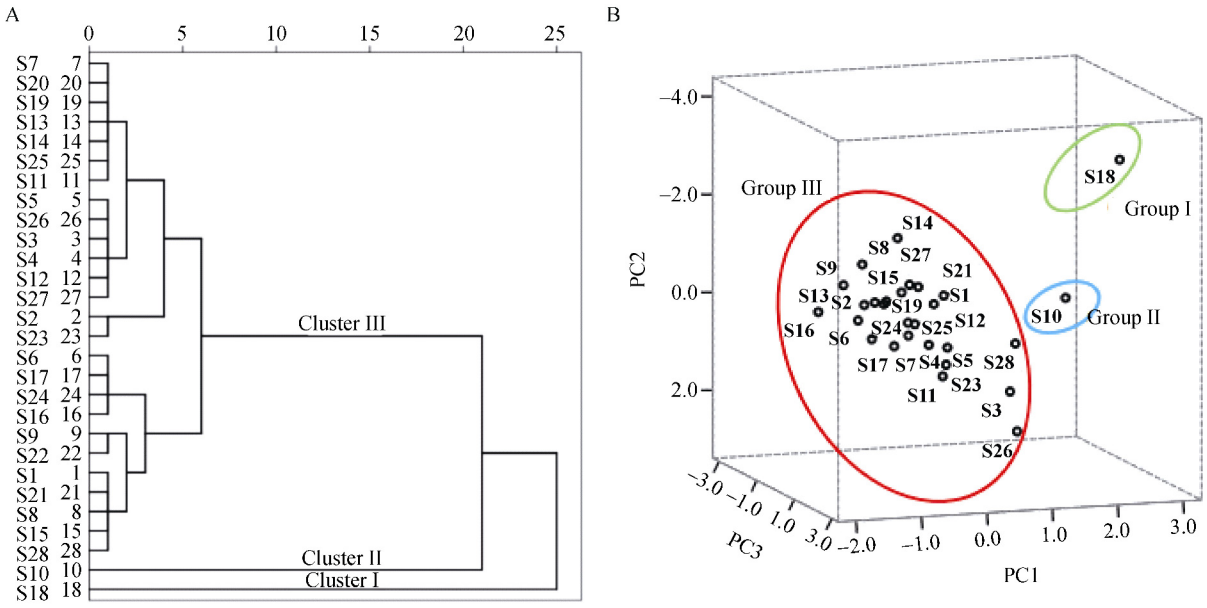
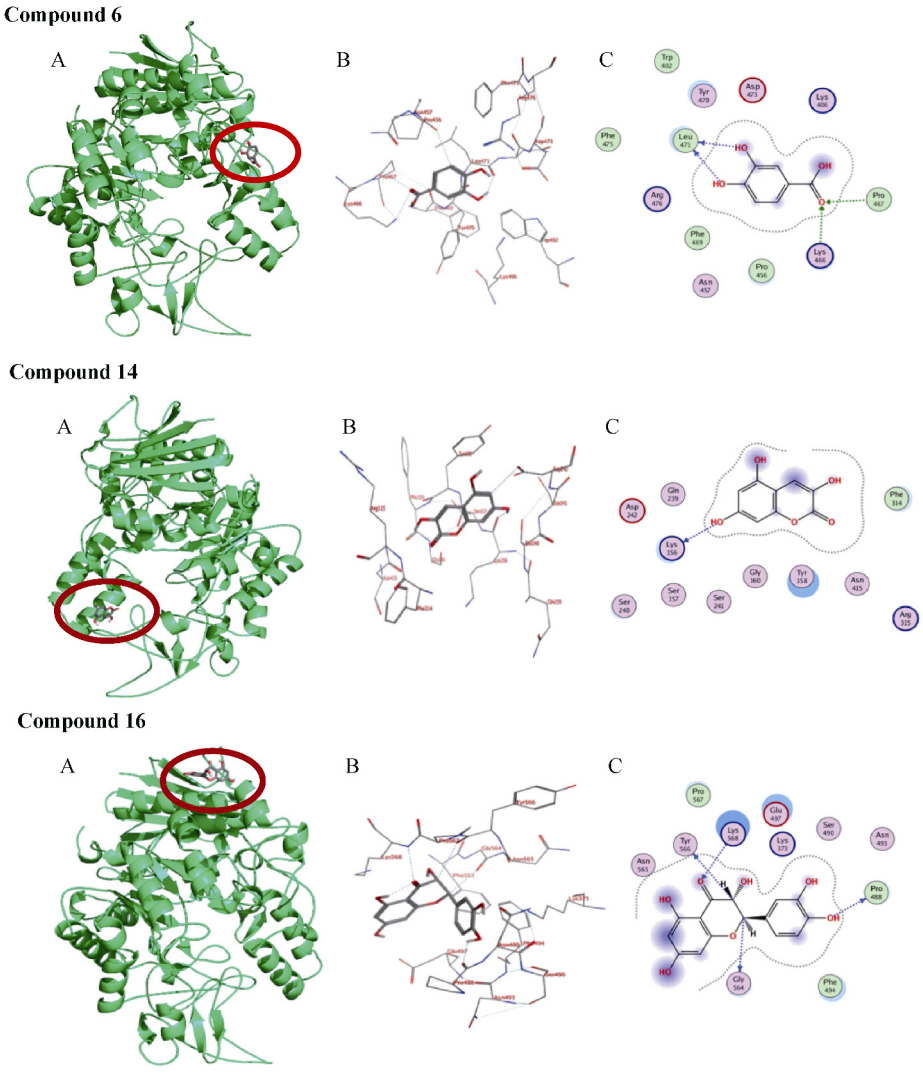


