

Comparison of the Amino-Acid Content in Pharmacopuncture Extracts Taken from a Scorpion's Body and from Its Tail

Jin-Ho Lee, Joon-Shik Shin, Eun-Hya Chi, In-Hee Lee*

Jaseng Medical Foundation, Seoul, Korea

Key Words

amino acid, *buthus martensii karsch*, herbal medicine, pharmacopuncture, scorpion, scorpion tail

Abstract

Objective: This study was conducted to investigate the amino-acid compositions of pharmacopuncture extracts taken from the body and from the tail of *Buthus martensii Karsch*, which are frequently prescribed in Oriental medicine.

Methods: Amino acids in hot water and 70% ethanol extracts taken from the scorpion's whole body and from its tail were screened by using high performance liquid chromatography (HPLC). The experiments were performed with linearity, precision and accuracy.

Results: The results of the amino-acid-composition analysis showed that the *Buthus martensii Karsch* extracts contained various amino acids such as aspartic acid, histidine, alanine, tyrosine, and cystine. The amino-acid analysis showed that the hot water extract was more beneficial than the ethanol extract, except for histidine. The amino acids from the tail and the body of the scorpion were compared, and the concentration of aspartic acid in the extract from the scorpion's tail was two times that found in the extract from its body. The

results of validation experiments were all satisfactory.

Conclusion: Studies on the ingredients in extracts from a scorpion other than buthotoxin may demonstrate that the antiepileptic efficacy, anticancer activity, anti-thrombotic action and analgesic effect are enhanced. Using only the tail of the scorpion when pharmacopuncture is dispensed may be beneficial because the extracts from the tail of the scorpion have higher potency than those from the whole body.

1. Introduction

Buthus martensii Karsch (BMK)(scorpiones : *Buthidae*), which is used in Oriental medicine, is produced by soaking in boiling water or boiling brine and then drying. The head, upper body and front of the abdomen of scorpion have a flat and long oval shape. The back of the abdomen is tail-like curve, which, in an adult, is approximately 6-7 cm in length and has a greenish-brown color. The abdomen and legs are a yellowy tan, and the tip of the tail is brown colored. On cutting the abdomen, one can see back ~ brown-yellow substances inside. The inside of tail is empty. The scorpion is smooth and lighter, and it has a salty taste and a fishy smell [1]. As another name, the Oriental medicine is called *Garza*, *Jeonchung*, *Jeonza*, *Dubaek*, or *Jubuchung* [2]. If only the scorpion's tail is

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used, the Oriental medicine is called *Garmi* or *Galcho* [3]. The scorpion produces a nerve poison, a toxic protein buthotoxin (= katusotoxin), whose chemical properties and pharmacological action is similar to those of snake neurotoxins [1]. Hydroxylamine coexists with lecithin, triethylamine, betaine, taurine, cholesterol, stearic acid, palmitic acid, etc. in the venom of a scorpion [1, 4].

Buthus martensi Karsch herbal acupuncture, as a traditional Korean immunosuppressive agent, has been widely used in the treatment of immune-related diseases, rheumatoid arthritis, and satisfactory results have been obtained [5]. As to pharmacological actions, *Bmk I4* and *Bmk I6*, *Bmk Ang P1* and *BMK dITAP3* are known to have analgesic effects [6]. Conventionally, scorpions have been boiled and braised and boiled scorpions are known to help blood pressure (BP) motor inhibition and to lower blood pressure by dilating the blood vessels. However, the blood pressure is increased by noradrenaline secretion in distal end of the sympathetic nerve end [7]. In addition, the coercive and the antibacterial actions of scorpions have been studied.

