Optimization of the Extraction Process for Fermented *Rhus verniciflua* Stokes Using Response Surface Methodology

Hyeon Ju Jeong¹, Ju-Hee Park¹, and Myong-Jo Kim^{2,*}

¹Samsung Herb Medicine Agricultural Co., Chuncheon, Gangwon 24341, Korea

²Department of Applied Plant Sciences, Kangwon National University, Chuncheon, Gangwon 24341, Korea

Received June 10, 2015
Revised September 4, 2015
Accepted September 15, 2015
Published online February 29, 2016

*Corresponding Author
Tel: +82-33-250-6413
Fax: +82-33-244-6410
E-mail: kimmjo@kangwon.ac.kr

pISSN 1226-7708
eISSN 2092-6456

© KoSFoST and Springer 2016

Abstract Extraction conditions for extraction yield (Y_1), total phenolic content (TPC, Y_2), and inhibition rate in human gastric carcinoma cells (Y_3) were optimized using response surface methodology for fermented *Rhus verniciflua* stokes (FRV). Optimal extraction conditions for effective use of FRV as a biofunctional material in food and pharmaceutical industries were investigated. Independent variables were extraction temperature ($X_1=25-105^\circ\text{C}$), extraction time ($X_2=4-20$ h), and the solvent to solid ratio ($X_3=10-30$ mL/g). A second order polynomial model satisfactorily fitted experimental data with coefficient of determination (R^2) values of 0.9613, 0.9851, and 0.8038, respectively, for Y_1 , Y_2 , and Y_3 . Optimal conditions for the highest extraction yield of 2.37%, a total phenol content (TPC) value of 113.75 mg of tannic acid equivalents (TAE)/g, and an inhibition rate of 72.61% were derived at $X_1=89.95^\circ\text{C}$, $X_2=9.25$ h, and $X_3=25$ mL/g.

Keywords: response surface methodology, central composite design, fermented *Rhus verniciflua* stoke, polyphenol

Introduction

Response surface methodology (RSM) is a mathematical and statistical experimental protocol that is widely used for optimization of factors involved in industrial processing for food, biology, and chemistry (1). The main advantages of RSM are reduction in the number of experimental measurements, simple evaluation of statistical relationships, and identification of interactions between variables (2,3). Therefore, RSM is less laborious and time consuming than other approaches that require optimization (4). Different RSM methods of a central composite design (CCD) and a Box-Behnken design (BBD) are widely used. CCD is popular due to suitability for a second-order polynomial model and is a powerful and common experimental design, in addition to minimal time requirements and efficiency, compared with other designs (5). Equations used to describe relationships between response variables are affected by test variables.

Rhus verniciflua stokes of family Anacardiaceae, commonly known as lacquer trees, is used as a traditional herbal medicine in Korea, Japan, and China. *R. verniciflua* stokes has been used to treat gastritis, stomach cancer, diabetes, and inflammatory diseases and has been reported to exert antioxidant (6), antiinflammatory (7,8), antirheumatoid arthritis (9), and anticancer (10,11) activities. However, application is limited as a food ingredient due to the presence of urushiol, which

causes allergic reactions (12). Therefore, removal of urushiol is important for use of the plant in the food industry. Processing using organic solvents, heat, and enzymes has been attempted for removal of urushiol. In this study, *R. verniciflua* stokes was used to remove urushiol produced by *Fomitella fraxinea* (13). Fermented *R. verniciflua* stokes (FRV) extracts have been reported to have antioxidant, antiinflammatory, anticancer, and neuro-protective effects (14-16). Phytochemical studies have revealed that FRV contains phenolic acids including gallic acid, protocatechuic acid, and lantabetulic acid, and flavonoids including sulfuretin, fustin, fisetin, and butein (17,18). These phenol compounds with a wide variety of biological properties are interesting targets for optimization of extraction from natural products (19,20). Phenolic compounds have been shown to exert anticancer, antiinflammatory, antiviral, antimicrobial, and antioxidant physiological activities (21).

