TANG [HUMANITAS MEDICINE]

Original Article

Validation of chrysophanol and cordycepin as marker compounds for standardization of a new herbal mixture AST2017-01

Jae-Bum Jang[1,*], Tae-Young Kim[2], Hyun-Ja Jeong[3], Hyung-Min Kim[4,*]

[1]Department of Pharmaceutical Engineering, Hoseo University, 20, Hoseo-ro 79beon-gil, Baebang-eup, Asan, Chungcheongnam-do 31499, Republic of Korea; [2]Gahwa Well Food Co. Jinchun-gun, Chungbuk, 27856, Republic of Korea; [3]Department of Food Science & Technology and Research Institute for Basic Science, Hoseo University, 20, Hoseo-ro 79beon-gil, Baebang-eup, Asan, Chungcheongnam-do 31499, Republic of Korea; [4]Department of Pharmacology, College of Korean Medicine, Kyung Hee University, 26, Kyungheedae-ro, Dongdaemun-gu, Seoul, 02447, Republic of Korea

ABSTRACT

Rumex crispus (RC) or *Cordyceps militaris* (CM) has been used traditionally to treat various diseases and has been also consumed as a functional food made by humanitas medicine concept. We prepared a new herbal mixture, AST2017-01 which is mainly composed of processed (Beopje)-RC (P-RC) and -CM (P-CM). This study aims to validate marker compounds (chrysophanol and cordycepin) in P-RC and P-CM and water extracted-RC and -CM using liquid chromatography-tandem mass spectrometry. In addition, we analyzed contents of chrysophanol and cordycepin in AST2017-01. The linarites of chrysophanol and cordycepin were obtained in calibration curve with a coefficient of correlation of 0.999. The results showed that the concentrations of chrysophanol and cordycepin in P-RC and P-CM were almost 1.70 and 1.23 fold higher than that in RC and CM, respectively. Furthermore, contents of chrysophanol and cordycepin in the AST2017-01 are approximately 0.13% and 0.028%, respectively. In conclusion, these results indicate that chrysophanol and cordycepin were validated as marker compounds in the AST2017-01.

Keywords *Rumex crispus*, *Cordyceps militaris*, liquid chromatography-tandem mass spectrometry, chrysophanol, cordycepin

INTRODUCTION

Standardization of medicinal herb is very important parts for the development and production of health functional foods and medicinal drugs. Medicinal herbs have obtained their efficacy and safety through long-term ingestion experience. However, qualities of medicinal herbs depend on a natural product-producing center, soil, collection time, and cultivation conditions. And contents of compound in medicinal herbs are difference by various extraction methods. The quality control method based on the content of a marker compound is usefully used to scientifically demonstrate the functionality and safety of medicinal herbs and a marker compound can be used as an index of quality control by establishing standardization for functional foods or medicinal drugs (KFDA, 2007).

Rumex crispus (RC) belongs to the family Polygonaceae. In Korean medicine, this is widely used to treat inflammation, diarrhea, jaundice, disinfestation, and edema (Cho et al., 2016; Lee et al., 2013). Recently, Orbán-Gyapai et al. (2014) reported that RC has neuroprotective and neurorestorative properties. The marker compound of RC is chrysophanol (Qian et al., 2008).

Cordyceps militaris (CM), a species of the fungal genus Cordyceps, have been generally used as a traditional tonic in East Asia and China (Won and Park, 2005; Das et al., 2010; Yue et al., 2013). CM shows anti-cancer and anti-inflammatory properties (Rao et al., 2010; Ruma et al., 2014; Lee et al., 2015;Song et al., 2016). Cordycepin, one of the marker compound and major bioactive components of CM, has anti-allergic inflammatory and anti-oxidant properties (Kim et al., 2006; Yoou et al., 2016, 2017).

