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# 액체크로마토그래피 질량분석법(LC/MS)과 다변량분석법에 의한 원산지별 쑥갓의 대사체 다양성 분석

건국대학교 생명공학과 : 김지영, 임채성, 구강모, 임순성[1], 김정상[2], 윤정한[1], 이충환*

# Ditermination of Metabolomic Differences between Geographical Origin of *Chrysanthemum coronarium* L. by Liquid Chromatography Mass Spectrometry (LC/MS) and Multivariate Analysis

Department of Bioscience and Biotechnology, Konkuk University, Seoul, Korea
[1]Department of Food Science and Nutrition, Hallym University, Chuncheon
[2]Department of Life and Food Science and Animal Science and Biotechnology, Kyungpook National University, Daegu

Jiyoung Kim, Chae Sung Lim, Kang Mo Ku, Soon Sung Lim[1], Jong Sang Kim[2], Jung Han Yoon[1] and Choong Hwan Lee*

## Objectives

The purpose of this study was to compare the secondary metabolites of *Chrysanthemum coronarium* L. from grown different geographical origins using LC/MS/MS and multivariate analysis.

## Materials and Methods

○ Materials

*Chrysanthemum coronarium* L. samples were collected in four different localities of Pocheon, Seoul, Yeoju and Youngin in Korea. The samples were freeze-dried to devide into two condition which uncooked and cooked at 95~100℃ for 10 min. The dried samples were extracted with two times aqueous 80% methanol and evaporated *in vacuo*. The aqueous phase were initially partitioned with ethyl acetate. Then the ethyl acetate extracts were separated by stepwise aqueous methanol eluted from solid phase extraction column.

○ Methods

Fractionated all solution were prepared to adjusted 1 mg/mL concentration for LC/MS analysis. Metabolites were separated by a reversed phase HPLC system on mobile phase consisted of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B). Column eluent was injected into a Varian 500MS electrospray iontrap mass spectrometry. Putative identification of peaks were further investigated using tandem mass spectrometry as data dependent scanning techniques. Data were collected and multivariate processed using SIMCA-P+ v 12.0 software.

Corresponding author: Choong Hwan Lee, E-mail:chlee123@konkuk.ac.kr, Tel:02-2049-6177

## Results

Thirty nine metabolites have been screened by LC/MS/MS in *Chrysanthemum coronarium* L extracts. Including 14 compounds were identified as caffeoylquinic acid, flavonoid analogues, and spiroketal enol ether groups from our library using the searchable parameter (retention time, *m/z* value, and MS/MS fragmentation pattern). All this profiling data were analyzed by statistical analysis. Using principle components analysis, we found that the 3 groups of the main contributors separated clustering. Two group clustering to whether it is cooking or not, its groups main components was similar. And the others clustering was presented separation according to specific geographical culture place such as mycosinol of pochun sample. Therefore, we presume that there is a correlation between place of origin and production of main metabolites such as mycosinol and dicaffeolyquinic aicd.

Table 1. Metabolite composition of *Chrysnathemum coronarium* L.

