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A Study on Physicochemical Characteristics of *Achyranthis Radix* Extract

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Abstract

Using the ethanol extract, *Achyranthis Radix*, various chemical characteristics were investigated. The nutritional compositions of the *Achyranthis Radix* extract were as follows;moisture 42.3%, crude protein 101.1%, crude fat 2.07%, ash 8.94%, and carbohydrate 36.5%. Among the free sugars, the maximum lactose concentration in the *Achyranthis Radix* extract was obtained at 0.0526 mg% and fructose, maltose, arabinose, and glucose were followed: 0.3654 mg%, 0.1160 mg%, 0.0365 mg% and 0.027 mg%, respectively. The total amino acid concentration of the *Achyranthis Radix* extract was 8908.3 mg% and concentrations of lysine, aspartic acid, glutamic acid, and arginine were 989.1 mg%, 954.4 mg%, 841.4 mg% and 763.2 mg%, respectively. Among various long chain fatty acids, the maximum concentrations of palmitic acid and linoleic acid were obtained at 47.8% and 31.058%, respectively. However, in the case of organic acid, only the oxalic acid and malic acid were determined. The potassium concentration in the *Achyranthis Radix* extract was relatively high and the concentrations of Ca, Mg, Fe, Na, Mn, and Zn were as follows: 275.3 mg%, 281.3 mg%, 119.4 mg%, 37.75 mg%, 10.43 mg% and 3.11 mg%, respectively. These results suggest that the *Achyranthis Radix* extract might have a possible positive effect for medical and edible purposes.

Key words : *Achyranthis Radix*, Sugar, Fatty acid, Amino acid.

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I INTRODUCTION

Achyranthis Radix is a perennial plant of the Amaranthaceae family and is an herbal medicine named for the Achytanthes japonica Leeille et Vaniot roots. It grows in various fields and street-sides within our country and has even been distributed to Japan and China. It grows to about between 60~150cm, has a rectangular shape with a green-colored trunkwith a particularly thick joint shaped like a cow knee. In addition, the flower blooms around August and seeds are oval-shaped and glossy with a dark-brownish in color and surrounded by a flowerbed[1,2]. They have been widely used as forms of oriental medicines or folk remedies to treat aches, Antihypertensive effects[3], rheumatism, eliminating arthralgia congestion, diuresis and other diseases [4], and young sprouts can also be eaten as herbs. The current research has been suggestive that medicinal and nutritional usage of such plants.

Under research on the physiological activities of *Achyranthis Radix*, *Achyranthis Radix* extracts within in vitro have been shown to possess inhibiting effects of cytochrome P450 drug metabolizing enzymes, Immune regulatory effects of *Achyranthis Radix*, Antimutagenic effects, anti-inflammatory effects for acute and subacute inflammation, effects on teeth and periodontal disease, anti-tumor effects on human cancer colon cells by increasing cisplantin induced cell toxicity[5], inhibitory effects to cathespin B existing in cases of senile dementia[6], whereas within research on in vivo, the protective effects on stem cells within CCl_4^- induced rats[7] and research on Rheumatoid Arthritis[8] are being reported. In addition, research on pharmacologicalactive compounds are being actively conducted, evidencing strong allergic and antitumoral activities and it is reported that AchyranthosideA methyl ester displays strong cytotoxic activities[9]. As already mentioned, various physiological substances have been found within *Achyranthis Radix*, but it is also current reality that research on the bodily effects of each such substance as well as research on drug interaction upon internal use is currently inadequate. Recently, there have beenactive research on developing nutritional products following discoveries that various physiological constituents acquired from certain animals, plants, and microorganisms within nature could serve as valid forms of disease prevention and treatment by helping to adjust physiological functions and maintaining homeostasis within humans[9].

