Identification of *Lepidium meyenii* (Walp.) based on spectra and chromatographic characteristics of its principal functional ingredients

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Abstract: *Lepidium meyenii* Walp. (maca), a perennial herbaceous plant with narrow distribution in the Andean region, was cultivated by local residents as early as 1600 BC. It has high nutritional value and multiple medicinal effects. The plant source of maca is now scarce because maca products are becoming increasingly popular in the world as dietary supplements. This means that studies on the identification of maca materials are now urgent. In present work, natural plants with similar appearance or medicinal effects, including maca, radish (*Raphanus sativus* L.), oriental ginseng (*Panax ginseng* C. A. Mey.), American ginseng (*Panax quinquefolium* L.) were investigated. Their alkaloid extracts of the hypogeal parts were analysed by FTIR and TLC. The essential oils (steam distillates) were analysed by GC/MS. Through comparison of the characteristics of their spectra and chromatograms, it was found that the functional ingredient extracts of maca have unique FTIR, TLC and GC/MS behaviours. The secondary amide group (–CH_2–NH–CO–CH_2–) and the phenyl structure in FTIR, the multiple spots at different R_F values colourised by modified Dragendorff's reagent, and the characteristic peaks produced by the major essential oil components (e.g. phenylacetonitrile, benzaldehyde, 3-methoxyphenylacetonitrile) in GC/MS are distinct. These behaviours can be applied to the identification of maca or maca products in the market.
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Keywords: *Lepidium meyenii*; identification; functional ingredients; FTIR; GC/MS

INTRODUCTION

Lepidium meyenii Walp. (Cruciferae), commonly known as maca, is a perennial herbaceous plant that grows in the Andean region at 3500–4450 m above sea level, where low temperatures and strong winds limit the growth of other crops.[1,2] It has been cropped there by local residents for centuries for its high nutritional value and medicinal effects.[2,3] In the early 1990s, the notion of returning to nature greatly pushed forward the discovery of South American folk herbs. At that time, maca, a mysterious plant, was being developed as a precious herb. It has multiple medicinal functions, such as anti-fatigue, fertility enhancement, sexual performance improvement, and menopausal symptoms treatment. Meanwhile, research on its chemical composition and biological activity mushroomed, especially for its principal functional ingredients, maca alkaloids, glucosinolates and benzyl isothiocyanates.[4–7]

In the 1960s, Chacón first separated four different maca alkaloids, and named them as macaina 1, 2, 3 and 4. Through pharmacological tests, she assumed these compounds may well contribute to its fertility enhancing effect.[8] Since the 1980s, systematic analysis on glucosinolates and isothiocyanates in maca were carried out successively, and it was believed that these bioactive substances played a direct role in fertility enhancement.[4,9,10] Macamides, a new kind of alkaloids with secondary amide group found lately, such as *N*-benzyl octanamide, *N*-benzyl-16-hydroxy-9-oxo-10*E*,12*E*,14*E*-octadecatrieneamide, and *N*-benzyl-9,16-dioxo-10*E*,12*E*,14*E*-octadecatrieneamide, provided a basis for revealing the mechanism behind the sexual ability enhancing activity.[5,11] Recently, two imidazole alkaloids with cytotoxic activity have been identified as 1,3-dibenzyl-4,5-dimethylimidazolium chloride named lepidiline A and 1,3-dibenzyl-2,4,5-trimethylimidazolium chloride named lepidiline B[7] (Fig. 1) and they were alkaloid-positive when tested with Dragendorff's reagent over silica gel thin-layer chromatogram (TLC) plates. Even though the biologically active principles of maca are not fully known, the promising functional ingredients, maca alkaloids (macamides, lepidilines especially), glucosinolates and

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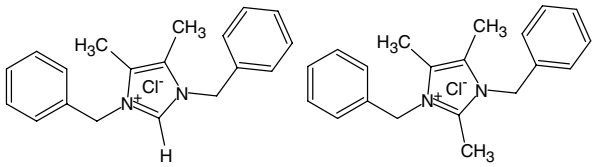

Figure 1. Structures of lepidiline A (left) and lepidiline B (right).

benzyl isothiocyanates, are therefore used as quality markers in most commercial powder or concentrated extract products of maca.