Fig. 3 Dendrogram of hierarchical cluster analysis (A) and principal component analysis scores plot (B) for twenty-eight batches of *V. paradoxa* nutshells from different regions



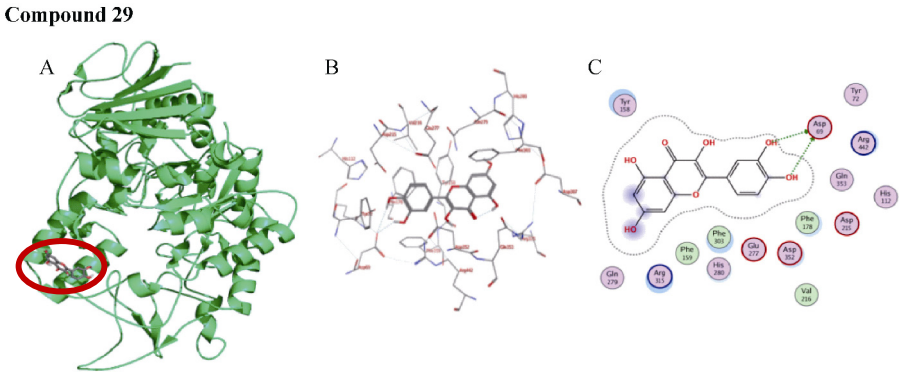


Fig. 4 Molecular docking of compounds with site of *S. cerevisiae* isomaltase (3A4A). Best docking pose found between *α*-glucosidase and compound (A); Docked pose showing interacting residues (B); Binding mode (C)

Principal components analysis

The aim of PCA is to reduce the dimensionality of multivariate data while preserving data from a higher to a lower dimensional space (called principal components) [38]. The full 28 × 20 (sample size × number of variables) data matrix was formed for PCA analysis. Three principal components (PC1 = 46.74%, PC2 = 20.85%, PC3 = 8.46%) were obtained when performing PCA using the complete data set, jointly accounting for 76.04% of the total variance, which were considered significant [39]. PCA 3D score plot of *V. paradoxa* nutshells from different origins was shown in Fig. 3B. From the scatter points, the twenty-eight *V. paradoxa* nutshell samples could be classified into three groups. Group I was formed by **S18**, group II by **S10**, and group III was marked by the rest of the samples, **S1**–**S9**, **S11**–**S17**, and **S19**–**S28**.

In summary, the results of SA, HCA and PCA were consistent and demonstrated an unignorable quality variation in the contents of common peaks between groups I, II and III, which indicated that the geographical origin, including longitude, altitude and elevation, were the major effects on quality difference of *V. paradoxa* nutshells.

Yields and total phenolic content

The yield percentage of twenty-eight sample extracts ranged from 3.10% to 6.92% (mean 5.04%) (Table 1). What's more, all samples contained high TPC (mean 190.08 mg GAE/g DW), and the sample from **S10** had the highest TPC (358.78 mg GAE/g DW) and the lowest TPC was the sample **S22** which was only 111.79 mg GAE/g DW. Besides, there was significant difference ($P < 0.01$) in yields and TPC between twenty-eight samples, which indicated that the geographical origin, including longitude, altitude and elevation, might be the major effects on quality difference of *V. paradoxa* nutshells.

Bioactivity evaluation

Antioxidant and α-glucosidase inhibitory activities of MeOH extracts

For the herbal medicine, different contents of chemical constituents might lead to significant differences in bioactivities. As shown in Table 4, all twenty-eight samples exhibited moderate-higher antioxidant capacity (DPPH: IC_{50} 6.16–15.17 μg·mL^{-1}, ABTS: 2.31–5.85 mmol TEs/g, and FRAP: 1.15–4.81 mmol TEs/g, respectively) compared with reference trolox (DPPH IC_{50} 6.43 μg·mL^{-1}). Among these, **S10** exhibited the most potent antioxidant capacity (DPPH: IC_{50} 6.16 μg·mL^{-1}, ABTS: 5.85 mmol TEs/g, and FRAP: 4.81 mmol TEs/g, respectively). It was observed the contents of phenols played a very important role in the antioxidative capacity of *V. paradoxa* nutshells. Besides, diabetes mellitus is closely related to oxidative stress and *α*-glucsidase is usually used to reduce the postprandial plasma glucose for patients with type II diabetes [16]. Therefore, *α*-glucosidase activity of *V. paradoxa* nutshell MeOH extracts was determined for screening potential anti-diabetic resources furtherly.