In order to develop new functional foods by nutritionalizing natural plants, a sitological and systematic scientific approach, such as experimenting on physiological responses, becomes necessary. In the case of *Achyranthis Radix*, there are wide-ranging possibilities because drugs can be easily. Consequently, this research has examined the possibilities on the development of new nutritional products through an analysis of the general and nutritional composition of Korean *Achyranthis Radix*.

II MATERIAL AND METHODS

2.1 Preparation of *Achyranthis Radix* extract

Hundred grams of dried *Achyranthis Radix* was added to 500mL of ethanol (80%), cut

by blender (Braun. MR 350 CA) for 2-3min. The extraction of *Achyranthis Radix* was carried out at 65 ℃for 3 hrs under refluxing condenser 3 times. The extract product was filtreated by Whatman filter paper (No. 2) and the solvent was removed by rotary vacuum evaporator at 40 ℃of water bath. The product was concentrated and stored at -70 ℃for sample.

2.2 Determination of crude protein and lipids concentration

The crude protein and crude fat of the samples were quantitated according to the AOAC method[10]. Briefly, the crude protein was measured by Kjeldahl method, and the crude fat was measured by the soxhelet extraction method using diethyl ether. Each sample was measured 3 times repeatedly, and the mean values are presented.

2.3 Determination of free sugar concentration

The free sugar concentration of sample was measured by Ion Chromatography (DX-600, DIONEX, USA) as follows. One gram of sample was added to 300ml of flask containing 50ml of ethanol (80%) and heated by heating mentle for 5hr at 75℃ The mixture was filtered by Whatman filter paper (No. 2). The liquid was concentrated by rotary vacuum evaporator and analysed.

2.4 Determination of amino acids concentration

Sample (0.5g) was added to 3mL of 6 N HCl and the hydrolysis was carried out for 24hr at 121 ℃ The mixture was then pressure-concentrated and 10mL of sodium phosphate buffer(pH 7.0) was added. One milliliter of solution was filtered by membrane filter(0.2 μM) and then analying by automatic amino acid analyzer (BIOCHROM 20, PHAMACIA ENGLAND)

2.5 Determination of long chain fatty acids concentration

After homogenization 5g of sample using warming blender, 10mL of chloform and 20mL of methanol were added and homogenized for 2 min. Ten milliliterof chloform was added to the sample and sprayed fro 30 sec. After filtration of the sample, the supernatant was removed and Na_2SO_4 (anhydrous) was added for dehydration. The sample was concentrated by rotary vacuum evaporator. The crude fat (100 mg) was disoluted into 5 mL of toluene. The methyl esterization using BF_3-MeOH was carried out. Fatty acids analysis using Gas Chromatography (GC-10A, SHIMADZU, JAPAN) was carried out. The analysis conditions are shown in Table 1.

Table 1. Conditions of GC for fatty acids analysis.

Items	Conditions
Instrument	Shimadzu GC-17A
Detector	Flame ionization detector
Column	HP-5
Column temperature	180℃ for 1min-200℃ for 5min
Injector temperature	250℃
Detector temperature	220℃
Split ratio	20:1

2.6 Determination of vitamins concentration

Five milligram of sample and 0.1g ascorbic acid was added to 50ml of glass centrifuge tube containing 5mL of ethanol, heated at 80 ℃for 10 min, and again

heated for 20min with addition of 0.25mL of KOH (50%) at 80 ℃. Twenty four milliliter of distilled water and 5mL of hexane was added and centrifuged for 20min at 1,150 ×g. Anhydrous Na_2SO_4 was added to supernatant for dehydrogenation and was concentrated by rotary vacuum evaporator. A sample was analysised by HPLC (LC-10AVP, SHIMADZU, JAPAN).

2.7 Determination of ion concentration

Five milligram of sample was burned for 24 hr and cooled for 30min. 4ml of the solution (HCl: distilled water= 0.5: 3.5) and 10ml of distilled water were added to mixture and heated. 100ml of sample was used for analysis of ion concentration at Ion Chromatography (DX-600, DIONEX, USA).