Although maca products have received high reputation in the world market, imbalance between the popularity of those products and the insufficiency on maca research is resulted from the narrow distribution of maca cultivation, the immediate lack of plant materials and the late setout on maca research in most countries. And the identification of the dried maca root, especially the powdered, which is its main edible part, is one of the urgent problems. Maca can enhance energy and stamina, so it is named 'Peruvian ginseng' by local residents. It is easy to be confused with oriental ginseng and American ginseng. Maca root also looks like that of the radish, a high-yield cheap crop planted in many countries. Furthermore, there is hardly any difference in appearance if the disparate dried roots are powdered. Consequently, it is very difficult for consumers, merchants, or even researchers who have little knowledge about maca to distinguish between dried maca powders and other plant powders. Obviously, the sensory indexes, such as smell, taste, colour and shape, are not powerful tools for accurate identification. In 2002, a study has reported that the high-performance liquid chromatography (HPLC) method was suitable for the qualitative and quantitative determination of the main macamides in maca.[12] However, the results were far from satisfactory. There are some deficiencies in the method; for example, the difficulty to order the necessary standard compounds, the insufficiency in HPLC peaks quantity, which limited its application to the identification of maca material, especially for commercial powder of dried maca. Therefore, more research and technology was needed to conveniently identify the materials and commercial products of maca.

Currently, Fourier transform infrared (FTIR) spectrometry, gas chromatography (GC) and TLC are widely accepted methods for the qualitative identification of herbs.[13–15] The aim of the present study was to appraise the suitability of these three methods for the identification of maca or maca products.

MATERIALS AND METHODS

Reagents and materials

Reagents

The reagents and organic solvents (95% ethanol, methanol, chloroform, sodium hydroxide, hydrochloric acid, ammonia, absolute diethyl ether, acetone, ethene diamine, anhydrous sodium sulfate, sodium carmellose, activated charcoal, ninhydrin, bismuth subnitrate, glacial acetic acid, potassium iodide) were of A.R. grade and purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China); phenylacetonitrile, benzaldehyde and 3-methoxyphenylacetonitrile (GC grade) from China Sigma Co. Ltd. (Beijing, China); potassium bromide (KBr) spectrograde powder and KBr window from Bruker (Ettlingen, German); silica gel (type G) for TLC from Qingdao Haiyang Chemical Co., Ltd. (Qingdao, Shandong, China).

Diethyl ether was redistilled at 50 °C for later use; anhydrous sodium sulfate was dried at 300 °C in a muffle furnace for 4 h and then kept cool in desiccators; double distilled water was prepared by ourselves.

Modified Dragendorff's reagent was as given by Kang *et al* in 2002.[16]

Materials

Dried maca root (*Lepidium meyenii* Walp., Cruciferae), kindly offered in April 2005 by Agro Naturales S.R.L. (Lima, Peru), was identified as *Lepidium meyenii* Walp. by the Biology Department Herbarium in the Universidad Nacional Agraria (Lima, Peru); commercial powder of dried maca root was purchased in April 2005 from Maca Biotechnology (Hubei) Co., Ltd. (Wuhan, Hubei, China), who purchased maca materials from Agro Naturales S.R.L.; fresh radish root (*Raphanus sativus* L., Cruciferae), oriental ginseng root (*Panax ginseng* C. A. Mey., Araliaceae) and American ginseng root (*Panax quinquefolium L.*, Araliaceae) were purchased in June 2005 from Wuhan (Hubei, China) supermarkets. All other plant specimens were authenticated in Wuhan Institute of Botany, Chinese Academy of Science (CAS).

Apparatus

VERTEX 70 FTIR spectrometer (Bruker Optics Inc., Ettlingen, Germany) with DTGS detector and OPUS workstation; Varian Saturn GC/MS (Palo Alto, CA, USA), consisting of a Varian 3900 GC with a split/splitless injector (GC capillary column DB-5, 30 m × 0.25 mm i.d., 0.25 μm film thickness), Varian 2100T ion trap mass spectrometer; Saturn GC/MS Workstation data system on a desktop PC.

Sample preparation

Preparation of dried sample powders

Fresh root samples of radish, oriental ginseng and American ginseng were washed with H_2O and evaporated for 12 h at 40 °C under vacuum (humidity 8–10%). These dried roots and dried maca root were chopped and milled, then were sieved (including the commercial powder of dried maca root) through a 75 μm mesh to obtain the minus sieve. The powders were evaporated for 4 h at 40 °C under vacuum (humidity 8%), sealed and kept in desiccators for later use.

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Preparation of alkaloid extracts

A 5.00 g portion of powdered maca root or other four powdered samples were extracted three times with 25 mL 95% EtOH (containing 0.5% HCl) for 30 min by sonication at room temperature. The extractive solutions were concentrated to dryness under reduced pressure at 40 °C, and then the resultant extracts were dissolved in 10 mL 2% HCl, decolourised with 0.1 g activated charcoal, centrifuged at 8000 × *g* for 10 min. The supernatants were adjusted to pH 12 with 14.3 mol L^{-1} NaOH solution, successively, extracted with $CHCl_3$ (3 × 15 mL). The $CHCl_3$ layers were dehydrated with 5 g anhydrous Na_2SO_4, concentrated and brought to a volume of 1.0 mL with $CHCl_3$ as the alkaloid extract.