Urushiol, a lipid soluble compound, is not dissolved in FRV water extracts (17), which have been reported to exhibit higher extraction yields than acetone and ethanol extracts (22). Thus, in this study, optimal extraction conditions for FRV water extracts were determined for use in food industry. Production parameters of extraction temperature, time, and the solvent to solid ratio were optimized as modifiers for maximum extraction yield, total phenolic content (TPC), and the FRV inhibition rate in human gastric carcinoma cell (AGS) cells using RSM with a CCD.

Materials and Methods

Chemicals and reagents Folin-Ciocalteu's phenol reagent (FC), sodium carbonate (SC), tannic acid (TA), and 3-(4, 5-dimethyl thiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Roswell Park Memorial Institute medium (RPMI)-1640, fetal bovine serum (FBS), and 100 U/mL penicillin A and 100 µg/mL streptomycin were obtained from HyClone Laboratories (Logan, UT, USA). All reagents used in this study were of analytical grade.

Preparation for extraction and yield measurement FRV was provided by the Fermentation and Food Processing Division of the National Academy of Agricultural Science, Rural Development Administration (Jeonju, Korea) and was stored at −20°C (DY-60F; LASSELE Co., Ltd., Seoul, Korea) prior to use. A biological approach for removal of urushiol from *R. verniciflua* stokes using *Fomitella fraxinea* was reported in a previous study (13). The FRV material was cut into 2×2 cm pieces and fermented for 14 days at 21°C to produce FRV, followed by drying in an oven at 50°C.

Dried and finely powdered FRV (1 g) was subjected to extraction using distilled water with solvent to solid ratios from 10:1 to 30:1 mL/g and subjected to extraction for different times from 4 to 20 h at constant temperatures varying from 25 to 105°C. Water extracts were filtered and dried using a vacuum evaporator (NE-Series; EYELA, Tokyo, Japan). FRV extracts were evaporated to dryness using freeze-drying (FD8508; IIShin Lab Co., Ltd., Seoul, Korea) at −72°C for 36 h and weighed (AL204; Mettler Toledo Inc., Columbus, OH, USA). The FRV extraction yield (Y_1) was expressed as a percentage based on g of extract/g of dry weight.

Total phenolic content (TPC) assay TPC values of FRV extracts were measured according to the FC procedure described by Taga *et al.* (23) with modification. Diluted extracts (1 mL) were reacted with 500 µL of FC (Sigma-Aldrich Co.), 1 mL of 35% SC (Sigma-Aldrich Co.), and 7.5 mL of distilled water. After maintaining FRV extract samples at room temperature for 1 h, samples were vortexed (SLV-6; Seoulin Bioscience, Seoul, Korea). Absorbance was measured at 760 nm using a UV-Vis spectrophotometer (V-530; Jasco, Tokyo, Japan). TPC values were quantified using a calibration curve obtained based on measuring the absorbance of TA (Sigma-Aldrich Co.) standard. The concentration was expressed as mg of tannic acid equivalents (TAE) per g of extract.

Cell culture AGS was obtained from the Korea Cell Line Bank (Seoul, Korea). Cells were cultured in RPMI-1640 medium (HyClone Laboratories) supplemented with 10% FBS and antibiotics (HyClone Laboratories), and were maintained at 37°C in a 5% CO₂ humidified incubator (NU-5510G; NuAire Inc., Plymouth, MN, USA).

Cell viability Cell viability assays were performed using MTT

reagent (Sigma-Aldrich Co.). Cells were cultured in a 96-well plate (30096; SPL Life Sciences, Pocheon, Korea) at a concentration of 1×10^4 cells/well. After 24 h of conditioning, the culture medium was aspirated, and cells were exposed to a sample concentration of 1 mg/mL for 24 h. Subsequently, 10 µL of MTT dye (5 mg/mL) was added to cultures, followed by incubation (NuAire Inc.) for 4 h at 37°C. The index of cell viability was calculated based on measurement of the optical density of color produced by MTT dye reduction at 540 nm (Model 680; Bio-Rad Inc., Hercules, CA, USA).

