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*Full Length Research Paper*

# Quality evaluation of different products derived from *Ganoderma*

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**Quality assessment of commercial products derived from *Lingzhi* was performed based on both their small (triterpenes) and macro (polysaccharides) molecular bioactive components by using high-performance thin-layer chromatography (HPTLC) and high-performance size-exclusion chromatography-evaporative light scattering detector (HPSEC-ELSD). Enzyme (α-amylase) digestion was employed for avoiding the interference, induced by starch added in formula preparation, on the analysis of polysaccharides during HPSEC-ELSD analysis. The results showed the quality variation among *Lingzhi* products were obvious. The developed method is simple, rapid, reliable and suitable for evaluation of quality of *Lingzhi* and their products.**

**Key words:** *Ganoderma*, polysaccharides, triterpenes, high-performance thin-layer chromatography (HPTLC), high-performance size-exclusion chromatography (HPSEC).

## INTRODUCTION

Mushrooms are important source of nutrient supplement as well as medicine (Wani et al., 2010). *Ganoderma*, also called *Lingzhi* (meaning “spirit plant”) in Chinese or *Reishi* in Japanese, is a genus of wood decay polypore fungus highly regarded in traditional Chinese medicine (TCM). Its usage can trace back to two millennium years ago. *Shen Nong Ben Cao Jing*, the earliest book on materia medica in the world, stated that *Ganoderma lucidum* can be used for enhancing “vital energy” and promoting “longevity” (Paterson, 2006; Halpern, 2007). Nowadays, two species of *Ganoderma*, *G. lucidum* and *Ganoderma sinense*, are officially considered as *Lingzhi* since 2000 edition of Chinese Pharmacopoeia. Generally, triterpenes and polysaccharides are extensively studied and considered as main bioactive ingredients in *Lingzhi*. Indeed, triterpenes and polysaccharides from *Lingzhi* have been shown their multiple pharmacological effects,including anti-oxidant (Smina et al., 2011), anti-tumor (Gao et al., 2011; Jedinak et al., 2011; Jiang et al., 2011), anti-platelet aggregation (Shimizu et al., 1985) and complement inhibitory (Seo et al., 2009) activities. Therefore, triterpenes were measured as marker for quality evaluation of *Lingzhi* (Su et al., 2001; Gao et al., 2004; Wang et al., 2006; Zhao et al., 2006; Fu et al., 2009; Zhao et al., 2009; Yan et al., 2010; Liu et al., 2011; Ding et al., 2010). Although chemical variation between *G. lucidum* (GL) and *G. sinense* (GS) have been reported (Zhao et al., 2006; Guan and Li, 2010), aqueous extracts of GL and GS were shown similar potency to increase lymphocyte growth (Yue et al., 2006). Therefore, how to evaluate the quality of *Lingzhi* should be carefully considered.

At present, *Lingzhi* is one of the most popular herbal products (Ulbricht et al., 2010), and a lot of commercial *Lingzhi* products such as herbal tea, capsule and powder can be found in the market. To date, there is no report for the quality evaluation of different products derived from *Lingzhi.* In this study, the quality of different commercial products derived from *Lingzhi* was evaluated based on

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**Table 1.** Summary of Lingzhi products.

| Sample no. | Dosage form | Manufacturer | Batch no. |
|---|---|---|---|
| P1 | Capsule | Good Harvestes | - |
| P2 | Capsule | Vita Green | HKNLV351 |
| P3 | Capsule | Wai Yuen Tong | 0509142 |
| P4 | Capsule | Peking Medicine | 26100 |
| P5 | Capsule | Chi Chun Tang | 40026 |
| P6 | Capsule | Nihon Vitamin | 0081 |
| P7 | Capsule | Good Harvestes | 7672758 |
| P8 | Capsule | Peking Medicine | 2041110 |
| P9 | Capsule | Nihon Vitamin | 10805 |
| P10 | Capsule | Peking Medicine | 26097 |
| P11 | Tablet | Dialian Merro | 20070401 |
| P12 | Capsule | Purapharm | A05557-03 |
| P13 | Capsule | Hunan Zhengqing | 0609203 |
| P14 | Capsule | Hang Ming Tong | 10400712 |
| P15 | Capsule | Boscogen | FH0581F |
| P16 | Capsule | Oriental Inter | 0407013 |
| P17 | Capsule | Nihon Vitamin | 05602 |

both triterpenes and polysaccharides by using high-performance thin-layer chromatography (HPTLC) and high-performance size-exclusion chromatography-evaporative light scattering detector (HPSEC-ELSD), respectively.