| No. | RT | Mode | M.W. | SP_CCB | SP_CC | SM_CCB | W_CCB | MH_CCB | MH_CC | GN_CCB | GN_CC | GD_CCB | GD_CC | Mass fragmentation | Formular | Chemical name | Re |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.812 | M-H | 354 | * | * | | * | * | * | * | * | | * | 353, 191 | C16H18O9 | Diaffeolyquinic acid | 1 |
| 2 | 10.456 | M-H | 430 | * | | | | | | | | | | 429 | | NI | |
| 3 | 10.596 | M-H | 610 | * | * | | * | * | * | * | * | * | * | 609, 301, 271, 255, 179, 151 | C27H30O16 | Quercetin 3-rutinoside; Rutin | 3 |
| 4 | 10.928 | M-H+46 | 295 | * | * | * | * | * | * | * | * | * | * | 294, 187 | | NI | |
| 5 | 10.977 | M-H | 516 | * | * | | | | * | | * | | * | 515, 353, 191, 179, 173 | C25H24O12 | Dicaffeolyquinic acid | 1 |
| 6 | 11.91 | M-H | 516 | * | * | | * | * | * | * | * | * | * | 515, 353, 191, 179, 173 | C25H24O13 | Dicaffeolyquinic acid | 1 |
| 7 | 12.252 | M-H | 516 | * | * | | * | * | * | * | * | * | * | 515, 353, 191, 179, 173 | C25H24O14 | Dicaffeolyquinic acid | 1 |
| 8 | 12.862 | M-H | 616 | * | * | * | * | * | * | * | * | * | * | 615, 515, 453, 353, 191 | C29H28O15 | Succinoyl dicaffeolyquinic acid | 1 |
| 9 | | | 616 | | | | | | * | | | | * | 615, 515, 453, 353, 191 | C29H28O15 | Succinoyl dicaffeolyquinic acid | 1 |
| 10 | 15.092 | M-H | 328 | | * | * | * | | * | * | * | | * | 327, 309, 291, 239, 229, 211, 171 | C21H28O3 | Pyrethrin I | 5 |
| 11 | 15.1 | M-H | 578 | * | | | | | | | | | | 577, 299, 225 | C27H30O14 | Apigenin Neohesperidoside | |
| 12 | 15.251 | M+H | 250 | * | * | * | * | * | * | * | * | * | * | 251, 233, 223, 203, 195, 175 | C13H14O3S | 2-(5-Methylsulfinyl-2-pentyn-4-enylidene)-1,6-dioxaspiro[4.4]non-3-ene | 4 |
| 13 | 15.881 | M-H | 330 | * | * | * | * | * | * | * | * | * | * | 329, 311, 293, 229, 211.171 | C21H30O3 | Pyrethrin I; 4',5'-Dihydro | 5 |
| 14 | 16.291 | M+H | 250 | * | * | * | * | * | * | * | * | * | * | 251, 233, 223, 203, 195, 175 | | 2-(5-Methylsulfinyl-2-pentyn-4-enylidene)-1,6-dioxaspiro[4.4]non-3-ene | 4 |
| 15 | 16.847 | M-H-18 | 708 | * | | * | | | * | | * | | * | 707,683, 625, 457 | | NI | |
| 16 | 17.001 | M+H | 688 | | | | | | * | | * | | * | 687, 641, 611, 579, 490, 444 | | NI | |
| 17 | 18.015 | M+H | 250 | * | | * | | | | | | * | * | 251, 233, 223, 203, 195, 175 | C13H14O3S | 2-(5-Methylsulfinyl-2-pentyn-4-enylidene)-1,6-dioxaspiro[4.4]non-3-ene | 4 |
| 18 | 18.028 | M-H | 330 | * | * | * | * | * | * | * | * | * | * | 329, 311, 293, 229, 211.171 | | NI | |
| 19 | 18.646 | M-H | 546 | * | | * | | | | | | | | 545, 502, 455, 429, 313 | | N.I; derivatives of M.W.530 | |
| 20 | 20.096 | M+H | 256 | * | * | * | * | * | * | * | * | * | * | 279, 197, 167, 139 | C15H12O4 | Mycosinol Ac (7E-) | 6 |
| 21 | 20.661 | M+H | 200 | * | * | * | * | * | * | * | * | * | * | 200, 183, 153, 129, 115 | C13H12O2 | Tonghaosu (7E-) | 6 |
| 22 | 20.883 | M-H | 676 | * | * | * | * | * | * | * | * | * | * | 675, 645, 593, 538, 483, 397, 375, 278 | | NI | |
| 23 | 21.393 | M+H | 256 | * | * | * | * | * | * | * | * | * | * | 279, 197, 167, 139 | C15H12O4 | Mycosinol Ac (7Z-) | 6 |
| 24 | 22.282 | M+H | 200 | * | * | * | * | * | * | * | * | * | * | 200, 183, 153, 129, 115 | C13H12O2 | Tonghaosu (7Z-) | 6 |
| 25 | 22.604 | M-H+46 | 517 | * | | * | * | * | * | * | * | * | * | 502 | | NI | |
| 26 | 23.616 | M-H+46 | 514 | * | | * | * | * | * | * | * | * | * | 277 | | NI | |
| 27 | 23.641 | M-H+47 | 514 | | | | | * | * | * | * | * | * | 513, 277 | | NI | |
| 28 | 23.969 | M-H | 538 | | | * | | | | | | | | 537, 291, 273, 247, | | NI | |
| 29 | 24.465 | M-H | 670 | | | * | | | | | | | | 869, 808 | | NI | |
| 30 | 25.384 | M-H+46 | 519 | | * | * | * | * | | | | * | | 564, 504 | | NI | |
| 31 | 25.674 | M-H | 506 | | | * | * | | | * | | | | 505, 277, 259, 233 | | NI | |
| 32 | 26.649 | M-H | 809 | | | * | | | | | | | | 807, 661, 545 483 | | NI | |
| 33 | 28.801 | M-H | 838 | * | | * | | | | | | | | 837, 787, 561 | | NI | |
| 34 | 31.698 | M-H | 608 | * | | * | | | | * | | | | 609, 521, 507, 419, 405, 383 | C30H24O14 | Apigenin 7-(4-caffeoylglucuronide) | 2 |
| 35 | 33.901 | M-H | 806 | * | | * | | | | | | | | 805, 743, 526 | | NI | |

CC: Rare crysanthemum coronarium L.
CCB: steamed Crysanthemum coronarium L.
SP:spring of pochun, SM: summer of pochun, W: winter of pochun,
MH: mohyun, GN: ganam, GD: gangdong
NI: not identify


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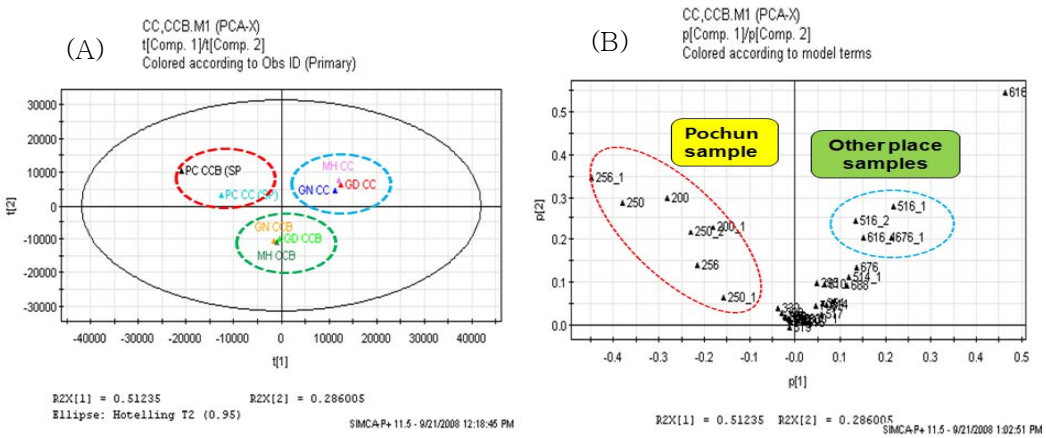


Figure 1. PCA score plots (A) and loading plots (B) for *Chrysanthemum coronarium* L metabolites from the different geographical origin.