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Analysis of Polyphenols from Jujube Stone by HPLC

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Abstract. An analysis of polyphenols from jujube stone was carried out by High Performance Liquid Chromatography (HPLC) method. The chromatographic conditions were as follow: chromatographic column: Create C18(200×4.6mm); flow rate: 1.0mL/min; injection volume: 20μL; detection wavelength: 254nm dural-wavelength detection, simultaneously; mobile phase: 100% acetonitrile (A phase)-2% acetic acid ultrapure water solution (B phase), gradient elution system. The results showed that the composition of the polyphenol was very complicated in the test conditions, which showed that the polyphenols were composed of trans cinnamic acid, chlorogenic acid, 4-hydroxy benzoic acid, caffeic acid, catechin, gallic acid, etc.

Keywords: Jujube stone, polyphenol, HPLC.

1. Introduction

As a fruit of Jujube tree, a kind of Rhamnaceae Zizyphus Mill, jujube is originated in China. It is the country's unique species. Most of its processing and the leftovers jujube stone, the bioactive substances, however, were wasted away or burned off as fuel. As a result, the unreasonable use has caused a tremendous waste of resources [1]. Polyphenol, which widely exists in plant body, is a compound derived from shikimic acid pathway and phenylalanine metabolic pathway [2]. It has the aromatic ring structure combining with one or more hydroxyl. Plant polyphenols were of anti-oxidation [3], [4], anti-allergic effect, anti-mutagenesis and anti-radiation function, it can remove free radicals, inhibit cell toxin, etc [5], [6]. The interest in health effects of polyphenols has been exemplified by the 1st and 2nd "International Conference on Polyphenols and Health" held in France and America [7]. In a word, the research about the bioactivity of plant polyphenols has further revealed its glorious prospects [8]. This study aims to provide a new way for seeking bioactivity from natural plant materials. A preliminary research for polyphenol in jujube stone was investigated by HPLC method, using the leftovers of processing and after-eating-Chinese dates for test materials.

2. Materials and Methods

2.1. Materials and Reagents

Jujube stone was provided winter jujube nuclear of Henan Xinzheng. AB-8 macroporous absorbent resin were purchased from Chemical Plants of Nankai university. Acetonitrile (HPLC level) was purchased from the United States Fisher company. Acetic acid (HPLC level) was purchased from Tianjin Kemiou Chemical Reagent Co., Ltd. Chlorogenic acid, caffeic acid, ferulic acid and rutin, phloridzin, phloretin, trans cinnamic acid, gallic acid it, leather grain, 4 - hydroxy benzoic acid, - coumaric acid, (+) - catechin, (-) – epicatechin were purchased from the American Sigma Company.

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2.2. Instruments and Equipments

RE-52AA rotary evaporator was purchased from Shanghai Yarong Biochemical Instrument Factory. SZ - 93 automatic dual pure water distiller was purchased from Shanghai Yarong Biochemical Instrument Factory. FB - 10T solvent filter was purchased from Tianjin Auto Science Company. Liquid chromatography column,Create C18 5 μ m 200 x 4.6 mm was purchased from Dalian Kerui Company. Quaternion gradient liquid chromatographic system was purchased from the American DIONEX company. MA series of separation chromatography was purchased from Shanghai Huxi Analysis Instrument Factory. SHB -III circulating water type multifunction vacuum pumpwas purchased from Zhengzhou Great Wall trade co., LTD.

2.3. Sample preparation

Polyphenols was exact from jujube stone powder using 60% ethanol. Then the ethanol was recovered by rotary evaporator. The exacts was first adsorbed by AB-8 macro-porous resin, and eluted with 10%, 20%, 30%, 40%, 50%, 60% ethanol in sequence. The obtained different effluent was collected, concentrated and was filtered through 0.45 μm filtration membrane.

2.4. Condition and Method of Chromatographic analysis

Condition and Method of Chromatographic analysis is as follows: Chromatographic Column Create C18 (200 x 4.6 mm) chromatographic column; Velocity: 1.0 mL/min; Sample Volume (quantitative ring sample injection) : 20 μ L; Detection Wavelength: 254nm & 280nm dural-wavelength detection, simultaneously; Mobile Phase: 100% acetonitrile (A phase) - 2% acetic acid ultrapure water solution (B phase), gradient elution system. Grads eluting program are shown in Table 1.