Choi et al. reported that methanol (MeOH) extraction had an inhibitory effect on nitric-oxide and cytokine production in lipopolysaccharide-activated Raw 264.7 cells [8]. Cho et al. reported that the anti-arthritic effects of *Buthus martensi Karsch* herbal acupuncture inhibited the expression of nitric-oxide synthase and the production of nitric oxide in interleukin-1-induced human chondrocytes [9]. In recent years, research has been focused on the Korean medicine ingredients used to manufacture pharmacopuncture. Pharmacopuncture, or herbal acupuncture (integrated acupuncture and herb therapy), is one of the new acupuncture therapies widely used in traditional East Asian medicine [10]. Pharmacopuncture is much more effective than acupuncture alone, and its effects vary with the herbs used [11].

In the past, the scorpion's tail was thought to be more potent. However, in recent years, the entire scorpion has typically been used even though no differences between the effects of the entire scorpion and its tail have been reported [1]. However, the text *Bonchogangmok* states that the end of the tail has higher potency, and Taiwanese Pharmacopoeia specifies the separate use of the entire scorpion and its tail.

Therefore, the present study analyzed the differences in the amino acids in the tail and the full body of a scorpion. The differences were obtained by comparing the amino acids 70% ethanol and hot water extracts.

2. Material

The carefully-selected scorpion used in this experiment was purchased from *Dong-Kyoung* General Trading Co. As standards, 17 kinds of amino acids (p/n 5061-3330), L-alanine, L-arginine, L-aspartic acid, L-cystine, L-glutamic acid, glycine, L-histidine hydrochloride monohydrate, L-isoleucine, L-leucine, L-lysine hydrochloride, L-methionine, L-phenylalanine, L-proline, L-serine, L-threonine, L-tyrosine and L-valine, were obtained from Agilent (USA) and were kept sealed at 4°C before use. HPLC- photodiode array detector (PDA) (Waters 600 series, USA) and a column (Eclipse plus RR-C18, 4.6 × 150, 3.5 μm, Agilent, USA) were used in this study. Borate buffer (p/n 5061-3339, Agilent, USA), ortho-hthalaldehyde (OPA) (p/n 5061-3335, Agilent, USA), and 9-fluorenyl-methyl chloroformate (FMOC) (p/n 5061-3337, Agilent, USA) were used for amino acid derivatization. Acetonitrile (J. T. Baker, USA) and pure water (J. T. Baker, USA) were used for the HPLC. Other high purity reagents were used as solvents for the separation of the standard and the sample extracts.

3. Method

3.1. Preparation of extracts

3.1.1. Hot water extracts

The scorpion (dry weight: 300 g) was loaded at the bottom of a reactor, and water (3 L) was added to the reactor for injection into the impeller, the upper part of the reactor, the bottom of reactor and the cooling pipe (reflux). The substance was boiled at 105°C for 3 h. After the extract had been filtered by using 3-μm filter paper (*Hyundai* Micro, Korea), it was concentrated under a reduced pressure by using a rotary evaporator (EYERA, Japan). Ethanol (75%) was added to the residue, the solution was stirred for 60 min, and a precipitate was allowed to form for 24 h. After the extract had been filtered using 3-μm filter paper (*Hyundai* Micro, Korea), it was concentrated under a reduced pressure by using a rotary evaporator (EYERA, Japan). The extracts were then filtered using 1-μm filter pad (*Hyundai* Micro, Korea), and the same procedure was used for both 85% ethanol and 95% ethanol. The product was concentrated under a reduced pressure, and it was dissolved in 200 ml of water for injection. The solution was freeze-dried in a freeze dryer (*Il-shin* Bio Base, Korea), and the resulting product was stored at -80°C before use.

3.1.2. Ethanol (70%) extracts

The scorpion (dry weight: 300 g) was loaded at the bottom of a reactor, and 70% ethanol (3 L) was added to the reactor for injection into the impeller, the upper part of the reactor, the bottom of reactor and the cooling pipe (reflux). The substance was boiled at 105°C for 3 h. After the extract had

been filtered using 3- μm filter paper (Hyundai Micro, Korea), it was concentrated under reduced pressure by using a rotary evaporator (EYERA, Japan). Ethanol (75%) was added to the residue, the solution was stirred for 60 min, and a precipitate was allowed to form for 24 h. After the extract had been filtered using 3- μm filter paper (Hyundai Micro, Korea), it was concentrated under reduced pressure by using a rotary evaporator (EYERA, Japan). The extracts were then filtered by using 1- μm filter pad (Hyundai Micro, Korea), and the same procedure was used for both 85% ethanol and 95% ethanol. The product was concentrated under a reduced pressure, and it was dissolved in 200 ml of water for injection. The solution was freeze-dried in a freeze dryer (I-shin Bio Base, Korea), and the resulting product was stored at -80°C before use.