## MATERIALS AND METHODS

### Chemicals, reagents and materials

Ethyl acetate, *n*-butanol, chloroform, dichloromethane and petroleum ether were of analytical grades from UNI-CHEM d.o.c. (Belgrade, Serbia and Montenegro), while acetic acid and formic acid were obtained from Luoyang Chemical Reagent Factory (Luoyang, China). HPLC grade methanol and anhydrous ethanol (Merck, Darmstadt, Germany) were used for sample preparation. Ammonium acetate and sodium acetate from Riedel-de Haën were used for buffer solution. Deionized water was prepared by Millipore Milli Q-Plus system (Millipore, Billerica, MA, USA). Ganoderic acid A, $C_2$, F, H, ergosterol, α-amylase, phosphoric acid (85%), and ninhydrin (A.C.S. reagent) were purchased from Sigma–Aldrich (St Louis, MO, USA).

Different samples of *G. lucidum* (GL) and *G. sinense* (GS) were collected from nine provinces in China, that is, GL01, GL02-GL04, GL05, GL06-GL08 and GS01-GS04, GS05, GL09 and GS06, GS 07, GL10 and GS08, GS09 were from Zhejiang, Shandong, Guizhou, Anhui, Macao, Beijing, Guangxi, Hunan and Sichuan, respectively. *Lingzhi* products were purchased from local market (Macao), listed in Table 1. Voucher specimens were deposited at Institute of Chinese Medicinal Sciences, University of Macau, Macao, China.

### Preparation of standard or test solution

Standard mixture solution for HPTLC analysis was prepared by dissolving accurate amount of samples in methanol. The concentrations of Ganoderic acid A, Ganoderic acid $C_2$, Ganoderic acid F, Ganoderic acid H and ergosterol were 0.4, 0.9, 0.9, 1.1 and 0.4 mg/ml, respectively. Iodine-potassium iodide solution for detection of starch was prepared with 0.5 g of potassium iodide and 0.15 g iodide in 50 ml ethanol.

### Extraction of triterpenes

Powder of *Lingzhi* (1.0 g) were immersed in 20 ml ethanol and refluxed in a Syncore parallel reactor (Büchi, Switzerland) for 30 min at 78°C and stirred at 200 rpm. And then the extract solution was centrifuged at 4,000 rpm for 10 min (Allegra X-15R, Beckman Coulter, Fullerton, CA).

The supernatant was evaporated to dryness under vacuum using rotary evaporator. The residue was dissolved in 2 ml methanol. After filtering through a 0.45 µm nylon membrane filter (Whatman, Maidstone, U.K.), the extract was used for HPTLC analysis. Extraction of *Lingzhi* products (200 mg) were treated as aforementioned instead with 3 ml ethanol.

### Extraction of polysaccharides

Powder of *Lingzhi* products (1.0 g) were immersed with 30 ml of water and refluxed in a Syncore parallel reactor (Büchi, Switzerland) for 1 h at 100°C with stirring at 200 rpm. And then the extract solution was centrifuged at 4,500 rpm for 10 min. An aliquot of 20 ml supernatant was withdrawn and evaporated to dryness. The residue was dissolved in 5 ml water. Then ethanol was added to a final concentration of 80% (v/v) for precipitation. After staying for 1 h at 4°C, centrifugation (4,000 rpm for 10 min) was performed. The precipitate was evaporated to dryness on water bath (60°C), and the crude polysaccharides were finally obtained.

### Enzymatic digestion

Crude polysaccharides from *Lingzhi* products were dissolved in 5 ml hot water (60°C). After centrifugation, the supernatant was transferred to a 5 ml volumetric flask and brought up to the volume with water to get polysaccharide solution. Polysaccharides (100 µl

of the solution) were digested by the addition of equal volume of α-amylase (12.5 U/ml), 30 µl of sodium acetate buffer (1 M, pH = 7.0) and 70 µl of distilled water.

In addition, polysaccharide without addition of α-amylase and α-amylase without addition of polysaccharides handled in the same way were used as control. After degradation for 24 h on a water bath of 40°C, a small volume of digested polysaccharides solution was tested with iodine-potassium iodide solution. If blue color appeared, the same amount of α-amylase was added for further digestion until the color reaction was negative. Finally, the mixture was heated at 80°C for 30 min to terminate the digestion. After filtration through a 0.45 µm nylon membrane, solutions were used for HPSEC analysis.