Preparation of essential oil extracts

A 5.00 g portion of powdered maca root or other four powdered samples were extracted by steam distillation as previously described,[17] and the essential oils in the distillate were extracted three times with redistilled diethyl ether (3 × 50 mL). The diethyl ether layers were then dehydrated with 5 g anhydrous Na_2SO_4, concentrated and the volume brought to 1.0 mL with diethyl ether as the essential oil extract.

Analytical methods

FTIR spectroscopy characterisation

Absorption FTIR spectra of each powdered sample, dried to constant weight in an infrared dryer, were obtained using the KBr disk technique, namely 3 mg powdered sample was mixed with 200 mg KBr powder and pressed into a KBr disk for FTIR scan. Subsequently, absorption FTIR spectra of each liquid alkaloid extract sample was obtained by the liquid coating technique, namely 0.05 mL liquid sample was dropped onto a KBr window and the solvent was evaporated off in the infrared dryer to give a thin film for FTIR scan. The spectra were recorded in the 4000–400 cm^{-1} range with a 30-scan data accumulation at a resolution of 4 cm^{-1}. The results are presented in Figs 2–4.

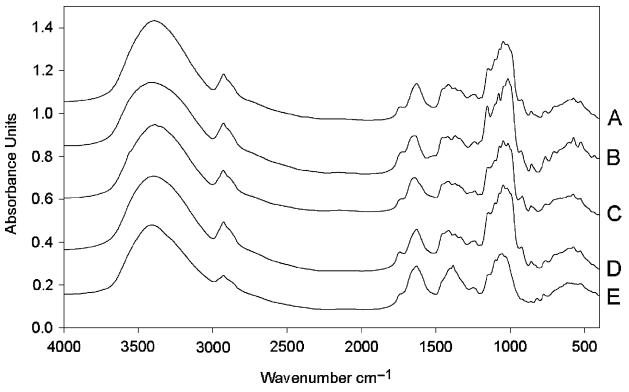

Figure 2. The FTIR spectrogram of different powdered samples. (A) Powdered maca root; (B) commercial maca powder; (C) powdered radish root; (D) powdered oriental ginseng root; (E) powdered American ginseng root.

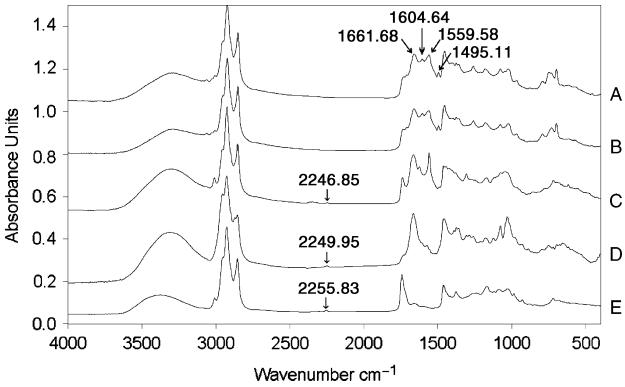

Figure 3. The FTIR spectrogram of different alkaloid extract samples. (A) Powdered maca root; (B) commercial maca powder; (C) powdered radish root; (D) powdered oriental ginseng root; (E) powdered American ginseng root.

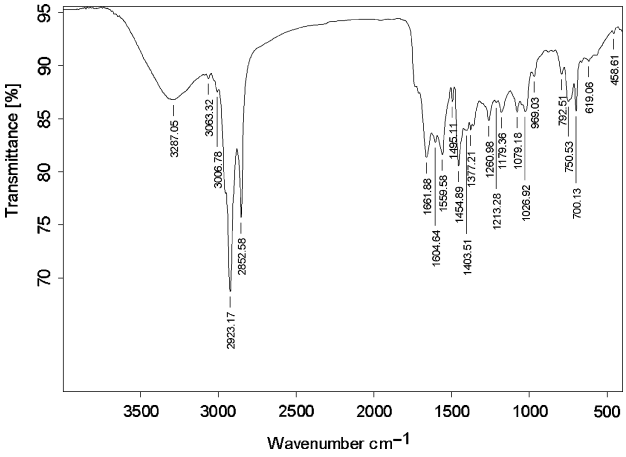

Figure 4. The FTIR spectrum of alkaloid extract of powdered maca root (Fig. 3A) marked with detailed peak positions data.

TLC characterization

To separate the alkaloid constituents in each alkaloid extract samples, TLC was carried out on 20 × 20 cm^2 glass plates covered with a 0.25 mm layer of silica gel. The plate was scored with 2 cm lanes, heated at 105 °C for 1 h before use, and allowed to cool to room temperature in desiccators before spotting with 10 μL alkaloid extract solutions. The chromatogram was developed over a distance of 15 cm in a glass TLC chamber of 25 × 25 × 10 cm in size at 30 °C. After development using acetone/methanol/water/ethene diamine (10:40:25:1) as a solvent system, the TLC plate was sprayed with Dragendorff's modification reagent. The alkaloid-positive spots in chromatogram were recorded by digital camera (Sony717, Tokyo, Japan). The results are presented in Fig. 5. Furthermore, preparative TLC was used to scrap out the main spots of alkaloid extracts from powdered maca root by the above-mentioned method. After extracting the alkaloids in the main spots with methanol, liquid chromatography–mass spectrometry (LC–MS) analysis was carried out for supplemental information. (We plan to describe the detailed methods and results in another paper.)