Experimental design and statistical analysis Optimization of FRV extraction was achieved using a CCD with five levels and three variables generated with Minitab statistical software (version 17.2; Minitab Inc., State College, PA, USA). CCD matrices consisted of six central points, eight factorial points, and six axial points (two axial points on the axis of each design variable at a distance of ± 2 from the design center), resulting in sixteen sets of experimental runs. The three independent variables selected were extraction temperature (°C) (X_1), extraction time (h) (X_2), and solvent to solid ratio (mL/g) (X_3). Actual and coded levels of the independent variables used in the experimental design are shown in Table 1. Extraction yield, TPC, and the inhibition rate in AGS cells were dependent responses.

Experimental data were subjected to an empirical second-order polynomial regression analysis using the Minitab statistical software (Minitab Inc.) and presented as:

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{1 \leq i < j \leq k} \beta_{ij} X_i X_j + \varepsilon$$

where Y is response function; β_0 is intercept, and β_i , β_{ii} , and β_{ij} are regression coefficients of the linear, quadratic, and interactive terms, respectively. Accordingly, x_i , x_i^2 , and $x_i x_j$ are coded independent variables, and k is the number of variables. Statistical significance of coefficients in the regression equation was determined using the analysis of variance (ANOVA) function of Minitab statistical software (Minitab Inc.). The fit of the polynomial model equation to responses was evaluated based on the coefficient of determination (R^2) and F test of Minitab statistical software (Minitab Inc.). Statistical significance was defined as $p < 0.05$.

Results and Discussion

Fitting the model Relationships between response functions and process parameters were identified using a CCD involving 3 factors. Extraction conditions of yield, TPC, and the inhibition rate in AGS cells were evaluated. Predicted and experimentally measured responses for each independent variable are listed Table 2. Amongst the experiments conducted in this study, experiment #14 (temperature=85°C, time=8 h, and solvent to solid ratio=25 mL/g) provided the highest extraction yield of 2.37%. Experiment #11 (temperature=65°C, time=12 h, and solvent to solid ratio=10 mL/g) produced the lowest

Table 1. Range of coded and actual values for a central composite design

Independent variables	Code units	Coded levels				
		-2	-1	0	1	2
Extraction temperature (°C)	X_1	25	45	65	85	105
Extraction time (h)	X_2	4	8	12	16	20
Solvent to solid ratio (mL/g)	X_3	10	15	20	25	30

Table 2. Central composite design with experimental and predicted values for extraction yield, total phenolic content, and the inhibition rate

Run	Extraction conditions			Response 1 (Y_1) Extraction yield (%)		Response 2 (Y_2) TPC (mg of TAE/g)		Response 3 (Y_3) Inhibition rate (%)	
	X_1 , Extraction temperature (°C)	X_2 , Extraction time (h)	X_3 , Solvent to solid ratio (mL/g)	Experimental mean (SD)	Predicted	Experimental mean (SD)	Predicted	Experimental mean (SD)	Predicted
1	85(1)	8(-1)	15(-1)	1.64 (0.12)	1.629	103.67 (1.17)	99.55	64.57 (5.71)	59.98
2	65(0)	12(0)	20(0)	1.93 (0.21)	1.943	74.49 (5.45)	78.16	57.68 (6.91)	47.12
3	45(-1)	8(-1)	25(1)	1.89 (0.11)	2.009	50.71 (2.76)	49.08	36.41 (6.15)	33.49
4	85(1)	16(1)	25(1)	2.15 (0.44)	2.299	108.48 (1.82)	104.7	56.25 (0.90)	62.29
5	65(0)	12(0)	20(0)	1.95 (0.19)	1.943	80.42 (1.37)	78.16	41.23 (1.41)	47.12
6	45(-1)	16(1)	15(-1)	1.19 (0.16)	1.229	56.64 (3.13)	58.48	33.66 (4.02)	38.5
7	25(-2)	12(0)	20(0)	1.53 (0.11)	1.523	46.38 (2.90)	43.02	33.01 (2.98)	30.45
8	105(2)	12(0)	20(0)	2.29 (0.20)	2.303	126.97 (6.41)	131.73	72.81 (0.47)	70.71
9	65(0)	20(2)	20(0)	1.88 (0.06)	1.843	77.45 (5.37)	76.25	59.47 (6.89)	47.94
10	65(0)	12(0)	30(2)	2.24 (0.09)	2.123	74.51 (3.52)	76.78	61.96 (5.14)	58.2
11	65(0)	12(0)	10(-2)	0.64 (0.09)	0.763	68.38 (3.24)	67.51	38.72 (1.28)	37.82
12	65(0)	4(-2)	20(0)	1.85 (0.09)	1.893	69.40 (4.63)	72.01	42.32 (3.27)	49.17
13	45(-1)	8(-1)	15(-1)	1.41 (0.10)	1.254	51.21 (2.94)	53.59	33.96 (3.35)	35.59
14	85(1)	8(-1)	25(1)	2.37 (0.06)	2.324	108.58 (4.61)	105.34	69.60 (1.29)	69.43
15	45(-1)	16(1)	25(1)	1.89 (0.13)	1.894	59.23 (3.79)	61.95	40.18 (4.06)	49.43
16	85(1)	16(1)	15(-1)	1.82 (0.19)	1.694	90.68 (2.51)	90.91	35.23 (3.81)	42.82

