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Purification of Aconitine, The Main Ingredient of Aconitum Ciliare Decaisne Using Alkaline Compounds

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Abstract

Object: Aconiti tuber is used after its toxicity is reduced by using various processing methods, Suchi (Processing. The processing to use medicine of plants, animals, minerals in Korean and Chinese medicine. It use lots of method like dividing, softening, detoxication. It can use to get the better treatment and a new effect.), because it is very toxic. It has been used to treat arthritis, neuralgia, stroke, diabetes and cold sensation as folk remedies. Processing (Suchi) of medicinal plants is one of the processing methods for reducing of toxicity or improving of effect on medicinal plants.

Methods: In this study, we attempted to clarify the change of the aconitine content on Aconitum ciliare Decaisne by the new processing methods, Suchi. Dry Aconitum ciliare Decaisne roots were processed by the 5-step processing methods, and changes in contents of aconitine were compared and analyzed by using High Performance Liquid Chromatography-Electrospray Ionization-Mass-Spectrometer (HPLC-ESI-MS).

Results: Experiment results, aconitine content of Aconitum ciliare Decaisne was 1,802 ng/g before first step Suchi, 130.2 ng/ml after the first Suchi, 681.1 ng/ml after the second Suchi, 228.5 ng/ml after the third Suchi, 97.7 ng/ml (ppb) after the fourth Suchi, and 21.6 ng/ml (ppb) after the fifth Suchi. It was found that the contents of aconitine were substantially decreased when more Suchi processes were performed.

Conclusion: As the results, we could decrease the contents of aconitine which was a main ingredient of Aconitum ciliare Decaisne by using Korean rice wine residues, Korean rice wine, and milk.

Keywords: Aconitum ciliare Decaisne; Aconitine; Reducing of toxicity; Alkaline compound; Detoxification.

Introduction

Aconitum ciliare Decaisne belongs to the family Ranunculaceae, the genus Aconitum sp. and is a perennial plant. It originates in Korea, Northeastern China and Russia [1,2]. About 40 species of Aconitum sp. are currently growing across the country [2,3]. Among them, about 25 species are being used as the source of aconitine. Typical examples include Aconitum triphyllum Nakai, Aconitum sibiricum ROIR, Aconitum uchyamai NAKAI, Aconitum kusnezofii REICHB. And Aconitum ciliare Decaisne. It has been known to be effective in relieving pains, strengthening the heart and heating insulation in Oriental herbal remedies and folk remedies in Korea and China. It is relatively commonly used to enhance analgesic effects on arthritis and

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neuralgia and to strengthen vital essence [4]. Among ingredients of Aconitum sp., diterpene alkaloids such as aconitine, hyp-aconitine, mesaconitine and deoxyaconitine have toxic substances [5,6]. Alkaloids may cause adverse effects such as reduced blood pressure, Raynaud's phenomenon and ventricular fibrillation [7]. This herb and its alkaloids are very toxic. LD50 for mice is 0.3 mg aconitine per Kg (s.c.) or 17.42 g of prepared aconite root/Kg (oral) or 3.5 g/Kg (I.V.). Toxic symptoms include bradycardia and irregular rhythm. The specific adverse effect depends on how the aconite preparation is prepared and how long it is decocted. Patients usually lose sensation in the mouth and tongue after drinking the Fu Zi decoction. Nausea and vomiting may occur, as well as spasm of extremities and cardiac arrhythmias. The intestinal absorption of the alkaloids is relatively fast. Because of this, gastric lavage is recommended in case of overdose [8].

Aconiti tuber is used after its toxicity is reduced by using various processing methods, Suchi (Processing. The processing to use medicine of plants, animals, minerals in Korean and Chinese medicine. It use lots of method like dividing, softening, detoxication. It can use to get the better treatment and a new effect.), because it is very toxic. It has been used to treat arthritis, neuralgia, stroke, diabetes and cold sensation as folk remedies [9-15]. Because of alkaloids such as aconitine included in Aconitum ciliare Decaisne, acute poisoning has been reported [16-18]. Poisoning has been reported after ingestion of as little as 0.2 mg aconitine. 3-4 mg of aconitine is lethal to human. Due to poisoning of aconitine, it has been used in Korean folk remedies after its toxicity is reduced by boiling Aconitum ciliare Decaisne together with pollack or pig legs [19]. Toxicity of Aconitum ciliare Decaisne is reduced over time of heating [20]. When it is heated in the micro oven for 5 min, mesaconitine is reduced. When it is heated for 10 min, aconitine is reduced. In addition, when it is treated with micro wave after it is immersed in 1 % $NaHCO_3$ solution, the toxicity is reduced [19]. Likewise, various methods such as immersion in alkaline solution, steam under high pressure after immersion in alkaline solution [21] or treatment with micro wave [22] have been studied in Korea.