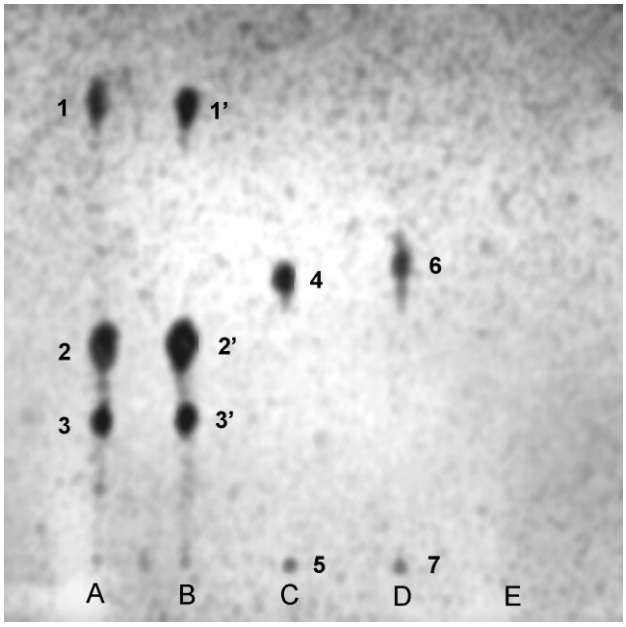

Figure 5. The TLC chromatogram of different alkaloid extract samples obtained by spraying with Dragendorff's modification reagent to visualize the various components. (A) powdered maca root; (B) commercial maca powder; (C) powdered radish root; (D) powdered oriental ginseng root; (E) powdered American ginseng root.

GC chromatography and GC/MS characterisation

Firstly, each essential oil sample was analysed by GC (Varian 3900 GC). A 5 μL sample was injected without splitting onto the capillary column using helium (He) as carrier gas (1.2 mL min^{-1}). A programmed temperature run was applied with initial column temperature of 35 °C for 2 min hold, raised to 215 °C at a rate of 15 °C min^{-1} for 16 min final temperature hold. The injector temperature was 220 °C and flame ionization detector (FID) temperature 250 °C with the 1:50 split ratio. The % relative area (%RA) of individual components of the oil are expressed as % peak area relative to total peak area (TA). Secondly, the main fraction in the essential oil of powdered maca root was analysed by gas chromatography–mass spectrometry (GC/MS). The MS (electron impact ionisation) conditions were: ionisation energy, 70 eV; mass range, m/z = 33–400 u; scan rate, 2 scans s^{-1}; electron multiplier voltage, 2.4 kV. Injector temperature was 200 °C and transfer line temperature 260 °C. Identification of the main components was performed by a comparison of the individual peaks with Wiley computer mass library, and by a comparison of their relative retention times with those of the authentic compounds. The results are presented in Figs 6 and 7.

RESULTS

FTIR results

As shown in Figs 2 and 3, the samples of different plant species have their own special characteristics. However, the difference in Fig. 3 is much more obvious than in Fig. 2. The reason is that the difference in Fig. 2 originates from the total chemical components of the plant species under study. Nevertheless, the main chemical components are nearly the same in those plants. Although the functional ingredients may vary greatly, they are most probably disguised by those main components in the FTIR spectrum because of their small proportions in the chemical composition.

We recommend that the functional ingredients (alkaloids) be extracted first, prior to subjecting them to FTIR identification. It can be easily seen in Fig. 3 that samples A and B from the same plant species have very close FTIR spectrum characteristics, such as peak positions, peak height and spike, both in functional group region (4000–1330 cm^{-1}) and fingerprint region (below 1330 cm^{-1}). The functional ingredient FTIR spectra of samples C, D and E show great differences from sample A or B, indicating different compositions of the ingredients of each alkaloid extracts. For instance, Fig. 3 shows clearly that the small peaks (marked by arrows on curves C, D and E) at 2246.85 cm^{-1}, 2249.95 cm^{-1} and 2255.83 cm^{-1} may be assigned to nitrile C=N stretching (2260–2240 cm^{-1}). These characteristics are not very noticeable in spectrum A and B of maca, however, because no C=N group is present in the currently known maca alkaloid structures. Furthermore, the special structure macamides or lepidilines possesses, such as the secondary amide group ($-CH_2-NH-CO-CH_2-$), conjugated carbon–carbon double bond ($-C=C-C=C-$) and phenyl group show many characteristic peaks at 1661.68 cm^{-1}, 1604.64 cm^{-1}, 1559.58 cm^{-1} and 1495.11 cm^{-1} in Fig. 3 (marked by arrows on curve A) and Fig. 4. In addition, a mass of characteristic peaks in the fingerprint region can serve as a fingerprint for identification of a given sample. Consequently, the FTIR spectrum of the alkaloid extract of powdered maca root is an efficient and exact method for the identification of maca root and its commercial powders. To our knowledge, this is the first report of FTIR identification of maca.

