International Journal of Scientific Research in Science, Engineering and Technology
Print ISSN: 2395-1990 | Online ISSN : 2394-4099 (www.ijsrset.com)
doi : https://doi.org/10.32628/IJSRSET21824

Study on the Optimization for the Extraction Process of Andrographolide by Ultrasonic Assisted Extraction

KiBong Kim[1], IlJin Kim[1], SongLok Uh[1], YongIl Seo[1], CholKwang Li[1], UnHyang Lyeom[2], KwangRyel Hwang[2]
[1]Pyongyang Jang Chol Gu University of Commerce, Pyongyang, Democratic People's Republic of Korea
[2]Pyongyang Technology College of Food and Commodity, Pyongyang, Democratic People's Republic of Korea

Article Info
Volume 8 Issue 2
Page Number : 08-17

Publication Issue :
March-April-2021

Article History
Accepted : 01 March 2021
Published : 07 March 2021

ABSTRACT

Because andrographis has a long history of folk medicine in Southeast Asian co untries with antibacterial, antipyretic, antineoplastic, anti-inflammatory, cardi oprotective effect and so on, the areas where andrographolia is introduced and cultivated are mainly concentrated. Espically andrographolide, neoandrograph olide, andrographiside, homoandrographolide, andrographane, andrographani n, andrographone and andrographosterol that is concentrated in the leaves of t he plant play the main medical role of andrographis. Of the above chemicals, a ndrographolide is the major bioactive constituent responsible for variety of acti vities. Leaves and stems of plant are used for extracting active phytochemicals; roots are used rarely. In this study, improved the extractability of andrographol ide from the A. paniculata by ultrasonic assisted extraction, the optimum condi tion for extracted the andrographolide is determined by response surface experi ment and detected the extractability of andrographolide under the optimum co ndition. By single factor analysis and response surface experiments, the optimu m conditions for extracted the andrographolide were the extraction solution co ncentration 62.8%, the ultrasonic extraction time 59min, the ratio of solid to li quid 1:10.5 and the extraction temperature 62°C. Investigate the andrographoli de extractability in the optimun extraction condition by HPLC were increased f rom 2.27% to 3.28%.

Keywords: Andrographis, Extraction, Andrographolide, Response Surface Expe riments, Ultrasonic Assisted Extraction

I. INTRODUCTION

Andrographis paniculata (Burm.f.) Wall. ex Nees, (En glish name— King of Bitters) is an herbaceous plant. I t belongs to Acanthaceae family, it has a long history of folk medicine in Southeast Asian countries and is o ne of the most representative "Great Southern Medici ne". It is native to India and Sri Lanka, and it is also fo und in abundance in Asian countries such as India, Pa kistan, Malaysia and Indonesia. It is cultivated extensi vely in China and Thailand, the East and West Indies, and Mauritius. Andrographis paniculata Nees, named as “Chuan Xin Lian” in Chinese, is a traditional Chine se medicine to treat sore throat, flu, and upper respira

tory tract infections. Prior researchers have deepened on andrographis's research since they recognized that the andrographis is an important substance that gives the main biological activity of life. It is normally grow n from seeds and grows in pine, evergreen and decidu ous forest areas, and also along roads and in villages [1]. Andrographis paniculata (AP) possesses many me dicinal benefifits due to the presence of various terpe ne compounds such as andrographolide (AND), 14-de oxy-11, 12-didehydroandrographolide (DDA), neoand rographolide (NEA), and andrograpanin[2]. In additio n to andrographolide induces cell cycle arrest and apo ptosis, and reduces migration, invasion, and metallopr oteinase expression in human rheumatoid fibroblast-li ke synoviocytes[3]. In the many kinds of traditional C hinese medicine preparation of the main raw material, AP can be used in respiratory tract infection, and gast roenteritis, hypertension, malaria, there are "Chinese medicine antibiotics," said. Phytochemical investigati ons on this genus have indicated that diterpenoids are the major constituents [4-6], such as andrographolide, isoandrographolide, neoandrographolide, and bisandr ographolides A-C. Furthermore, some of them showe d a variety of pharmacological effects, including antivi ral [7], anti-proliferation [8], and anti-inflammatory a ctivities [9-11], as well as activation on TRPV channel s [12]. And the bioactives are present in medicinal pla nts but in low concentration and hence a proper extra ction method needs to be developed to extract maxim um bioactives from plants. Extraction of andrographol ide from A. paniculata has been investigated in the pa st focusing mainly on conventional solvent extraction. Various modern extraction techniques such as micro wave-assisted extraction (MAE), supercritical fluid ext raction (SCFE) and ultrasonic assisted extraction (UA E) have also been developed recently for its efficient e xtraction [13]. But the study to optimized ultrasonic a ssisted extractive process of andrographolide by respo nse surface experiments was not carried out previousl y. Therefore in this study, optimized the ultrasonic as sisted extractive process of andrographolide by respon se surface experiment and detected the extractability of andrographolide under the optimum condition by HPLC.