All extracts showed strong *α*-glucosidase inhibitory activity with IC_{50} values ranging from 8.76 to 30.68 μg·mL^{-1}, which were significantly higher than the reference acarbose (IC_{50} 119.71 μg·mL^{-1}). Meanwhile, **S10** exhibited the highest inhibitory activity (IC_{50} 8.76 μg·mL^{-1}). Considering phenolic constituents as an important compound group that contributed to the bioactivities of *V. paradoxa* nutshell, bioactive components of *V. paradoxa* nutshell MeOH extract were isolated and purified by bioassay-guided method and main characteristic compounds were quantified. The correlation was also discussed between TPC and bioactivities in the following section.

Antioxidant and α-glucosidase inhibitory activities of fractions and isolated compounds

Due to the good bioactivities of CH_2Cl_2 and AcOEt fractions, the isolated compounds **VP-1**–**VP-21** were made for further antioxidant and *α*-glucosidase inhibitory activities evaluation and the results were summarized in Table 5. The CH_2Cl_2 and AcOEt fractions showed obvious antioxidant and *α*-glucosidase inhibitory activities (for CH_2Cl_2 fraction DPPH: IC_{50} 10.48 μg·mL^{-1}, ABTS: 4.19 mmol TEs/g, FRAP: 3.56 mmol TEs/g and *α*-glucosidase: IC_{50} 9.03 μg·mL^{-1}, respectively; for AcOEt fraction DPPH: IC_{50} 9.10 μg·mL^{-1}, ABTS: 5.62 mmol TEs/g, FRAP: 3.71 mmol TEs/g and *α*-glucosidase: IC_{50} 4.81 μg·mL^{-1}, respectively), and the *n*-BuOH and H_2O frac-

tions exhibited moderate antioxidant activity (for *n*-BuOH fraction DPPH: IC_{50} 39.44 μg·mL^{-1}, ABTS: 0.99 mmol TEs/g, FRAP: 0.63 mmol TEs/g and *α*-glucosidase: IC_{50} 32.40 μg·mL^{-1}, respectively; for H_2O fraction DPPH: IC_{50} 65.39 μg·mL^{-1}, ABTS: 0.87 mmol TEs/g, FRAP: 0.37 mmol TEs/g and *α*-glucosidase: IC_{50} 12.12 μg·mL^{-1}, respectively) compared with reference trolox (DPPH IC_{50} 6.43 μg·mL^{-1}) and acarbose (IC_{50} 119.71 μg·mL^{-1}).

Table 4 The antioxidant (DPPH, ABTS and FRAP) *α*-glucosidase inhibitory activities of twenty-eight *V. paradoxa* nutshell MeOH extracts (Mean ± SD, *n* = 3)

Sample No.	Antioxidant activity			*α*-glucosidase IC_{50} (μg·mL^{-1})
	DPPH IC_{50} (μg·mL^{-1})	ABTS (mmol TEs/g)	FRAP (mmol TEs/g)	
S1	13.59 ± 1.10	3.51 ± 0.12	2.72 ± 0.15	18.42 ± 0.55
S2	8.16 ± 0.48	4.74 ± 0.07	3.62 ± 0.09	8.94 ± 0.21
S3	7.91 ± 0.47	4.43 ± 0.24	3.60 ± 0.10	15.65 ± 0.14
S4	8.58 ± 0.31	4.51 ± 0.09	3.37 ± 0.07	11.85 ± 0.25
S5	10.69 ± 0.64	4.76 ± 0.11	3.48 ± 0.09	10.63 ± 0.32
S6	15.00 ± 1.21	2.31 ± 0.07	2.27 ± 0.11	23.26 ± 0.51
S7	11.62 ± 1.09	3.84 ± 0.10	3.75 ± 0.29	11.05 ± 0.87
S8	12.63 ± 0.75	3.34 ± 0.24	2.37 ± 0.02	19.12 ± 0.34
S9	14.62 ± 0.97	3.70 ± 0.06	1.98 ± 0.07	30.68 ± 1.43
S10	6.16 ± 0.36	5.85 ± 0.21	4.81 ± 0.24	8.76 ± 0.56
S11	12.25 ± 1.13	3.49 ± 0.11	3.19 ± 0.21	13.23 ± 0.26
S12	8.64 ± 0.58	3.72 ± 0.27	3.50 ± 0.08	10.68 ± 0.14
S13	13.81 ± 1.28	3.76 ± 0.17	3.05 ± 0.13	11.90 ± 0.01
S14	12.51 ± 0.75	3.35 ± 0.10	3.05 ± 0.09	18.52 ± 0.15
S15	13.68 ± 1.16	3.59 ± 0.24	2.39 ± 0.09	14.02 ± 1.35
S16	15.17 ± 1.31	3.16 ± 0.27	1.93 ± 0.05	26.68 ± 0.75
S17	14.39 ± 1.25	3.27 ± 0.06	2.41 ± 0.11	21.35 ± 0.03
S18	11.56 ± 0.69	4.59 ± 0.06	3.60 ± 0.12	15.68 ± 0.13
S19	12.24 ± 1.21	4.39 ± 0.05	2.98 ± 0.28	13.43 ± 0.42
S20	11.91 ± 0.93	4.91 ± 0.04	3.21 ± 0.09	14.99 ± 0.24
S21	13.11 ± 0.94	3.88 ± 0.07	2.08 ± 0.13	25.57 ± 1.36
S22	15.02 ± 0.90	2.37 ± 0.05	1.15 ± 0.05	17.36 ± 0.12
S23	8.01 ± 0.48	4.98 ± 0.12	3.89 ± 0.28	9.95 ± 0.23
S24	13.96 ± 1.35	3.55 ± 0.18	2.47 ± 0.03	19.45 ± 0.02
S25	13.04 ± 1.26	3.85 ± 0.07	2.91 ± 0.03	15.33 ± 0.16
S26	7.11 ± 0.42	4.88 ± 0.13	3.77 ± 0.07	10.97 ± 0.51
S27	9.22 ± 0.55	4.69 ± 0.09	3.23 ± 0.19	10.17 ± 0.24
S28	12.06 ± 1.03	4.49 ± 0.15	2.78 ± 0.21	16.62 ± 0.69
Trolox	6.43 ± 0.38			
Acarbose				119.71 ± 7.55