TLC results

The TLC chromatograms of the alkaloid extracts of different samples show that maca has more types of alkaloids than the others and of a higher content. As shown in Fig. 5 and Table 1, the difference between maca and other plant species is clear in the number of saffron yellow spots visualised by Dragendorff's modification reagent and the R_F values.

In addition, the alkaloids corresponding the spots 2 and 3 in Fig. 5 was accumulated gradually by several preparative TLC operations, extracted with methanol and further determined by LC–MS. Two known imidazole alkaloids (lepidiline A and lepidiline B; Fig. 1), and one unreported homologic imidazole alkaloid, lepidiline C, were confirmed.

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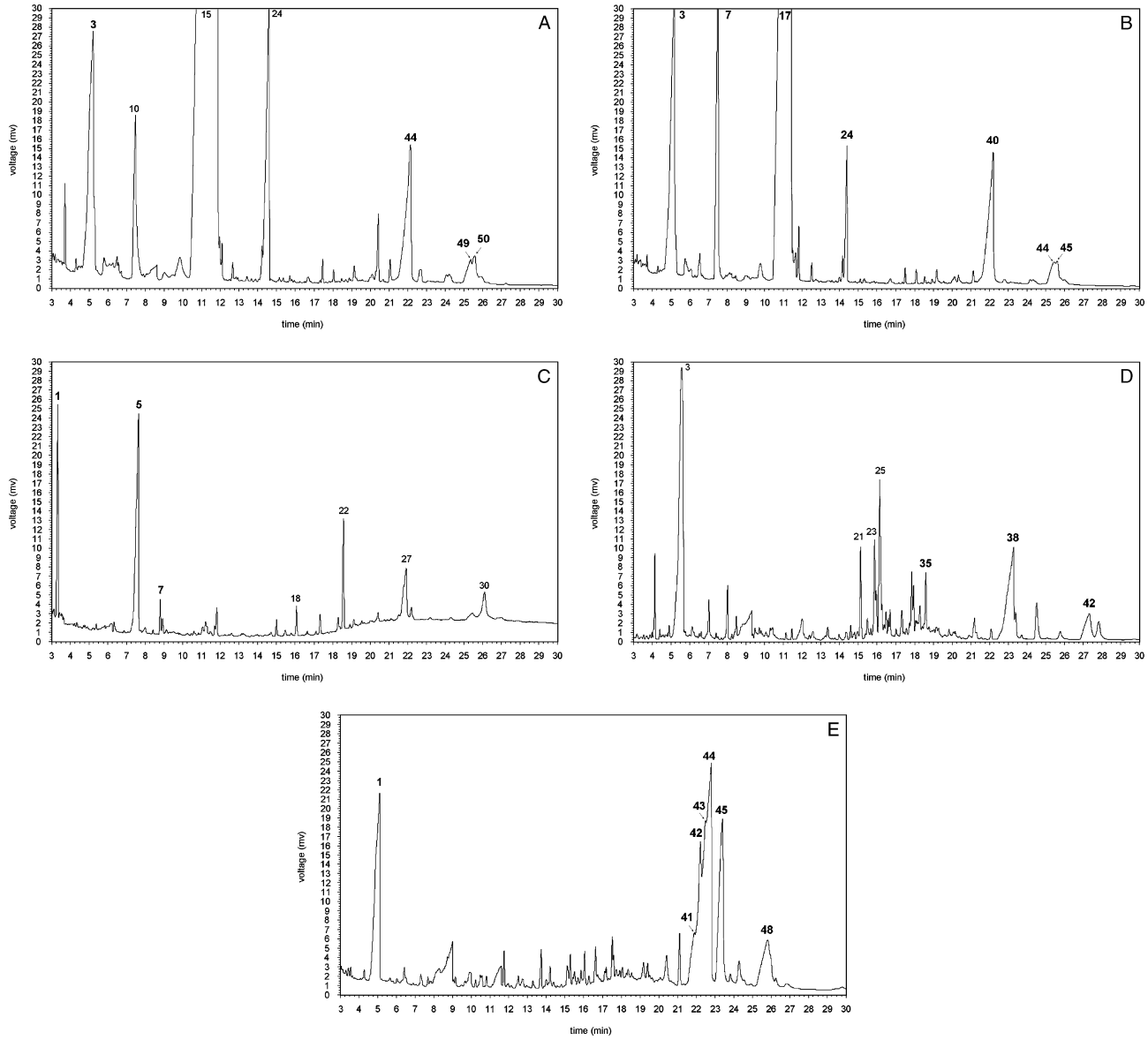

Figure 6. The GC chromatograms of different essential oil samples. (A) powdered maca root; (B) commercial maca powder; (C) powdered radish root; (D) powdered oriental ginseng root; (E) powdered American ginseng root.