3.1.3. Extracts of the scorpion's tail

The scorpion's tail (dry weight: 300 g) was treated in the same way for both the 70% ethanol extract and the water extract.

3.2. Sample preparation for the HPLC analysis of amino acids

If an amino-acid analysis is to be conducted using ordinary HPLC, the sample needs derivatization. After borate buffer had been added to each standardized sample and the scorpion extract had been stirred well, OPA and FMOc were added and stirred. The standardized samples, which had been derivatized as above, were then diluted in a stepwise manner into five concentrations, which were then spun for 15 s and used after having been filtered through a 0.45- μm membrane syringe filter. Mobile phase A (with phosphoric acid) was used as the dilution solution.

3.3. HPLC analysis

The conditions for each HPLC analysis of the amino acids were based on those in Refs. 12 and 13. The column temperature and the UV wavelength were 40°C and 262 nm, respectively. Mobile phase A consisted of pure water with 10-mM Na_2HPO_4 , 10-mM $\text{Na}_2\text{B}_4\text{O}_7$, and 5-mM NaN_3 and was calibrated to pH 8.2. Mobile phase B consisted of acetonitrile, methanol, and water mixed at a ratio of 45:45:10. The flow rate was 1.5 mL/min. The conditions of the analysis are shown in Table 1.

3.4. Validation of the method

3.4.1. Linearity

The peak area ratio was measured using the standardized amino-acid samples that had been weighed into five different concentrations. The calibration curve was created using the regression equation obtained in the form $y = ax + b$ (with x being the peak area and y the sample concentration).

The linearity of the created calibration curve was determined using the value of R^2 . When the value of R^2 exceeded 0.99, the calibration curve was used to evaluate the contents of the ingredients. The sum of the area values of 17 types of amino acids was used in the verification process.

3.4.2. Precision

In order to observe any subtle intra- and inter-day changes, we divided the standardized samples into five various concentrations, and we examined them five times a day for three days. The precision was evaluated using the relative standard deviation from the measured values. The sum of the area values of 17 types of amino acids was used in the verification process.

3.4.3. Accuracy

The results obtained from injecting the standardized samples at three various concentrations three times were substituted into the calibration curve, and the resultant value was compared with the true value to assess the accuracy: 90%-100%.

$$\text{Recovery}(\%) = \frac{\text{Measured concentration}}{\text{Theory of concentration}} \times 100$$

4. Result

4.1. Preparation of extracts

Dried scorpion, 300 g, was extracted by means of hydrothermal extraction and then immersed in ethanol at a concentration from 75% to 95%. This was then freeze-dried into 30.0 g of powder. The closing rates are as shown

Table 1 Operating conditions of HPLC for amino acid analysis

	Time (min)	Flow (mL/min)	Mobile Phase	
			A (10-mM Na_2HPO_4 , 10-mM $\text{Na}_2\text{B}_4\text{O}_7$, 5-mM NaN_3 , pH 8.2)	B (Acetonitrile: Methanol: H_2O = 45:45:10)
Gradient Profile	0		98	2
	1.5		98	2
	60		43	57
	60.1	1.5	0	100
	63.5		0	100
	63.6		98	2
	75		End	End

in Table 2. In addition, 300 g of dried scorpion was extracted under 70% ethanol three times and then immersed in ethanol at a concentration from 75% to 95%. This was then freeze-dried into 30.0 g of powder. The closing rates are as shown in Table 2. The extracts from the scorpion's tail were obtained in the same way as the hot water and the 70% ethanol extracts. Dried scorpion's tail, 300 g, was extracted in the same way as the hot water extracts, was then immersed in ethanol at a concentration from 75% to 95%, and was freeze-dried into 28.1 g of powder. The closing rates are shown in Table 2.