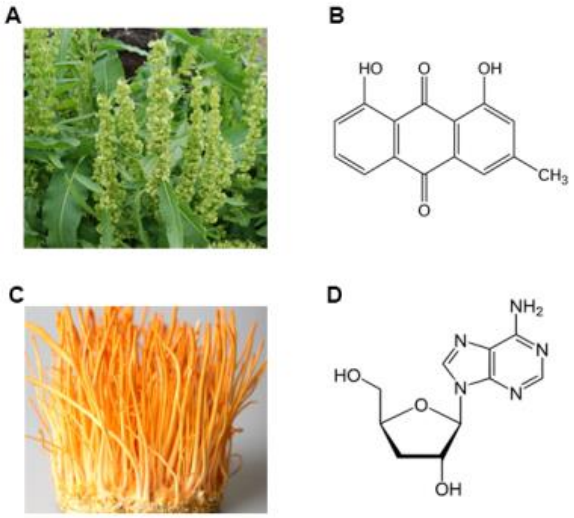

Fig. 1. Plants and structure of marker compounds. (A) *Rumex crispus*. (B) Structure of chrysophanol. (C) *Cordyceps militaris*. (D) Structure of cordycepin.

*Correspondence: Jae-Bum Jang, Hyung-Min Kim
E-mail: jaebum@hoseo.edu, hmkim@khu.ac.kr.
Received June 27, 2017; **Accepted** August 21, 2017; **Published** August 31, 2017
doi: http://dx.doi.org/10.5667/tang.2017.0013

In Korean medicine, various processing methods (Beopje, 法製) have been applied to herbal medicines in order to improve their therapeutic effects and safety in clinical trials. The processing methods for herbal medicines such as toasting, boiling in honey, steaming, and dipping or soaking in alcohol, water, or vinegar can increase their desirable effect, and decrease toxicities and side effects (Kim et al., 2002; Lee et al., 2003). Studies investigating changes in biological activities and chemical components upon processing were carried out for several medicinal herbs (Doui et al., 2010; Lee et al., 2010; Shin et al., 2003). In this respect, we applied the specific process to the RC and CM and prepared a new healthful herbal mixture, AST2017-01 which is mainly composed of processed-RC (P-RC) and processed-CM (P-CM). In this study, we identified the changes of chrysophanol and cordycepin in non-processed and processed herbs and validated chrysophanol and cordycepin as the marker compounds in AST2017-01 using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

MATERIALS AND METHODS

Preparation of RC and CM

AST2017-01 was provided by Gahwa Well Food Co. (Chungbuk, Republic of Korea). RC and CM were boiled with distilled water (DW) at 80℃ for 3 h. P-RC and P-CM were processed by Korean traditional method, Beopje. Dried RC and CM were prepared in order of wash, steam, dehydrated, parch, and then dehydrate. The crude extracts (RC, CM, P-RC, P-CM, and AST2017-1) were filtered and concentrated *in vacuo* at 60°C. And then these were lyophilized. The extract yields of herbs were about 15 - 20 % (w/w). The powders were dissolved in DW and filtered using a 0.22 μm syringe filter and kept at 4℃. Chrysophanol (purity: ≥ 98%) and cordycepin (purity: ≥ 95) were purchased from Sigma Chemical Co.(St. Louis, MO, USA) and prepared by dissolving it in dimethyl sulfoxide and DW, respectively and then diluting with DW.

Table 1. Analytical conditions of LC-MS/MS for analysis of chrysophanol

Column	Thermo Syncronis HILIC column (150 X 2.1mm, 5 μm)	
Column temp.	25°C	
Mobile phase	Methanol / 0.1%formic acid = 85/15(v/v)	
Flow rate	200 μl /min	
Injection volume	5 μl	
Autosampler temp.	25°C	
Detector (MS/MS)	**Native** ion mode, MRM mode	
	Target Compound(m/z)	253.1→224.9
	DP. :	-77.09
	EP	-10.96
	CE	-37.95
	CXP:	-15.75

Analysis of chrysophanol and cordycepin

The chrysophanol and cordycepin were analyzed using LC-MS/MS (LC: 1290Infinity; Agilent Technologies, Richardson, TX, USA; MS/MS: API 4000; Applied Biosystems, Foster City, CA, USA). RC and P-RC were extracted with ethyl acetate and filtered. CM and P-CM were dissolved in DW. Analytic conditions were summarized in Table 1 and Table 2. The concentration was analyzed using Analyst software (version 1.4.2; Applied Biosystems).