Table 5 Antioxidant (DPPH, ABTS and FRAP) and *α*-glucosidase inhibitory activities of the MeOH extract (S15) and fractions of *V. paradoxa* nutshell (Mean ± SD, *n* = 3)

Extract or Fraction	Antioxidant activity			*α*-glucosidase IC_{50} (μg·mL^{-1})
	DPPH IC_{50} (μg·mL^{-1})	ABTS (mmol TEs/g)	FRAP (mmol TEs/g)	
MeOH Extract	13.62 ± 0.09	3.36 ± 0.02	2.47 ± 0.13	17.09 ± 0.36
PE Soluble Fr.	324.92 ± 0.78	0.32 ± 0.02	0.06 ± 0.00	26.04 ± 0.48
CH_2Cl_2 Soluble Fr.	10.48 ± 0.55	4.19 ± 0.11	3.56 ± 0.31	9.03 ± 0.90
EtOAc Soluble Fr.	9.10 ± 0.32	5.62 ± 0.33	3.71 ± 0.05	4.81 ± 0.05
n-BuOH Soluble Fr.	39.44 ± 0.05	0.99 ± 0.01	0.63 ± 0.03	32.40 ± 0.16
H_2O Soluble Fr.	65.39 ± 1.12	0.87 ± 0.00	0.37 ± 0.01	12.12 ± 0.42
Trolox	6.43 ± 0.38			
Acarbose				119.71 ± 7.55

Among the isolated compounds tested (Table 6), compounds **VP-1**–**8**, **VP-12**, **VP-15** and **VP-20** exhibited moderate-higher DPPH radical-scavenging activity (IC_{50} 5.55–87.35 μmol·L^{-1}), and compounds **VP-1**, **VP-4**–**8, VP-12, VP-15** and **VP-20** showed potent radical-scavenging activity in the range of 1.02–4.29 μmol·L^{-1} Trolox/μmol·L^{-1} by ABTS analysis. Meanwhile, compounds **VP-1** and **VP-4**–**8** displayed ferric reducing power (1.09–3.53 μmol·L^{-1} Trolox/μmol·L^{-1}) by FRAP test. Besides, compounds **VP-2**–**10** exhibited strong *α*-glucosidase inhibitory activity (IC_{50} 7.70–87.17 μmol·L^{-1}). It was noteworthy that (2*R*, 3*R*)-(+)-taxifolin (DPPH: IC_{50} 17.48 μmol·L^{-1}, ABTS: 1.72 μmol·L^{-1} Trolox/μmol·L^{-1}, FRAP: 1.43 μmol·L^{-1} Trolox/μmol·L^{-1} and *α*-glucosidase: IC_{50} 26.79 μmol·L^{-1}, respectively) and quercetin (DPPH: IC_{50} 12.78 μmol·L^{-1}, ABTS: 1.62 μmol·L^{-1} Trolox/μmol·L^{-1}, FRAP: 2.46 μmol·L^{-1} Trolox/μmol·L^{-1} and *α*-glucosidase: IC_{50} 20.87 μmol·L^{-1}, respectively) showed more potent antioxidant and *α*-glucosidase inhibitory activities compared with reference trolox (IC_{50} 25.69 μmol·L^{-1}) and acarbose (IC_{50} 185.42 μmol·L^{-1}). Protocatechuic acid exhibited lower antioxidant and *α*-glucosidase inhibitory effects (DPPH: IC_{50} 21.67 μmol·L^{-1}, ABTS: 1.02 μmol·L^{-1} Trolox/μmol·L^{-1} and FRAP: 0.83 μmol·L^{-1} Trolox/mol·L^{-1}, respectively) than (2*R*, 3*R*)-(+)-taxifolin and quercetin. There was minor antioxidant activity (DPPH, ABTS and FRAP) of 3, 5, 7-trihy- droxy-coumarin observed, yet it exhibited higher *α*-glucosidase inhibitory activity (IC_{50} 43.28 μmol·L^{-1}) than acarbose. The results revealed that four characteristic components with high contents could be the main active constituents in *V. paradoxa* nutshell extracts.