Table 1. TLC results of different alkaloid extract samples visualised by Dragendorff's modification reagent

Sample	RF ($\overline{X} \pm$ SD)	Description of spots
A: Powdered maca root	R_F 1 = 0.822 ± 0.012	SY; O
	R_F 2 = 0.386 ± 0.013	SY; VO
	R_F 3 = 0.250 ± 0.011	SY; VO
B: Commercial maca powder	R_F 1′ = 0.820 ± 0.011	SY; O
	R_F 2′ = 0.391 ± 0.010	SY; VO
	R_F 3′ = 0.251 ± 0.011	SY; VO
C: Powdered radish root	R_F 4 = 0.512 ± 0.010	SY; VO
	R_F 5 = 0.003 ± 0.001	SY; UO
D: Powdered oriental ginseng root	R_F 6 = 0.540 ± 0.017	SY; O
	R_F 7 = 0.002 ± 0.001	SY; UO
E: Powdered American ginseng root	None	

VO, very obvious; O, obvious; UO, unobvious; SY, saffron yellow.

As a commonly accepted means for obtaining the physico-chemical properties of given plant materials, TLC is very simple and cost effective, and it is particularly valuable in the identification of maca when experimental conditions are limited.

Gas chromatography results

In Fig. 6 and Table 2 it can be seen that samples A and B, both of which are distilled from maca materials, are very similar in essential oils content (compared by the total peak area), retention time, peak size and shape of the main components in the oils, while other essential oil samples have remarkable variations in the gas chromatograms. We find that the peaks at retention time (RT) 11.862 min (identified by GC/MS as phenylacetonitrile, a decomposed product of glucosinolates in maca, accounting for over 70% of the total peak area), RT 5.208 min (benzaldehyde), RT 22.147 min (3-methoxyphenylacetonitrile) are the

