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# Using Orthogonal Arrays to Obtain Efficient and Reproducible Extraction Conditions of Geniposide and Genipin in Gardenia Fruit with Liquid Chromatography-Mass Spectrometry Determinations

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## ABSTRACT

The conditions to efficiently and reproducibly extract geniposide and genipin in Gardenia fruit powders were established using Taguchi experimental design approaches to improve extraction recovery. Geniposide, the major iridoid glycoside component of Gardenia fruits, which has been recognized to have choleretic effects, is transformed to Genipin in animals. In this study, the extraction conditions of geniposide and genipin such as solvent types, solvent acidities and boiling times were investigated under the guidance of experimental designs to obtain the highest content in extracts determined with liquid chromatography-mass spectrometry (LC-MS) methods. Herbal powders ground from Gardenia fruits, *Gardenia jasminoides* Ellis and *Gardenia jasminoides* Ellis var. *grandiflora* Nakai, were used. This paper has successfully demonstrated the use of Taguchi orthogonal arrays to obtain proper extraction conditions of geniposide and genipin in the above fruits. These design methods are capable of obtaining the optimum extraction conditions in only nine trials in which three factors, namely solvent type, boiling time and solvent acidity are investigated. The results show that solvent acidity is the influencing factor in achieving adequate contents and the other factors only have minor influences. The highest content obtained is similar to the reported data. In addition, using Taguchi orthogonal arrays within four experimental trials has identified that the extraction of ground fine powders results in more reproducible recovery than that of coarse ones.

Key words: orthogonal array, Geniposide, Genipin, LC-MS, Gardenia fruit

## INTRODUCTION

Gardenia fruit (zhi-zi in Chinese) has been used to treat hepatic pain due to cirrhosis and abdominal pain due to dysentery, diuretics and laxatives. Zhi-zi is also externally applied in the treatment of trauma for choleretic and homeostatic purposes[1,2]. Gardenia fruit is the fruit of *Gardenia jasminoides* Ellis (*Rubiaceae*). The protocols to extract and purify the ingredients of Gardenia fruit raw material and the analytical procedures to determine the extracted components have been reviewed by Wang *et al*.[3] Geniposide is transformed to genipin by intestinal bacteria in animals[4,5]. Genipin has also been reported to show several biological activities[6-8]. These two ingredients also exist in *Gardenia jasminoides* Ellis var. *grandiflora* Nakai.

Recent publications reported methods using isocratic or gradient liquid chromatography with electrospray tandem mass spectrometry (LC-MS/MS) to determine geniposide with ultra-trace detection limits (2 ng/mL)[9,10]. In addition, more sensitive gradient LC-MS/MS methods using Taguchi approaches are developed by our group to improve the detection limits by one order of magnitude[11].

Taguchi experimental design methods based on orthogonal array have been a popular technique to reduce the number of mandatory trials used in the quality engineering[12]. In addition to listing various conditions of each investigated parameter in one column, the orthogonal table also lists the interactions between investigated

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parameters in other columns. When one interaction is proved negligible, the interaction column of this weak interaction can be used for another investigated parameter. For example, in the orthogonal table of three parameters, there are seven columns, which represent three parameters, three interactions between any two parameters, and one interaction between three parameters. When these three parameters have no interactions, four interactions columns can be used for other four investigated parameters. Therefore this orthogonal table can be used for a maximum of seven investigated parameters. Since the numbers of required trials remain the same (eight trials) as studying three parameters, the numbers of experiments to investigate more parameters can be reduced efficiently. Taguchi methods can also be applied in analytical validation to improve method robustness[13]. More recently, there are other analytical applications such as finding proper experimental conditions to develop sample preparation methods and chip-based devices[14,15]. Besides, our group has applied Taguchi orthogonal array designs to optimize the gradient LC-MS/MS conditions to achieve higher sensitivity[16].

