Quantitative Analysis of Marker Compounds and Matabolic Profiling of *Zanthoxylum piperitum* (Chopi) According to Different Parts and Harvest Times

Hyejin Hyeon[1], Eun Bi Jang[1], Yoonji Lee[2], Sung Hye Han[3], Baek Kwang Yeol[4], Su Young Jung[5], Ki Sung Shin[6], and Weon-Jong Yoon[7]*

[1]Researcher, [2]Senior researcher, and [7]Team leader, Jeju Biodiversity Research Institute, Jeju Technopark, Seogwipo 63608, Republic of Korea
[3]Researcher, [4]Research fellow, [5]Director, [6]Presedent, JoheunChinguDeul Corp., Jeju 63359, Republic of Korea

ABSTRACT

Zanthoxylum piperitum ("chopi" in Korean) has been used as traditional medicinal plants with high anti-inflammatory, antioxidant, and antifungal activities. The aims of the study were to identify marker compounds and to investigate metabolites variation of chopi according to different parts and harvest times. Every month from June to September, chopi were harvested with three different parts: leaves, leaf-twig mixtures, twigs. Using liquid chromatography-tandem mass spectrometry (LC-MS/MS), two main marker compounds (quercitrin and quercetin-3-*O*-glucoside) were characterized in 70% ethanol extracts of chopi. Quantification of the two marker compounds were subsequently conducted by high performance liquid chromatography (HPLC), representing that contents of these compounds were higher in leaves and leaf-twig mixtures rather than twigs. For the comprehensive analysis of metabolites associated with production of marker compounds, 35 primary metabolites were identified using gas chromatography-mass spectrometry (GC-MS). Multivariate analysis results represented that plant parts were main contributors to the separation of chopi. However, significant differences were not observed between leaves and leaf-twig mixtures samples. The partial least square (PLS) predictive model revealed that monosaccharides (fructose, galactose, glucose, mannose, xylose) and branched-chain amino acids (isoleucine, valine, leucine) were important determinants for the production of marker compounds together with alanine, inositol, GABA, and theronic acid. This study could be extended to stabilize and utilize chopi as an industrial material, as well as to find good candidates with various nutritional traits.

INTRODUCTION

➢ *Zanthoxylum piperitum*

- ❖ *Zanthoxylum piperitum* ("chopi" in Korean) has been utilized as traditional medicine due to their physiological bioactivities and phytochemicals.
- ❖ Since chemical characteristics largely depend on plant parts and harvest times, quality assessments are required for utilizing chopi as industrial materials.
- ❖ However, few studies has been reported for the quality evaluation of chopi by analyzing its various chemical compounds.

OBJECTIVES

➢ Investigation of marker compounds in chopi

- ❖ To identify major chemical constituents of chopi, 70% ethanol extracts of chopi were analyzed using LC-MS/MS technique.

➢ Metabolic profiling of chopi according to parts and harvest periods

- ❖ Together with marker compounds, primary metabolites such as amino acids, organic acids, and sugars were analyzed to compare various chemical characteristics of chopi.
- ❖ Univariate and multivariate analysis were implemented to investigate overall patterns and to create predictive models associated with the levels of marker compounds.

METHODS

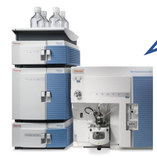
1. Plant material preparation

(Harvested in)
June
July
August
September

Zanthoxylum piperitum

Leaves | Leaf-twig mixtures | Twigs

2. Investigation of maker compounds



LC-MS/MS analysis
(LCQ-Fleet, Thermo scientific)

- ❖ Compounds were identified by comparing retention time and fragmentation patterns with those of standards.

3. Metabolic profiling

GC-MS analysis
(7890A-5975C, Agilent)

HPLC analysis
(U3000, Thermo scientific)

4. Statistical analysis

- ❖ Principal component analysis (PCA)
- ❖ Hierarchical cluster analysis (HCA)
- ❖ PLS predictive model

