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Optimization of Extraction Condition of Methyl Jasmonate-treated Wild Ginseng Adventitious Root Cultures using Response Surface Methodology

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Abstract – The usage of wild ginseng (*Panax ginseng* C.A. Meyer) has been limited due to short supply and high price. Therefore, sufficient production as well as efficient extraction of mountain ginseng are required for the development as products. In this study, wild ginseng adventitious root cultures were prepared for efficient production with advantages of fast growth and stable production. Treatment of methyl jasmonate (MJ) to wild ginseng adventitious root cultures increased the extraction yield and antioxidative activity. Further investigation on effect of extraction conditions suggested the importance of ethanol concentration on antioxidative activity and extraction yield of MJ-treated wild ginseng adventitious root cultures. Optimized extraction condition of MJ-treated wild ginseng adventitious root cultures for maximum extraction yield and antioxidative activity was determined using response surface methodology with three-level-three-factor Box-Behnken design (BBD). Extraction of 1 g MJ-treated wild ginseng adventitious root culture with 30 ml of 9% ethanol at 30 ºC produced 310.2 mg extract with 71.0% antioxidative activity at 100 μg/ml. Taken together, MJ-treated wild ginseng adventitious root culture is valuable source for wild ginseng usage and optimized extraction condition can be used for the development of functional products or folk remedies.

Keywords – Wild ginseng adventitious root cultures, Methyl jasmonate, Optimized extraction condition, Antioxidative activity, Extraction yield

Introduction

Panax ginseng C.A. Meyer (Araliaceae), commonly called ginseng, is one of the most widely used traditional herbs. Its roots have been used as a tonic to enhance immune response and consequent health and longevity for over 2000 years in Oriental countries (Tang and Eisenbrand, 2011). They also exert wide ranges of pharmacological effects including anti-cancer, anti-inflammatory, anti-diabetic, anti-fatigue and neuroprotective activities (Ru *et al*., 2015; Wang *et al*., 2016; Park *et al*., 2016; Patel and Rauf, 2017).

Ginseng is a perennial plant that grows slowly and has a long production cycles, 4 - 6 years. The bioactive constituents of ginseng reach desirable quality when the ginseng has matured (Chung *et al*., 2016; He *et al*., 2016). Ginseng grows in wild environment or is cultivated on farm. Cultivated ginseng is systematically grown on farm under the control of growth condition and harvested after 4 - 6 year cultivation. On the contrary, wild ginseng, also called mountain ginseng in Korea, grows in the wild and deep in the mountain with little sunshine and fluctuating temperature without man's intervention. Due to the difference of environmental condition and genotypes, wild ginseng and cultivated ginseng have different composition and biological activities. The concentration of ginsenosides and amino acids in wild ginseng are much higher than those of cultivated ginseng. Wild ginseng also reported to have enhanced host defense components and biological activities (Pan *et al*., 2013; Sun *et al*., 2016).

Although wild ginseng has a great benefit, its usage has been limited because of short supply and high price. Therefore, sufficient production is required for the development as products. As a preparation of wild ginseng, tissue culture system is considered a valuable tool to achieve rapid and stable production of excellent individual (Ruffoni *et al*., 2010; Murphy *et al*., 2014). In this study, wild ginseng adventitious root cultures were prepared for

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efficient production with advantages of fast growth and stable production with high antioxidant activity. Effect of elicitor and extraction conditions on antioxidant activity and extraction yield were also analyzed. For the development of wild ginseng as functional products, extraction procedure is indispensable. The content of active constituents in extract together with their biological activity are highly affected by extraction conditions such as extraction solvent, extraction time, extraction temperature and ratio of sample/solvent. For this reason, extraction conditions was optimized for maximal antioxidant activity and extraction yield using response surface methodology (RSM) to secure efficiency in quality and economic aspects.