Table 6 Antioxidant (DPPH, ABTS and FRAP) and *α*-glucosidase inhibitory activities of compounds isolated from *V. paradoxa* nutshell (Mean ± SD, *n* = 3)

Compounds	Antioxidant activity			*α*-glucosidase IC_{50} (μmol·L^{-1})
	DPPH IC_{50} (μmol·L^{-1})	ABTS (μmol·L^{-1} Trolox/μmol·L^{-1})	FRAP (μmol·L^{-1} Trolox/μmol·L^{-1})	
VP-1	19.52 ± 0.07	1.17 ± 0.06	0.92 ± 0.10	> 100
VP-2	23.18 ± 0.23	0.91 ± 0.05	0.84± 0.02	> 100
VP-3	29.27 ± 0.05	0.73 ± 0.06	0.69 ± 0.03	> 100
VP-4	32.76 ± 0.13	0.85 ± 0.02	0.78 ± 0.11	37.29 ± 1.57
VP-5	17.48 ± 1.22	1.22 ± 0.04	1.13 ± 0.04	26.79 ± 1.01
VP-6	12.78 ± 0.59	1.62 ± 0.08	2.46 ± 0.13	20.87 ± 0.94
VP-7	19.74 ± 0.52	1.38 ± 0.02	1.26 ± 0.04	35.78 ± 1.62
VP-8	23.03 ± 0.03	1.18 ± 0.05	1.03 ± 0.04	28.78 ± 1.49
VP-9	> 100	0.25 ± 0.02	0.13 ± 0.00	87.17 ± 1.30
VP-10	> 100	0.52 ± 0.02	0.14 ± 0.01	43.20 ± 1.91
VP-11	> 100	0.03 ± 0.00	0.01 ± 0.00	> 100
VP-12	21.67 ± 0.88	1.02 ± 0.02	0.87 ± 0.02	> 100
VP-13	> 100	0.07 ± 0.01	0.01 ± 0.00	> 100
VP-14	> 100	0.02 ± 0.00	0.01 ± 0.00	> 100
VP-15	42.88 ± 0.40	0.43 ± 0.00	0.32 ± 0.00	> 100
VP-16	> 100	0.08 ± 0.01	0.01 ± 0.00	> 100
VP-17	> 100	0.04 ± 0.00	0.01 ± 0.00	> 100
VP-18	> 100	0.06 ± 0.00	0.03 ± 0.00	> 100
VP-19	> 100	0.14 ± 0.00	0.19 ± 0.01	> 100
VP-20	87.35 ± 2.17	0.20 ± 0.01	0.03 ± 0.00	> 100
VP-21	> 100	0.01 ± 0.00	0.28 ± 0.01	> 100
Trolox	25.69 ± 1.52			
Acarbose				185.42 ± 1.17

Pearson correlation analysis

Pearson correlation analysis was carried out to reflect the correlation between chemical component contents and bioactivities of *V. paradoxa* nutshell extracts, and the most significant correlation between chemical component contents and bioactivities how highest the contribution degree of association was.

The correlation between the antioxidant (DPPH, ABTS and FRAP) and *α*-glucosidase inhibitory activities and TPC was evaluated (Table 7), and the results showed significant negative or positive correlation (r = –0.937, 0.872, 0.918 and –0.696 at the level of 0.01) between TPC and DPPH, TPC and

ABTS, TPC and FRAP and TPC and α-glucosidase inhibitory activity, respectively. For pure compounds, (2*R*, 3*R*)-(+)-taxifolin and 3, 5, 7-trihydroxycoumarin significantly correlated with DPPH, ABTS and FRAP at $P < 0.01$ or 0.05 level ($r = –0.854$ and –0.386), while protocatechuic acid and quercetin showed no significant correlation with DPPH, ABTS and FRAP ($P > 0.05$ level). Only (2*R*, 3*R*)-(+)-taxifolin correlated with α-glucosidase inhibitory activity significantly at $P < 0.01$ level ($r = –0.817$). 3, 5, 7-Trihydroxycoumarin also contributed to the α-glucosidase inhibitory activity significantly. It was noteworthy that protocatechuic acid and quercetin with higher contents and better bioactivities didn't significantly relate to antioxidant or α-glucosidase inhibitory activities, we inferred that the factor of high level of (2*R*, 3*R*)-(+)-taxifolin contributed to this case, certainly with the synergistic reaction of compounds. Moreover, antioxidant activity (DPPH, ABTS and FRAP) and α-glucosidase inhibitory activity also were significantly correlated with each other ($r = 0.731$, –0.606, and –0.766 at the level of 0.01), which supported the close connection between them.