RESULTS

➢ LC-MS/MS analysis

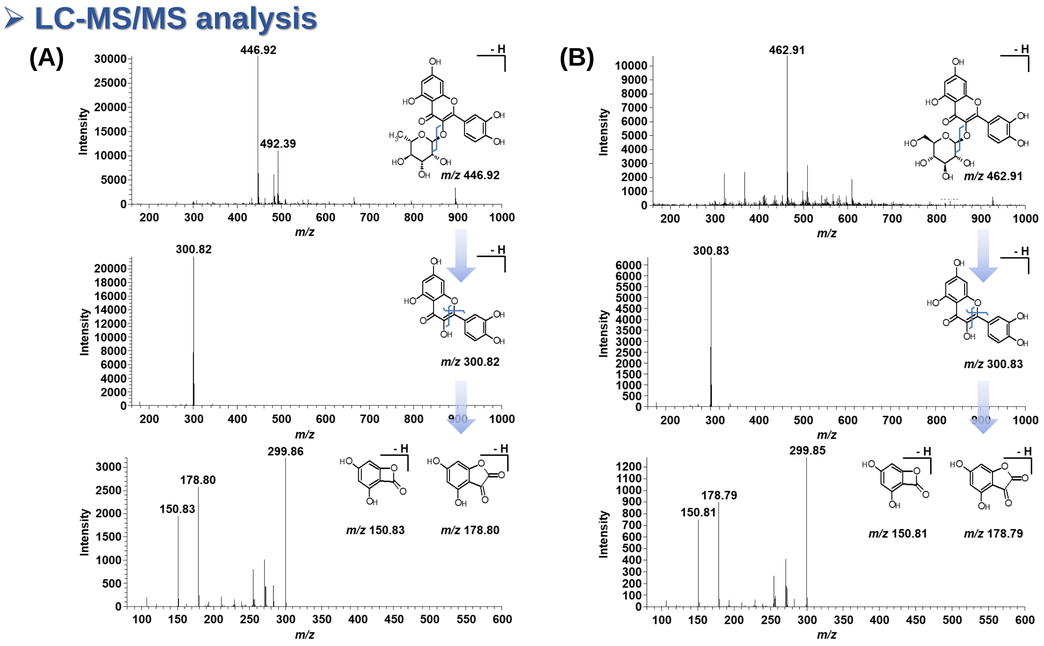


Figure 1. Fragmentation pathway proposed for (A) quercitrin and (B) quercetin-3-*O*-glucoside contained in 70% ethanol extracts of chopi, employing electrospray ionization mass spectrometry at negative ionization mode.

➢ PCA

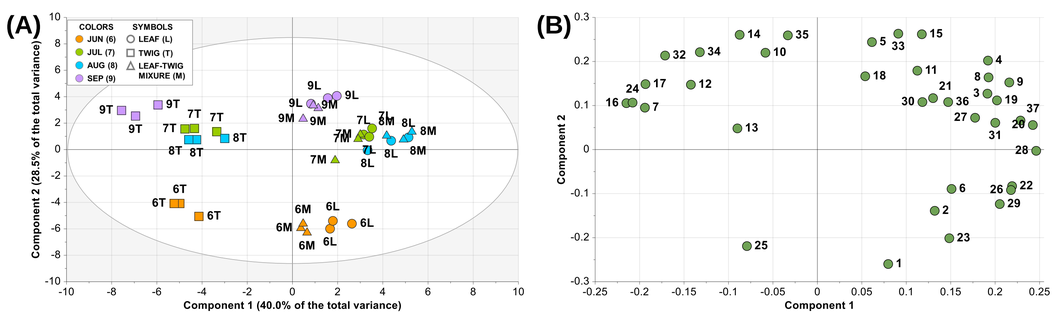
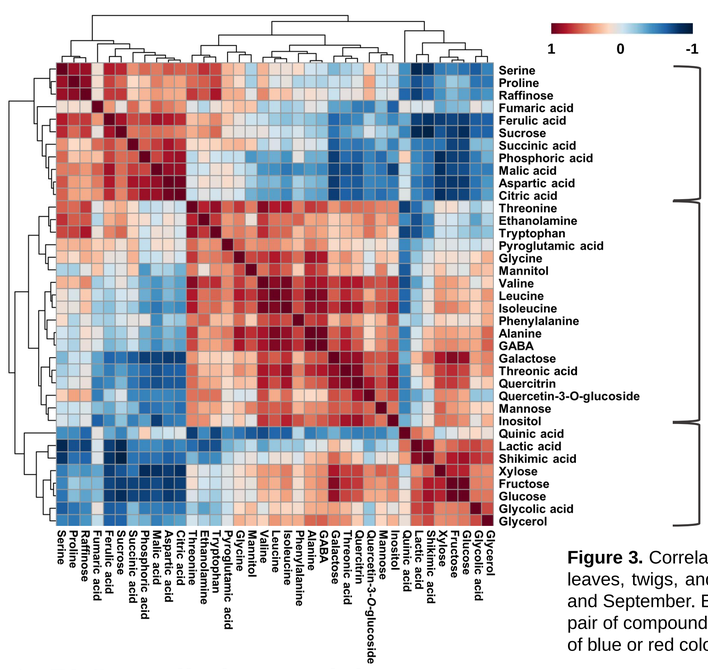