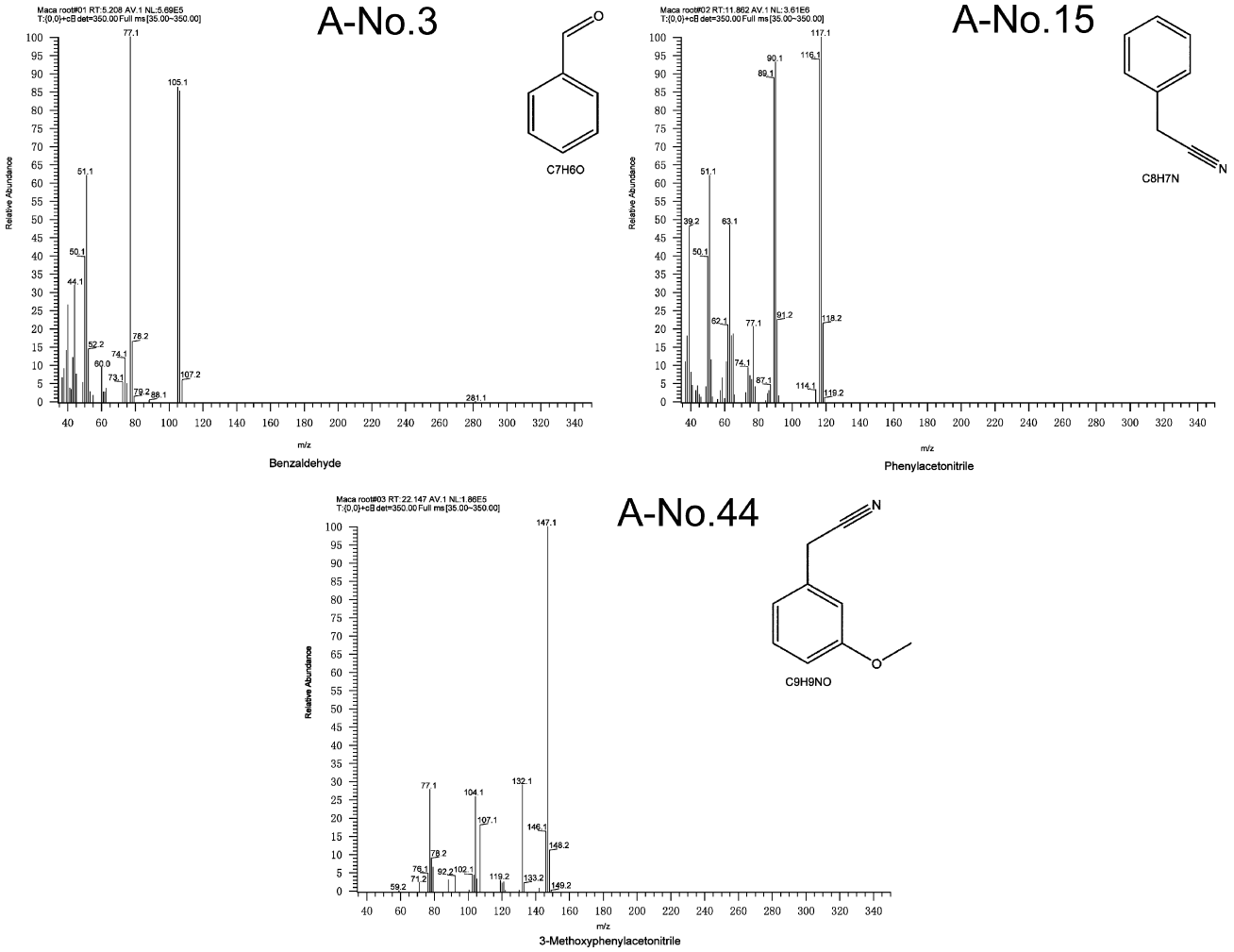

Figure 7. The MS results of the most main components in the essential oils of powdered maca root (A-No.3: benzaldehyde, RT 5.208 min; A-No.15: phenylacetonitrile, RT 11.862 min; A-No.44: 3-methoxyphenylacetonitrile, RT 22.147 min).

most significant characteristics in maca essential oils (GC/MS result see Fig. 7, which provides the MS spectra and corresponding chemical structures of those compounds). In particular, the uppermost peak at RT 11.862 min is not seen in any of the other materials, which may be the most important distinction.

The above-mentioned main components were also observed in recent GC/MS study on the essential oils of the aerial parts of maca,[10] which may suggest that the essential oil components in maca samples are stable. Furthermore, Table 2 lists the RTs and RAs of each sample for more detailed comparison. The most remarkable peaks (seven peaks) are marked by a superscript c behind their RT data, and it is found that two kinds of maca materials have nearly the same characteristics in both the RT and RA of the seven peaks. GC of maca essential oils is very convenient and accurate, and thus complementary to the identification of maca by FTIR and TLC.

DISCUSSION

In this study, the powdered maca root and commercial maca powder can be exactly identified by the FTIR and TLC analysis of its alkaloid extract and/or the GC/MS analysis of its essential oil extract. Besides the general characteristics of the spectra or chromatograms, the alkaloid extracts clearly show the secondary amide group, conjugated carbon–carbon double bond and phenyl group in FTIR. There are more types of alkaloid (several imidazole alkaloids mostly) in maca as indicated by those spots in TLC visualised by Dragendorff's modification reagent. The GC and GC/MS analysis of the essential oils of maca indicate that phenylacetonitrile, benzaldehyde, 3-methoxyphenylacetonitrile are the main components in the oils. The analytical techniques can be applied to identify maca and its processed powder (especially commercial powder of dried maca root) even without the standard samples of its unique chemical profiles and the complicated separation process.

The spectroscopic and chromatographic analytical techniques mentioned above cannot be used to assess the quality of maca material, because they had not utilized the in-depth research on the quantitative analysis of the principal functional ingredients of maca, which had been realized in HPLC method.[5] However, if further improved, for example, by acquiring more FTIR and/or GC/MS data for various grade of maca; combining FTIR spectroscopy with soft independent

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Table 2. Gas chromatography results of the different essential oil samples