III RESULTS AND DISCUSSION

For propose of basic data for health functional food and drug adjuvant, the compositions of sugars, amino acids, fatty acids, organic acids, mineral, and ions using *Achyranthis Radix* extract were investigated.

Table 2. General compositions of *Achyranthis Radix*.

Item / Sample	Moisture (%)	Crude protein (%)	Crude fat (%)	Ash (%)	Carbohydrate (%)
Achyranthis Radix	42.38	10.13	2.07	8.94	36.48

Table 2 was compositions of moisture, crude protein, crude fat, ash, and carbohydrate of *Achyranthis Radix* extract. The concentrations of moisture, crude protein, crude fat, ash, and carbohydrate were 42.38, 10.13, 2.07, 8.94, and 36.48, respectively.

Table 3. Compositions of free sugars in *Achyranthis Radix.*

Free sugar	Content (mg%)
D-Manitol	0.0023
D-Arabinose	0.0365
D-Glucose	0.0270
D-Fructose	0.3654
D-Lactose	0.5260
D-Ribose	0.0023
D-Maltose	0.1160

Table 3 is compositions of free sugars in *Achyranthis Radix* extract. Among various free sugars, the lactose and fructose concentration was high and their concentrations were 0.0526 mg% and 0.3654mg%, respectively. On the other hand, the concentration of D-maltose, D-arabinose, and D-glucose was low. In the case of D-Ribose and D-Manitol, they were very low. Table 4 is compositions of free and total amino acids in *Achyranthis Radix* extract. 17 kinds of amino acids werecomposed and total amino acids concentration was 8908.252 mg%. Among various amino acids, the concentration of amino acids is order of lysine, aspartic acid, glutamic acid, arginine and their concentrations ranged of 763.219 - 989.079 mg%. In the case of essential amino acids, they were order of lysine, arginine, methionine isoleucine threonine, histidine, and valine. On the other hand, In the case of non-essential amino acids, they were 1734.513 mg%. The ratio of essential amino acids against total amino acids was 45.75%.

Table 4. Compositions of free amino acids in *Achyranthis Radix.*

Amino acid	Content (mg%)
Aspartic acid	954.368
Threonine	456.187
Serine	605.377
Glutamic acid	841.359
Proline	526.178
Glycine	200.613
Alanine	81.759
Valine	25.659
Methionine	717.268
Isoleucine	559.047
Leucine	646.047
Tyrosine	723.059
Phenylalanine	420.954
Histidine	398.079
Lysine	989.079
Arginine	763.219
Total A.A.	8,908.252
Total E.A.A.	4232.612
E.A.A. %	45.75

Table 5. Compositions of fatty acids of *Achyranthis Radix.*

Fatty acid	Composition (%)
Palmitic acid (C16:0)	31.058
Oleic acid (C18:1n9c)	21.142
Linoleic acid (C18:2n6c)	47.800
Polyunsaturated fatty acid/ saturated fatty acid	1.53

Table 5 is compositions of polyunsaturated fatty acid and saturated fatty acid of *Achyranthis Radix*. Among various Polly fatty acids, palmitic acid (C16:0) concentration was high, 31.058%, in the case of polyunsaturated fatty acids, oleic acid (C18:1n9c) and Linoleic acid (C18:2n6c) concentration were 21.142 and 47.800, respectively.

Table 6. Compositions of vitamin A and E in *Achyranthis Radix.*

Vitamins	Content (mg%)
A	0.0187
E	0.0004

The ratio of polyunsaturated fatty acid and saturated fatty acid was 1.53%. As shown in Table 6, concentrations of vitamin A and E in *Achyranthis Radix* extract were low. Vitamin A and B concentrations were 0.0187mg% and 0.0004mg%, respectively.