When neither effect of experimental factors is affected by the other, these two effects are orthogonal. In an orthogonal array, any two columns of numbers have the orthogonality property that the sum of the products of their respective terms is equal to zero. The estimated effects based on those columns are orthogonal. Orthogonal array techniques, originally developed to control errors in an experiment, have been adapted by Taguchi methods to determine the effect of a control factor under study not only on the average result but also the variation from the average result. Therefore, using Taguchi array as experimental design guidance, the influence of each factor on the extraction studied such as solvent type, extraction boiling time and solvent acidity[17,18] can be estimated. These results are able to provide the conditions for the highest extraction efficiency.

Previous publications of Gardenia fruit extraction studies using trial-and-error approaches concluded that both geniposide and genipin have similar extraction content when extracted with either water or alcohol-based solvents (methanol or ethanol)[17,18]. The extraction contents using basic solvents are not adequate and are significantly improved using acidic solvents. In addition, boiling time has negligible effect on the extraction content. The above systematical methods are tedious and sometimes overlook important factors. This paper employs Taguchi design methods to efficiently reduce the numbers of experimental trials. Moreover. the extraction contents using the conditions obtained from the designs are expected to be similar or even improved. Orthogonal array designs can be also used to obtain extraction conditions for high reproducibility. Under the conditions of neutral or acidic extraction solvents and boiling times of 5 min or less, the studies suggest that fine Gardenia fruit powders result in more reproducible extraction recovery in general. In order to compare with previous studies, the conditions to extract geniposide and genipin from different species of Gardenia fruit were studied.

## MATERIALS AND METHODS

### I. *Materials*

Geniposide and genipin standards were obtained from the Medical and Pharmaceutical Industry Technology and Development Center (Taipei, Taiwan). The Gardenia fruits, *Gardenia jasminoides* Ellis (*Rubiaceae*) and *Gardenia jasminoides* Ellis var. *grandiflora* Nakai, were purchased from a Chinese herbal medicine shop in Tainan, Taiwan. The fruit particles were in their original granule sizes or ground to finer sizes (0.2 to 0.5 mm i.d.) The extraction solvent, methanol, was obtained from J. T. Baker (Phillipsburg, NJ, USA). The acidities of extraction solvents were adjusted with sodium hydroxide (NaOH) or hydrochloric acid (HCl) solutions. NaOH and concentrated HCl (12%) were obtained from Showa (Tokyo, Japan) and Tedia (Fairfield, OH, USA), respectively. Ammonium acetate was purchased from Showa (Tokyo, Japan). Acetonitrile was obtained from J. T. Baker. Deionized (DI) water was prepared with Milli-Q Ultrapure water purification system (Millipore, Bedford, MA, USA).

### II. *Analytical Apparatus*

Geniposide and genipin standards were dissolved in injection solvents and an injection volume of 20 μL was used. The elution liquid was driven with a ProStar 220 solvent delivery system (Varian). A triple quadrupole mass spectrometer system (Quattro Ultima$^{TM}$, Micromass, UK) was used. The mass spectrometer was equipped with a Z-spray electrospray ionization source. Typical source conditions were as follows: Capillary, 3.0 kV; source temperature, 110°C; desolvation temperature, 200 to 350°C; cone gas flow, 150 L/h; and desolvation gas flow, 550 L/h. The first stage of the quadrupole mass analyzer was set to follow the ion at *m/z* 406.2 to detect the cationic adduct of geniposide with ammonium ions. Similarly, the loss of water ion fragment of genipin at *m/z* 209.0 was followed to detect the protonated adducts. These ions are the same as those reported previously[10,11]. The photomultiplier voltage was set at 650 V. Ion signals are recorded at positive selected-ion-monitoring (SIR) mode with the following conditions: Interchannel delay, 0.02 s; mass span, 0.02 amu; dwell time, 0.1 or 0.2 s; and recording time, 5 min. The mass spectrometer was controlled with Masslynx 3.5 to acquire the LC-MS/MS chromatograms.