4.2. HPLC analysis

4.2.1. Amino acid analyses of the extracts obtained from the scorpion's tail and its entire body by using hydrothermal extraction

The results of the HPLC analyses of the contents of amino acids in the extracts derived from the scorpion's tail and its entire body by using hydrothermal extraction are as shown in Table 3. Seventeen kinds of amino acids in both extracts were identified, and the total amino acid contents in the extracts from the tail and the entire body were 162.8392 mg/g and 163.0755 mg/g, respectively. This study revealed that the 17 types of amino acids contained aspartic acid, histidine, alanine, tyrosine and cystine in large amounts: 26.6787, 18.4964, 11.4944, 28.8083 and 20.7936 mg/g, respectively, in the extract from the scorpion's entire body, and 45.632, 19.9854, 13.0293, 21.8338 and 12.311 mg/g in the extract from the scorpion's tail. The contents of the other amino acids are shown in Fig. 1. Most amino acids were found in similar amounts in the extracts from the scorpion's entire body and the extracts from its tail, but the extract from the scorpion's tail contained 71% more aspartic acid than the extract from the entire body.

4.2.2. Amino acid analyses of the extracts obtained from the scorpion's tail and its entire body by using the 70% ethanol extraction method

The results of the HPLC analyses of the contents of amino acids in the extracts derived from the scorpion's tail and its entire body by using 70% ethanol extraction are as shown in Table 3. Seventeen kinds of amino acids in both extracts were identified, and the total amino acid contents in the extracts from the tail and the entire body were 93.4242 mg/g and 103.4810 mg/g, respectively. This study revealed that the 17 types of amino acids contained aspartic acid, histidine, alanine, tyrosine and leusine in large amounts, with the contents being 21.4016, 26.7793, 5.7639, 8.7088 and 5.4464 mg/g, respectively, in the whole-body extracts, and 36.5224, 27.4986, 6.5252, 6.5438 and 5.7001 mg/g in the tail extracts. The contents of the other

amino acids are shown in Fig. 1. Most amino acids were found in similar amounts in the extracts from the scorpion's entire body and the extracts from its tail, but the extracts from the scorpion's tail contained 71% more aspartic acid than the extracts from the entire body. A comparison between the two extraction methods revealed

Table 2 Yield of freeze-dried powder according to the extraction solvent

Extraction Solvent	Extraction Site	Freeze-dried Powder	
		Whole Body	Tail
Hot Water (%)		10.00	9.38
70% Ethanol (%)		10.20	10.00

Table 3 Analysis of amino-acid compositions of the hot water and the 70% ethanol extracts from a scorpion

Amino Acid	Solvent Extraction		Amino Acid / Extraction (mg / g)			
			Hot Water		70% Ethanol	
	Whole body	Tail	Whole body	Tail	Whole body	Tail
Aspartic acid	26.6787	45.632	21.4016	36.5224		
Glutamic Acid	6.5378	6.4511	2.9918	2.9521		
Serine	5.6087	4.9509	1.3293	1.1744		
Histidine	18.4964	19.9854	26.7793	27.4986		
Glycine	1.0192	1.2495	0.4211	0.5163		
Threonine	8.8898	6.5689	3.6435	2.7884		
Arginine	3.6769	2.4788	1.0232	0.6894		
Alanine	11.4944	13.0293	5.7639	6.5252		
Tyrosine	28.8083	21.8338	8.7088	6.5438		
Cystine	20.7936	12.311	6.381	3.7419		
Valine	3.6998	2.5216	1.3262	0.8854		
Methionine	4.3595	3.1486	1.7467	1.2511		
Phenylalanine	1.7913	1.5558	0.6443	0.5331		
Isoleucine	3.6859	3.0203	1.0112	0.8254		
Leusine	7.5804	7.9572	5.4464	5.7001		
Lysine	0.9685	3.1195	0.5194	1.6724		
Proline	8.75	7.2618	4.2865	3.6610		
Total	162.8392	163.0755	93.4242	103.4810		