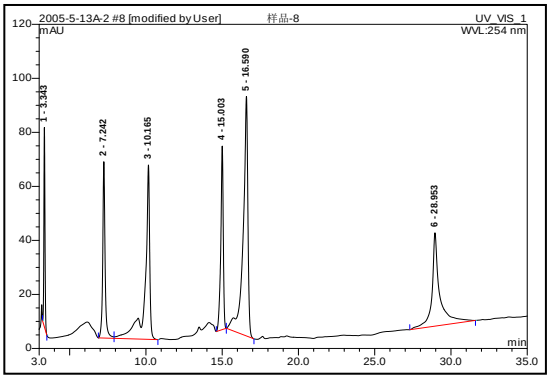
Table 1: Grads eluting program

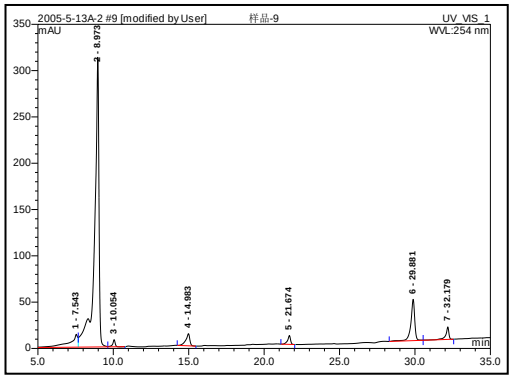
Time (min)	Acetoinitrile (%)	acetic acid (%)
0	10	90
25	30	70
35	50	50
40	10	90
45	10	90

3. Results

3.1. Analysis results of standards

In this study, 13 polyphenol standards were used and their elution profile are shown in fig 1.