II. METHODS AND MATERIAL

2.1 Materials

① Materials

The Andrographis paniculata (Burm.f.) Nees were use d in this study were purchased from a local supermark et in Heilongjiang Province, China in 2020. 7.

② Reagents

Andrographolide standard (Aldrich 365645-100 mg, 9 8 % HPLC grade) was produced from Sigma-Aldrich i n 2020. 7.

Acetonitrile and ethanol were purchased from XinShe ng biological thechnology Co. Ltd in Heilongjiang Pro vince, China in 2020. 7.

③ Instruments

QYTC type ultrasonic cleaning machine

HPLC (Agilent 1100)

MS205DU type electronic analytical balance

④ Period for experiment

From Augest 2020 to December 2020, an experiment was conducted at Northeast Agricultural University of China.

2.2 Method

① Chromatographic condition and system adaptabilit y test

Agilent TC-C18 column (4.6 mm×250 mm, 5 um), mo bile phase: acetonitrile-water (38:62), flow rate: 1.0 m L /min, column temperature was 25 ℃, sample size w as 10 μL, detection wavelength was 225 nm, new andr ographolide 210 nm, dehydrated andrographolide 254 nm. The theoretical plate number should be no less t han 2000 according to the peak of andrographolide, d ehydrated andrographolide and neandrographolide [1 3].

② Preparation of reference solution

An appropriate amount of andrographolide, dehydrated andrographolide and neandrographolide was weighed accurately, and methanol was added to prepare a mixed solution containing 0.1 mg for each 1 mL.

③ Preparation of test solution

Sample was accurately weighed at 10g, and the extracted solution was extracted by different extraction methods is maken at 100 mL. Take 5mL of superneutral alumina column, constant volume with 50% ethanol to 100 mL, shake well, get.

④ Single factor experiments

The effects of different extraction methods, different concentration of ethanol, different ultrasonic extration time, different ratio of solid to liquid and different extraction temperature on the total content of diterpenoid lactones (the sum of andrographolide, dehydrated andrographolide and neandrographolide) of andrographolide were investigated.

⑤ Response surface experiment

Based on the results obtained from single factor analysis, optimization was carried out by using Response surface experiment to study the effect of four independent variables [X_1: concentration of ethanol, X_2: ultrasonic extration time, X_3: ratio of solid to liquid, X_4: extraction temperature]. The Surface Response Methodology (RSM) is applied to the central composite design (CCD) for the adaptation of a second order polynomial by the least square technique. Below equation is used to determine the effects of test variables to the searched responses (extractability) and the correlation between variables.

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i\leq j}^{k} \beta_{ij} X_i X_j + \sum_{i=1}^{k} \beta_{ii} {X_i}^2 + \epsilon \quad (1)$$

where Y shows the dependent variable, β_0, β_0, β_i, β_{ii} and β_{ij} represent the regression coeffificients for constant, linear, quadratic, and interactive effects, respectively; Xi and Xj denote the independent variables.

The effects of the factors on the response were expressed as surface and contour plots to visualize the relationship between the response and the independent variables and to acquire the optimal conditions of the process.

2.3 Statistical analysis

All experiments were triplicated. Analysis of variance (ANOVA) of the results was performed using Design-Expert version 11. The statistical signifificance of the model terms was determined by calculating the F-value at confidence levels of 95% ($P < 0.05$) and 99% ($P < 0.01$). To determine the differences between the control sample and the optimum condition extractability, SPSS version 26 and the independent T-test were used.