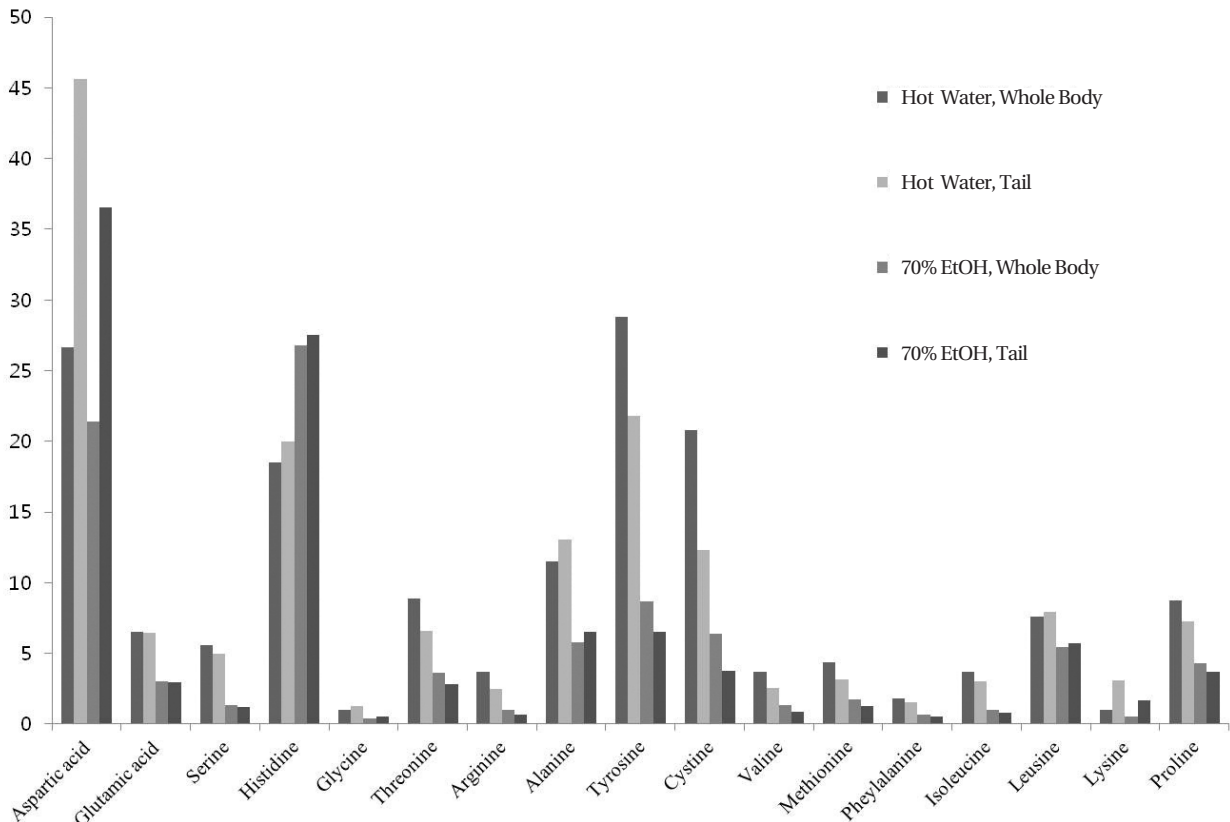


Figure 1 Comparison of the amino acid compositions of the hot water extract and the 70% ethanol extract.

