

**Comprehensive extraction method integrated with NMR metabolomics: a new bioactivity screening method for plants, adenosine A1 receptor binding compounds in *Orthosiphon stamineus* benth**

ABSTRACT

A large number of plant metabolites has provided as an incomparable chemical source for drug development. However, the wide range of the polarity of metabolites has been a big obstacle for full use of the chemical diversity. The initial step conventional extraction method by a single solvent does not make use of all the metabolites contained in plants. Also, it takes a long time to confirm the target activity of a single compound because of tedious separation steps. To solve the problem, a new extraction method coupled to NMR-based metabolomics is applied to identify bioactive natural products. A comprehensive extraction method consisting of a continuous flow of solvent mixtures through plant material was developed to provide extracts with a wider chemical variety than those yielded with a single solvent extraction. As the model experiment, <sup>1</sup>H NMR spectra of the extracts obtained from the comprehensive extraction of *Orthosiphon stamineus* were subjected to multivariate data analysis to find its adenosine A1 binding activity. On the basis of the results, two flavonoids from a large number of chemicals were clearly verified to show the adenosine A1 binding activity without any further purification steps. This method could provide a solution to the major drawbacks of natural products in drug development.

**Keyword:** NMR metabolomics; *Orthosiphon stamineus*; Comprehensive extraction; Adenosine A1 receptor