Molecular characteristics of extractives of *Hylocereus undulates* stems

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Abstract: As one of traditional and dominant species of famous fruits in South China, *Hylocereus undulates* is considered as the important fruit resources, however, the constituent properties of *Hylocereus undulates* stems have not been known. Therefore, the molecular characteristics of extracts from *Hylocereus undulates* stems are studied to further utilize the resources. The result showed that there were many toxic substances in the extracts of *Hylocereus undulates* stems, suggesting that *Hylocereus undulates* stems should not be used as food. FTIR spectra showed that there were many active groups in the extracts of *Hylocereus undulates* stems, suggesting that *Hylocereus undulates* stems contain many bioactive substances. The result indicated that the extracts of *H. undulates* stems have huge potential resources.

Keywords: Plant extractives, *Hylocereus undulates* stems, GC-MS, FTIR.

INTRODUCTION

Hylocereus undulates, is a very famous fruit in many countries. It was widely planted in Hainan, Guangxi, Guangdong, Fujian, Yunnan and other provinces of south China (Masyahit *et al.,* 2009). There are rich nutrition and unique features in *H. undulates* fruit which contain vegetative albumin, anthocyanidin, rich vitamin and water-solubility fiber. *H. undulates* fruit, which taste sweet and has a high nutritional value, carry a certain curative effect fruit health-care nutrient food for rich vitamin B1, B2, B3 and vitamin C, carotene, anthocyanin, calcium, phosphorus, iron and soluble dietary fiber. However, it could prevent constipation, promote eye health, increase bone density, promote cell membrane growth, whiten skin, and so on (Tel-zur *et al.,* 2004; Lichtenzveig *et al.,* 2000). For example, Anthocyanins, the widely distributed plant polyphenols, were one class of flavonoid compounds, and were antioxidant flavonoids that protected many body systems (Tsuda *et al.,* 2000; Meiers *et al.,* 2001). So, *H. undulates* fruit was further studied to promote the utilization.

Now more and more farmers in China plan to cultivate *H. undulates* wide areas. The cultivated area has been more than 100000 mu. After *H. undulates* fruit was harvested, many stems were abandoned by a large number. However, only fruits of *H. undulates* had been utilized as bioactive resources (Hongchen *et al.,* 2012; Wanxi *et al.,* 2013a; 2013b; 2013c; 2013d; Le *et al.,* 2014). Plant extracts consist of a large variety of low molecular mass compounds, and plant extracts could have many bioactive effects (Hongchen *et al.,* 2012; Zhu-Ping *et al.,* 2013; Dongli *et al.,* 2014; Wanxi *et al.,* 2013a; 2013b; 2013c; 2013d; 2012a; 2014c). To further utilize *H. undulates* stems as biomedical resources, the molecular characteristics of plant extracts were investigated and analyzed by optimized extracting techniques.

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MATERIALS AND METHODS

Materials

Fresh *H. undulates* stems were collected from the Forest Farm of Central South University of Forestry and Technology, P. R. China. The fresh stems were crushed to pieces and kept in vacuum. Benzene, methanol, petroleum ether, acetic ether and ethanol (chromatographic grade) were prepared for the subsequent experiments. Cotton bag and cotton thread were all extracted in benzene/ethanol solution for 12 h. The benzene-ethanol solution was mixed according to $V_{ethanol}$ /$V_{benzene}$ 2 double.

Experiment methods

Single extraction

Weighed 2 pieces of stems, each was about 20g (0.1mg accuracy) and finally parceled by using the cotton bag and tied by using cotton thread, and signed. Extraction was carried out in 350ml solvents by the Foss method for 6 hours. Solvents were methanol and benzene/ethanol solution ($V_{ethanol}$ /$V_{benzene}$=2), respectively. Methanol extraction and benzene/ethanol extraction were done under the condition of 75°C and 95°C, respectively. After extraction, the stems extracts were obtained by evaporation in 60°C -70°C air.

Sequential extraction

Weighed 3 pieces of stems, each of 20g (1.0mg accuracy), and finally parceled by using the cotton bag and tied by using cotton thread, and signed. Extraction was carried out by large-caliber Soxhlet according to different orders combined by methanol → benzene/ ethanol → petroleum ether/acetic ether (SE-petroleum ether/acetic ether), petroleum ether/acetic ether → methanol → benzene/ ethanol(SE- benzene/ ethanol), benzene/ ethanol → petroleum ether/acetic ether → methanol(SE- methanol), respectively. After extraction, the stems extracts were obtained by evaporation in 60°C -70°C air.