Values are mean $\pm$ SD ($n=3$).

Table 3. Analysis of variance (ANOVA) for the fitted quadratic polynomial model for optimization of extraction parameters

Source	Extraction yield ($R^2=0.9613$)					TPC ($R^2=0.9851$)					Inhibition rate ($R^2=0.8038$)				
	SS ¹⁾	DF	MS	F value	p value	SS	DF	MS	F value	p value	SS	DF	MS	F value	p value
Model	2.80	9	0.31	16.55	0.001	8422.37	9	935.82	44.02	0.00	2403.21	9	267.02	2.73	0.117
Lack of fit	0.11	5	0.02	112.61	0.071	109.96	5	21.99	1.25	0.588	451.1	5	90.22	0.67	0.725
Pure error	0.00	1	0.00			17.58	1	17.58			135.34	1	135.34		

¹⁾SS, sum of squares; DF, degrees of freedom; MS, mean square

extraction yield of 0.64%. The extract from experiment #8 (temperature =105°C, time=12 h, and solvent to solid ratio=20 mL/g) had the highest TPC of 126.97 mg/TAE g, and the extract from experiment #3 (temperature=45°C, time=8 h, and solvent to solid ratio=25 mL/g) had the lowest TPC of 50.71mg/TAE g. The extract from experiment #8 (temperature= 105°C, time=12 h, and solvent to solid ratio=20 mL/g) had the highest inhibition rate in AGS cells of 72.81%, and the extract from experiment #13 (temperature=45°C, time=8 h, and solvent to solid ratio=15 mL/g) had the lowest at 33.96%. The extraction process was thus optimized to obtain maximum desirable responses.

Fitting the quadratic model to data is shown in Table 3. ANOVA results indicated that the contribution of the quadratic model was

significant ($p<0.05$) for response of the dependent variables of extraction yield, TPC, and inhibition rate. ANOVA indicated a good model performance with R^2 values of 0.9613, 0.9851, and 0.8038 for extraction yield, TPC, and the inhibition rate in AGS cells, respectively. F values of 16.55, 44.02, and 2.73 for extraction yield, TPC, and the inhibition rate, respectively. p -Value indicated significance of the extraction yield, and the TPC value generated from the model was higher than the 95% confidence level (Table 3).

Response surface analysis of extraction yield Extraction temperature, time, and solvent to solid ratio significantly ($p<0.05$) contributed to the extraction yield. The regression equation was:

$$Y_1 (\%) = -2.51 + 0.0118X_1 + 0.0292X_2 + 0.2913X_3 - 0.000019X_1^2 \\ - 0.00117X_2^2 - 0.00500X_3^2 + 0.000281X_1X_2 - 0.000150X_1X_3 \\ - 0.00113X_2X_3$$

The quadratic relationship between the extraction yield and the extraction factors had a good regression coefficient of $R^2=0.9613$. High F values with low p values always lead to more significant correspondence amongst independent variables. X_1 , X_3 , and X_3^2 were significant ($p<0.05$), whereas X_2 , X_1^2 , X_2^2 , X_1X_2 , X_1X_3 , and X_2X_3 were not significant ($p>0.05$).