Table 2. Analytical conditions of LC-MS/MS for analysis of cordycepin

Column	Thermo Syncronis C_{18} column (150 X 2.1mm, 5 μm)	
Column temp.	25°C	
Mobile phase	Methanol / 0.1%formic acid = 85/15(v/v)	
Flow rate	200 μl /min	
Injection volume	5 μl	
Autosampler temp.	25°C	
Detector (MS/MS)	**Positive** ion mode, MRM mode	
	Target Compound(m/z)	252.1→136.1
	DP. :	66.03
	EP	10.89
	CE	21.45
	CXP:	7.09

RESULTS

Analysis of chrysophanol and cordycepin by LC-MS/MS

The concentrations of chrysophanol (a marker compound of RC) and cordycepin (a marker compound of CM) (Fig. 1) were analyzed using LC-MS/MS. Figure 2 showed calibration curves (Fig. 2A) and LC-MS/MS spectrum of marker compounds (Fig. 2B). The linarites of chrysophanol and cordycepin were obtained in calibration curve with a coefficient of correlation of 0.999 (Figs. 2A and B). LC-MS/MS patterns of standard and marker compounds were showed in Figure 3. The analytical results for chrysophanol and cordycepin are abbreviated in Table 3. Results on the chemical compositions of RC, P-RC, CM, and P-CM showed that the contents of chrysophanol were about 0.8166 mg/g and 1.3842 mg/g in RC and P-RC, respectively and the concentrations of cordycepin were about 1.2029 mg/g and 1.4821 mg/g in CM and P-CM, respectively (Table 3). The concentrations of chrysophanol and cordycepin in P-RC and P-CM were almost 1.23 and 1.70 fold higher than that in RC and CM, respectively.

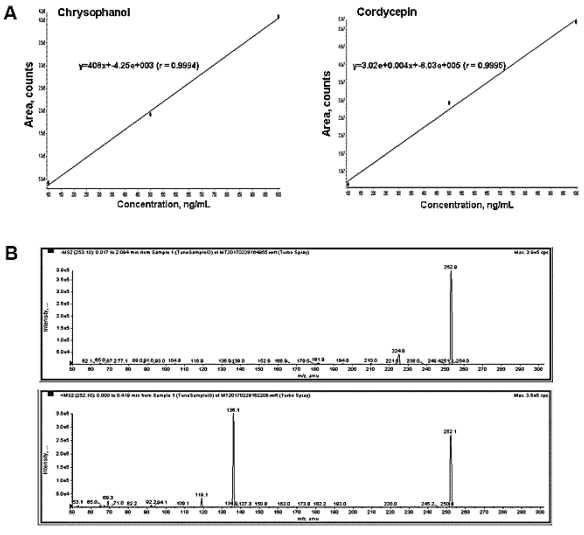

Fig. 2. Calibration curves and LC-MS/MS spectrum of marker compounds. (A) Calibration curve. (B) LC-MS/MS spectrum of chrysophanol (upper) and cordycepin (lower).

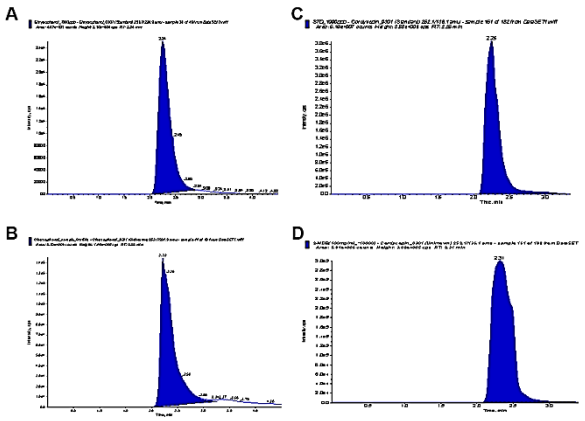

Fig. 3 LC-MS/MS pattern of standard and marker compounds. (A) Chrysophanol standard. (B) Chrysophanol in processed-RC (P-RC). (C) Cordycepin standard. (D) Cordycepin in processed-CM (P-CM).

Table 3. Contents of marker compounds by LC-MS/MS.

Samples	Chrysophanol (mg/g)	Cordycepin (mg/g)
Rumex crispus	0.8166	
Processed-*Rumex crispus*	1.3842	
Cordyceps militaris		1.2029
Processed-*Cordyceps militaris*		1.4821

Contents of chrysophanol and cordycepin in the AST2017-01 by LC-MS/MS

Contents of chrysophanol and cordycepin in AST2017-01 were determined using LC-MS/MS. The analytical results for chrysophanol and cordycepin are abbreviated in Table 4. Results on the chemical composition of AST2017-01 showed that the chrysophanol was about 0.13% and cordycepin was about 0.028%.