According to Ungok Herbology [23], aconitine is used as uncooked fruit or spending some medicine, but it should be used after it is processed through Poje (This is classical method of making medicines, and called as 'quick frying and roasting', 'making the drugs', 'processing the drug', 'managing the drugs', and 'purifying and cutting'. The word of 'processing the drugs' is used in broad concept including.) as it has been done in aconiti tuber. After aconitine is soaked in cold water for 3-5 days, it is boiled in the liquorice drop. Swaegeon (This is a method of processing the drug and called "Sae" and means drying the medicinal substance in the sun.) is performed until white core disappears. Alternatively, after aconitine is soaked in ginger soup for 3-4 days, it is steamed and then hastily parched by Muhwa (Degree of the strength of fire whether it is weak or mild.). In addition, water 10 times as much as aconitine is added and boiled for 10-14 hours until white core disappears. After that, it is heated by Munhwa until water is almost evaporated. It is dried under sunlight or honggeon (Drying of medicinal stuff by heating with fire.) is performed. Pharmacological effects of aconitine in Aconitum sp. are as follows: enhancement in the cardiovascular system, improvement in congestive heart failure, analgesic effects, anti-inflammatory effects and enhancement in the endocrine system, central nervous system, and peripheral nervous system [24]. Because adverse effects are caused by toxic substances in aconitine, it is often not used in herbal medicine despite its various pharmacological effects. Many studies have been conducted on toxicity reduction or detoxification (Mudok) of toxic substances in alkaloids such as aconitine, mesaconotine and hypaconitine in Aconiti tuber. However, methods to substantially reduce toxicity have not been developed yet. Studies on changes in contents of aconitine determined by Suchi processes have been reported as follows. Kitagawa et al. [25] quantified the contents of aconitum alkaloids by using HPLC and absorbance measurement. According to Korean Pharmacopoeia and Korean Herbal Pharmacopoeia commentary [7], a titration method is described as the quantification method of aconiti tuber. It has been used as the method to analyze alkaloids such as aconitine in aconiti tube-based medicinal herbs.

Thus, the purpose of this study was to determine contents of aconitine alkaloids in Aconitum ciliare Decaisne by various new processing methods, Suchi. Changes in contents of aconitine were compared and analyzed by using High Performance Liquid Chromatography-Electrospray Ionization-Mass-Spectrometer (HPLC-ESI-MS).

Materials and methods

After Chinese dry Aconitum ciliare Decaisne roots were purchased and fine roots were removed, dry aconiti tuber was used in the experiment.

Suchi process of aconiti tuber

Dry Aconitum ciliare Decaisne roots were processed by the following methods (Figure 1). For the first step Suchi process, after about 50 g ginger was added in about 3 l Korean rice wine residues, 600 g dry Aconitum ciliare Decaisne roots were added and mixed. It was kept at room temperature for 5 days. After Aconitum ciliare Decaisne roots were collected, they were washed five times with water. For the second step Suchi process, after about 50 g ginger was added in about 3 l Korean rice wine, dry Aconitum ciliare Decaisne roots obtained from the first process was added and kept at room temperature for 5 days. After Aconitum ciliare Decaisne roots were collected, they were washed five times with water. For the third step Suchi process, the second process was repeated with Aconitum ciliare Decaisne roots. For the fourth step Suchi process, Aconitum ciliare Decaisne roots were added in about 3 l 100 % milk and kept at room temperature for 5 days. Aconitum ciliare Decaisne roots were washed five times with water. For the final step Suchi process, the fourth process was repeated. Aconitum ciliare Decaisne roots obtained from the fifth process were dried at room temperature for 4 days and then were analyzed.

Reagent and equipment

Reference material, aconitine, was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Its purity was over 98.0%. Methanol, acetonitrile and D.I. water for LC-MS analysis were purchased from J.T. Baker (Phillipsburg, NJ, USA). Formic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). For separation and detection of Aconitine, UPLC (Waters, Milford, MA, USA) was used and it consisted of pump, digasser, column oven and autosampler. LC-MS (Waters, Milford, MA, USA) equipped with Electrospray Ionization (ESI) source was used as a mass spectrometer. Waters Mass Lynx software (version 4.1, Milford, MA, USA) was used for data analysis.