	A: Maca root		B: Maca root		C: Red radish root		D: Oriental ginseng		E: American ginseng root	
Number	RT	RA	RT	RA	RT	RA	RT	RA	RT	RA
1	3.705	0.25	3.727	0.10	3.327[c]	13.15	4.135	1.61	5.077[c]	13.26
2	4.285	0.06	4.304	0.05	5.378	0.37	4.892	0.26	6.435	0.39
3	5.208[c]	4.76	5.193[c]	9.11	6.173	1.46	5.572[c]	29.74	7.313	0.25
4	5.787	0.20	5.757	0.38	6.330	0.88	6.127	0.65	8.148	0.94
5	6.073	0.16	6.048	0.13	7.635[c]	33.89	8.020	1.49	8.252	0.90
6	6.267	0.15	6.540	0.30	7.992	0.74	8.483	0.75	8.763	2.47
7	6.487	0.15	7.510[c]	5.13	8.803[c]	2.16	8.722	0.48	8.950	1.73
8	6.545	0.09	7.933	0.04	8.927	0.92	8.812	0.46	9.920	0.76
9	6.712	0.04	8.138	0.13	9.125	0.58	8.902	0.67	10.500	0.38
10	7.462[c]	1.88	8.245	0.06	11.050	0.90	9.135	1.31	10.828	0.19
11	8.608	0.34	8.370	0.03	11.218	1.44	9.238	0.95	11.545	1.55
12	9.007	0.12	8.460	0.04	11.462	0.46	9.308	0.88	11.772	0.58
13	9.435	0.08	8.968	0.04	11.707	0.62	9.482	0.45	12.527	0.33
14	9.837	0.54	9.033	0.06	11.817	1.86	10.298	0.44	12.745	0.30
15	11.862[c]	81.04	9.137	0.02	14.705	0.34	10.413	0.54	13.308	0.22
16	11.947	0.09	9.770	0.36	15.000	1.11	11.457	0.24	13.738	0.63
17	12.088	0.15	11.428[c]	72.46	15.468	0.52	11.990	1.78	14.222	0.35
18	12.663	0.09	11.497	0.29	16.072[c]	1.88	12.583	0.52	15.143	0.65
19	12.838	0.03	11.653	0.35	16.642	0.36	13.368	0.47	15.303	0.51
20	13.417	0.03	11.825	0.41	17.328	1.37	14.592	0.45	15.445	0.19
21	13.660	t	12.515	0.14	18.290	0.93	15.127[c]	2.79	15.522	0.32
22	13.868	t	13.992	0.04	18.580[c]	7.92	15.483	0.97	15.720	0.24
23	14.222	0.18	14.167	0.23	18.920	0.26	15.865[c]	3.45	15.873	0.33
24	14.607[c]	3.56	14.402[c]	1.59	19.132	0.64	15.950	1.70	16.070	0.66
25	15.143	0.02	15.108	0.03	20.422	0.94	16.133[c]	6.39	16.308	0.15
26	15.350	t	15.327	0.05	21.523	0.75	16.258	0.99	16.647	0.85
27	15.707	0.03	16.725	0.09	21.928[c]	10.98	16.477	0.87	17.147	0.14
28	15.818	t	17.357	t	22.195	1.52	16.600	0.69	17.218	0.20
29	15.942	t	17.503	0.12	25.447	2.47	16.693	1.10	17.538	0.54
30	16.655	0.02	18.100	0.12	26.107[c]	8.57	17.318	1.37	17.613	0.29
31	16.712	0.03	18.548	0.06	–	–	17.737	0.64	17.755	0.12
32	17.462	0.09	18.945	0.04	–	–	17.853	2.29	17.947	0.10
33	18.042	0.05	19.188	0.16	–	–	17.942	1.49	18.077	0.15
34	18.467	t	19.317	0.03	–	–	18.293	1.30	18.302	0.10
35	18.688	t	19.565	0.02	–	–	18.600[c]	2.80	18.393	0.19
36	18.870	t	20.033	0.05	–	–	21.198	1.06	18.583	0.09
37	18.957	t	20.140	0.13	–	–	22.088	0.43	19.205	0.56
38	19.150	0.11	20.348	0.14	–	–	23.287[c]	14.64	19.428	0.38
39	19.972	0.03	21.138	0.19	–	–	23.392	1.31	20.448	1.38
40	20.100	0.09	22.197[c]	5.11	–	–	24.515	2.46	21.152	0.95
41	20.300	0.06	22.818	0.15	–	–	25.777	0.89	21.978[c]	5.29
42	20.428	0.38	24.172	0.10	–	–	27.347[c]	4.32	22.267[c]	10.09
43	21.058	0.15	24.327	0.16	–	–	27.835	1.82	22.548[c]	12.13
44	22.147[c]	3.21	25.450[c]	0.95	–	–	–	–	22.792[c]	12.68
45	22.662	0.11	25.627[c]	0.62	–	–	–	–	23.412[c]	11.23
46	22.712	0.08	25.992	0.15	–	–	–	–	23.852	0.73
47	24.062	0.10	–	–	–	–	–	–	24.338	1.58
48	24.205	0.15	–	–	–	–	–	–	25.882[c]	8.53
49	25.427[c]	0.56	–	–	–	–	–	–	26.335	1.08
50	25.567[c]	0.42	–	–	–	–	–	–	26.842	0.94
51	25.877	0.14	–	–	–	–	–	–	–	–
tA	9 680 746.234		5 326 387.403		667 122.983		1 524 337.227		2 058 242.318	

RT = retention time (min) on a DB-5 column; RA = % of relative area (peak area relative to total peak area, %); t = trace (RA < 0.02%). TA = total peak area in mm^2.

[c] characteristic peak.

modelling of class analogy (SIMCA) clustering analysis method;[18] analysing the developed TLC plates with thin-layer chromatography scan (TLCS) method continuously;[19] and assessing the similarity of GC fingerprints artificially or automatically with computer,[20] they can be applied to the quantitative analysis and quality appraisal of the raw material and products of maca. This is of great significance in

future studies on the quality control, transplant and deep process of maca, for example.

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