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Improving quality control of yucca extracts used as food additives by screening antimicrobial activity using NMR metabolomics

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Abstract

Yucca schidigera is mainly distributed in southwestern US and the northern desert of Mexico. Its extract is widely used as a food additive for its antimicrobial activity. However, this antimicrobial activity is subject to significant variability across production lots. Yucca extracts are natural products and their composition is affected by their cultivation area and weather. Manufacturer deal with natural products such as food additives pay particularly close attention to quality control. In the present study, NMR metabolomics methods were used to screen the antimicrobial activity of yucca extracts. Yucca extracts were subjected to principal component analysis (PCA) and categorized on a score plot of their ^{1}H NMR spectral data according to their antimicrobial activity. Furthermore, hierarchical cluster analysis (HCA) was also used to classify yucca extracts based on their antimicrobial activity. Classification using PCA and HCA was dependent upon saponin content, particularly that of schidigera-saponin A1 and D1, which was further confirmed by HPLC analysis of the yucca extracts. We demonstrated that NMR-based metabolomics is a potentially useful tool to use in combination with conventional quality control methods for yucca extracts used as food additives. We envisage this method as tool for initially screening the extracts prior to carrying out the officially recommended quality control tests.

Keywords: *Yucca schidigera*, NMR metabolomics, antimicrobial activity, schidigera-saponins A1 and D1.

Introduction

Yucca schidigera, belonging to the Yucca genus (Agavaceae) is mainly distributed in southwestern US and the northern desert of Mexico. [1] The commercial extract of yucca is widely used as a food additive. Yucca extract is a GRAS (generally recognized as safe) product and is approved for use by the US Food and Drug Administration (FDA). [2] In Japan the extracts of yucca (whole of *Y. schidigera* and *Y. brevifolia*) are listed as 'Yucca Foam Extract' in Japan's Specifications and Standards for Food Additives, 9th Edition (JSFA-IX). [3] In Japan, yucca extracts are added to foods to extend their shelf life. A previous study reported that yucca extracts contain steroidal saponins that exert antimicrobial activities. [4]

Owing to their use as food additives, yucca extracts have received much attention from researchers. However, based on our experience, its antimicrobial activity can vary significantly among production lots. The content and composition of yucca extracts are affected by the environment they are cultured in and the local weather conditions, similar to other natural medicinal plants. Therefore, quality control of yucca extracts is crucial to

ensure their beneficial properties when used as a food additive. However, quality control of antimicrobial activity of each sample using conventional analytical methods, such as disc diffusion assay, is impractical because they are labor-intensive and time-consuming. Therefore, we evaluated the efficacy of using metabolomics for quality control analysis of yucca extracts prepared from *Y. schidigera*. Although yucca foam extracts used as food additives include the whole plant of *Y. schidigera* and *Y. brevifolia* according to JSFA-IX, [3] we focused on the stem of Y. *schidigera* in the present study, based on their availability.

Metabolite fingerprinting using nuclear magnetic resonance (NMR) spectroscopy can be used to generate valuable metabolite signatures for distinguishing complex plant extracts. The advantage of ^{1}H NMR spectroscopy over other metabolomic techniques is that the signal intensity depends only on the molar concentration of compounds in the test solution, which enables direct comparisons of the concentrations of all compounds in the sample, and thus, a more robust analysis. Previously, we demonstrated that an NMR-based metabolomics approach was able to distinguish between *Glycyrrhiza* species (*G. glabra*, *G. uralensis*, and *G. inflata*), [5] and furthermore, was able to identify the habitats of *Sophora flavescens* in Japan and China. [6]

In the present study, an NMR-based metabolomics approach using ^{1}H NMR spectral measurements and multivariate analysis was used to screen the antimicrobial activities of

yucca extracts. We demonstrated that quality control of yucca extracts can be performed using NMR-based metabolomics. Antimicrobial activity of yucca extracts depends on types and contents of steroidal saponins which are contained in yucca extracts. As NMR metabolomics can reveal metabolite signature and molar concentration of contents, NMR metabolomics is suitable for quality evaluation of yucca extracts. According to official recommendations, [3] quantitative analysis of yucca saponin is conducted as follows. The sapogenins formed through the hydrolysis of yucca saponin are quantified by reacting with 4-methy benzaldehyde through colorimetric methods. Although this is the officially recommended method for quality checking yucca extracts, NMR metabolomics may provide an effective initial screening technique for yucca quality control. We evaluated the efficacy and usefulness of this method as preliminary study for evaluation of yucca extracts.