Experimental

Plant material – Adventitious root cultures of wild ginseng (*P. ginseng*) were produced from a 100-year-old wild ginseng through callus culture as we described previously (Yu *et al*. 2000). The root cultures were proliferated in a 5 L airlift balloon type bioreactor containing 4.0 L Murashige and Skoog (MS) liquid medium (3/4 strength) supplemented with 5.0 mg/L IBA, 0.1 mg/L kinetin, and 5% (w/v) sucrose for seven weeks. One week before harvest, 100 μM MJ were added to the culture as an elicitor. These roots explants were dried after collection from culture and were used for further experiments.

Optimization using response surface of methodology – A Box-Behnken design (BBD) with three variables and three levels was used to optimize the extraction conditions for maximum antioxidant activity and extraction yield of wild ginseng adventitious root cultures. Ethanol concentration (X_1), extraction temperature (X_2) and solvent/sample ratio (X_3) were chosen for independent variables, meanwhile antioxidant activity and extraction yield were as dependent responses. The variables were coded at three levels (-1, 0, and, 1) and the complete design was consisted of 15 experimental points including three replication of the center points whose variables were all coded as zero (Table 1).

Regression analysis was performed according to the experimental data. Y is the response, β_0, β_i, β_{ii}, β_{ij} are the coefficients for intercept, linearity, square and interaction, respectively and the mathematical model can be explained by the following equation

$$: Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} X_i^2 + \sum_{1 \le i \le j}^{3} \beta_{ij} X_i X_j$$

Optimized extraction condition was derived from RSM and optimal condition was confirmed by the extraction and measurement of antioxidant activity and extraction yield.

Evaluation of antioxidant activity – The antioxidant activity was evaluated by measuring the free radical

Table 1. A Box-Behnken design for extraction factors on antioxidant activity and extraction yield of wild ginseng adventitious root cultures

Run	Actual variables			Observed values	
	Ethanol concentration (%)	Extraction Temperature (ºC)	Solvent/sample ratio	Antioxidant activity[a] (%)	Yield (mg extract/g sample)
1	100	50	20	53.6	48.2
2	100	30	20	51.8	31.5
3	50	50	30	65.5	317.4
4	50	30	30	65.4	297.6
5	100	40	30	54.5	40.5
6	50	40	20	60.1	253.7
7	0	50	20	65.0	219.4
8	50	40	20	58.0	250.8
9	50	30	10	60.8	199.8
10	0	30	20	69.6	246.0
11	50	50	10	62.0	213.8
12	0	40	30	67.3	247.3
13	100	40	10	52.3	29.6
14	0	40	10	68.2	85.1
15	50	40	20	58.9	247.2

[a] Antioxidant activity (%) was measured at 100 μg/mL.

scavenging activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, extracts prepared from different extraction conditions were mixed with freshly prepared DPPH solution. After shaking, the reaction mixtures were stand for 30 min at room temperature in dark places. The radical scavenging activity was determined by measuring the absorbance at 517 nm. The relative radical scavenging activity (%) was calculated as [1 – absorbance of solution with sample and DPPH / absorbance of solution with DPPH] × 100.

Result and Discussion

Antioxidant activity and extraction yield of MJ-untreated and MJ-treated wild ginseng adventitious root culture extract – Ginseng is traditionally used as a tea or an alcoholic beverage with ethanol for the health promoting effects. Therefore, antioxidant activity and extraction yield of wild ginseng adventitious root cultures were investigated. In plant tissue cultures, elicitors were used for enhancing the production of bioactive constituents (Kim *et al*., 2007; Rahimi *et al*., 2015). Therefore, wild ginseng adventitious root cultures were treated with methyl jasmonate (MS) as an elicitor and the antioxidant activity and extraction yield of MJ-treated wild ginseng adventitious root cultures were also analyzed. Extraction of 1 g wild ginseng adventitious root cultures with 50% ethanol yielded 88.2 mg extract with IC_{50} value of 75.9 μg/mL. The amount of extract prepared from 1 g MJ-treated wild ginseng cultures was 152.4 mg with IC_{50} value of 38.4 μg/mL. Several elicitors have been reported to increase secondary metabolite such as saponins and phenolic compounds in cultures of *P. ginseng*. Treatment of elicitors also increased the biological activity (Jeong *et al*., 2005; Hu *et al*., 2007). In our present study, HPLC chromatogram of MJ-treated wild ginseng adventitious root cultures showed similar pattern to that of MJ-untreated cultures, however, some minor additional peaks were observed, as shown in Fig. 1. In addition, MJ-treated wild ginseng adventitious root cultures produced high yield with better antioxidant activity compared to MJ-untreated samples. Therefore, MJ-treated wild ginseng adventitious root cultures were chosen for further experiment.