### III. *Standard Curves of Geniposide and Genipin*

Small aliquots of geniposide or genipin stock solution were spiked into mobile phase solutions to prepare standards at 10, 30, 50, 100, 300, 500 and 1000 ng/mL, respectively to establish standard curves. These standards were loaded into the LC column and their peak intensities were detected by the electrospray mass spectrometer. The mobile phase solvents for the elution of geniposide and genipin were the mixture of acetonitrile and ammonium acetate solution (0.16 mM) in the ratio of 13 : 87 and the mixture of methanol and de-ionized

water in the ratio of 40 : 60 respectively. The extracts from the ground powders were diluted by 200-fold or 1000-fold with the mobile phase solvents prior to analysis.

IV. *Experimental Design*

Taguchi $L_9$ ($3^4$) orthogonal array was used to evaluate how investigated factors affect the extraction contents of geniposide or genipin in the powders of Gardenia fruits, *Gardenia jasminoides* Ellis (*Rubiaceae*) and *Gardenia jasminoides* Ellis var. *grandiflora* Nakai. As there were three factors to investigate, the orthogonal array was modified to the spreadsheet format of Table 1, which contained factors A to D. Factor A represents the types of extraction solvents. Factor B represents the boiling times for extraction. Factor C represents the acidities of the extraction solvents. Factor D is an unassigned factor, which reflects the random variability. The values of average, range (the difference between the highest and lowest average values) and effect (range/average × 100%) of each factor can be easily calculated with this worksheet.

V. *Using Taguchi Tables to Investigate Extraction Recovery*

Similar previous publications[16,17], level 1, level 2 and level 3 of factor A (type of extraction solvent), were methanol, mixture of methanol and de-ionized water (1 : 1), and de-ionized water, respectively. Level 1, level 2 and level 3 of factor B (extraction boiling time) were 0, 5 and 15 min, respectively. Level 1, level 2 and level 3 of factor C (solvent acidity) were basic (0.1 M NaOH; pH 13), neutral and acidic (0.1 M HCl; pH 1), respectively.

VI. *Using Taguchi Tables to Investigate Extraction Reproducibility*

Using the fine fruit powders, the conditions for extraction reproducibility were investigated with the neutral and acidic aqueous extraction solvents when the boiling times were 0 and 5 min. Taguchi orthogonal table $L_4$ ($2^3$) was selected to design experiments. Level 1 and level 2 of factor A (extraction boiling time) were 0 and 5 min, respectively. Level 1 and level 2 of factor B (extraction solvent acidity) were neutral and acidic aqueous solvents, respectively. Level 1 and level 2 of factor C (coarseness of fruit powder) were the original and ground fruit particles, respectively. The number of replicates for each experiment were three to six. The reproducibility is represented with the S/N value, equal to $10 \times \log([x]^2/\sigma^2)$, where [x] and σ are the average and standard deviation, respectively.

**Table 1.** The experimental design table in spreadsheet format using Taguchi orthogonal array $L_9$ ($3^4$) to develop geniposide or genipin extraction methods. Three factors, namely extraction solvent type, boiling time and extraction solvent acidity, are investigated

| Experiment number | Content (mg/g) | Factor A | | | Factor B | | | Factor C | | | Factor D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Extraction solvent type | | | Boiling time (min) | | | Extraction solvent acidity | | | Unassigned | | |
| | | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 |
| 1 | | | | | | | | | | | | | |
| 2 | | | | | | | | | | | | | |
| 3 | | | | | | | | | | | | | |
| 4 | | | | | | | | | | | | | |
| 5 | | | | | | | | | | | | | |
| 6 | | | | | | | | | | | | | |
| 7 | | | | | | | | | | | | | |
| 8 | | | | | | | | | | | | | |
| 9 | | | | | | | | | | | | | |
| Total | | | | | | | | | | | | | |
| Number of values | | | | | | | | | | | | | |
| Average | | | | | | | | | | | | | |
| Range* | | | | | | | | | | | | | |
| Effects = (Range/Average) × 100% | | | | | | | | | | | | | |

* Range is the difference between the highest and lowest average values.