Material and methods

Plant materials

Stems of *Y. schidigera* were purchased from Desert King (San Diego, CA, USA) in over a period of 4 years (2014–2017). Voucher specimens (J4132: MP122016-1, J4133: MP002099-1, J4134: MP072014-1, J4135: MP092014-1, J4136: MP042014-1, J4137:

MP012015-1, J4154: MP012015-2, J4155: MP012017-1, J4156: MP012017-2) were deposited at Maruzen Pharmaceuticals Co., Ltd. The plants were identified by Prof. Chen B of the South China Botanical Garden.

Preparation of extracts for NMR analysis and HPLC analysis

Each of nine stems of *Y. schidigera* were extracted with water for 3 h under reflux. The water extracts were evaporated to give dried residues. These residues (200 mg) were loaded onto a Sep-Pak C18 Plus Short Cartridge (360 mg, Waters, Milford, MA) and separated by washing with H_2O, then 50% aqueous MeOH, and eluted with MeOH (4 mL for each step). The methanol fractions were dissolved in pyridine-d_5 (Cambridge Isotope Laboratories, Inc., Tewksbury, MA) at a concentration of 10 mg/mL for NMR measurements. The water extracts of yucca were also dissolved in 40% aqueous acetonitrile for HPLC analysis. Samples were filtered through a 0.45 μm membrane before HPLC analysis.

HPLC apparatus and chromatographic conditions

HPLC analysis was performed using an Agilent 1100 Series HPLC System (Agilent Technologies, Santa Clara, CA) composed of a binary pump equipped with a degasser,

autosampler, column compartment, and PL-ELS2100 detector (Polymer Laboratories). Data were recorded using the Agilent OpenLAB CDS ChemStation (C.01.07. SR1 113) software. The ELSD (Evaporative Light Scattering Detector) conditions were set as follows: evaporative temperature at 70 °C, nebulizer temperature at 50 °C, and gas flow at 1.6 L/min. The HPLC column was a COSMOSIL(R) 2.5C18-MS-Ⅱ packed column (100 × 3.0 mm i.d., Nacalai Tesque Inc., Nakagyo-ku, Kyoto, Japan). The sample injection volume was 10 μL, the flow rate was 0.4 mL/min, and the column temperature was maintained at 40 °C. The mobile phases were: A (0.1% formic acid in water, v/v) and B (acetonitrile containing 0.1% formic acid, v/v). The gradient program was 40–70% solvent B over 14 min. Schidigera-saponin A1, D1, D5, and F1 were used as authentic samples. These authentic samples were previously isolated from yucca by us. Schidigera-saponin A1, D1, D5, and F1 in each yucca extract were quantified using an absolute calibration curve method. The linearity of the method was evaluated by a calibration curve in the range of 5 to 500 μg/ml.

Evaluation of antimicrobial activity

Inhibitory activity against different microbes that cause food degradation (*Pichia anomala*, *Saccharomyces cerevisiae*, and *Candida albicans*) was determined using an

agar dilution method. These microbes were obtained from Department of Molecular Biotechnology, Graduate School of Advanced Sciences of Matter, Hiroshima University (HUT Culture Collection), Biological Resource Center, Nation Institute of Technology and Evaluation (NITE) (NBRC), and Microbe Division/Japan Collection of Microorganisms, RIKEN BioResource Research Center (RIKEN BRC JCM), respectively. In brief, 0.5 mL of a test sample diluted to different concentration was mixed with 9.5 mL of culture medium (potato dextrose agar; Nissui Pharmaceutical Co., Ltd., Taito-ku, Tokyo, Japan). Culture media containing test samples were poured into Petri dishes, onto which each microorganism culture was streaked. After an appropriate incubation period (72 h, 25 °C), the inhibitory activity of the sample was assessed as the minimum inhibitory concentration (MIC, μg/mL), which is the lowest concentration at which no growth was observed. MIC values were computed in JMP Pro 13 (SAS Institute Inc., Cary, NC) to plot histograms.

NMR spectroscopy

^{1}H NMR spectra were recorded at room temperature on a 400-MHz Agilent 400MR-vnmrs 400 spectrometer (Agilent Technologies). Each spectrum consisted of 65,536 complex data points and had a spectral width of 6,410.3 Hz. They were obtained through

16 scans with a repetition time of 5.0 s and a relaxation delay of 1.50 s per scan. The detection pulse flip angle was set at 45°.