#### TLC procedures

HPTLC was performed on plates coated with nano-silica gel 60 with fluorescent indicator UV 254 (Macherey-Nagel, Germany). Sample and standards were spotted on the plates as 8 mm bands by a Desaga (Germany) AS 30 sample applicator. The plate was developed with dichloromethane–ethyl acetate–petroleum ether-formic acid-ethanol 8:3:9:0.8:0.5 (v/v) with a distance of 95 mm, then imaged and photographed at 254 and 365 nm, respectively, before colorization with vanillin-$H_2SO_4$ solution (1 g vanillin dissolved in 100 ml 5% $H_2SO_4$ ethanol solution).

The colorization was performed at 110°C on a YOKO-XR plate heater (Wuhan YOKO technology Ltd., China) until spots colorized clearly; the plate was then covered with a transparent glass and photographed.

#### HPSEC-ELSD analysis

Polysaccharides or polysaccharides after enzymatic digestion were analyzed by using an Agilent 1100 series LC system (Agilent Technologies, Palo Alto, CA, USA) coupled with Alltech 2000 ES evaporate light scattering detection (ELSD, W. R. Grace & Co.-Conn. , USA) with modification of chromatographic conditions (Guan and Li, 2010).

In brief, a Tosoh TSK G-3000PWXL column (300 mm × 7.8 mm, i.d., 7 µm, Tosoh Bioscience, Tokyo, Japan) was used at 30°C with injection volume of 10 µl. Isocratic elution was operated with 20 mmol/L ammonium acetate aqueous solution at a flow-rate of 0.6 ml/min. The parameters of ELSD were set as follows: The drift tube temperature was 110°C and nebulizer nitrogen gas flow-rate was at 3.0 L/min, impact off mode.

## RESULTS AND DISCUSSION

### Quality evaluated by HPTLC

HPTLC is conventionally used for authentication of *Lingzhi* due to the method has advantages such as simple and multiple channel operation, as well as lower price of instrument (Chinese Pharmacopoeia, 2010). However, in order to improve the resolution, ethyl acetate and petroleum ether were used.

Formic acid was also added to reduce tailing effect but the organic phase became cloudy. So the volume of methanol was optimized to get clear liquid. Finally, the optimum mobile phase was as follow: dichloromethane–ethyl acetate–petroleum ether-formic acid-ethanol 8:3:9:0.8:0.5 (v/v).

TLC chromatograms, imaged at UV 254 nm (middle), UV 365 nm (right) and colorization (left) with vanillin-$H_2SO_4$ solution, of different samples of *G. lucidum* (Figure 1A) and *G. sinense* (Figure 1B) from different locations were shown in Figure 1.

The results showed that both *G. lucidum* and *G. sinense* contained ergosterol, a specific component of fungal cell membrane. But triterpenes were only found in *G. lucidum* rather than *G. sinense*, which were in accordance to our previous report (Zhao et al., 2006). Same species of *Ganoderma* from different locations showed high homogeneity.

Figures 1C and D showed TLC chromatograms of 17 commercial products of *Lingzhi*, which indicated their chemical profiles were significantly different. P2, P12 and P15 showed comprehensive characters of *G. lucidum* though the bands in P15 were very light. P1, P3, P6, P11, P13, P14 and P17 also showed partial characters of *G. lucidum*, which might suggest the problem in production. In addition, TLC chromatograms of P4, P8 and P10 from the same manufacturer seemingly had high similarity.

### Quality evaluated by HPSEC-ELSD

Polysaccharides in different *Lingzhi* products were analyzed by using HPSEC-ELSD. In order to avoid the interference of starch added in formula preparation, polysaccharides before and after enzymatic (α-amylase) digestion were tested. Appearance of new peaks after α-amylase digestion suggested that starch widely existed in *Lingzhi* products (Figure 2). The difference of polysaccharides in *Lingzhi* products was obvious. Especially, P4, P5, P7, P8, P10, P11, P15 and P16 almost contained little polysaccharides except starch. P3, P12, P13 and P14 showed obvious polysaccharides profiles.

## Conclusion

It is the first time to evaluate the quality of commercial *Lingzhi* products based on small molecules such as triterpenes and ergosterol and macromolecules such as polysacchairdes.