III. RESULTS AND DISCUSSION

3.1 The results of single factor experiments

① Selection of ethanol concentration

10g of sample was accurately weighed and soaked with 100 mL of 30%, 40%, 50%, 60%, 70%, 80%, and 90% for 1 h, respectively, and then extracted by ultrasonic cleaning machine for 1h and the extraction temperature 60℃. Total extractability of diterpenoid lactones were calculated under different ethanol concentrations, and the results were shown in Figure 1.

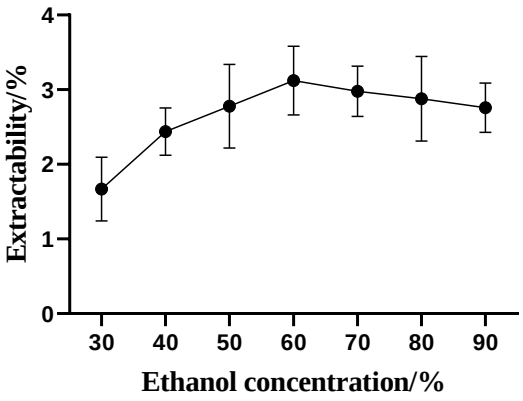

Figure 1. Comparison of total diterpene lactones extracted with different ethanol concentrations

As can be seen from Figure 1, with the increase of ethanol concentration, diterpene lactones extractability gradually increased, reaching the highest when the concentration was 60%, and then the content decreased slightly but remained at a stable level. Therefore, 60% ethanol was selected as the extraction solvent.

② Selection of extraction time

10 g of sample was accurately weighed and soaked in 100 mL 60% ethanol for 1 h, respectively, after ultrasonic extraction for 30, 60, 90, 120 and 150min, the extractability of diterpenoids extracted under different ultrasonic time was calculated, and the results were shown in Figure 2.

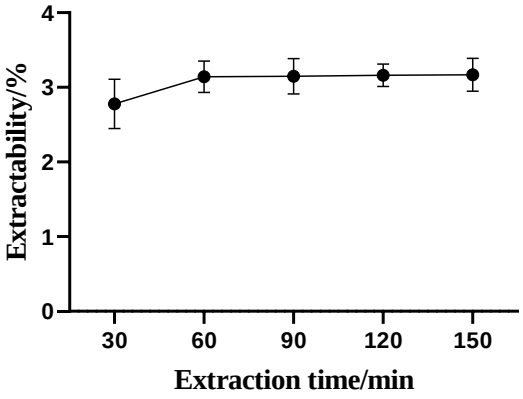

Figure 2. Comparison of total diterpene lactones extracted at different ultrasonic times

As can be seen from Figure 2, the total extractability of diterpenoids gradually increased with the extension of extraction time, and difference between extractability is rare after 1 h. Considering the economy and high efficiency of the extraction process, ultrasonic extraction time of 1 h was selected.

③ Selection of ratio of solid to liquid

10 g of sample was accurately weighed and ultrasonically extracted with 60% ethanol at 50, 100, 200 and 250 mL for 1 h, respectively. The total extractability of diterpene lactones at different ratio of solid to liquid was calculated, and the results were shown in Figure 3.

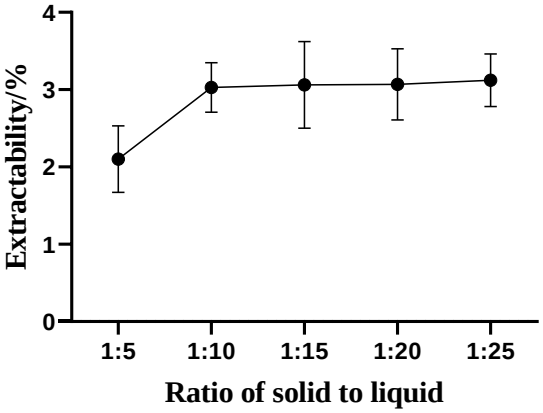

Figure 3. Comparison of total content of diterpene lactones extracted
by different ratio of solid to liquid

It can be seen from Figure 3, that the total extractability of diterpenoids gradually increased with the increase of ratio of solid to liquid, and difference between extractability is rare after 1:10, considering the extraction process. The ratio of solid to liquid is selected 1:10.