GC/MS condition

Each 0.5 mg stems extracts was analyzed by online linked GC/MS (gas chromatograph/ mass spectrometer), respectively. The GC/MS analysis was the same or similar as documental literature (Wanxi *et al.,* 2011; 2012a; 2012b; 2012c; 2013a; 2013b; 2013c; 2013d; Dongli *et al.,* 2014).

FTIR analysis

The extracts samples were recorded on a Thermo Nicolet FT-IR spectrometer (Thermo Fisher Nicolet, 670FT-IR). Thirty-two scans were collected per sample at a spectral resolution of 4 cm^{-1} and the collected spectra were normalized against air. The spectral range was from 4000 to 500 cm^{-1}(Zhu-Ping *et al.,* 2013; Lansheng *et al.,* 2013a; 2013b; Yong-Chang *et al.,* 2014; Ling-Ping *et al.,* 2014; Qiu *et al.,* 2014; Wanxi *et al.,* 2014a; 2014b; 2014c).

RESULTS

During five solvent extractives (methanol, benzene/alcohol, SE-petroleum ether/acetic ether, SE-benzene/ethanol, SE-methanol) were obtained respectively. The total ion chromatograms of three solvent extractives by GC/MS were shown in fig. 1, respectively. The FTIR spectra of extractives of *H. undulates* stems are shown in fig. 2.

ANALYSES

Molecular properties of H. undulates stems extractives

Analyzing the MS data, the NIST standard MS map by computer, open-published books and papers, than components and their contents were identified. Relative content of each component was counted by area normalization.

According to GC/MS result, 1 component was identified from methanol extracts (LD-101) of *H. undulates* stems. The result showed that the main components were benzene.

According to GC/MS result, 7 components were identified from benzene/ethanol extracts (LD-103) of *H. undulates* stems. The result showed that the main components were malic acid (9.581%), dibutyl phthalate (20.429%), 7-pentadecyne (11.638%), tricyclo[6.3.3.0] tetradec- 4-ene,10,13-dioxo- (24.03%), 1,2-benzenedicar - boxylic acid, mono(2-ethylhexyl) ester (3.227%), 1,2-benzisothiazol-3-amine tbdms (4.849%), benzo[h] quinoline, 2,4-dimethyl- (26.246%).

According to GC/MS result, 3 components were identified from SE-benzene/ethanol extracts (LD-107) of *H. undulates* stems. The result showed that the main components were benzene (95.702%), dibutyl phthalate (2.894%), 1,2-benzenedicarboxylic acid, mono(2-ethylhexyl) ester (1.404%).

According to GC/MS result, 2 components were identified from SE-benzene/ethanol extracts (LD-108) of *H. undulates* stems. The result showed that the main components were dibutyl phthalate(23.473%), 2,4,6-cycloheptatrien-1-one, 3,5-bis-trimethylsilyl- (76.527%).

According to GC/MS, 1 component was identified from SE-methanol extracts (LD-109) of *H. sundulates* stems. The result showed that the main components were benzene.

Group properties of H. undulates stems extractives

The spectra in fig.2 were assigned as follows, the signals observed at 3417 cm^{-1} related to -OH stretching vibrations. The peaks at 1720–1740 cm^{-1}, 1460 cm^{-1}, 1370 cm^{-1}, 1235 cm^{-1}, 1205 cm^{-1}, 1160 cm^{-1}, 1050 cm^{-1}, and 1030 cm^{-1} were the C=O stretching vibration of acetyl xylan, C-H bending vibration of chitosan, CO-OR stretching vibration of acetyl xylan, O-H in-plane bending vibration of hemicelluloses, C-O-C stretching vibration of hemicelluloses, C-O stretching vibration of acetyl xylan, and the C-O stretching vibration of hemicelluloses, respectively. The absorbance of peaks at 3440-3372 cm^{-1} increased from 0.165 to 0.575 in LD-108 extracts. The peak at 2850 cm^{-1} appeared only in LD-109 extracts and LD-107 extracts, and the peak at 1600 cm^{-1} did not appear only in the two extracts. The absorbance of peaks at 1725–1736 cm^{-1} reduced from 0.775 to 0.535 in LD-109 extracts, the absorbance of peaks at 1462 cm^{-1} and 1380 cm^{-1} were only in LD-107 extracts, the absorbance of peaks at 1400-1407 cm^{-1} increased from 0.621 to 0.330 in LD-108 extracts, the absorbance of peaks at 1219-1283 cm^{-1} reduced from 0.642 to 0.387 in LD-107 extracts, the absorbance of peaks at 1103-1176 cm^{-1} reduced from 0.729 to 0.271 in LD-103 extracts, the absorbance of peaks at 1040-1072 cm^{-1} increased from 0.552 to 0.349 in LD-107 extracts and not in LD-101 extracts. The above five extracts contained different active groups.