Variation of yield with extraction temperature, time, and solvent to solid ratio is presented in Fig. 1A. These factors affected the extraction efficiency. A linear increase in extraction yield with temperature is shown in Fig. 1A(a) and 1A(b). An increase in extraction temperature can improve the extraction yield due to dispersion and absorption of solutes and reduction in cell wall integrity (24,25). The extraction yield increased as the time increased from 4 to 20 h. High temperatures produced a curved effect similar to extraction time, perhaps because with a longer exposure time, a liquid could diffuse and penetrate FRV soluble materials. Zhu and Liu (26) reported that the extraction yield of crude polysaccharides from pomegranate peels increased at high extraction temperatures and with long durations. The extraction yield increased with a high solvent to solid ratio (Fig. 1A(c)). The solvent to solid ratio is another factor that influences the efficiency and selectivity of an extraction solution. If the solvent to solid ratio is too low, extraction is incomplete, whereas too much solvent can result in a high processing cost (26). The FRV yield continued to increase as the solvent to solid ratio increased, perhaps because the solvent to solid ratio increased diffusion and enhanced desorption of FRV from cells. Optimum conditions for a maximum extraction yield of 2.45% were an extraction temperature of 100.46°C for 12.21 h with a solvent to solid ratio of 24.62 mL/g.

Response surface analysis of the total phenolic content The coefficient of determination for TPC ($R^2=0.9851$) and the relationship between TPC and temperature, time, and solvent to solid ratio are shown in Table 3. The quadratic polynomial equation with significant terms was:

$$Y_2 (\text{mg of TAE/g}) = 9.4 + 0.351X_1 + 2.52X_2 - 0.01X_3 + 0.00576X_1^2 \\ - 0.0630X_2^2 - 0.0601X_3^2 - 0.0422X_1X_2 + 0.0258X_1X_3 + 0.0999X_2X_3$$

X_1 was the most significant factor for TPC ($p<0.05$). TPC was affected by temperature and time, with linear relationships between TPC and both factors shown in Fig. 1B(a). Temperature and time both operated independently on TPC (Table 3). Temperature and time are important factors for increasing the solubility and diffusion rate of components, such as phenolic contents (20,27,28). In accordance with the increase in temperature to improve the extraction rate, the extraction time required for maximum recovery of phenols decreased. Increasing the temperature likely promoted compound movement

Table 4. Response under optimal conditions of an extraction temperature of 89.95°C, a time of 9.25 h, and a solvent to solid ratio of 25 mL/g

Responses	Predicted value
Extraction yield (%)	2.37
TPC (mg of TAE/g)	113.75
Inhibition rate (%)	72.61

and increased membrane permeability of FRV phenolic compounds, potentially destroying plant cell components. In addition, all phenolic compounds may not be stable at high temperatures. Thus, high temperature extraction may be more appropriate for sample with a high proportion of thermally stable phenolic compounds. The length of solute exposure indicates the time required for a solvent to penetrate into a dry compound, dissolve a solute, and diffuse out of the compound (29).

When the solvent to solid ratio increased from 10 to 30 mL/g, the TPC value also increased by about 8.2% (at $X_1=65^\circ\text{C}$ and $X_2=12$ h) (Table 2), likely due to an increased amount of solvent that better penetrated cells allowing recovery of more phenolic compounds at a higher solvent to solid ratio (30,31). The optimum conditions for maximum total phenolic extraction of 133.80 mg of TAE/g were an extraction temperature of 104.07°C for 10.97 h with a solvent to solid ratio of 21.72 mL/g.

Response surface analysis of the inhibition rate The coefficient of determination for the inhibition rate in AGS cells ($R^2=0.8038$) and the relationship between response and extraction factors are shown in Table 3. The quadratic polynomial equation with significant terms for the FRV inhibition rate, omitting not significant terms, was:

$$Y_3 (\%) = 12.5 + 0.66X_1 + 1.57X_2 - 2.23X_3 + 0.00216X_1^2 + 0.022X_2^2 \\ + 0.0089X_3^2 - 0.0721X_1X_2 + 0.0214X_1X_3 + 0.125X_2X_3$$