Manufacturing of standard solution and test liquid

For separation and detection of Aconitine, the reference material was dissolved in methanol and prepared in the concentra-

tion of 100 µg/ml. It was stored at 4°C and it was diluted prior to use. In addition, for LC-MS quantitative analysis, 0.1 g of sample powder was added in 1 ml of methanol and then extracted by using ultrasonic wave for 5 min. After 0.1 ml of extract was diluted in 10 ml of methanol, it was filtered through 0.22 µm membrane.

HPLS and LC-MS analysis condition

For separation and detection of Aconitine from Aconitum ciliare Decaisne roots, ACQUITY TQD LC-MS (Agilent) combined with mass spectrometry was used. Aconitine was isolated by using Kinetex 2.6 u C8 100A column. The column temperature was maintained at 45°C. Mobile phase A was distilled water containing 0.1% formic acid and mobile phase B was acetonitrile containing 0.1 % formic acid. Volume flow rate of the mobile phases A and B was 0.5 ml/min. Injection volume was 0.1 µl. In addition, mass of each ingredient was detected in cation and anion modes. For optimal mass detection, capillary voltage and extract voltage was 3.3 kV and 3 V, respectively. Source temperature was 120°C. RF lens were 0.3 V. Desolvation temperature was 300°C. Volume flow rate of desolvation gas, cone gas and collision gas was 600 l/h, 50 l/h and 0.14 ml/min, respectively. Quantitative analysis was performed by using the multiple reaction monitoring method.

For analysis on changes in the contents of aconitine included in Aconitum ciliare Decaisne roots before and after Suchi process was performed, LC-MS conditions were shown in Table 1: Fragmentor voltage 70, Collision energy 20 and Ionization mode ESI.

Calibration curve

A calibration curve was created for calculating peak area and concentration, once aconitine standard solutions were diluted in 1, 10, 100 and 1000 ppb. The linearity (linear function) was determined by calculating a correlation coefficient (R^2) on the calibration curve.

Results and discussion

Stock solutions of aconitine were prepared in 1, 10, 100 and 1,000 ppb to create a calibration curve. TIC (Total ion chromatogram) MRM and MRM chromatogram were measured at each concentration in the MRM (Multiple Reaction Monitoring) mode of ESI (Electrospray Ionization method) by using LC-MS. Results were shown in Figure 2. Areas and concentration of MRM chromatogram of standard solutions were calculated by using variables (peak areas). Results were shown in Table 2. Calculated areas of aconitine were 124 at 1 ppb, 1,085 at 10 ppb, 8,735 at 100 ppb and 59,023 at 1,000 ppb, respectively. A calibration curve was created as shown in Figure 3. A correlation coefficient (R^2) was 0.998, which was close to 1 and represented good linearity (linear function). The regression equation was y=107.82x + 7.6648, where x represented concentration of aconitine and y represented peak area.

LC-MS analysis was used to determine the contents of aconitine in samples after each step Suchi process was performed. MRM conditions of aconitine for quantitative analysis in LC-MS were as follows: after m/z 646.2 (precursor ion) → 586 (product ion) was set, the contents of aconitine were measured after each step Suchi process was performed. The results of analysis were shown in Figure 4. In each Figure, A was TIC (Total ion chromatogram) and represented the results obtained by elution after mixing water and 0.1 % formic acid. B represented

Figure 1: Processing procedure of aconitine detoxification from root of *Aconitum ciliare.*

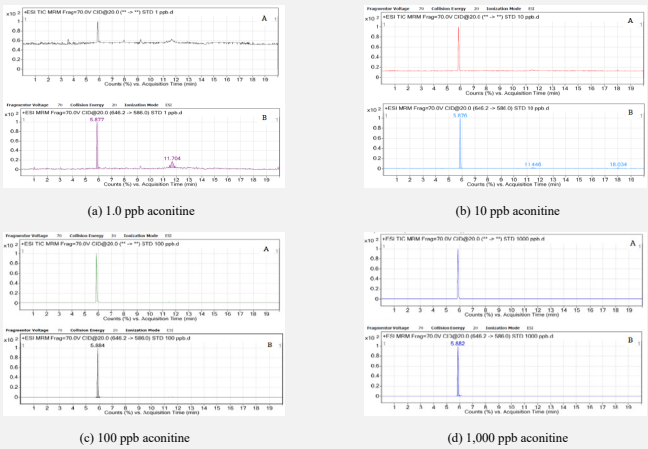


Figure 2: LC-ESI-MS chromatogram of depending on the various aconitine concentrations (RT, 5.876min) in the positive ion mode: **(A)** Total ion chromatogram, **(B)** multiple reaction monitoring chromatogram. **(a)** 1.0 ppb aconitine, **(b)** 10 ppb aconitine, **(c)** 100 ppb aconitine, **(d)** 1000 ppb aconitine).