Table 7 Pearson's correlation coefficients (*r*) between chemical component contents and bioactivities.

	TPC	Protocatech-uic acid	3, 5, 7-Trihydrox-ycoumarin	(2*R*, 3*R*)-(+)-Taxifolin	Quercetin	DPPH	ABTS	FRAP	α-glucosidase
TPC	1								
Protocatechuic acid	0.272	1							
3,5,7-Trihydroxy-coumarin	0.442*	0.560**	1						
(2*R*, 3*R*)-(+)-Taxifolin	0.884**	0.119	0.164	1					
Quercetin	0.324	0.685**	0.746**	0.173	1				
DPPH	–0.937**	–0.136	–0.386*	–0.854**	–0.258**	1			
ABTS	0.872**	0.248	0.472*	0.802**	0.344	–0.805**	1		
FRAP	0.918**	0.193	0.374*	0.841**	0.349	–0.856**	0.810**	1	
α-glucosidase	–0.696**	0.038	–0.152	–0.817**	–0.168	0.731**	–0.606**	–0.766**	1

*Correlation is significant at the 0.05 level; **Correlation is significant at the 0.01 level.

Molecular docking of four characteristic compounds

Due to the high contents and good α-glucosidase inhibitory activity of four characteristic compounds, they could be the major active constituents responsible for the majority of medical effects of *V. paradoxa* nutshell. Thus, their binding mode to the allosteric site of *S. cerevisiae* isomaltase was investigated by a docking simulation, which was conducted to better understand the the mechanism of α-glucosidase inhibition of *V. paradoxa* nutshell resources and the interaction between the compound and the protein. The molecular docking results were shown in Fig. 3, which included best docking pose with the lowest docking energy (A), docked pose showing interacting residues (B) and binding mode (C). From the docking results, it could be obtained that the selected docked energies for the protocatechuic acid, 3, 5, 7-trihydroxycoumarin, (2*R*, 3*R*)-(+)-taxifolin and quercetin were –5.13, –6.32, –6.55 and –7.00 kcal/mol, respectively. This result displayed that the quercetin and (2*R*, 3*R*)-(+)-taxifolin molecules had larger binding affinities towards a-glucosidase of *S. cerevisiae* isomaltase, supporting for their α-glucosidase inhibitory activity, which reflected the inhibition mainly depended on the binding modes and binding affinities of the molecules to the enzyme [40]. Besides, it could be seen that the binding site between the α-glucosidase and four molecules was stabilized by hydrogen bond, and protocatechuic acid docked to α-glucosidase was stabilized by four hydrogen bonds to Leu471, Pro467 and Lys466, which for 3, 5, 7-trihydroxycoumarin was stabilized by one hydrogen bonds to Lys156. The (2*R*, 3*R*)-(+)-taxifolin–α-glucosidase interactions was stabilized by four hydrogen bonds to Pro488, Gly564, Lys568 and Tyr566, which for quercetin was stabilized by two hydrogen bonds to Asp69. Namely, the four characteristic compounds inhibited α-glucosidase by blocking the interaction between four ligands and the receptor at the binding site, and the high binding affinity was mainly ascribed to the hydrogen bonding [41-42]. Thus, the docking study method employed was adequate and effective, and the α-glucosidase inhibitory activity could be a result of a synergistic effect between different phenolic constituents in *V. paradoxa* nutshell.

Conclusions

In conclusion, it is the first time that we carried out research on the *V. paradoxa* nutshell waste and this study revealed all *V. paradoxa* nutshell samples from seven sub-Saharan countries could be used as cheap natural antidiabetic herbal source, specially rich in (2*R*, 3*R*)-(+)-taxifolin. Future research would deeply probe into the hypoglycemic mechanism of the target component and further *in vivo* experiments are also needed to evaluate. Besides, the synergy effect between different chemical constituents in *V. paradoxa* nutshell samples remained to be studied, which would establish foundation for prospect of resource utilization.

References

[1] Yang Z, Wang Y, Wang Y, *et al*. Bioassay-guided screening and isolation of *α*-glucosidase and tyrosinase inhibitors from leaves of *Morus alba* [J]. *Food Chem*, 2012, **131**(2): 617-625.

[2] Shahidi F, Ambigaipalan P. Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects-A review [J]. *J Funct Foods*, 2015, **18**: 820-897.

[3] Hoofnagle JH, Wright EC. Weight loss from green tea extracts [J]. *Clin Nutr*, 2016, **35**(1): 238-238.

[4] Oh S, Shintani R, Koike S, *et al*. Ginkgo fruit extract as an additive to modify rumen microbiota and fermentation and to mitigate methane production [J]. *J Dairy Sci*, 2017, **100**(3): 1923-1934.

[5] Morteza S, Saeedi K, Shahnavaz M. Comparison of antioxidant activity of extract from roots of licorice (*Glycyrrhiza glabra* L.) to commercial antioxidants in 2% hydroquinone cream [J]. *J Cosmet Sci*, 2003, **54**(6): 551-558.

