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Identification of immuno-modulatory compounds from several type of rice brans and vegetables by metabolomics approach

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Plant metabolite has provided as an incomparable source for bioactive compounds. However, the wide range of the polarity of metabolites present in plants has been a big obstacle for its full use of the chemical diversity. Identification of active compounds needs tedious extraction and separation steps. To solve the problem, a new extraction method coupled to NMR- and fluorescence spectroscopy-based metabolomics was 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, which is suitable for metabolomics-based work.

In the first part of our research, several type of rice brans and vegetables extracts were tested for histamine release inhibitory activity using RBL 2H3 cells line. Vegetable x showed the highest inhibitory activity. Comprehensive extraction in combination with NMR based metabolomics for the vegetable x showed that compound having chemical shift of 3.14 ppm are probably responsible for the activity. Based on ^{1}H NMR and 2D NMR data, this signal came from arginine. The OPLS data obtained from 3-D Fluorescence Spectra Measurement (Fluorescence Fingerprint) supported this conclusion. Our data was also supported by previous report that compound with strong histamine release inhibitor from vegetable x was a type of peptide/protein.

In the second part of our research, we screened our samples for several cytokines modulatory activity (IL-2, IL-4, IL-5, IL-10, IL-12p40 and IFN-γ). The screening results showed that Green Torbangun suppressed IFN-γ but it showed no effect in IL-2 secretion, thus we chose to undergo NMR based OPLS analysis for IFN-γ inhibitory activity of Green Torbangun fractions obtained from comprehensive extraction. The results indicated that triterpene acids and flavonoids were the active compounds. The most active signals were probably came from pomolic acid and methyl 3-epimaslinate which were reported to be present in Green Torbangun. However these compounds were not commercially available. We tested the other 4 available triterpenes (ursolic acid, oleanolic acid, maslinic acid and tormentic acid) and a flavonoid (eriodictyol) for IFN-γ and IL-2 inhibitory activities. The tested concentrations were 5, 10, and 20 μM. Oleanolic acid and eriodicytiol also showed IFN-γ inhibitory activity with eriodictyol had the stronger activity than oleanolic acid. IL-2 secretion and cells viability was not affected by the two compounds. On the other hand, ursolic acid inhibited secretion of not only IFN-γ but also IL-2. The data of Fluorescence Fingerprint based OPLS analysis also supported this finding. However, the compounds with the strongest active markers remained unidentified. As previously mentioned, OPLS of NMR data predicted that the compounds probably pomolic acid or methyl 3-maslinate but this prediction needs to be confirmed by isolating them from Green Torbangun and testing their IFN-γ inhibitory activity. In this research, comprehensive extraction coupled to NMR- and Fluorescence Fingerprint -based metabolomics were shown to be an effective tool to screen biological active compounds from natural sources.