NMR data reduction procedures and pattern recognition analysis

Each NMR spectrum was divided into 350 regions (0.04 ppm wide) from 0.00–14.00 ppm. Each segment of spectral region was integrated. The remaining integral values for each spectrum were normalized over 100 total summed integrals to compensate for any differences in concentration among yucca extracts. Spectral processing was performed using ALICE2 for Metabolome version 5.0 (JEOL, Tachikawa, Tokyo, Japan). Multivariate analyses such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) were conducted using JMP Pro 13 (SAS Institute Inc.).

Results and discussion

Antimicrobial activities against three microbes ((a) *Pichia anomala*, (b) *Saccharomyces cerevisiae*, and (c) *Candida albicans*) of the nine different yucca water extracts were shown in Table 1. The nine yucca extracts were classified into three groups (strong, middle, and weak) according to their antimicrobial activity (Fig. 1).

All 350 variables in the bucketed regions were equally accounted for in the datasets.

PCA models were depicted as score plots and consisted of two synthetic variables: principal component (PC) 1 (the greatest data variance) and PC2 (the second greatest data variance, orthogonal to PC1). The PCA score plot clearly showed that highly active samples (J4132 and J4133) were considerably different from weakly active samples (J4137) (Fig. 2). The PC1, PC2, and PC3 scores were 27.7%, 21.2%, and 12.3%, respectively. The values of PC1 and PC2 were used to distinguish the yucca extracts. The sum of PC1 and PC2 was 48.9%, which was sufficient to identify yucca extracts.

Similarly, the yucca extracts were grouped according to their antimicrobial activities on an HCA constellation dendrogram (Fig. 3). All samples were divided into two main clusters (top and bottom clusters). Samples located in the bottom right cluster exhibited strong antimicrobial activity (J4132 and J4133). In contrast, the sample in the top left cluster exhibited weak antimicrobial activity (J4137).

We previously reported that the antimicrobial activities of yucca extracts were exerted by saponins [7]. Therefore, we aimed to confirm whether saponin content related with the results of the PCA and HCA analyses of yucca extracts. Table 2 summarizes the content of schidigera-saponins A1, D1, D5, and F1 in the yucca extracts (Scheme 1). A1 and D1 were higher in J4132 and J4133 than in the other samples, and both these samples exhibited high antimicrobial activities. In contrast, the lowest content of these saponins

was observed in J4137, which represented the weakest antimicrobial activity. These results indicated that yucca extracts were classified by PCA and HCA based primarily on schidigera-saponin A1 and D1. By the way J4135 was located in the upper cluster but was far from all other groups, indicating that this sample was substantially different from the other samples. The presence of schidigera-saponin F1 at a much higher concentration in J4135 compared with the other samples might explain why this sample was located far from the others on the constellation dendrogram.

Conclusions

In the present study, we tested the efficacy of NMR-based metabolomics analysis for quality control of yucca extracts used as food additives. NMR-based metabolomics coupled with PCA and HCA were able to discriminate yucca extracts based on their antimicrobial activity. Use of NMR-based metabolomics for quality control of yucca extracts has the potential to significantly reduce the labor and cost associated with quality control testing. Conclusive quality evaluations of yucca extracts should be conducted according to officially recommended methods. However, NMR-based metabolomics may represent a useful tool for screening the raw material of yucca extracts. Furthermore, this method does not require authentic samples, which gives it an advantage over other

methods, such as HPLC analysis. In general, quality control of bioactive natural materials containing large numbers of compounds is difficult without evaluating bioactivity because bioactive components are not clear in many cases. Therefore, comprehensive analyses, such as metabolomics, rather than targeted analyses, might be suitable for quality control of natural materials.

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Figure legends

Fig. 1 Histogram of MIC values of yucca extracts. The histogram was plotted by determining minimum inhibitory concentration (MIC) of yucca extracts. (a) *Pichia anomala*, (b) *Saccharomyces cerevisiae*, and (c) *Candida albicans*

Fig. 2 PCA score plot. PCA score plot of *yucca schidigera*, in which 350 variables were equally accounted for in the datasets. The PCA score plot clearly showed that highly active samples (J4132 and J4133) were considerably different from weakly active samples (J4137).

Fig. 3 Constellation dendrogram. Constellation dendrogram using the ^{1}H NMR spectral data of yucca extracts was illustrated by hierarchical cluster analysis. The yucca extracts were grouped according to their antimicrobial activities on an HCA constellation dendrogram.