④ Selection of extraction temperature

10 g of sample was accurately weighed and soaked in 100 mL 60% ethanol in 40, 50, 60, 70, 80℃ of extraction temperature for 1 h, respectively. The total extractability of diterpene lactones at different extraction temperature was calculated, and the results were shown in Figure 4.

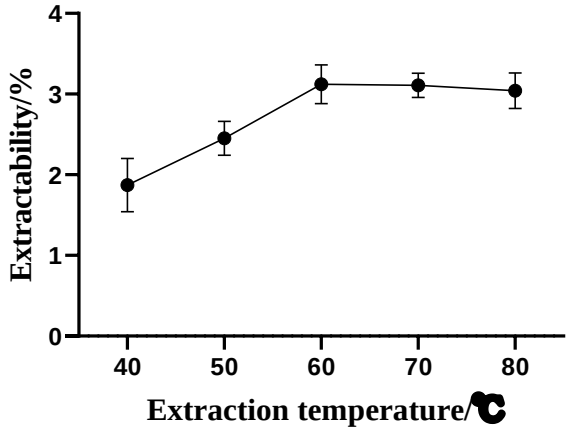

Figure 4. Comparison of total content of diterpene lac tones extracted by different extraction temperature

It can be seen from Figure 4, that the total extractabili ty of diterpenoids gradually increased with the increa se of extraction temperature, and the growth slowed d own after 60℃, considering the extraction process. T he extraction temperature is selected 60℃.

3.2 The results and analysis of Response surface experiment

The range of the independent variables and their corr esponding levels is shown in Table 1.

Table 1. The range of the independent variables and their corresponding levels

Code		Coded levels				
Independent variables	Symbol	-2	-1	0	+1	+2
Ethanol concentra tion/%	X_1	40	50	60	70	80
Extraction time/min	X_2	40	50	60	70	80
Ratio of solid to li quid	X_3	8	9	10	11	12
Extraction temper ature/℃	X_4	20	40	60	80	100

The results of central composite design (CCD) using the Design-Expert 11 software are shown in Table 2.

Table 2. Central composite design and Extractability

Runs	Factors				Extractability /%
	X_1:Ethanol content/%	X_2:Extract Time/min	X_3: Ratio of solid to liquid	X_4:Extract Temperature/℃	
1	50	50	9	40	2.81
2	70	50	9	40	2.86
3	50	70	9	40	2.82
4	70	70	9	40	2.84
5	50	50	11	40	2.93
6	70	50	11	40	3.17

7	50	70	11	40	2.97
8	70	70	11	40	3.15
9	50	50	9	80	3.09
10	70	50	9	80	2.9
11	50	70	9	80	3
12	70	70	9	80	2.83
13	50	50	11	80	3.13
14	70	50	11	80	3.16
15	50	70	11	80	2.94
16	70	70	11	80	3.03
17	40	60	10	60	2.83
18	80	60	10	60	3
19	60	40	10	60	2.93
20	60	80	10	60	3.01
21	60	60	8	60	2.83
22	60	60	12	60	3.04
23	60	60	10	20	2.92
24	60	60	10	100	3.1
25	60	60	10	60	3.26
26	60	60	10	60	3.3
27	60	60	10	60	3.29
28	60	60	10	60	3.23
29	60	60	10	60	3.26

※ Center points is 5

The Design-Expert version 11 software was used to conduct square error analysis on the data in Table 2, and th e results were shown in Table 3. Taking ethanol concentration (X_1), extraction time (X_2), ratio of solid to liquid (X_3) and extraction temperature (X_4) as the influencing factors, and the extractability(Y) as the response value, t he multiple quadratic regression equation for optimization for the extraction process of Andrographolide by ult rasonic assisted extraction was as below equation:

$$Y=3.268+0.025X_1-0.013X_2+0.073X_3+0.037X_4+0.051X_1X_3-0.045X_1X_4-0.008X_2X_3-0.03X_2X_4-0.028X_3X_4-0.085X_1^2-0.071X_2^2-0.08X_3^2-0.061X_4^2 \quad (2)$$

The correction coefficient of the model R^2=0.9523 and R_{Adj}^2 =0.9046 indicate that the model has good fitting deg ree and small experimental error, so the model is suitable