Effect of extraction variables on antioxidant activity and extraction yield – The content of active constituents in extract together with their biological activity are highly affected by extraction conditions (Herzi *et al*., 2013; Jeong *et al*., 2014). Therefore, effects of extraction conditions of wild ginseng adventitious root cultures on extraction yield and antioxidant activity were analyzed. RSM is the statistical analysis, which can figure out relationship between each factor and values with only a few point of factors. Therefore, RSM is used for the analysis of extraction conditions.

Extraction variables for RSM were selected as extraction solvent (X_1, ethanol concentration in water, 0, 50 and 100%), extraction temperature (X_2, 30, 40 and 50 ºC) and solvent/sample ratio (X_3, 10, 20 and 30) based on the preliminary study and complete with 15 experimental points was set up (Table 1).

Antioxidant activity and extraction yield of wild ginseng adventitious root cultures extracts prepared from

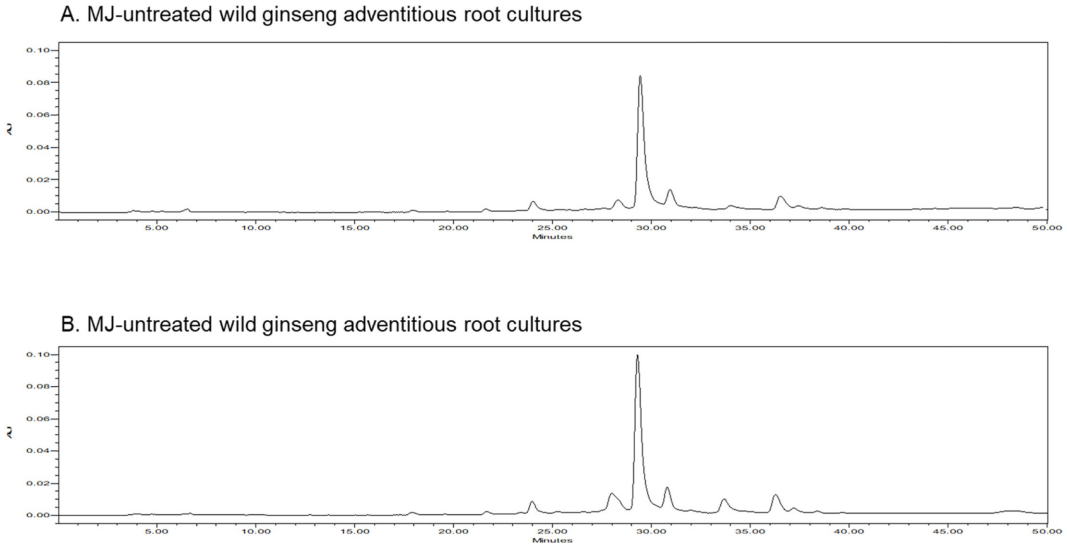

Fig. 1. HPLC chromatograms of MJ-untreated and MJ treated wild ginseng adventitious root cultures.