Table 7. Compositions of organic acids in *Achyranthis Radix.*

Oragnic Acid	Content (mg%)
Oxalic acid	1.049
Tartaric acid	-
Citric acid	-
Malic acid	0.058
Formic acid	0.002
Succinic acid	0.001
Furmaric acid	0.001

Table 7 is compositions of organic acids in *Achyranthis Radix* extract. Organic acids were consisted of oxalic acid, malic acid, formic acid, succinic acid, and furmaric acid. Especially, the oxalic acid concentration was 1.049, which was about 18 fold higher than of malic acid. In the case of formic acid, succinic acid, and furmaric acid, they were ranged of 0.002 and 0.001mg%.

Table 8. Compositions of minerals in *Achyranthis Radix.*

Mineral	Content (mg%)
Ca	275.26
Fe	119.44
K	2588
Mg	281.3
Mn	10.43
Cu	0.75
Na	37.75
Zn	3.11

Table 8 is compositions of minerals in *Achyranthis Radix* extract. The minerals were consisted of Ca, Fe, K, Mg, Mn, Cu, Na, and Zn, respectively. The potassium concentration was highest in the *Achyranthis Radix* extract. Especially, this concentration was about 10 fold higher than that of Ca. The minerals concentrations Ca, Mg, and Fe were 275.26, 281.3, and 119.44mg%, respectively. On the other hand, in the case of Zn, Cu, Mn and Na, they were very low. The cation and anion concentrations of *Achyranthis Radix* extract are shown in Table 9 and 10.

Table 9. Compositions of cations in *Achyranthis Radix*.

Cation	Content (mg%)
Lithium(Li^+)	0.011
Sodium(Na^+)	0.031
Ammonium(NH_4^+)	0.010
Potassium(K^+)	0.613
Mgnesium(Mg_2^+)	0.011
Calcium(Ca^{2+})	0.011

Table 10. Compositions of anions in *Achyranthis Radix*.

Anion	Content (mg%)
Fluoride(F^-)	-
(Br-)	0.002
Chloride(Cl^-)	0.065
Nitrite(NO_2^-)	0.005
Nitrate(NO_3^-)	0.343
Phosphate(PO_4^{3-})	0.148
Sulfate(SO_4^{2-})	0.259

Lithium (Li^+), sodium (Na^+), ammonium (NH_4^+), potassium (K^+), magnesium (Mg_2^+), calcium (Ca^{2+}), and lithium (Li^+) were detected. Especially, the potassium (K^+) concentration was 0.613mg%, which was about 20 fold higher than that of sodium (Na^+). However, magnesium (Mg_2^+), calcium (Ca^{2+}), ammonium (NH_4^+), and lithium (Li^+) concentrations were very low. On the other hand, in the case of anion, the nitrate (NO_3^-) concentration was highest. The sulfate (SO_4^{2-}), phosphate (PO_4^{3-}), and chloride (Cl^-) concentration were 0.259 mg%, 0.148mg%, 0.065mg%, respectively. In the case of fluoride (F^-), it was not detected.

From these results, the extract of domestic *Achyranthis Radix* has value to use as medicinal herbs. Now, we are investigating the antioxidant activity in vitro and antioxidant capacity, Lipid metabolism and antithrombogenic capacity in vivo using the ethanol extract of *Achyranthis Radix*.

IV. CONCLUSION

In order to study the biological activities using the ethanol extract using *Achyranthis Radix*, various chemical positions such as sugar, amino acid, fatty acid, organic acid, mineral, and ions were measured. Maltose was major in free sugars, proline in amino acids, linoleic acid in fatty acids, oxalic acid in organic acids, calcium in minerals, K^+ in cations and Cl^- in anions, respectively. These results suggest that ethanol extract of *Achyranthis Radix* have a possible positive effect on a good data of investigating the biological activities. Now, we are investigating the antioxidant activity in vitro and antioxidant capacity, lipid metabolism and antithrombogenic capacity in vivo using the ethanol extract of *Achyranthis Radix*.

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