The results suggested the quality homogeneity of *Lingzhi* products was poor. Considering the significant difference in polarity of triterpenes and polysaccharides, chemical variation of *Lingzhi* products may attribute to: (1) Variation of preparation techniques, and (2) Variation of raw materials.

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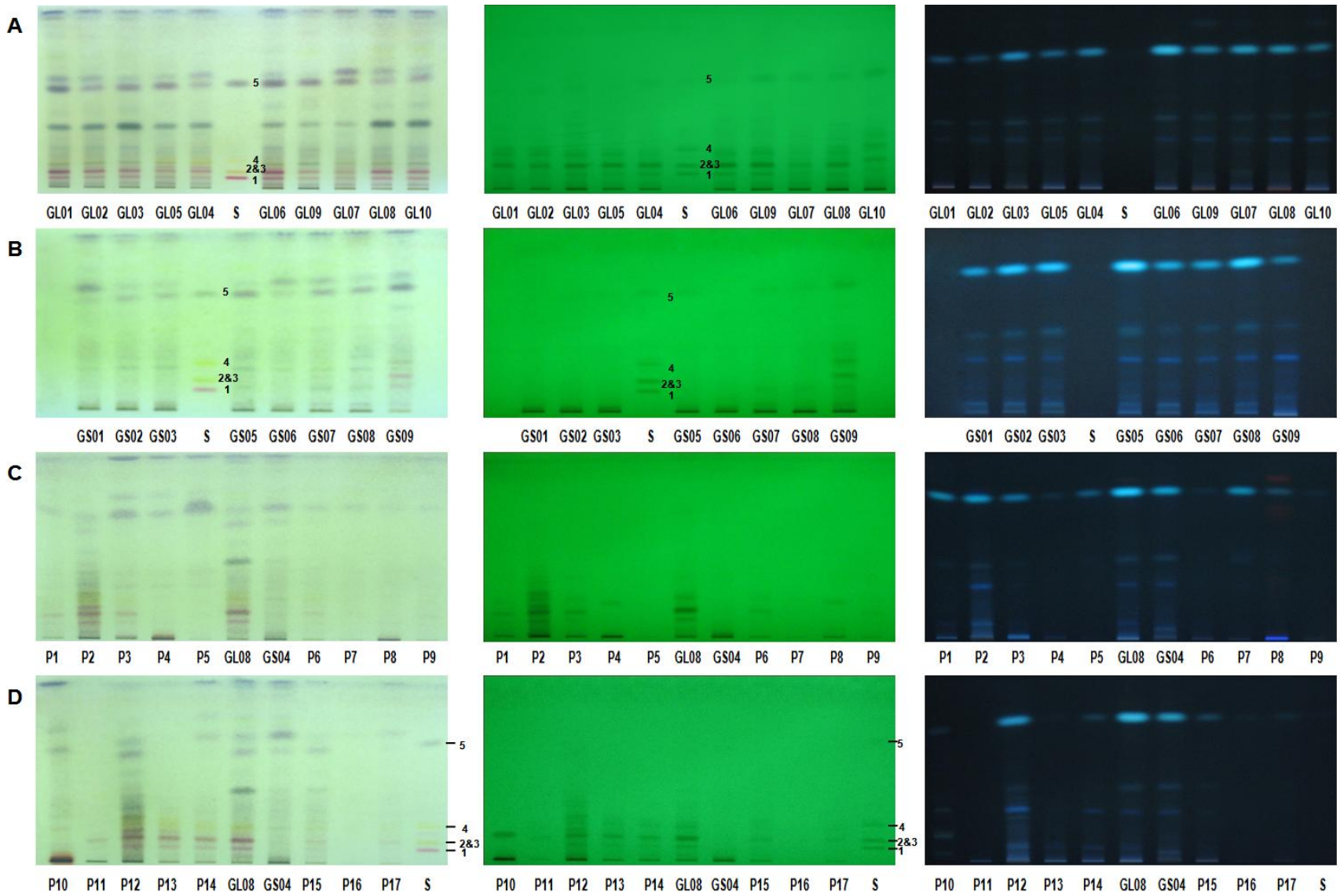


**Figure 1.** HPTLC profiles of *G. lucidum* (A), *G. sinense* (B) and *Lingzhi* products (C and D) observed under 254 nm (middle), 365 nm (right) and colorization with vanillin-$H_2SO_4$ solution (left). GL01, GL02-GL04, GL05, GL06-GL08 and GS01-GS04, GS05, GL09 and GS06, GS 07, GL10 and GS08, GS09 were different samples of *G. lucidum* (GL) and *G. sinense* (GS), respectively, from Zhejiang, Shandong, Guizhou, Anhui, Macao, Beijing, Guangxi, Hunan and Sichuan; P1-P17 were same as in Table 1; S, mixed standards (1, Ganoderic acid $C_2$; 2 and 3, Ganoderic acid A and H; 4, Ganoderic acid F; 5, ergosterol).