Table 2. Regression coefficients and their significances in the second-order polynomial regression equation for antioxidant activity and extraction yield

	Coefficient	Standard error	*t* value	*p* value
[Antioxidant activity]				
Intercept	59.000	0.822	71.786	<0.001
X_1	-7.238	0.503	-14.380	<0.001
X_2	-0.188	0.503	-0.373	0.725
X_3	1.175	0.503	2.335	0.067
X_1^2	-0.925	0.741	-1.249	0.267
X_2^2	1.925	0.741	2.598	0.048
X_3^2	2.500	0.741	3.375	0.020
X_1X_2	1.600	0.712	2.248	0.074
X_1X_3	0.775	0.712	1.089	0.326
X_2X_3	-0.275	0.712	-0.386	0.715
[Extraction yield]				
Intercept	250.567	12.284	20.398	<0.001
X_1	-81.000	7.522	-10.768	<0.001
X_2	2.988	7.522	0.397	0.708
X_3	46.813	7.522	6.223	0.002
X_1^2	-135.408	11.073	-12.229	<0.001
X_2^2	21.117	11.073	1.907	0.115
X_3^2	-14.533	11.073	-1.313	0.247
X_1X_2	10.825	10.638	1.018	0.357
X_1X_3	-37.825	10.638	-3.556	0.016
X_2X_3	1.450	10.638	0.136	0.897

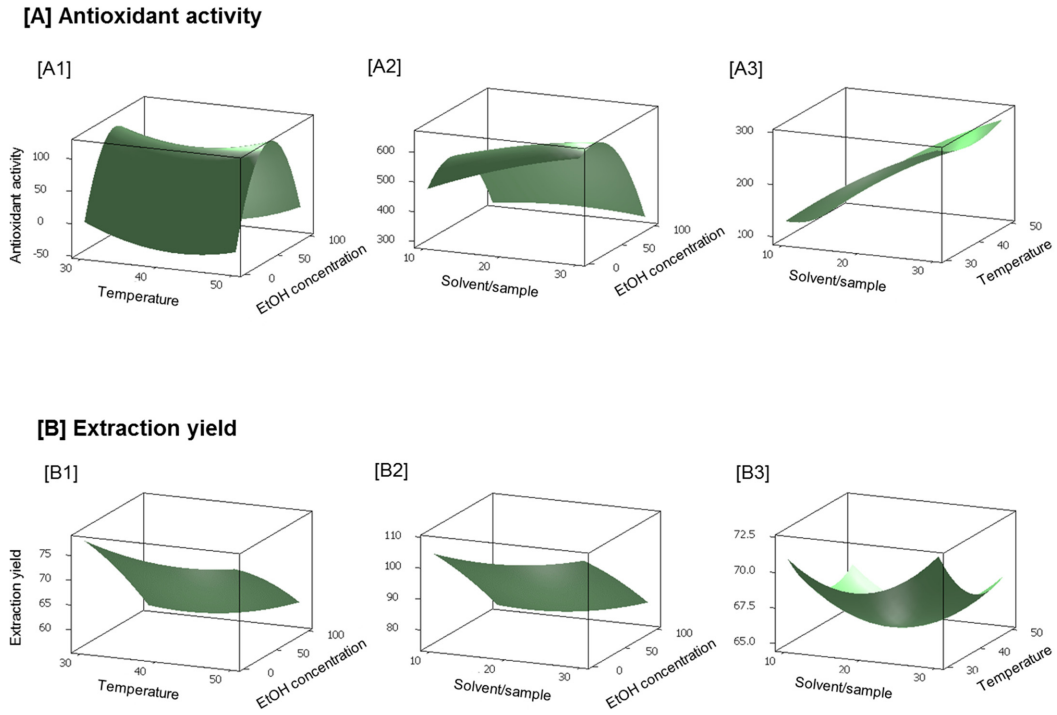

Fig. 2. Response surface plots of extraction variables on [A] antioxidant activity and [B] extraction yield of wild ginseng adventitious root cultures.

different extraction conditions were measured. As shown in Table 1, extraction yield was greatly affected depending on extraction conditions, ranging from 29.6 to 317.4 mg extract from 1 g dried sample. However, antioxidant activity (%) was ranged from 51.8 to 69.6%, which showed good antioxidant activity in all experimental points.