Table 4. Contents of marker compounds in AST2017-01 by LC-MS/MS.

Samples	Chrysophanol (%)	Cordycepin (%)
AST2017-01	0.13	0.028

DISCUSSION

Medicinal herb contains a variety of nutrition and diterpenes, triterpenes, vitamins, essential amino acids, sesquiterpenes, anthraquinones, phytosterols, glycosidic derivatives of flavonoids, caffeoylquinic acid derivatives, minerals, and trace elements,. It has various biological properties. Although drugs can alleviate the symptoms of various diseases, research efforts are currently focusing on functional foods and medicinal herbs that possess the ability to reduce the clinical symptoms of these diseases (Ryu et al., 2015; Seo et al., 2015). Many active components derived from medicinal herb have recently fascinated attention for their potential use as drugs or functional foods for treating and preventing various diseases (Ha et al., 2014). However, to develop medicinal herb as a health functional food or drug, it is also necessary to analyze the marker compound. RC contains chrysophanol, saponin, tannins, flavonoids, oil, and emodin (Jeong et al., 2006). A variety of compounds (cordycepin, cordycepic acid, nucleosides, polysaccharides, ergosterol, and other compounds) have been isolated from CM (Yue et al., 2012). In this study, we showed that contents of chrysophanol and cordycepin in P-RC and P-CM were higher than RC and CM. In addition, we found that AST2017-01 contains chrysophanol and cordycepin as marker compounds. In conclusion, we suggested that chrysophanol and cordycepin were validated as marker compounds in the AST2017-01.

ACKNOWLEDGEMENTS

This work was supported by Korea Institute of Planning and Evaluation for Technology in Food, Agriculture, Forestry and Fisheries (IPET) through High Value-added Food Technology Development Program, funded by Ministry of Agriculture, Food and Rural Affairs (MAFRA) (116169-3).

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES

Cho EJ, Um SI, Han JH, Kim B, Han SB, Jeong JH, Kim HR, Kim I, Whang WK, Lee E, Sohn UD. The cytoprotective effect of Rumex Aquaticus Herba extract against hydrogen peroxide-induced oxidative stress in AGS cells. Arch Pharm Res. 2016;39:1739-1747.

Das SK, Masuda M, Sakurai A, Sakakibara M. Medicinal uses of the mushroom Cordyceps militaris: current state and prospects. Fitoterapia 2010;81:961-968.

Doui M, Kakiuchi N, Mikage M. Chemical differences between steamed rhubarbs with or without pre-processing with liquor. J Trad Med. 2010;27:109-114.

Ha BG, Yonezawa T, Son MJ, Woo JT, Ohba S, Chung UI, Yagasaki K. Antidiabetic effect of nepodin, a component of Rumex roots, and its modes of action in vitro and in vivo. Biofactors. 2014;40:436-447.

Jeong GT, Lee KM, Park DH. Study of Antimicrobial and Antioxidant Activities of Rumex crispus Extract. Korean Chem. Eng. Res. 2006;44:81-86.

KFDA. Guideline for standard of health functional food. Korea Food & Drug Administration. Seoul, Korea. 2017: 6-13.

Kim HG, Shrestha B, Lim SY, Yoon DH, Chang WC, Shin DJ, Han SK, Park SM, Park JH, Park HI, Sung JM, Jang Y, Chung N, Hwang KC, Kim TW. Cordycepin inhibits lipopolysaccharide-induced inflammation by the suppression of NF-kappaB through Akt and p38 inhibition in RAW 264.7 macrophage cells. Eur J Pharmacol. 2006;545:192-199.

Kim JS, Kim HJ, Ma JY, Kim JM. Studies on the processing of herbal medicines (II)-HPLC analysis of standard compounds of unprocessed and processed herbal medicines. Korean J Pharmacogn. 2002;33:305-307.

Lee DH, Kwon HW, Kim HH, Lim DH, Nam GS, Shin JH, Kim YY, Kim JL, Lee JJ, Kwon HK, Park HJ. Cordycepin-enriched WIB801C from Cordyceps militaris inhibits ADP-induced [Ca(2+)] i mobilization and fibrinogen binding via phosphorylation of IP 3R and VASP. Arch Pharm Res. 2015;38:81-97.