Table 3. Analysis of variance of central composite design (CCD)

Source	Sum of Squares	df	Mean Square	F-value	p-value	significant
Model	0.6611	14	0.0472	19.96	< 0.0001	**

X_1-Ethanol content	0.0145	1	0.0145	6.13	0.0267	*
X_2-Extract Time	0.0040	1	0.0040	1.69	0.2143	
X_3- Ratio of solid to liquid	0.1276	1	0.1276	53.94	< 0.0001	**
X_4-Extract Temperature	0.0330	1	0.0330	13.95	0.0022	**
X_1X_2	6.250E-06	1	6.250E-06	0.0026	0.9597	
X_1X_3	0.0431	1	0.0431	18.20	0.0008	**
X_1X_4	0.0333	1	0.0333	14.08	0.0021	**
X_2X_3	0.0011	1	0.0011	0.4465	0.5149	
X_2X_4	0.0150	1	0.0150	6.34	0.0246	*
X_3X_4	0.0127	1	0.0127	5.35	0.0364	*
X_1^2	0.1876	1	0.1876	79.27	< 0.0001	**
X_2^2	0.1318	1	0.1318	55.71	< 0.0001	**
X_3^2	0.1661	1	0.1661	70.22	< 0.0001	**
X_4^2	0.0974	1	0.0974	41.17	< 0.0001	**
Residual	0.0331	14	0.0024			
Lack of Fit	0.0300	10	0.0030	3.90	0.1007	not significant
Pure Error	0.0031	4	0.0008			
Cor Total	0.6942	28				

※ ** means that it has a very significant effect on the results($P<0.01$)

* means that it has a significant effect on the results ($P<0.05$)

As can be seen Table 3, the model F-value of 19.96 with a low probability P-value of less than 0.0001 indicted h igh signifificance of the model. The lack of fit for an F-value of 3.90 meant that this term was not signifificantly relative to the pure error, the nonsignifificant value of lack fit (>0.05) showed that the quadratic model was val id for this study. From the results in Table 3, the ratio of solid to liquid and the extract temperature had high si gnificant effects on the total extractabilty of diterpenoids($p<0.01$), then the ethanol content had significant effe cts($P<0.05$), and the extract time had no significant effects($P>0.05$).