Multiple regression analysis (Table 2) on the experiment data yielded the second-order polynomial regression

equations as follows:

Antioxidant activity (%) = $59.00 - 7.24X_1 - 0.19X_2 - 1.18X_3 - -0.93X_1^2 - 1.92X_2^2 - 2.50X_3^2 + 1.60X_1X_2 + 0.78\ X_1X_3 - 0.28X_2X_3$

Extraction yield (%) = $250.57 - 81.00X_1 + 2.99X_2 + 46.81X_3 - 135.41X_1^2 + 21.12X_2^2 - 14.53X_3^2 + 10.83\ X_1X_2 - 37.83X_1X_3 + 1.45X_2X_3$

Among extraction variables, ethanol concentration (X_1) showed the most significant effect on antioxidant activity with p-value of <0.001 (Table 2). Extraction yield was also affected by ethanol concentration (X_1) and solvent/sample ratio (X_3). The linear terms of ethanol concentration (X_1) and solvent/sample ratio (X_3), the quadratic term of extraction temperature (X_1^2) and interaction term of ethanol concentration and solvent/sample ratio (X_1X_3) showed significant effect on extraction yield. However, extraction temperature showed relatively little effect on antioxidant activity and extraction yield of wild ginseng adventitious root cultures.

Consistent with regression coefficients and their significances, three dimensional response surface plots of antioxidant activity and extraction yield also showed the dramatic effect of ethanol concentration on extraction yield and antioxidant activity of wild ginseng adventitious root cultures (Fig. 2). On fixed temperature at 40 ºC or solvent/sample ratio as 20, antioxidant activity was improved as ethanol concentration reduced (Fig. 2.A). On the aspect of extraction yield, quadratic pattern of temperature and solvent/sample ratio was observed at fixed ethanol concentration (Fig. 2.B).

Taken together, antioxidant activity and extraction yield were greatly affected by ethanol concentration and solvent/sample ratio, whereas extraction temperature exerted relatively little effect.

Optimization of extraction condition and verification – Due to the importance of extraction condition on antioxidant activity and extraction yield based on our present study, optimization for extraction condition is required for maximum efficacy. Therefore, optimized extraction condition for maximum antioxidant, optimized extraction condition for maximum extraction yield and optimized extraction condition for both maximum antioxidant and extraction yield were deduced using RSM. Maximum antioxidant activity was expected when 1 g wild ginseng adventitious root culture was extracted by 30 mL water at 30 ºC which predicted 72.2% antioxidant activity. Extraction of 1 g sample under this extraction condition yielded 288.8 mg extract with 74.1% antioxidant activity. On the other hands, maximum extraction yield was expected when 1 g wild ginseng adventitious root culture was extracted by 30 mL of 26.1% ethanol at 30 ºC which predicted 330.5 mg extract. Extraction of 1 g sample under this extraction condition yielded 323.4 mg extract with 71.6% antioxidant activity. Extraction condition for maximum antioxidant activity and extraction yield were optimized as extraction of 1 g wild ginseng adventitious root culture with 30 mL of 9.2% ethanol at 30 ºC which predicted 315.0 mg extract with 71.0% antioxidant activity. Extraction of 1 g sample under this extraction condition yielded 310.2 mg extract with 72.6% antioxidant activity, which was well matched with predicted values (Table 3).

Conclusion

Although the great value of wild ginseng, its usage has been limited due to its supply and subsequent high price. In our present study, we suggested wild ginseng adventitious root cultures as efficient production. Extract of wild ginseng adventitious root cultures showed antioxidant activity. In addition, MJ-treated sample improved antioxidant activity and extraction yield. Extraction conditions, especially extraction solvent and solvent/sample ratio greatly affected antioxidant activity and extraction yield of wild ginseng adventitious root cultures. Our study also provided the optimized extraction conditions for the

Table 3. Predicted and observed values of antioxidant and extraction yield under differential optimized condition

Extraction condition			Predicted		Observed	
Ethanol concentration (%)	Extraction temperature (ºC)	Solvent/sample ratio	Antioxidant activity	Extraction yield	Antioxidant activity	Extraction yield
[Optimized for maximum antioxidant activity]						
0.0	30.0	30.0	72.2	-	74.1	288.2
[Optimized for maximum extraction yield]						
26.1	30.0	30.0	-	330.5	71.6	323.4
[Optimized for maximum activity and extraction yield]						
9.2	30.0	30.0	71.0	315.0	72.6	310.2

maximum antioxidant activity and extraction yield of wild ginseng adventitious root cultures as extraction of 1 g wild ginseng adventitious roots with 30 mL of 9.2% ethanol at 30 ℃ which yielded 310.2 mg extract with 72.6% antioxidant activity. These results will give a strong support for economic efficiency for the preparation of wild ginseng adventitious root cultures.