**Table 2.** The extraction content of geniposide in *Gardenia jasminoides* Ellis under the experimental design conditions indicated in Table 1B. (DI stands for deionized water)

| Experiment number | Content (mg/g) | Factor A | | | Factor B | | | Factor C | | | Factor D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Extraction solvent type | | | Boiling time (min) | | | Extraction solvent acidity | | | Unassigned | | |
| | | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 |
| | | MeOH | MeOH/ DI | DI | 0 | 5 | 15 | Basic | Neutral | Acidic | | | |
| 1 | 28.3 | 28.3 | | | 28.3 | | | 28.3 | | | 28.3 | | |
| 2 | 54.1 | 54.1 | | | | 54.1 | | | 54.1 | | | 54.1 | |
| 3 | 62.1 | 62.1 | | | | | 62.1 | | | 62.1 | | | 62.1 |
| 4 | 57.9 | | 57.9 | | 57.9 | | | | 57.9 | | | | 57.9 |
| 5 | 55.6 | | 55.6 | | | 55.6 | | | | 55.6 | 55.6 | | |
| 6 | 1.83 | | 1.83 | | | | 1.83 | 1.83 | | | | 1.83 | |
| 7 | 54.3 | | | 54.3 | 54.3 | | | | | 54.3 | | 54.3 | |
| 8 | -0.15 | | | -0.15 | | -0.15 | | -0.15 | | | | | -0.15 |
| 9 | 62.9 | | | 62.9 | | | 62.9 | | 62.9 | | 62.9 | | |
| Total | 376.88 | 144.5 | 115.33 | 117.05 | 140.5 | 109.55 | 126.83 | 29.98 | 174.9 | 172 | 146.8 | 110.23 | 119.85 |
| Number of values | 9 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Average | 41.9 | 48.2 | 38.4 | 39 | 46.8 | 36.5 | 42.3 | 9.99 | 58.3 | 57.3 | 48.9 | 36.7 | 40 |
| Range | | 9.8 | | | 10.3 | | | 48.31 | | | 12.2 | | |
| Effects (%) | | 23.4 | | | 24.6 | | | 115 | | | 29.1 | | |

## RESULTS AND DISCUSSION

### I. *Validation of LC-MS Methods to Determine Geniposide and Genipin*

The standard curves showing signal intensity relations (y) *versus* concentrations in ng/mL (x) were obtained using the linear regression method. The standard curves were y = 50x + 87.5 and y = 32x + 447 for geniposide and genipin, respectively. The $R^2$ values of geniposide and genipin were 0.9996 and 0.9995, respectively.

### II. *The Use of Taguchi Design Methods to Establish Extraction Conditions that Enhance the Content of Geniposide in the Fruit of Jasminoides*

Table 2 shows the extraction content of geniposide in the fruit of *jasminoides* under various extraction conditions assigned in the Taguchi array. The highest content (62.9 mg/g) was obtained when the solvent type was de-ionized water, the boiling time was 15 min and the solvent acidity was neutral. In Table 2, only the effect of solvent acidity (115%) is larger than the effect of the unassigned factor (29.1%), reflecting the random variability. In other words, the effects of solvent types and boiling times are relatively minor. Some determined content values were negative, they were less than 0.5% of the highest value (62.9 mg/g) and to be neglected. The extraction factor A (solvent type) and factor B (boiling time) show no obvious variations. The extraction factor C (solvent acidity) indicates that the geniposide content exhibited a significant increase when acidic solvent was used compared to basic solvent, which is consistent with previous studies[16,17].

### III. *Geniposide in the Fruit of Jasminoides Var. Grandiflora Nakai*

Table 3 shows the extraction contents of geniposide in the fruit of *jasminoides* var. *grandiflora*. The highest content (89.9 mg/g) was obtained when solvent type was de-ionized water, the boiling time was 15 min and the solvent acidity was neutral. This extraction content was somewhat higher than that of *jasminoides*. Similar results were reported previously[16,17]. In Table 3, only the effect of solvent acidity (114%) was larger than the effect of unassigned factor (51%), reflecting the random variability. In contrast, the effects of solvent type and boiling time, which are 25% and 6% respectively, are minor. The extraction factor A (solvent type) and factor B (boiling time) show no obvious variations. The extraction factor C (solvent acidity) indicates that the geniposide content also exhibited a significant increase when the basic extraction solvent turned to neutral and acidic. These results are also consistent with previous studies[16,17].