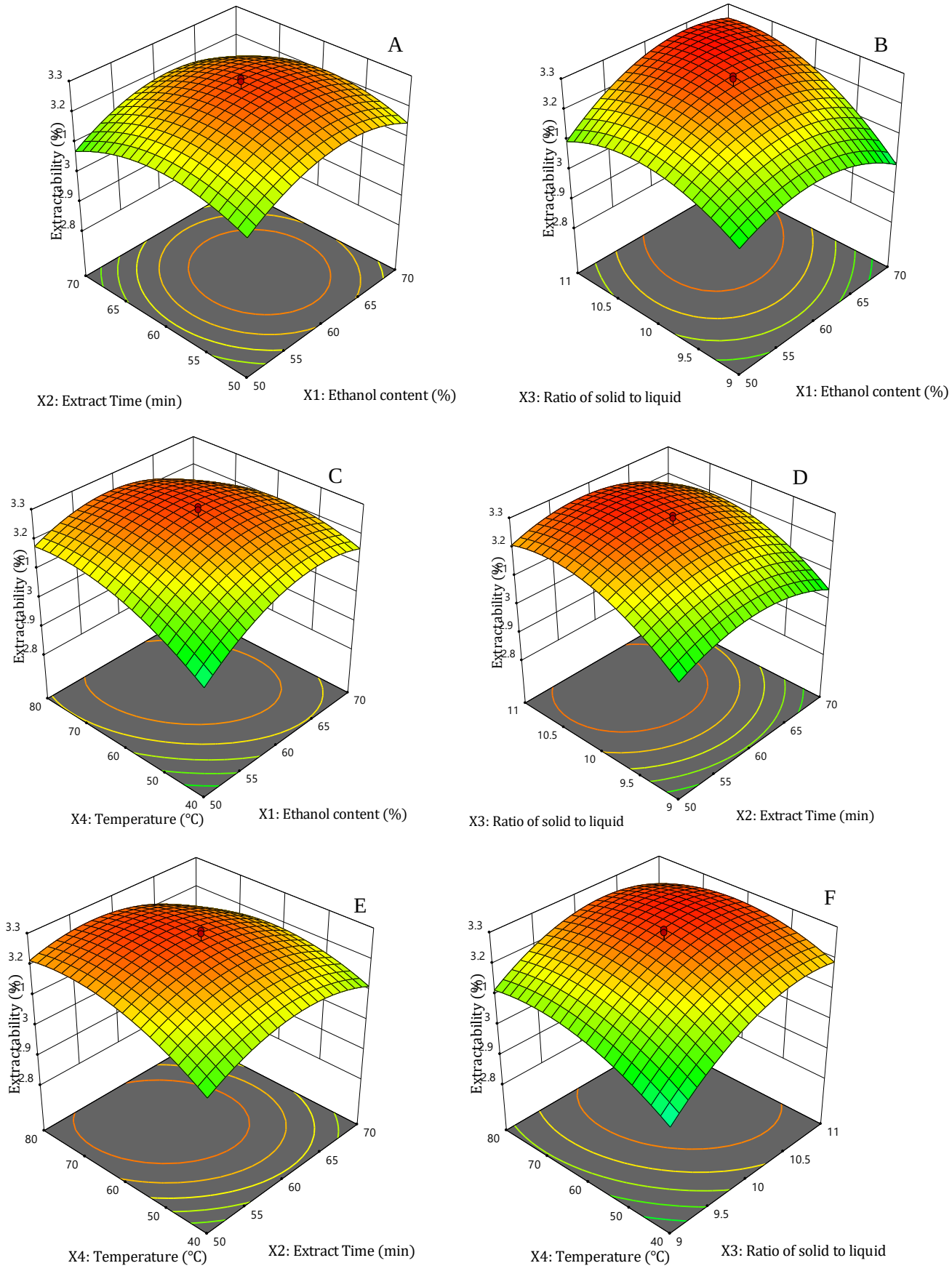

Figure 5. Response surface plots and contour lines of effects of interaction between each factor

As can shown Figure 5B and 5C, the surface on the interaction between each factor in this is the steepest, next steep is the surface on the interaction between each factor in Figure 5E and 5F. Therefore, the interinfluence of the ethanol content and the ratio of solid to liquid and the interinfluence of the ethanol content and the extraction temperature on the extractability is high significant, next significance is the interinfluence of the extraction temperature and the extraction time and the interinfluence of the extraction temperature and the ratio of solid to liquid. Thourgh the optimizing of AP extraction processing by response surface experiment, determined that the optimal condition of its process is the extraction solution concentration 62.8%, the extraction time 59min, the ratio of solid to liquid 1:10.5 and the extraction temperature 62℃. Under the optimum condition, the actual extratability is 3.21%, which was not much different from the theoretical value of 3.29%, were increased from extractability 2.27% in previous extractive method to 3.28%.

IV.DISCUSSION

The extractability of andrographolide from the *A. paniculata* by ultrasonic assisted extraction is improved, the optimum condition for extracted the andrographolide is determined by response surface experiment and detected the extractability of andrographolide under the optimum condition. As be shown Table 3 and Figure 5, the main factors affecting the extractability of AP were tested by response surface experiment, and the optimum extraction condition was determined. According above table and figure, the ratio of solid to liquid and the extract temperature had high significant effects on the total extractabilty of diterpenoids($p<0.01$), then the ethanol content had significant effects($P<0.05$), and the extract time had no significant effects($P>0.05$), and the interinfluence of the ethanol content and the ratio of solid to liquid and the interinfluence of the ethanol content and the extraction temperature on the extractability is high significant, next significance is the interinfluence of the extraction temperature and the extraction time and the interinfluence of the extraction temperature and the ratio of solid to liquid. Thourgh the optimizing of AP extraction processing by response surface experiment, determined that the optimal condition of its process is the extraction solution concentration 62.8%, the extraction time 59min, the ratio of solid to liquid 1:10.5 and the extraction temperature 62℃. Under the optimum condition, the actual extratability is 3.28%, which was not much different from the theoretical value of 3.29%, were increased from extractability 2.27% in previous extractive method to 3.28%.