the results obtained by elution after mixing MeCN and 0.1 % formic acid. At the time, the volume flow rate of extract was 0.5 ml/min. Aconitine was isolated at the retention time was about 5.87 min. Results of chromatogram analysis on TIC and MRM of LC-MS were shown in Figure 4. Results of quantitative analysis of aconitine were shown in Table 3.

Figure 4a showed LC-MS chromatogram results of Aconitum ciliare Decaisne root samples before the Suchi was performed. In this Figure, A showed TIC (Total Ion Chromatogram) results, representing that several ingredients were detected, and B showed MRM (multiple reaction monitoring) results, representing that aconitine was detected at the retention time of 5.875 min. As the results of quantification of aconitine, peak area (632.4→572) was 202 and 1,802 ng/g was detected. Figure 4b showed LC-MS chromatogram results of the sample after the first step Suchi was performed once. Retention time was 5.869 min. As the results of quantification of aconitine, peak area (616.2→556.1) was 148 and 1,302 ng/g was detected. It was found that the content of aconitine was decreased after the first step Suchi was performed. However, the content of aconitine was 6,811 ng/g after the second step Suchi was performed. It was increased (Figure 4c), but it was decreased down to 2,285 ng/g after the third step Suchi was performed (Figure 4d). In addition, the content of aconitine was 977 ng/g after the fourth step Suchi was performed (Figure 4e). After the fifth step Suchi was performed, the content was 216 ng/g (Figure 4f). As the results, contents of aconitine were gradually decreased after the third Suchi was performed. After the fifth Suchi, the content was substantially decreased. It was found that the contents of aconitine were decreased after Suchi was performed several times. The Suchi process in this study was different from that reported by Kim et al. (2002) [26], but similar results were shown. When differences in contents were compared between two studies, the content was about 10 times as low as that reported in Kim et al. (2002) [26]. It is required to compare various aspects rather than contents of aconitine.

Table 1: Analytical condition of LC/MS-Positive mode.

Activity	**Condition**		
Column	Kinetex 2.6u C8 100A		
Detector	G1367D		
Mobile phase	A: Water + 0.1% formic acid B: MeCN + 0.1% formic acid		
	Time (min)	**A (%)**	**B (%)**
	0	95	5
	1	95	5
	10	0	100
	12	0	100
	15	95	5
	20	95	5
Flow rate	0.5 mL/min		
Ion source	ESI		
Gas	Sheath gas (N_2, 40 Arb) Ion sweep gas (N_2, 15 Arb)		
Spray voltage	(+) ve 4000V		
Collision gas	Argon 1.5 m torr		

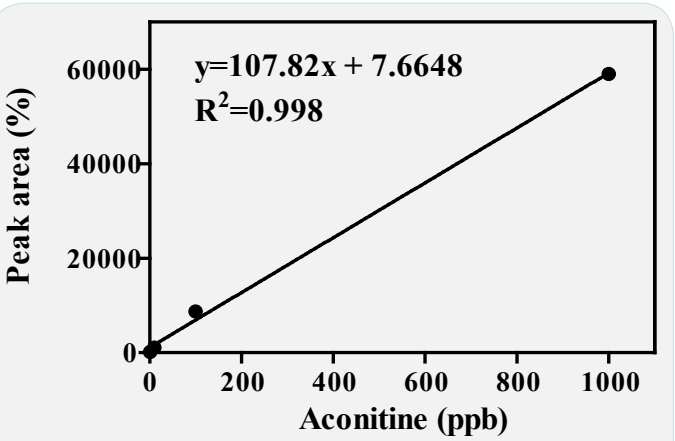
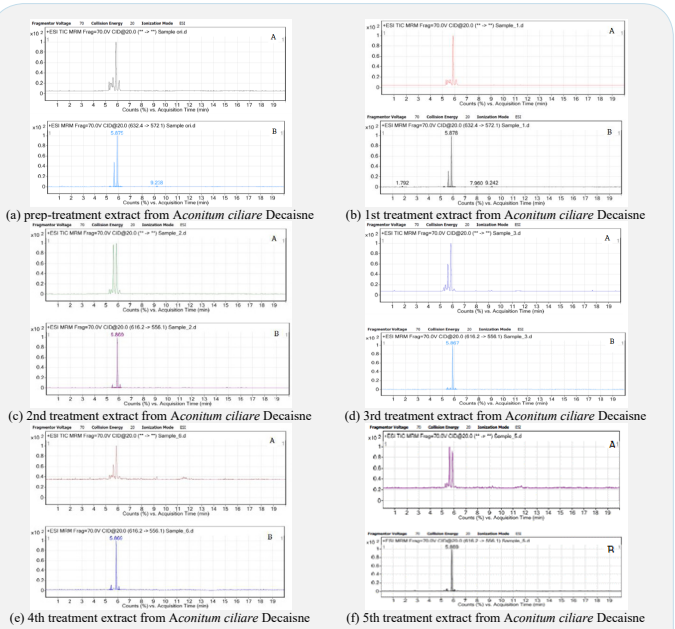