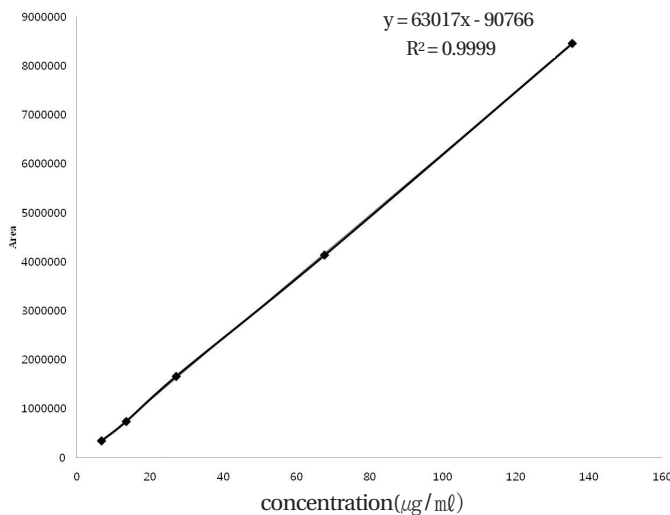


Figure 2 Calibration curve for the concentration of amino acids.

that, in general, more amino acids, with the only exception being histidine for which a larger amount was extracted through the 70% ethanol extraction method, were extracted through the hydrothermal extraction method.

4.3. Validation of the method

Standardized solutions were produced for each amino acid in concentrations of 6.774, 13.548, 27.096, 67.74 and 135.48 $\mu\text{g}/\text{ml}$ and were analyzed using the established procedures for HPLC amino-acid analysis. As shown in Fig. 2, the calibration curve was plotted with a linearity (R^2) of 0.9999 (x = concentration, y = peak area). The intra-day precision was confirmed by measuring the standardized amino-acid sample solutions with concentrations 6.774, 13.548, 27.096, 67.74, 135.48 $\mu\text{g}/\text{ml}$ five times. A high precision of 0.05%-0.33% was obtained, as shown in Table 4. The inter-day precision was confirmed by repeated measurements using each amino-acid standard at the same concentrations once per day for three days. A high precision of 0.37%-0.54% was obtained, as shown in Table 5. The accuracy was confirmed by measuring three times the standardized amino-acid samples with concentrations of 6.774, 27.096 and 135.48 $\mu\text{g}/\text{ml}$ and then converting the result to the original concentration by using the calibration curve shown in Fig. 2. The average of the accuracies for each of the standardized samples at the various concentrations was high (96%-99%, Table 6).

Table 4 Peak areas of the sums of amino acids analyzed in five different concentrations for determining the intra-day precision

Measurement	Dilution($\mu\text{g}/\text{m}\ell$)				
	6.774	13.548	27.096	67.74	135.48
1	343296	742592	1657733	4135473	8461516
2	343072	739841	1650244	4133290	8471459
3	342988	740659	1644178	4119987	8464977
4	342874	741158	1656223	4134456	8446569
5	343111	738549	1653493	4136599	8421597
Mean	343068.20	740559.80	1652374.20	4131961.00	8453223.6
S.D.	156.43	1504.78	5396.69	6804.65	19899.51
Intra-day Precision (%)	0.05	0.20	0.33	0.16	0.24

Table 5 Peak areas of the sums of amino acids analyzed in five different concentrations for determining the inter-day precision

Measurement	Dilution($\mu\text{g}/\text{m}\ell$)				
	6.774	13.548	27.096	67.74	135.48
1st Day	343296	742592	1657733	4135473	8461516
2nd Day	339658	746552	1655980	4112547	8447459
3rd Day	341157	740598	1643821	4141589	8401369
Mean	341370.33	743247.33	1652511.33	4129869.67	8436781.33
S.D.	1828.36	3030.61	7576.92	15310.37	31463.07
Inter-day Precision (%)	0.54	0.41	0.46	0.37	0.37

Table 6 Calculated concentrations of the sums of amino acids analyzed in three different concentrations for accuracy