[6] Sepúlveda J, Pinto R, Garcés R, *et al*. Use of *Stevia rebaudiana* purified extract as a sweetener on pediatric oral formulations [J]. *Int J Pharmaceut*, 2018, **536**(2): 518-519.

[7] Qi X, Zhang Z, Xu Y. Effects of Ginkgolide B on action potential and calcium, potassium current in guinea pig ventricular myocytes [J]. *Acta Pharmacol Sin*, 2004, **25**(2): 203.

[8] Yang MH, Wang CH, Chen HL. Green, oolong and black tea extracts modulate lipid metabolism in hyperlipidemia rats fed high-sucrose diet [J]. *J Nutr Biochem*, 2001, **12**(1): 14-20.

[9] Pan XJ, Liu HZ. Microwave-assisted extraction of glycyrrhizic acid from *Licorice* root-effect of the property of solution on extraction of GA [J]. *J Chem Ind Eng (China)*, 2000, **51**(S1): 240-243.

[10] Siddique AB, Rahman SMM, Hossain MA, *et al*. Phytochemical screening and comparative antimicrobial potential of different extracts of *Stevia rebaudiana* Bertoni leaves [J]. *Asian Pac J Trop Dis*, 2014, **4**(4): 275-280.

[11] Akihisa T, Kojima N, Katoh N, *et al*. Triacylglycerol and triterpene ester composition of shea nuts from seven African countries [J]. *J Oleo Sci*, 2011, **60**(8): 385-391.

[12] Aboaba S, Akande A, Flamini G. Chemical composition and toxicity of essential oil of *Vitellaria paradoxa* (C.F. Gaertner.) from Nigeria [J]. *J Essent Oil-Bear Plants*, 2014, **17**(1): 126-130.

[13] El-Mahmood AM, Doughari JH, Ladan N. Antimicrobial screening of stem bark extracts of *Vitellaria paradoxa* against some enteric pathogenic microorganisms [J]. *Afr J Pharm Pharmacol*, 2008, **2**: 89-94.

[14] Zhang J, Kurita M, Ebina K, *et al*. Melanogenesis-inhibitory activity and cancer chemopreventive effect of glucosylcucurbic acid from shea (*Vitellaria paradoxa*) kernels [J]. *Chem Biodiversity*, 2015, **12**(4): 547-558.

[15] Akihisa T, Kojima N, Katoh N, *et al*. Triterpene alcohol and fatty acid composition of shea nuts from seven African countries [J]. *J Oleo Sci*, 2010, **59**(7): 351-360.

[16] Choi CI, Lee SR, Kim KH. Antioxidant and *α*-glucosidase inhibitory activities of constituents from *Euonymus alatus twigs* [J]. *Ind Crops Prod*, 2015, **76**: 1055-1060.

[17] Li H, Zhang D, Tan LH, *et al*. Comparison of the antioxidant properties of various solvent extracts from *Dipsacus asperoides* and identification of phenolic compounds by LC-ESI-QTOF-MS-MS [J]. *S Afr J Bot*, 2017, **109**: 1-8.

[18] Zhang DY, Luo M, Wang W, *et al*. Variation of active constituents and antioxidant activity in pyrola [*P. incarnata* Fisch.] from different sites in Northeast China [J]. *Food Chem*, 2013, **141**(3): 2213-2219..

[19] Uvarani C, Jaivel N, Sankaran MK, *et al*. Axially chiral biscarbazoles and biological evaluation of the constituents from *Murraya koenigii* [J]. *Fitoterapia*, 2014, **94**: 10-20.

[20] Yuan T, Wan C, Liu K, *et al*. New maplexins F–I and phenolic glycosides from red maple (*Acer rubrum*) bark [J]. *Tetrahedron*, 2012, **68**(4): 959-964.

[21] Li Q, Wang X, Chen J, *et al*. Antioxidant activity of proanthocyanidins-rich fractions from *Choerospondias axillaris* peels using a combination of chemical-based methods and cellular-based assay [J]. *Food Chem*, 2016, **208**: 309-317.

[22] Zhuang B, Bi ZM, Wang ZY, *et al*. Chemical profiling and quantitation of bioactive compounds in Platycladi Cacumen by UPLC-Q-TOF-MS/MS and UPLC-DAD [J]. *J Pharm Biomed Anal*, 2018, **154**: 207-215.

[23] Sun H, Liu M, Lin Z, *et al*. Comprehensive identification of 125 multifarious constituents in Shuang-huang-lian powder injection by HPLC-DAD-ESI-IT-TOF-MS [J]. *J Pharm Biomed Anal*, 2015, **115**: 86-106.

[24] Díaz-De-Cerio E, Gómez-Caravaca AM, Verardo V, *et al*. Determination of guava (*Psidium guajava*, L.) leaf phenolic compounds using HPLC-DAD-Q-TOF-MS [J]. *J Funct Foods*, 2016, **22**: 376-388.

[25] Trautvetter S, Koelling-Speer I, Speer K. Confirmation of phenolic acids and flavonoids in honeys by UPLC-MS [J]. *Apidologie*, 2009, **40**(2): 140-150.