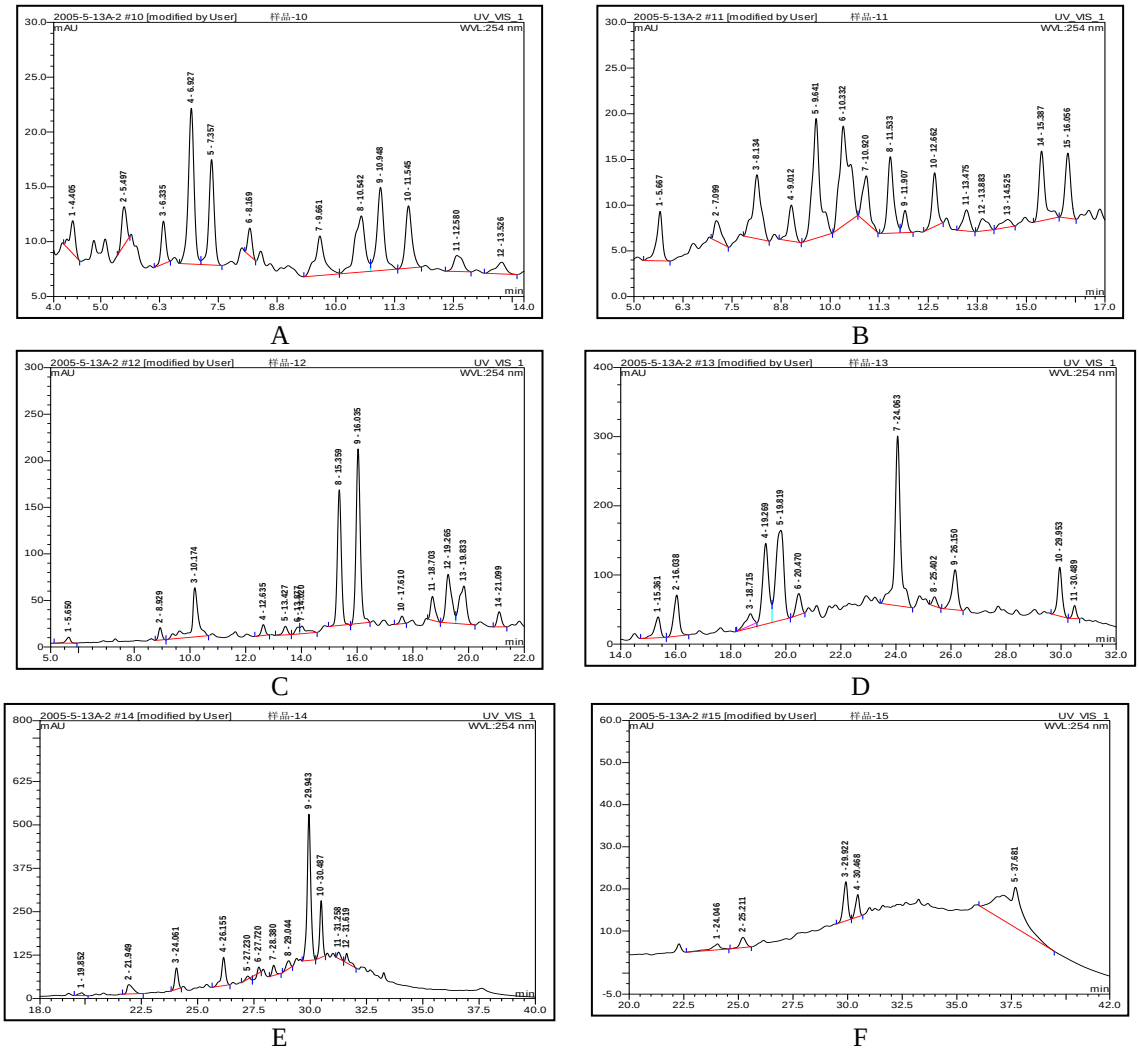


1.gallic acid,2.chlorogenic acid,3.caffeic acid,4.rutin,5. ferulic acid,6.quercetin（upper）
1.catechin,2.4-hydroxybenzoic acid,3. (-)-epicatechin,4. *p*-coumaric acid,5.phloridzin ,6.trans-cinnamic acid,7.phloretin（lower）

Fig. 1: Chromatography of mixed standards

3.2. Results of the polyphenol composition in ethanol gradient eluent

Crude polyphenol extractant was loaded onto column and eluted with solvent containing t 10%, 20%, 30%, 40%, 50%, and 60% ethanol.The elution profiles are shown in fig 2.



A.10% ethanol; B. 20% ethanol; C.3 0% ethanol; D. 40% ethanol;E. 50% ethanol;F. 60% ethanol

Fig. 2: HPLC chromatography of eluting solution containing different concentrations ethanol

Firstly, as is shown in figure 2 A and B, there are a lot of chromatographic peak, and the peaks are mostly within the retention time of 3 ~17min range. While there was no peak after 17min; Secondly, the peak in figure 2 C is concentrated in 5 ~ 25 min range, but it appeared inactive after 25 min; Finally, the peak in figure 2 D, E and F have appeared slowly. Basically, there is no peak before 14min, and it gradually delayed backward. What's more, in figure 2 F, the peak did not appear until the retention time reached 21min. These findings indicated that, with the increase of concentration of ethanol for gradient elution, the complexity of eluent composition had gradually decreased. The peakwas also lower as well. Furthermore, both retention time and peak height were gradually backwards, which reflected that in different concentration, there were significant difference in solubility of the adsorptive on resin column , and the polarity varied from each other, too.

4. conclusion

According to the HPLC analysis results, it can be seen that the composition of polyphenol in the jujube stone was very complicated. At present, with the preliminary analysis under test conditions of polyphenols standards, the polyphenols could be identified as trans cinnamic acid, chlorogenic acid, 4-hydroxy benzoic acid, caffeic acid, catechin, gallic acid, etc.In addition, the greater peak appeared at the retention time of 6.927 min, 7.357 min, 8.133 min, 15.359 min, 16.309 min, 24.063 min etc., and further research is needed to determine these specific substances.

5. References

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