X_1 was the significant factor ($p<0.05$) for the FRV inhibition rate. The inhibition rate in AGS cells was affected by temperature, time, and solvent to solid ratio (Fig. 1C). The relationship between response and the extraction factors of temperature and time, and cumulative effects on the response are shown in Fig. 1C(a). An increase in the inhibition rate was observed with an increasing temperature, but time did not have a significant ($p>0.05$) effect. When the temperature was increased from 25 to 105°C, the inhibition rate also increased by approximately 54.66% (at $X_2=12$ h and $X_3=20$ mL/g) (Table 2), likely attributable to antioxidant rich polyphenol contents (32-34). Inhibition rate on AGS cells are affected by extraction conditions of the temperature and solvent to solid ratio (Fig. 1C(b)). The response increased with an increasing temperature at a high solvent to solid ratio. The highest inhibition rates were observed at a high temperature of 105°C and a high solvent to solid ratio of 30 mL/g. The extraction time and solvent to solid ratio are shown effects on inhibition rate by their interactive (Fig. 1C(c)). Significant ($p<0.05$) increases in the response were observed at high solvent to solid ratios, whereas overheating always reduced the inhibition rate. Optimum conditions

Fig. 1. Response surface plots of (A) extraction yield, (B) total phenolic contents, and (C) inhibition rate of fermented *Rhus verniciflua* stokes showing effects of temperature, time, and the solvent to solid ratio. ¹⁾ X_1 , temperature; X_2 , time; X_3 , solvent to solid ratio.

Fig. 2. Overlapping contour plot for simultaneous optimization of extraction yield, total phenolic content, and inhibition rate from fermented *Rhus verniciflua* stokes under optimized extraction conditions. The plot shows the effect of extraction temperature and time.

for a maximum inhibition of 79.78% were an extraction temperature of 99.24°C for 8.70 h at a solvent to solid ratio of 23.11 mL/g.

Optimization of responses and verification of the model Numerical optimization of extraction parameters was performed using Minitab statistical software based on initial experimental results. Optimum extraction conditions for extraction yield, total phenolic contents, and the inhibition rate in AGS cells from FRV were obtained using a CCD and RSM. Extraction temperatures of 99.24–104.07°C for an extraction time in a range of 8.70–12.21 h at high solvent to solid ratios of 21.72–24.62 mL/g resulted in an optimal extraction yield of 2.45%, a TPC value of 133.80 mg of TAE/g, and an inhibition rate in AGS cells of 79.78% for FRV.

Prediction of a set of optimum conditions for the three response variables was also accomplished using the desirability function approach. A total desirability function value of 0.9399 was obtained within a range of 0 to 1, where 0 represents a completely undesirable response and 1 represents the most desirable response. At this desirability value, extraction from FRV using a temperature of 89.95°C, an extraction time of 9.25 h, and a solvent to solid ratio (Fig. 2) of 25 mL/g as modifiers resulted in an extract yield of 2.37%, a TPC

value of 113.75 mg of TAE/g, and an inhibition rate of 72.61% in AGS cells from FRV (Table 4). Optimal conditions for extraction produced extracts with the highest extraction yield, total phenolic contents, and inhibition rate in AGS cells using non-toxic solvents and, therefore, extracts were directly applicable for use in food and pharmaceutical industries. Identification of optimal conditions is important for industry and further studies into FRV applications as healthy and valuable materials are necessary.

Acknowledgments This study was supported by the Joint Research Project Agenda Program (Project Number: PJ009859) of the Rural Development Administration of Korea.

Disclosure The authors declare no conflict of interest.