[26] Willemse CM, Stander MA, De VA. Hydrophilic interaction chromatographic analysis of anthocyanins [J]. *J Chromatogr A*, 2013, **1319**: 127-140.

[27] Gómez-Romero M, Zurek G, Schneider B, *et al*. Automated identification of phenolics in plant-derived foods by using library search approach [J]. *Food Chem*, 2011, **124**(1): 379-386.

[28] Touati R, Santos SAO, Rocha SM, *et al*. Phenolic composition and biological prospecting of grains and stems of *Retama sphaerocarpa* [J]. *Ind Crops Prod*, 2017, **95**: 244-255.

[29] Kukula-Koch W, Koch W, Angelis A, *et al*. Application of pH-zone refining hydrostatic countercurrent chromatography (HCCC) for the recovery of antioxidant phenolics and the isolation of alkaloids from *Siberian barberry* herb [J]. *Food Chem*, 2016, **203**: 394-401.

[30] López-Gutiérrez N, Romero-González R, Plaza-Bolaños P, *et al*. Identification and quantification of phytochemicals in nutraceutical products from green tea by UHPLC-Orbitrap-MS [J]. *Food Chem*, 2015, **173**: 607-618.

[31] Hu X, Chen L, Shi S, *et al*. Antioxidant capacity and phenolic compounds of *Lonicerae macranthoides* by HPLC-DAD-QTOF-MS/MS [J]. *J Pharm Biomed Anal*, 2016, **124**: 254-260.

[32] Herchi W, Sawalha S, Arráez-Román D, *et al*. Determination of phenolic and other polar compounds in flaxseed oil using liquid chromatography coupled with time-of-flight mass spectrometry [J]. *Food Chem*, 2011, **126**(1): 332-338.

[33] García-Salas P, Gómez-Caravaca AM, Arráez-Román D, *et al*. Influence of technological processes on phenolic compounds, organic acids, furanic derivatives, and antioxidant activity of whole-lemon powder [J]. *Food Chem*, 2013, **141**(2): 869-878.

[34] Peña-Cerda M, Arancibia-Radich J, Valenzuela-Bustamante P, *et al*. Phenolic composition and antioxidant capacity of *Ugni molinae Turcz.* leaves of different genotypes [J]. *Food Chem*,

2017, **215**: 219-227.

[35] García-Villalba R, Carrasco-Pancorbo A, Oliveras-Ferraros C, *et al*. Characterization and quantification of phenolic compounds of extra-virgin olive oils with anticancer properties by a rapid and resolutive LC-ESI-TOF-MS method [J]. *J Pharm Biomed Anal*, 2010, **51**: 416-429.

[36] Wang S, Hao LJ, Zhu JJ, *et al*. Comparative evaluation of *Chrysanthemum Flos*, from different origins by HPLC-DAD-MS^n, and relative response factors [J]. *Food Anal Method*, 2015, **8**(1): 40-51.

[37] Xia Y, Liu F, Feng F, *et al*. Characterization, quantitation and similarity evaluation of *Codonopsis lanceolata* from different regions in China by HPLC-Q-TQF-MS and chemometrics [J]. *J Food Compos Anal*, 2017, **62**: 134-142.

[38] Li L, Zhao Y, Liu W, *et al*. HPLC with quadrupole TOF-MS and chemometrics analysis for the characterization of *Folium Turpiniae* from different regions [J]. *J Sep Sci*, 2013, **36**: 2552-2561.

[39] Zhou J, Yao L, Li Y, *et al*. Floral classification of honey using liquid chromatography-diode array detection-tandem mass spectrometry and chemometric analysis [J]. *Food Chem,* 2014, **145**: 941-949.

[40] Devipriya B, Kumaradhas P. Molecular flexibility and the electrostatic moments of curcumin and its derivatives in the active site of p300: a theoretical charge density study [J]. *Chem Biol Interact*, 2013, **204**(3): 153-156.

[41] Qi YJ, Zhao YM, Lu HN, *et al*. Exploring molecular flexibility and the interactions of Quercetin derivatives in the active site of α-glucosidase using molecular docking and charge density analysis [J]. *Comput Theor Chem*, 2016, **1094**: 55-68.

[42] Zhang J, Sasaki T, Li W, *et al*. Identification of caffeoylquinic acid derivatives as natural protein tyrosine phosphatase 1B inhibitors from *Artemisia princeps* [J]. *Bioorg Med Chem Lett*, 2018, **28**(7): 1194-1197.

Cite this article as: LI Da, XIAO Jian-Qi, LIU Wen-Yuan, ZHANG Chao-Feng, AKIHISA Toshihiro, ABE Masahiko, MASTERS Eliot-T., ZHAI Wei-Wei, FENG Feng, ZHANG Jie. *Vitellaria paradoxa* nutshells from seven sub-Saharan countries as potential herbal medicines for treating diabetes based on chemical compositions, HPLC fingerprints and bioactivity evaluation[J]. *Chin J Nat Med*, 2019, **17**(6): 446-460.