**Table 3.** The extraction content of geniposide in *Gardenia jasminoides* Ellis var. *grandiflora* Nakai under the experimental design conditions indicated in Table 1. (DI stands for deionized water)

| Experiment number | Content (mg/g) | Factor A | | | Factor B | | | Factor C | | | Factor D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Extraction solvent type | | | Boiling time (min) | | | Extraction solvent acidity | | | Unassigned | | |
| | | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 |
| | | MeOH | MeOH/ DI | DI | 0 | 5 | 15 | Basic | Neutral | Acidic | | | |
| 1 | 42 | 42 | | | 42 | | | 42 | | | 42 | | |
| 2 | 73 | 73 | | | | 73 | | | 73 | | | 73 | |
| 3 | 74.6 | 74.6 | | | | | 74.6 | | | 74.6 | | | 74.6 |
| 4 | 66.5 | | 66.5 | | 66.5 | | | | 66.5 | | | | 66.5 |
| 5 | 81.7 | | 81.7 | | | 81.7 | | | | 81.7 | 81.7 | | |
| 6 | 0.8 | | 0.8 | | | | 0.8 | 0.8 | | | | 0.82 | |
| 7 | 57.3 | | | 57.3 | 57.3 | | | | | 57.3 | | 57.3 | |
| 8 | 1.4 | | | 1.4 | | 1.4 | | 1.4 | | | | | 1.36 |
| 9 | 89.9 | | | 89.9 | | | 89.9 | | 89.9 | | 89.9 | | |
| Total | 487.18 | 189.6 | 149.02 | 148.56 | 165.8 | 156.06 | 165.32 | 44.18 | 229.4 | 213.6 | 213.6 | 131.12 | 142.46 |
| Number of values | 9 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Average | 54.1 | 63.2 | 49.7 | 49.5 | 55.3 | 52 | 55.1 | 14.7 | 76.5 | 71.2 | 71.2 | 43.7 | 47.5 |
| Range | | 13.7 | | | 3.3 | | | 61.8 | | | 27.5 | | |
| Effects (%) | | 25.3 | | | 6.1 | | | 114 | | | 50.8 | | |

**Table 4.** The extraction content of genipin in *Gardenia jasminoides* Ellis under the experimental design conditions indicated in Table 1. (DI stands for deionized water)

| Experiment number | Content (mg/g) | Factor A | | | Factor B | | | Factor C | | | Factor D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Extraction solvent type | | | Boiling time (min) | | | Extraction solvent acidity | | | Unassigned | | |
| | | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 |
| | | MeOH | MeOH/ DI | DI | 0 | 5 | 15 | Basic | Neutral | Acidic | | | |
| 1 | 5.8 | 5.8 | | | 5.8 | | | 5.8 | | | 5.8 | | |
| 2 | 11.5 | 11.5 | | | | 11.5 | | | 11.5 | | | 11.5 | |
| 3 | 12.6 | 12.6 | | | | | 12.6 | | | 12.6 | | | 12.6 |
| 4 | 11.5 | | 11.5 | | 11.5 | | | | 11.5 | | | | 11.5 |
| 5 | 12.6 | | 12.6 | | | 12.6 | | | | 12.6 | 12.6 | | |
| 6 | 0.4 | | 0.4 | | | | 0.4 | 0.43 | | | | 0.43 | |
| 7 | 12.4 | | | 12.4 | 12.4 | | | | | 12.4 | | 12.4 | |
| 8 | -0.18 | | | -0.18 | | -0.18 | | -0.18 | | | | | -0.18 |
| 9 | 12.9 | | | 12.9 | | | 12.9 | | 12.9 | | 12.9 | | |
| Total | 79.5 | 29.85 | 24.53 | 25.12 | 29.65 | 23.92 | 25.93 | 6 | 35.9 | 37.6 | 31.25 | 24.33 | 23.92 |
| Number of values | 9 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Average | 8.83 | 9.95 | 8.18 | 8.37 | 9.88 | 7.97 | 8.64 | 2 | 12 | 12.5 | 10.4 | 8.11 | 7.97 |
| Range | | 1.77 | | | 1.91 | | | 10.5 | | | 2.43 | | |
| Effects (%) | | 20 | | | 21.6 | | | 119 | | | 27.5 | | |