Figure 2. PCA (A) score and (B) loading plots for leaves, twigs, leaf-twig mixtures of chopi harvest at four different time points. Each plant parts was represented on the plot by a unique symbol, and each harvest time was represented on the plot by a unique color. Plot annotation 1, Lactic acid; 2, Glycolic acid; 3, Alanine; 4, Valine; 5, Ethanolamine; 6, Glycerol; 7, Phosphoric acid; 8, Leucine; 9, Isoleucine; 10, Proline; 11, Glycine; 12, Succinic acid; 13, Fumaric acid; 14, Serine; 15, Threonine; 16, Malic acid; 17, Aspartic acid; 18, Pyroglutamic acid; 19, GABA; 20, Threonic acid; 21, Phenylalanine; 22, Xylose; 23, Shikimic acid; 24, Citric acid; 25, Quinic acid; 26, Fructose; 27, Mannose; 28, Galactose; 29, Glucose; 30, Mannitol; 31, Inositol; 32, Ferulic acid; 33, Tryptophan; 34, Sucrose; 35, Raffinose; 36, Quercetin-3-*O*-glucoside; 37, Quercitrin.

➢ HCA



TCA cycle intermediates
: Citric acid, Malic acid, Fumaric acid, Succinic acid

Polysaccharides
: Sucrose, Raffinose

Flavonoids
: Quercitrin, Quercetin-3-*O*-glucoside

Branched-chain amino acids
: Isoleucine, Leucine, Valine

Aromatic amino acids
: Phenylalanine, Tryptophan

Sugar alcohol
: Mannitol, Inositol

Monosaccharides
: Glucose, Fructose, Xylose

Figure 3. Correlation matrix for 37 primary and specialized metabolites data for leaves, twigs, and leaf-twig mixtures of chopi harvested in June, July, August, and September. Each square indicates the Pearson's correlation coefficient of a pair of compounds, with the value of this coefficient represented by the intensity of blue or red colors, as indicated on the color scale.

➢ PLS predictive model

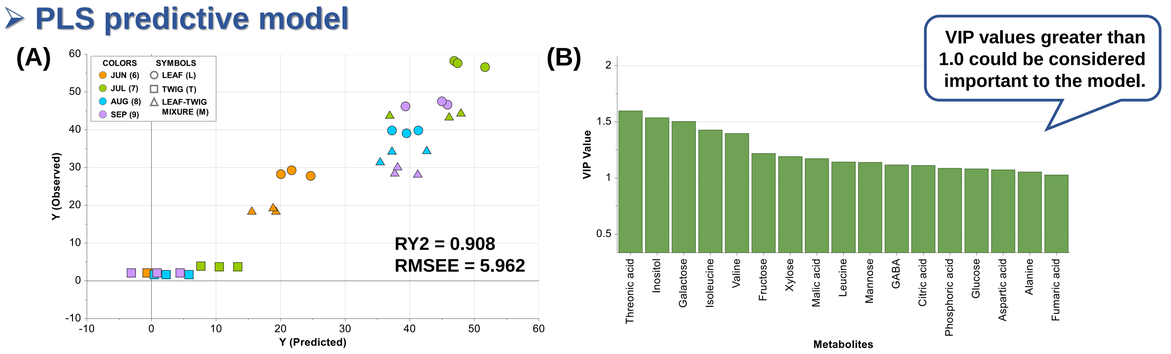


Figure 4. (A) The PLS predictive models constructed from the leaves, twigs, leaf-twig mixtures of chopi harvest at four different time points as training sets for predicting the ranks of marker compounds' contents on the basis of metabolic profiles of chopi samples. (B) The influence of variables used to create a marker compounds predictor for chopi.

- ❖ **Contents of marker compounds (quercitrin, quercetin-3-*O*-glucoside) in chopi were successfully predicted using quantitative data of primary metabolites in PLS predictive models.**

CONCLUSION

- ❖ **LC-MS/MS analysis results revealed that quercitrin and quercetin-3-O-glucoside were marker compounds in 70% ethanol extracts of chopi.**
- ❖ **Comprehensive primary and specialized metabolites profiling results demonstrated that chopi samples were mainly characterized by plant parts rather than harvest periods.**
- ❖ **Contents of marker compounds in chopi were well predicted on the basis of primary metabolite contents, and this model could be used as an alternative method to predict the quality of chopi.**

- ❖ **Acknowledgement: This work was supported by the SME Technology Development Support Project grant funded by the Ministry of SMEs and Startups (MSS) (No. S3226168).**