References

- Karvela E, Makris DP, Kalogeropoulos N, Karathanos VT. Deployment of response surface methodology to optimize recovery of grape (*Vitis vinifera*) stem and seed polyphenols. *Procedia Food Sci.* 1: 1686-1693 (2011)
- Alberti A, Zielinski AAF, Zardo DM, Demiate IM, Nogueira A, Mafra LI. Optimisation of the extraction of phenolic compounds from apples using response surface methodology. *Food Chem.* 149: 151-158 (2014)
- Yim HS, Chye FY, Koo SM, Matanjun P, How SE, Ho CW. Optimization of extraction time and temperature for antioxidant activity of edible wild mushroom, *Pleurotus porrigens*. *Food Bioprod. Process.* 90: 235-242 (2012)
- Giovanni M. Response surface methodology and product optimization. *Food Technol.-Chicago* 37: 41-45 (1983)
- Aybastier O, Isik E, Sahin S, Demir C. Optimization of ultrasonic-assisted extraction of antioxidant compounds from blackberry leaves using response surface methodology. *Ind. Crop. Prod.* 44: 558-565 (2013)
- Jung CH, Jun CY, Lee S, Park CH, Cho K, Ko SG. *Rhus verniciflua* stokes extract: Radical scavenging activities and protective effects on H₂O₂-induced cytotoxicity in macrophage RAW 264.7 cell lines. *Biol. Pharm. Bull.* 29: 1603-1607 (2006)
- Kim KH, Moon E, Choi SU, Kim SY, Lee KR. Polyphenols from the bark of *Rhus verniciflua* and their biological evaluation on antitumor and anti-inflammatory activities. *Phytochemistry* 92: 113-121 (2013)
- Kim JH, Shin YC, Ko SG. Integrating traditional medicine into modern inflammatory diseases care: Multitargeting by *Rhus verniciflua* stokes. *Mediat. Inflamm.* 2014: 154561 (2014)
- Choi J, Yoon BJ, Han YN, Lee KT, Ha J, Jung HJ, Park HJ. Antirheumatoid arthritis effect of *Rhus verniciflua* and of the active component, sulfuricin. *Planta Med.* 69: 899-904 (2003)
- Kim JH, Go HY, Jin DH, Kim HP, Hong MH, Chung WY, Park JH, Jang JB, Jung H, Shin YC, Kim SH, Ko SG. Inhibition of the PI3K-Akt/PKB survival pathway enhanced an ethanol extract of *Rhus verniciflua* Stokes-induced apoptosis via a mitochondrial pathway in AGS gastric cancer cell lines. *Cancer Lett.* 256: 197-205 (2008)
- Lee J, Chae J, Lee S, Kim K, Eo W, Kim S, Choi W, Cheon SH. The efficacy and safety of standardized allergen-removed *Rhus verniciflua* extract as maintenance therapy after first-line chemotherapy in patients with advanced non-small cell lung cancer. *Am. J. Chinese Med.* 41: 773-787 (2013)
- Epstein WL. Occupational poison ivy and oak dermatitis. *Dermatol. Clin.* 12: 511-516 (1994)
- Choi HS, Kim MK, Park HS, Yun SE, Mun SP, Kim JS, Sapkota K, Kim S, Kim TY, Kim SJ. Biological detoxification of lacquer tree (*Rhus verniciflua* stokes) stem bark by mushroom species. *Food Sci. Biotechnol.* 16: 935-942 (2007)
- Liu CS, Nam TG, Han MW, Ahn SM, Choi HS, Kim TY, Chun OK, Koo SI, Kim DO. Protective effect of detoxified *Rhus verniciflua* stokes on human keratinocytes and dermal fibroblasts against oxidative stress and identification of the bioactive phenolics. *Biosci. Biotech. Bioch.* 77: 1682-1688 (2013)
- Choi MJ, Lee SJ, Jang SH, Reza MA, Hong JH, Jung HK, Park SC. Biological activities and single oral dose toxicity in rat of fermented *Rhus verniciflua* extract. *Korean J. Vet. Res.* 50: 187-195 (2010)
- Sapkota K, Kim S, Kim MK, Kim SJ. A detoxified extract of *Rhus verniciflua* Stokes upregulated the expression of BDNF and GDNF in the rat brain and the human dopaminergic cell line SH-SY5Y. *Biosci. Biotech. Bioch.* 74: 1997-2004 (2010)
- Choi HS, Yeo SH, Jeong ST, Choi JH, Park HS, Kim MK. Preparation and characterization of urushiol free fermented *Rhus verniciflua* stem bark (FRVSB) extracts. *Korean J. Food Sci. Technol.* 44: 173-178 (2012)