Figure 3: Calibration curve of aconitine.



(a) prep-treatment extract from *Aconitum ciliare* Decaisne (b) 1st treatment extract from *Aconitum ciliare* Decaisne

(c) 2nd treatment extract from *Aconitum ciliare* Decaisne (d) 3rd treatment extract from *Aconitum ciliare* Decaisne

(e) 4th treatment extract from *Aconitum ciliare* Decaisne (f) 5th treatment extract from *Aconitum ciliare* Decaisne

Figure 4: LC-ESI-MS chromatogram of treatment extract from *Aconitum ciliare* Decaisne in the positive ion mode: **(A)** Total ion chromatogram, **(B)** multiple reaction monitoring chromatogram. **(a)** prep-treatment extract from *Aconitum ciliare* Decaisne, (b) 1st treatment extract from *Aconitum ciliare* Decaisne, **(c)** 2nd treatment extract from *Aconitum ciliare* Decaisne, **(d)** 3rd treatment extract from *Aconitum ciliare* Decaisne, **(e)** 4th treatment extract from *Aconitum ciliare* Decaisne, **(f)** 5th treatment extract from *Aconitum ciliare* Decaisne.

Table 2: The area compared to standard Aconitine concentration.

No	**Aconitine(ppb)**	**Peak area(%)**
1	1	124
2	10	1,085
3	100	8,735
4	1,000	59,023

Conclusion

The purpose of this study was to examine the changes in contents of aconitine known as the main ingredient of Aconitum ciliare Decaisne by a new Suchi process. 1, 10, 100 and 1,000 ppb aconitine standard solutions were used. TIC MRM and MRM chromatogram were measured at each concentration in MRM mode of ESI method by using LC-MS. Areas and concentrations of MRM chromatogram were analyzed by using variables (peak area) as follows: Calculated areas of aconi-

Table 3: Contents of aconitine in methanol extract of Aconitum ciliare DC.

Sample	Aconitine peak area 646.2→586	ppb	Xdilution factor Ippb, ng/ml)	ng/0.1 g Sample	ng/l g sample
Original	202	1.8	180.2	180.2	1802
1st treatment	148	1.3	130.2	130.2	1302
2nd treatment	742	6.8	681.1	681.1	6811
3rd treatment	254	2.3	228.5	228.5	2285
4th treatment	113	1.0	97.7	97.7	977
5th treatment	31	0.2	21.6	21.6	216

tine were 124 at 1 ppb, 1,085 at 10 ppb, 8,735 at 100 ppb and 59,023 at 1,000 ppb, respectively. A calibration curve was created. A correlation coefficient (R^2) was 0.998, which was close to 1 and represented good linearity (linear function). The regression equation was y=107.82x + 7.6648. Contents of aconitine which was a main ingredient of Aconitum ciliare Decaisne were measured by using LC-MS as follows: 130.2 ng/ml (ppb) after the first step Suchi, 681.1 ng/ml (ppb) after the second step Suchi, 228.5 ng/ml (ppb) after the third step Suchi, 97.7 ng/ml (ppb) after the fourth step Suchi, and 21.6 ng/ml (ppb) after the fifth step Suchi. It was found that the contents of aconitine were substantially decreased when more Suchi processes were performed. As the results, we could decrease the contents of aconitine which was a main ingredient of Aconitum ciliare Decaisne by using residues from unrefined rice wine, unrefined rice wine and milk.

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