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IV. *The Use of Taguchi Design Methods to Establish Extraction Conditions that Enhance the Content of Genipin*

Tables 4 and 5 show the extraction contents of genipin in the fruit of *jasminoides* and *jasminoides* var. *grandiflora,* respectively, under various extraction conditions assigned in the Taguchi arrays. The trends found in Tables 4 and 5 are similar to that in Tables 2 and 3. Some determined content values in Tables 4 and 5 were negative, they were less than 2% of the highest values (around 13 mg/g) and to be neglected.

**Table 5.** The extraction content of genipin in *Gardenia jasminoides* Ellis var. *grandiflora* Nakai under the experimental design conditions indicated in Table 1. (DI stands for deionized water)

| Experiment number | Content (mg/g) | Factor A | | | Factor B | | | Factor C | | | Factor D | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Extraction solvent type | | | Boiling time (min) | | | Extraction solvent acidity | | | Unassigned | | |
| | | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 | Level 1 | Level 2 | Level 3 |
| | | MeOH | MeOH/ DI | DI | 0 | 5 | 15 | Basic | Neutral | Acidic | | | |
| 1 | 4.63 | 4.63 | | | 4.63 | | | 4.63 | | | 4.63 | | |
| 2 | 9.41 | 9.41 | | | | 9.41 | | | 9.41 | | | 9.41 | |
| 3 | 12.9 | 12.9 | | | | | 12.9 | | | 12.9 | | | 12.9 |
| 4 | 10.7 | | 10.7 | | 10.7 | | | | 10.7 | | | | 10.7 |
| 5 | 11.6 | | 11.6 | | | 11.6 | | | | 11.6 | 11.6 | | |
| 6 | -0.12 | | -0.12 | | | | -0.12 | -0.12 | | | | -0.12 | |
| 7 | 8.50 | | | 8.50 | 8.50 | | | | | 8.50 | | 8.50 | |
| 8 | 0.01 | | | 0.01 | | 0.01 | | 0.01 | | | | | 0.01 |
| 9 | 13.0 | | | 13.0 | | | 13.0 | | 13.0 | | 13.0 | | |
| Total | 70.63 | 26.94 | 22.18 | 21.51 | 23.83 | 21.02 | 25.78 | 4.52 | 33.11 | 33 | 29.23 | 17.79 | 23.61 |
| Number of values | 9 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 | 3 |
| Average | 7.84 | 8.98 | 7.39 | 7.17 | 7.94 | 7.01 | 8.59 | 1.51 | 11 | 11 | 9.74 | 5.93 | 7.87 |
| Range | | 1.81 | | | 1.58 | | | 9.49 | | | 3.81 | | |
| Effects (%) | | 23.1 | | | 20.2 | | | 121 | | | 48.6 | | |

**Table 6.** The extraction content reproducibility of geniposide in *Gardenia jasminoides* Ellis var. *grandiflora* Nakai under the experimental design conditions of Taguchi orthogonal table $L_4(2^3)$. (DI stands for deionized water)