Resource properties of H. undulates stems extractives

There were many biomedicine components in the extracts of *H. undulates* stems. Because of its officinal value, malic acid, which was commonly found in tart fruits and drinks, tasted sour. Dibutyl phthalate was a pesticide to keep internal environment homeostasis. 3,5-bis-trimethylsilyl-2,4,6-cycloheptatrien-1-one was a functionalized conjugated carbocyclic compound with diversified reactivity. However, there were some toxic substances to maintain the balance of *H. undulates* stems.

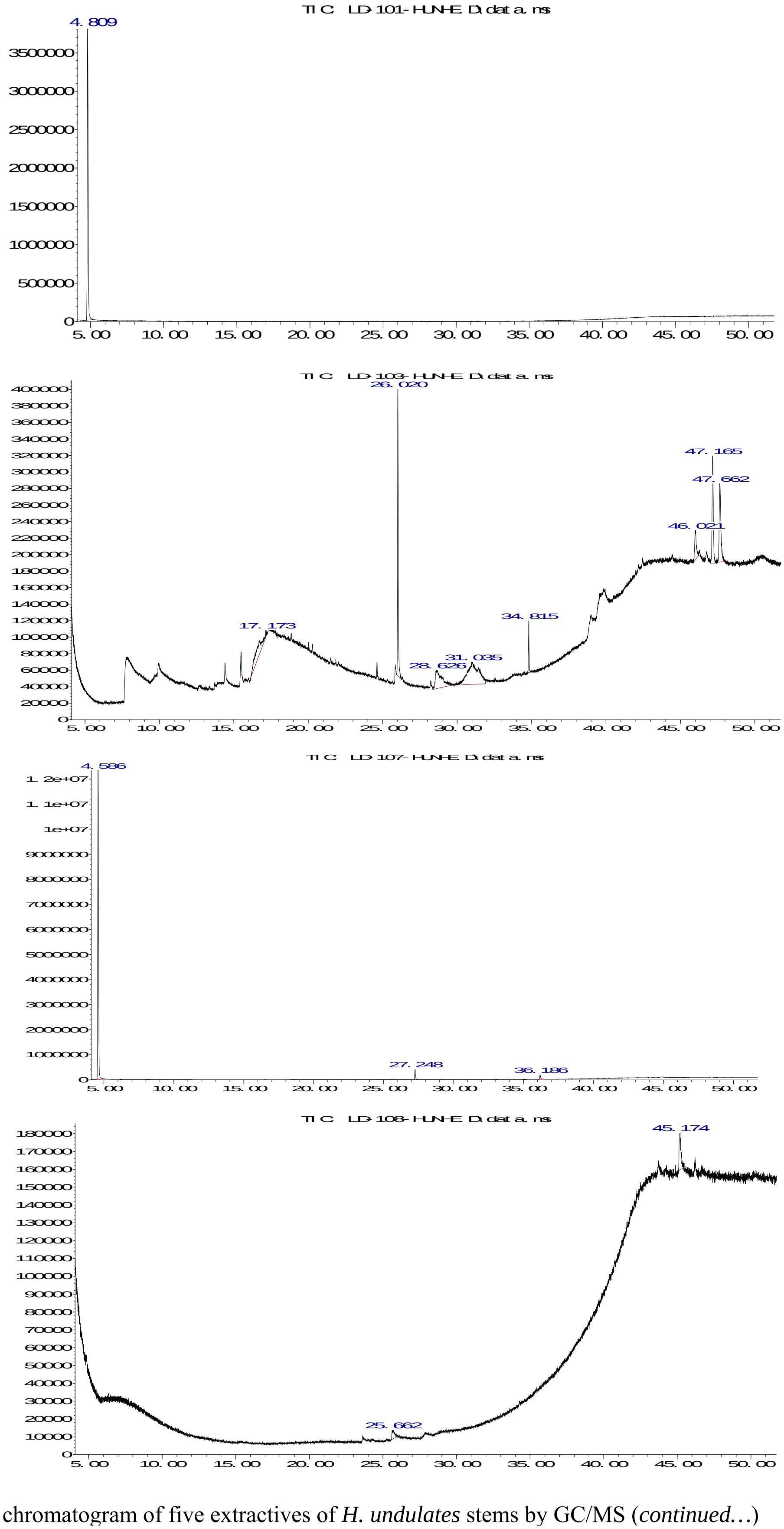

Fig. 1: Total ion chromatogram of five extractives of *H. undulates* stems by GC/MS (*continued…*)