- Shin SH, Koo KH, Bae JS, Cha SB, Kang IS, Kang MS, Kim HS, Heo HS, Park MS, Gil GH, Lee JY, Kim KH, Li Y, Lee HK, Song SW, Choi HS, Kang BH, Kim JC. Single and 90-day repeated oral dose toxicity studies of fermented *Rhus verniciflua* stem bark extract in Sprague-Dawley rats. *Food Chem. Toxicol.* 55: 617-626 (2013)
- Ghafoor K, Park J, Choi YH. Optimization of supercritical fluid extraction of bioactive compounds from grape (*Vitis labrusca* B.) peel by using response surface methodology. *Innov. Food Sci. Emerg.* 11: 485-490 (2010)
- Shi J, Yu J, Pohorly J, Young JC, Bryan M, Wu Y. Optimization of the extraction of polyphenols from grape seed meal by aqueous ethanol solution. *J. Food Agric. Environ.* 1: 42-47 (2003)
- Beara IN, Lesjak MM, Cetojević-Simin DD, Marjanović ZS, Ristić JD, Mrkonjić ZO, Mimica-Dukić NM. Phenolic profile, antioxidant, anti-inflammatory and cytotoxic activities of black (*Tuber aestivum* Vittad.) and white (*Tuber magnatum* Pico) truffles. *Food Chem.* 165: 460-466 (2014)
- Kim MK, Kim MJ, Upadhyaya J, Yong GR, Park HS. Biological activity of lacquer tree (*Rhus verniciflua* stokes) stem bark detoxified with *Fomitella fraxinea*. *J. Agric. Life Sci.* 45: 59-66 (2014)
- Taga MS, Miller EE, Patt DE. Chia seeds as a source of natural lipid antioxidants. *J. Am. Oil Chem. Soc.* 61: 928-931 (1984)
- Samavati V, Manoochehrizade A. *Dodonaea viscosa* var. *angustifolia* leaf: New source of polysaccharide and its anti-oxidant activity. *Carbohydr. Polym.* 98: 199-207 (2013)
- Liu JL, Zheng SL, Fan QJ, Yuan JC, Yang SM, Kong FL. Optimisation of high-pressure ultrasonic-assisted extraction and antioxidant capacity of polysaccharides from the rhizome of *Ligusticum huanxiong*. *Int. J. Biol. Macromol.* 76: 80-85 (2015)
- Zhu C, Liu X. Optimization of extraction process of crude polysaccharides from Pomegranate peel by response surface methodology. *Carbohydr. Polym.* 92: 1197-1202 (2013)
- Hemwimol S, Pavasant P, Shotipruk A. Ultrasound-assisted extraction of anthraquinones from roots of *Morinda citrifolia*. *Ultrason. Sonochem.* 13: 543-548 (2006)
- Liyana-Pathirana C, Shahidi F. Optimization of extraction of phenolic compounds from wheat using response surface methodology. *Food Chem.* 93: 47-56 (2005)
- Gan CY, Latiff AA. Optimisation of the solvent extraction of bioactive compounds from *Parkia speciosa* pod using response surface methodology. *Food Chem.* 124: 1277-1283 (2011)
- Pompeu DR, Silva EM, Rogez H. Optimisation of the solvent extraction of phenolic antioxidants from fruits of *Euterpe oleracea* using response surface methodology. *Bioresour. Technol.* 100: 6067-6082 (2009)
- Prasad KN, Yang E, Yi C, Zhao M, Jiang Y. Effects of high pressure extraction on the extraction yield, total phenolic content and antioxidant activity of longan fruit pericarp. *Innov. Food Sci. Emerg.* 10: 155-159 (2009)
- Sabzevari O, Galatic G, Moridania MY, Sirakia A, O'Brien PJ. Molecular cytotoxic mechanisms of anticancer hydroxychalcones. *Chem.-Biol. Interact.* 148: 57-67 (2004)
- Rodrigo G, Almanza GR, Cheng Y, Peng J, Hamann M, Duan RD, Akesson B. Antiproliferative effects of curcuphenol, a sesquiterpene phenol. *Fitoterapia* 81: 762-766 (2010)
- Al-Suede FS, KhadeerAhamed MB, Abdul Majid AS, Baharetha HM, Hassan LE, Kadir MO, Nassar ZD, Abdul Majid AM. Optimization of Cat's whiskers tea (*Orthosiphon stamineus*) using supercritical carbon dioxide and selective chemotherapeutic potential against prostate cancer cells. *Evid.-Based Compl. Alt.* 2014: 396016 (2014)