| Experiment number | S/N Ratio | Factor A | | Factor B | | Factor C | | Content (mg/g) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Boiling time (min) | | Solvent acidity | | Coarseness of fruit powder | | | | | | |
| | $10\log([x]^2/SD^2)$ | Level 1 | Level 2 | Level 1 | Level 2 | Level 1 | Level 2 | Replicate number | | | Average | SD |
| | | 0 | 5 | Neutral | Acidic | Original | Ground | #1 | #2 | #3 | [x] | |
| 1 | 36.62 | 36.62 | | 36.62 | | 36.62 | | 148.70 | 145.63 | 153.36 | 147.17 | 2.17 |
| 2 | 28.49 | 28.49 | | | 28.49 | | 28.49 | 228.74 | 242.97 | 226.98 | 232.90 | 8.77 |
| 3 | 28.05 | | 28.05 | 28.05 | | | 28.05 | 229.65 | 247.43 | 233.39 | 236.82 | 9.37 |
| 4 | 18.34 | | 18.34 | | 18.34 | 18.34 | | 170.54 | 192.05 | 150.62 | 171.07 | 20.72 |
| Total | 111.5 | 65.11 | 46.39 | 64.67 | 46.83 | 54.96 | 56.54 | | | | | |
| Average | 27.88 | 32.56 | 23.20 | 32.34 | 23.42 | 27.48 | 28.27 | | | | | |
| Range | | 9.36 | | 8.92 | | 0.79 | | | | | | |
| Effects (%) | | 33.58 | | 32.00 | | 2.83 | | | | | | |

Only extraction solvent acidity had significant influences, which again confirmed the published findings[16,17].

V. *The Stability of Geniposide and Genipin under Hydrolysis Conditions*

Although geniposide and genipin are prone to hydrolysis under either basic or acidic conditions, when incubated in acidic solvents (0.1 M HCl; pH 1) for 15 min to 2 h, the contents remained constant (95% or greater).

VI. *The Use of Taguchi Design Methods to Establish Extraction Conditions that Enhance the Reproducibility of Geniposide and Genipin*

Tables 6 and 7 contain the extraction reproducibility of geniposide and genipin in the fruit of *jasminoides* var. *grandiflora* respectively. Although the most reproducible recovery of geniposide (highest S/N value), was obtained when rough powder was extracted with neutral solvent and no boiling time, the average recovery was significantly lower than the average values of using fine powders. Besides, the most reproducible and highest recovery of genipin was obtained using fine powders with neutral solvent and boiling time of 5 min. The extraction reproducibility results of geniposide and genipin in the fruit of *jasminoides* in Tables 8 and 9 were similar to the results of those in the fruit of *jasminoides* var. *grandiflora*. Therefore, the reproducibility investigations suggest that fine powders are preferred.

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**Table 7.** The extraction content reproducibility of genipin in *Gardenia jasminoides* Ellis var. *grandiflora* Nakai under the experimental design conditions of Taguchi orthogonal table $L_4(2^3)$. (DI stands for deionized water)

| Experiment number | S/N Ratio | Factor A | | Factor B | | Factor C | | Content (mg/g) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Boiling time (min) | | Solvent acidity | | Coarseness of fruit powder | | Replicate number | | | | | | Average | SD |
| | $10\log([x]^2/SD^2)$ | Level 1 | Level 2 | Level 1 | Level 2 | Level 1 | Level 2 | | | | | | | | |
| | | 0 | 5 | Neutral | Acidic | Original | Ground | #1 | #2 | #3 | #4 | #5 | #6 | [x] | |
| 1 | 15.45 | 15.45 | | 15.45 | | 15.45 | | 11.18 | 7.69 | 12.76 | 11.15 | 11.25 | 12.93 | 11.16 | 1.88 |
| 2 | 18.91 | 18.91 | | | 18.91 | | 18.91 | 16.07 | 18.05 | 20.59 | 16.80 | 20.45 | 20.99 | 18.83 | 2.13 |
| 3 | 22.36 | | 22.36 | 22.36 | | | 22.36 | 22.03 | 18.75 | 22.34 | 18.80 | 20.91 | 21.43 | 20.71 | 1.58 |
| 4 | 19.62 | | 19.62 | | 19.62 | 19.62 | | 17.69 | 17.47 | 16.87 | 13.83 | 15.71 | 14.17 | 15.96 | 1.67 |
| Total | 76.34 | 34.36 | 41.98 | 37.81 | 38.53 | 35.07 | 41.27 | | | | | | | | |
| Average | 19.09 | 17.18 | 20.99 | 18.91 | 19.27 | 17.54 | 20.64 | | | | | | | | |
| Range | | 3.8 | | 0.36 | | 3.1 | | | | | | | | | |
| Effects(%) | | 19.96 | | 1.89 | | 16.24 | | | | | | | | | |