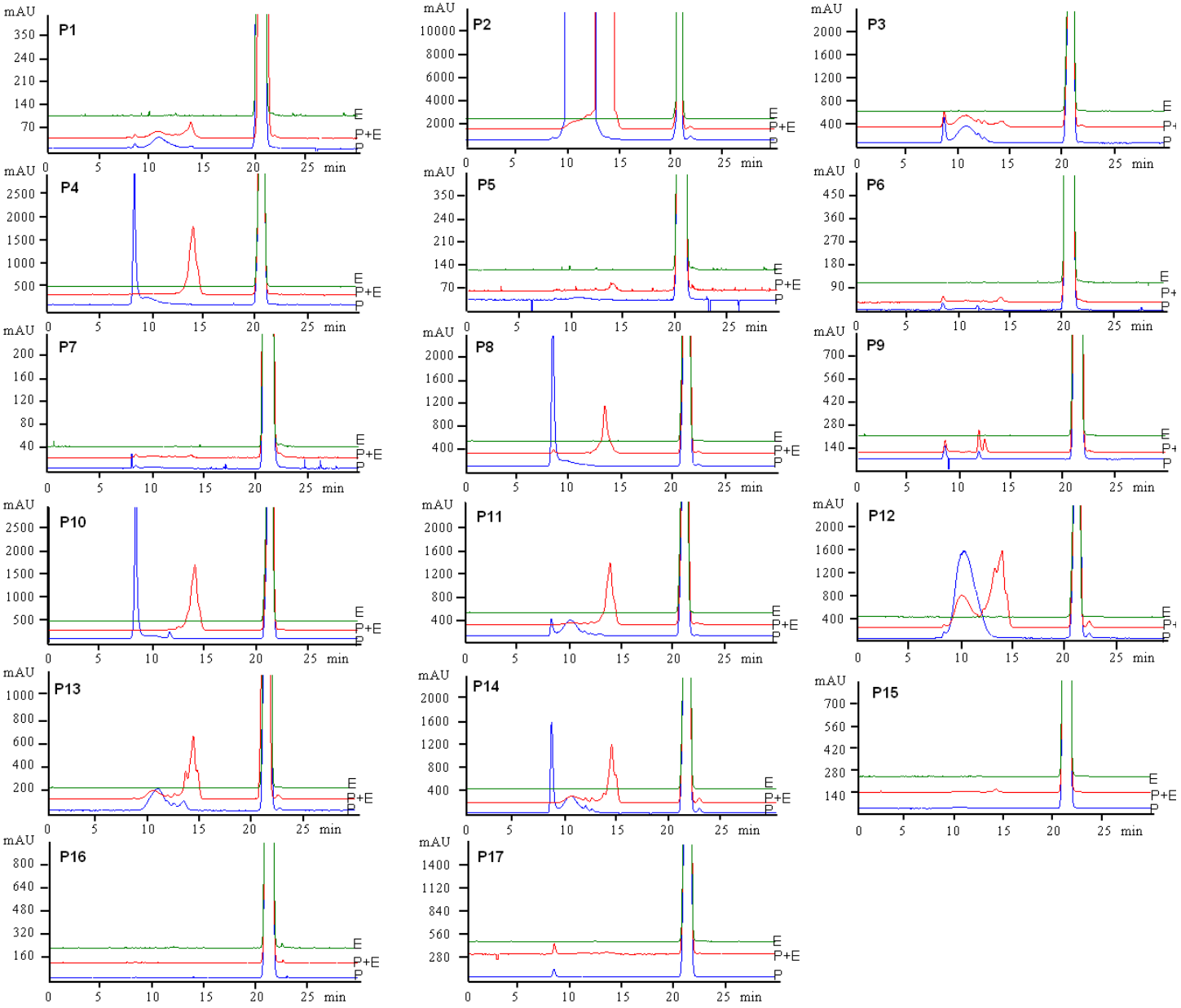


**Figure 2.** HPSEC profiles of polysaccharides from *Lingzhi* products (P) before and (P+E) after digestion with α-amylase (E).

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