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References

(1) Chung, I. M.; Lim, J. J.; Ahn, M. S.; Jeong, H. N.; An, T. J.; Kim, S. H. *J. Ginseng Res.* **2016**, *40*, 68-75.

(2) He, J. M.; Zhang, Y. Z.; Luo, J. P.; Zhang, W. J.; Mu, Q. *Nat. Prod. Commun*. **2016**, *11*, 739-740.

(3) Herzi, N.; Bouajila, J.; Camy, S.; Romdhane, M.; Condoret, J. S. *Food Chem*. **2013**, *141*, 3537-3545.

(4) Hu, F. X.; Zhong, J. J. *J. Biosci. Bioeng.* **2007**, *104*, 513-516.

(5) Jeong, G. T.; Park, D. H.; Ryu, H. W.; Hwang, B.; Woo, J. C.; Kim, D.; Kim, S. W. *Appl. Biochem. Biotechnol.* **2005**, *124*, 1147-1157.

(6) Jeong, J.Y.; Jo, Y. H.; Lee, K. Y.; Do, S. G.; Hwang, B. Y.; Lee, M. K. *Bioorg. Med. Chem. Lett*. **2014**, *24*, 2329-2333.

(7) Kim, O. T.; Bang, K. H.; Shin, Y. S.; Lee, M. J.; Jung, S. J.; Hyun, D. Y.; Kim, Y. C.; Seong, N. S.; Cha, S. W.; Hwang, B. *Plant Cell Rep*. **2007**, *26*, 1941-1949.

(8) Murthy, H. N.; Georgiev, M. I.; Kim, Y. S.; Jeong, C. S.; Kim, S. J.; Park, S. Y.; Paek, K. Y. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 6243-6254.

(9) Pan, H. Y.; Qu, Y.; Zhang, J. K.; Kang, T. G.; Dou, D. Q. *J. Ginseng Res*. **2013**, *37*, 355-360.

(10) Park, J. G.; Son, Y. J.; Aravinthan, A.; Kim, J. H.; Cho, J. Y. *J. Ginseng Res.* **2016**, *40*, 431-436.

(11) Patel, S.; Rauf, A. *Biomed. Pharmacother*. **2017**, *85*, 120-127.

(12) Rahimi, S.; Kim, Y. J.; Yang, D. C. *Appl. Microbiol. Biotechnol*. **2015**, *99*, 6987-6996.

(13) Ru, W.; Wang, D.; Xu, Y.; He, X.; Sun, Y. E.; Qian, L.; Zhou, X.; Qin, Y. *Drug Discov. Ther*. **2015**, *9*, 23-32.

(14) Ruffoni, B.; Pistelli, L.; Bertoli, A.; Pistelli, L. *Adv. Exp. Med. Biol*. **2010**, *698*, 203-221.

(15) Sun, H.; Liu, F.; Sun, L.; Liu, J.; Wang, M.; Chen, X.; Xu, X.; Ma, R.; Feng, K.; Jiang, R. *J. Ginseng Res*. **2016**, *40*, 113-120.

(16) Im, D.S.; Nah, S.Y. *Acta Pharmacol. Sin.* **2013**, *34*, 1367-1373.

(17) Wang, T.; Guo, R.; Zhou, G.; Zhou, X.; Kou, Z.; Sui, F.; Li, C.; Tang, L.; Wang, Z. *J. Ethnopharmacol*. **2016**, *188*, 234-258.

(18) Yu, K. W.; Gao, W. Y.; Son, S. H.; Paek, K. Y. *In Vitro Cell. Dev. Biol. Plant*. **2000**, *36*, 424-428.

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