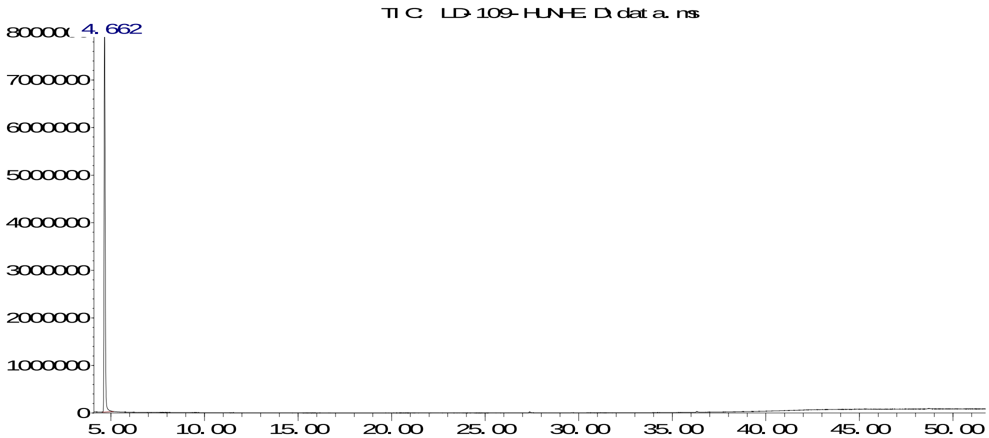

Fig. 1: Total ion chromatogram of five extractives of *H. undulates* stems by GC/MS

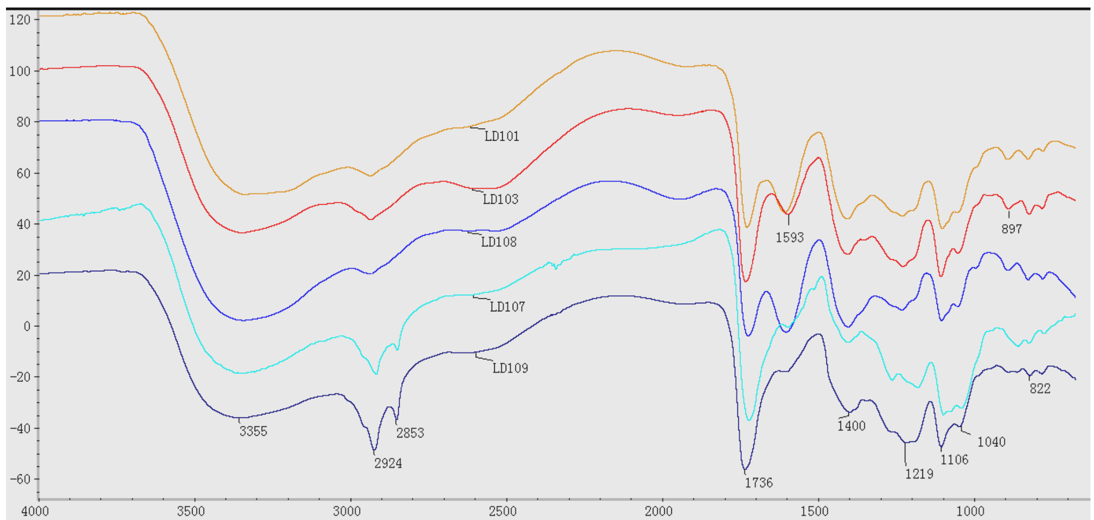

Fig. 2: Group characteristic of extractives of *H. undulates* Stems

CONCLUSION

The fruit of *H. undulates,* which was rich in nutrition and unique features, contained rare albumin anthocyanidin, rich vitamins and water-soluble dietary fiber. However, there were many toxic substances in the extracts of *H. undulates* stems, resulting that *H. undulates* stems should not be used as food. FTIR spectra showed there were much actives groups in the extracts of *H. undulates* stems, it implied that *H. undulates* stems contained many bioactive substances.

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