Lee JR, Jo MJ, Park SM, Kim SC, Park SJ. Establishment of UPLC method for analysis of liquiritigenin and studies on the processing of licorice for enhancement of liquiritigenin content. Korean J Orient Med Prescrip. 2010;18:145-154.

Lee MJ, Song HJ, Jeong JY, Park SY, Sohn UD. Anti-Oxidative and Anti-Inflammatory Effects of QGC in Cultured Feline Esophageal Epithelial Cells. Korean J Physiol Pharmacol. 2013;17:81-87.

Lee YM, Kim JS. Studies on the processing of herbal medicines (VI), HPLC analysis of standard compounds of unprocessed and processed herbal medicines. Korean J Orien Med. 2003;9:69-72.

Orbán-Gyapai O, Raghavan A, Vasas A, Forgo P, Hohmann J, Shah ZA. Flavonoids isolated from Rumex aquaticus exhibit neuroprotective and neurorestorative properties by enhancing neurite outgrowth and synaptophysin. CNS Neurol Disord Drug Targets. 2014;13:1458-1464.

Qian G, Leung SY, Lu G, Leung KS. Optimization and validation of a chromatographic method for the simultaneous quantification of six bioactive compounds in Rhizoma et Radix Polygoni Cuspidati. J Pharm Pharmacol. 2008;60:107-113.

Rao YK, Fang SH, Wu WS, Tzeng YM. Constituents isolated from Cordyceps militaris suppress enhanced inflammatory mediator's production and human cancer cell proliferation. J Ethnopharmacol. 2010;131:363-367.

Ruma IM, Putranto EW, Kondo E, Watanabe R, Saito K, Inoue Y, Yamamoto K, Nakata S, Kaihata M, Murata H, Sakaguchi M. Extract of Cordyceps militaris inhibits angiogenesis and suppresses tumor growth of human malignant melanoma cells. Int J Oncol. 2014;45:209-218.

Ryu HW, Song HH, Shin IS, Cho BO, Jeong SH, Kim DY, Ahn KS, Oh SR. Suffruticosol A isolated from Paeonia lactiflora seedcases attenuates airway inflammation in mice induced by cigarette smoke and LPS exposure. J Functional Foods. 2015;17:774-784.

Seo KH, Ryu HW, Park MJ, Park KH, Kim JH, Lee MJ, Kang HJ, Kim SL, Lee JH, Seo WD. Mangosenone F, a furanoxanthone from Garciana mangostana, induces reactive oxygen species-mediated apoptosis in lung cancer cells and decreases xenograft tumor growth. Phytother Res. 2015;29:1753-1760.

Shin YW, Kim DH, Kim NJ. Studies on the processing of crude drugs (VII)—on the constituents and biological activities of Gardeniae Fructus by processing. Korean J Pharmacogn. 2003;34:45-54.

Song J, Wang Y, Teng M, Zhang S, Yin M, Lu J, Liu Y, Lee RJ, Wang D, Teng L. Cordyceps militaris induces tumor cell death via the caspase dependent mitochondrial pathway in HepG2 and MCF 7 cells. Mol Med Rep. 2016;13:5132-5140.

Won SY, Park EH. Anti-inflammatory and related pharmacological activities of cultured mycelia and fruiting bodies of Cordyceps militaris. J Ethnopharmacol. 2005;96:555-561.

Yoou MS, Jin MH, Lee SY, Lee SH, Kim B, Roh SS, Choi IH, Lee MS, Kim HM, Jeong HJ. Cordycepin Suppresses Thymic Stromal Lymphopoietin Expression via Blocking Caspase-1 and Receptor-Interacting Protein 2 Signaling Pathways in Mast Cells. Biol Pharm Bull. 2016;39:90-96.

Yoou MS, Yoon KW, Choi Y, Kim HM, Jeong HJ. Cordycepin diminishes thymic stromal lymphopoietin-induced interleukin-13 production. Eur J Pharmacol. 2017;802:1-6.

Yue K, Ye M, Zhou Z, Sun W, Lin X. The genus Cordyceps: a chemical and pharmacological review. J Pharm Pharmacol. 2013;65:474-493.