DOCTORAL THESIS

Oligosaccharides approach for the qualitative analysis of dendrobium officinale polysaccharides

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ABSTRACT

Numerous of studies have reported that polysaccharides have many bioactivities including immune system modulation, anti-oxidative and anti-tumor activities. Because herbal materials are rich in polysaccharides, some of the herbs like *Dendrobium offcinale*, its polysaccharide marker (DOP) has been applied in the quality analysis of polysaccharides in *D. officinale* by high performance gel permeation chromatography (HPGPC). However, such polysaccharide marker is not presented in every herb, and DOP also has limitation in monitoring herb formula. In addition, even though the same herbal materials and the same extraction methods were used, different studies gave out different conclusion regarding the structure of polysaccharides. Therefore, in the current study, *D. officinale* was used as a case study to demonstrate the application of ABEE-labeled oligosaccharides approach on structure elucidation and quantification of polysaccharides in herbs and herb formulae.

First, the elucidation of polysaccharide structure remains challenging due to the lack of accurate analytical methods to determine the sequence and nature of glycosidic linkages. Oligosaccharide fragments from hydrolysis of polysaccharides are believed to provide accurate structure information, however, they are hard-to-separate and hard-to-detect. In the proposed method, the oligosaccharides generated from partial acid hydrolysis of DOP were labeled with p-aminobenzoic ethyl ester (ABEE), which made them separable and detectable by ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC-Q-TOF-MS-DAD). Subsequently, nine ABEE-labeled oligosaccharide fragments (dimer to decamer) were isolated and identified by MS sequencing and 2D-NMR, and were confirmed by methylation analysis. The results indicated that the backbone should be β-D-1,4-linked Man*p* chain instead of mixed mannose and glucose chain.

Second, two ABEE-labeled oligosaccharides namely, Te-Man-ABEE and Pen-Man-ABEE, were selected as chemical markers in the quantification of DOP in *D. officinale* and *D.officinale* (DO) products due to their high specificity in herb formula. The linear relationship between the content of these two markers and the content of DOP was successfully established. The linear relationship was further transformed to that between peak area of chemical markers and DOP content so that chemical markers were not necessary to be isolated for analysis. This linear relationship was systemically validated in terms of repeatability, precision and accuracy. The results showed that these two oligosaccharide markers presented a good linear relationship with DOP ($R^2 \geq 0.997$) in the range of 0.68-16.02 μg and also demonstrated satisfactory repeatability (RSD < 7.0%), and recovery (91.41% - 118.30%) in real sample determination. There was no significant difference between the results given by the two chemical markers as the RSD values were not more than 7.0%. While concerning the results given by the oligosaccharide-markers and the previously-published polysaccharide marker, the RSD value was not more than 6.4%.

In conclusion, this approach provided an efficient and reliable method to obtain accurate structure information of polysaccharides and quantify specific polysaccharide in herb formula. it is believed that ABEE-labeled oligosaccharides approach can be also applied in the analysis of other saccharide-dominant herbal materials which in turns helps to find out the bioactivity mechanism of polysaccharides.

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