Measurement	Dilution($\mu\text{g}/\text{m}\ell$)		
	6.774	27.096	135.48
1	6.69	6.69	135.51
2	6.71	6.71	134.29
3	6.15	6.15	135.94
Mean	6.51	6.51	134.91
Accuracy (%)	69.16	69.16	99.58

5. Conclusion

The Pharmacopoeia of Taiwan suggests that the scorpion's body and its tail be separated when it is used as medicine. *Bonchogangmok* also specifies that the medicinal efficacy is higher for the scorpion's tail than for its body. From this, one may infer the possibility of

a difference in the medicinal efficacy between a scorpion's tail and its body. Hence, this study attempted to develop an objective and scientific method for analyzing this difference between a scorpion's entire body and its tail by analyzing their amino-acid contents. A variety of amino acids from the scorpion's tail and its entire body were measured using HPLC, and their concentrations were then compared. In addition, hydrothermal extraction and 70% ethanol extraction were compared to each other in order to examine the differences between the solvent extraction techniques used. In order to ensure the legitimacy of the HPLC amino-acid analyses, we performed verification tests: linearity, accuracy, and precision. The extracts were purified through a step-by-step alcohol purification and fine-filter purification process so that they could be used in pharmacopuncture. The extracts were filtered using a 0.1- μm sterile filter in order to achieve high purity.

Amino acids can take various forms, depending on their chemical structure. Mainly, amino acids are present as components of proteins or as free amino acids. Small

amounts of amino acids are present as peptides or as complex proteins combined with sugars or lipids [14].

A total of 17 types of amino acids were analyzed in the extracts from the scorpion's entire body and from its tail. Amino acids such as aspartic acid, histidine, alanine, tyrosine, cystine, and leusine were identified in large amounts while glycine, phenylalanine, and lysine were identified in small amounts. Aspartic acid prevents body fatigue, protects the immune system by producing immunoglobulin and antibodies, and removes excess ammonia and toxins from the bloodstream, thereby preventing damage to the liver and the central nervous system (CNS) [15]. Valine contributes to muscle recovery after exercise, vitality of the spirit, the growth of new tissues, and the healing of wounds [16]. Arginine stimulates the secretion of growth hormones, regulates blood pressure, inhibits the proliferation of cancer cells, and enhances the immune system. In particular, when fats and fatty oils are added, arginine becomes softer, and emulsification is increased, which leads to effective antioxidant response [17]. Phenylalanine, which produces norepinephrine, activates thinking, improves the memory and learning ability, and decreases the appetite [18]. Isoleucine acts to maintain blood sugar and energy [19]. As previously mentioned, scorpions contain a large variety of amino acids with various medical benefits.

In this study, the extraction yield-rate of the 70% ethanol extraction method was higher than that of the hydrothermal extraction method, but the amino acid content was higher in the hydrothermal extracts than in the 70% ethanol extracts. The amount of histidine extracted through the 70% ethanol method was the only exception and was detected to be 41.0% higher. This study did not show any significant differences in the contents of the amino acids between the scorpion's tail and its body, but the content amount of aspartic acid was detected to be approximately 71% higher in the tail section. Although scorpions contain many ingredients other than amino acids [20], the results of the analysis of the 17 amino acids revealed that little difference in the contents existed between the scorpion's tail and its body and that the hydrothermal extraction method was more effective in extracting large amounts of amino acids than the 70% ethanol extraction method.

Often buthotoxin, a component of scorpion's venom, is extensively researched, leaving amino acids, fatty acids, etc. unexplored. If research on components of the scorpion other than buthotoxin is carried out consistently, not only could it be used in supporting the previously known medical efficacy of the scorpion, such as antiepileptic efficacy, anticancer activity, anti-thrombotic action and analgesic effect, but also it might uncover many

more efficacies that have yet to be discovered. As a direct means of physical administration, caution should be taken to maintain a high purity of ingredients when applying pharmacopuncture, and given the above points, use of only the tail of the scorpion would be desirable.

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