V. CONCLUSION

In this study, improved the extractability of andrographolide from the *A. paniculata* by ultrasonic assisted extraction, the optimum condition for extracted the andrographolide is determined by response surface experiment and detected the extractability of andrographolide under the optimum condition. By single factor analysis and response surface experiments, the optimum conditions for extracted the andrographolide were the extraction solution concentration 62.8%, the extraction time 59min, the ratio of solid to liquid 1:10.5 and the extraction temperature 62℃. Investigate the andrographolide extractability in the optimun extraction condition by HPLC were increased from 2.27% to 3.28%.

VI. REFERENCES

[1]. Patil R, Jain V. Andrographolide: A Review of Analytical Methods, Journal of Chromatographic Science, 2021, 59(2): 191-203.

[2]. Chia VV, Pang SF, Gimbun J. Mass spectrometry analysis of auxiliary energy-induced terpenes extraction from Andrographis Paniculata, Industrial Crops & Products, 2020, 155: 1-7.

[3]. Hancke Juan L. A double-blind, randomized, placebo-controlled study to assess the efficacy of Andrographis paniculata standardized extract (ParActin®) on pain reduction in subjects with knee osteoarthritis, Phytotherapy Research. 2019, 33(5): 1469-1479.

[4]. Balap A, Lohidasan S , Sinnathambi A. Pharmacokinetic and pharmacodynamic interaction of andrographolide and standardized extract of Andrographis paniculata (Nees) with nabumetone in wistar rats, Phytotherapy research, 2017, 31(1): 75-80.

[5]. Pramanick S, Banerjee S, Achari B. Andropanolide and isoandrographolide, minor diterpenoids from Andrographis paniculata: structure and X-ray crystallographic analysis, Journal of Natural Products, 2006, 69(3): 403-405.

[6]. Uchaipichat V. In vitro inhibitory effects of major bioactive constituents of Andrographis paniculata, Curcuma longa and Silybum marianum on human liver microsomal morphine glucuronidation: a prediction of potential herb-drug interactions arising from andrographolide, curcumin and silybin inhibition in humans, Drug Metabolism and Pharmacokinetics, 2018, 31(1): 67-76.

[7]. Wiart C, Kumar K, Yusof MY. Antiviral properties of ent-labdene diterpenes of Andrographis paniculata Nees, inhibitors of herpes simplex virus type 1, Phytotherapy research, 2005, 19(12): 1069–1070.

[8]. Yuan HH, Sun B, Gao F. Synergistic anticancer effects of andrographolide and paclitaxel against A549 NSCLC cells, Pharmaceutical Biology, 2016, 54(11): 2629–2635.

[9]. Gan L, Zheng YR, Deng LJ. Diterpenoid lactones with anti-inflammatory effects from the aerial parts of Andrographis paniculata, Molecules, 2019, 24(15): 1–17.

[10]. Kandanur SGS, amang N, Golakoti NR. Andrographolide: A natural product template for the generation of structurally and biologically diverse diterpenes, European Journal of medicinal chemistry, 2019, 176: 513–533.

[11]. Kim N, Lertnimitphun P, Jiang YW. Andrographolide inhibits inflammatory responses in LPS-stimulated macrophages and murine acute colitis through activating AMPK, Biochemical Pharmacology. 2019, 170: 1–17.

[12]. Gao SY. Wang D, Chai H. Unusual ent-labdane diterpenoid dimers and their selective activation of TRPV channels, Journal of organicchemistry, 2019, 84(21): 13595–13603.

[13]. Virendra Rathod, Priyanka Rao. Effect of Three Phase Extraction with Ultrasound on recovery and Antioxidant Activity of Andrographis paniculata, Journal of Biologically Active Products from Nature, 2015, 5(4): 264–275.

Cite this article as :

KiBong Kim, IlJin Kim, SongLok Uh, YongIl Seo, CholKwang Li, UnHyang Lyeom, KwangRyel Hwang, " Study on the Optimization for the Extraction Process of Andrographolide by Ultrasonic Assisted Extraction, International Journal of Scientific Research in Science, Engineering and Technology(IJSRSET), Print ISSN : 2395-1990, Online ISSN : 2394-4099, Volume 8, Issue 2, pp.08-17, March-April-2021. Available at doi : https://doi.org/10.32628/IJSRSET21824