**Table 8.** The extraction content reproducibility of geniposide in *Gardenia jasminoides* Ellis under the experimental design conditions of Taguchi orthogonal table $L_4$ ($2^3$). (DI stands for deionized water)

| Experiment number | S/N Ratio | Factor A | | Factor B | | Factor C | | Content (mg/g) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Boiling time (min) | | Solvent acidity | | Coarseness of fruit | | | | | | |
| | $10\log([x]^2/SD^2)$ | Level 1 | Level 2 | Level 1 | Level 2 | Level 1 | Level 2 | Replicate number | | | Average | SD |
| | | 0 | 5 | Neutral | Acidic | Original | Ground | #1 | #2 | #3 | [x] | |
| 1 | 18.84 | 18.84 | | 18.84 | | 18.84 | | 34.89 | 30.48 | 27.87 | 31.08 | 3.55 |
| 2 | 19.56 | 19.56 | | | 19.56 | | 19.56 | 64.81 | 58.96 | 52.46 | 58.74 | 6.18 |
| 3 | 22.08 | | 22.08 | 22.08 | | | 22.08 | 69.47 | 63.88 | 59.38 | 64.24 | 5.05 |
| 4 | 13.04 | | 13.04 | | 13.04 | 13.04 | | 63.46 | 45.11 | 43.00 | 50.52 | 11.25 |
| Total | 73.52 | 38.40 | 35.12 | 40.92 | 32.60 | 31.88 | 41.64 | | | | | |
| Average | 18.38 | 19.20 | 17.56 | 20.46 | 16.30 | 15.94 | 20.82 | | | | | |
| Range | | 1.64 | | 4.16 | | 4.88 | | | | | | |
| Effects (%) | | 8.92 | | 22.63 | | 26.55 | | | | | | |

**Table 9.** The extraction content reproducibility of genipin in *Gardenia jasminoides* Ellis under the experimental design conditions of Taguchi orthogonal table $L_4(2^3)$. (DI stands for deionized water)

| Experiment number | S/N Ratio | Factor A | | Factor B | | Factor C | | Content (mg/g) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Boiling time (min) | | Solvent acidity | | Coarseness of fruit powder | | | | | | |
| | $10\log([x]^2/SD^2)$ | Level 1 | Level 2 | Level 1 | Level 2 | Level 1 | Level 2 | Replicate No. | | | Average | SD |
| | | 0 | 5 | Neutral | Acidic | Original | Ground | #1 | #2 | #3 | [x] | |
| 1 | 16.97 | 16.97 | | 16.97 | | 16.97 | | 11.85 | 14.17 | 10.77 | 12.26 | 1.74 |
| 2 | 22.24 | 22.24 | | | 22.24 | | 22.24 | 20.55 | 18.30 | 21.27 | 20.04 | 1.55 |
| 3 | 21.31 | | 21.31 | 21.31 | | | 21.31 | 22.15 | 19.01 | 22.16 | 21.11 | 1.82 |
| 4 | 18.72 | | 18.72 | | 18.72 | 18.72 | | 19.29 | 15.70 | 16.08 | 17.02 | 1.97 |
| Total | 79.24 | 39.21 | 40.03 | 38.28 | 40.96 | 35.69 | 43.55 | | | | | |
| Average | 19.81 | 19.61 | 20.02 | 19.14 | 20.48 | 17.85 | 21.78 | | | | | |
| Range | | 0.41 | | 1.34 | | 3.93 | | | | | | |
| Effects (%) | | 2.07 | | 6.76 | | 19.84 | | | | | | |

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