Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2017; 9(1); 108-113

ISSN: 0975-4873

Research Article

Isolation and Identification of some Primary Metabolites, Micro- and Macroelements of *Aesculus hippocastanum* L. Seeds

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Received: 16th Nov, 16; Revised: 23rd Nov, 16; Accepted: 27th Dec,16; Available Online: 15th January, 2017

ABSTRACT
The qualitative composition and quantitative content of amino acids were studied by PC and HPLC methods. 15 free amino acids were identified in the endosperm and skin of *A. hippocastanum*. 16 bound amino acids were identified in the endosperm; 17 bound amino acids were identified in the skin. The GC/MS method shows that *A. hippocastanum* skin and endosperm contain 2 free sugars and 5 after hydrolysis. We can also distinguish specific sugars for seed endosperm which are not present in the skin of the seed: Rha, Fuc, and Sucrose. Specific sugars in the skin of the seed are Xyl and Man. The endosperm of seeds of *Aesculus hippocastanum* accumulates starch, WSPS, and lipophilic compounds. PS and HC are concentrated in the skin. The qualitative composition and quantitative content of 19 macro and microelements in skin and endosperm of seeds of *A. hippocastanum* was studied by atomic emission spectrophotometry. The skin of seeds of *A. hippocastanum* accumulates macro and microelements. The seeds do not accumulate toxic metals, and this enables their use as medicinal plant material. The results show big differences between endosperm and skin in their contents of primary metabolites and elements. Those differences depend on functions that the skin and endosperm are playing in plants, and they influence how medicines and food supplements might be created.

Keywords: *Aesculus hippocastanum* L. seeds, amino acids, monosaccharides, polysaccharides, lipophilic compounds, micro- and macroelements.

INTRODUCTION

Plant cell produces two types of metabolites: primary metabolites and secondary metabolites. Primary metabolites are involved directly in growth and metabolism. Secondary metabolites considered as end products of primary metabolism that are not involved in metabolic activity and typically act as chemical defense or to attract pollinators. Their absence does not cause adverse effects on the growth of the plant itself.

Primary metabolites comprise many different types of organic compounds, including, but not limited to, carbohydrates, lipids, proteins, and nucleic acids. They are found universally in the plant kingdom because they are the components or products of fundamental metabolic pathways or cycles such as glycolysis, the Krebs cycle, and the Calvin cycle[1-4].

Plant primary metabolites are widely used in different areas: food, pharmacy, medicines, and cosmetics. Proteins and amino acids are primary components of living organisms. Their presence in plants increases the food value of these plants or of protein-based compounds and extracts that could be isolated from them. Plant lipids are used as auxiliary and active substances in many drugs. Carbohydrates can serve main components of drugs and food supplements, as stipulated by their pharmacological action. Carbohydrates from plants are increasingly being considered as ecofriendly alternatives to the use of synthetic additives in many other products, including plastics, detergents, pharmaceutical tablets, gels, and others[4,5].

Nowadays, interest in the analysis of chemical composition of medicinal herbs is growing, owing to the continuing developments in nutrition and in biochemical surveys and mineral prospecting. Additionally, studies of plant raw materials not only characterize the active components but also seek scientific evidence of their therapeutic properties. Macro, micro, and trace elements are known to play a vital role in biological functions in plants and in human metabolic reactions. Moreover, trace elements play an important role in the formation of bioactive chemical constituents in medicinal herbs and accordingly are responsible for their medicinal and toxic properties[6,7].

Horse chestnut – *Aesculus hippocastanum* L. of Hippocastanaceae family – is a well-known plant, widely used in medicine and pharmacy. The study of its primary metabolites, as well as the macro- and microelements, will help to develop new medicines and food supplements.

MATERIALS AND METHODS

Plant material

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Horse chestnut seeds were collected in Kyiv region (Ukraine) in September 2015. The average temperature at harvest time was 14 °C. The raw material was dried in a well-ventilated, shaded place. The dried seeds were divided into two parts – skin and endosperm – which were milled to powder. Dried material samples were kept in a dry and dark place in multilayer paper bags at room temperature.

Determination of amino acids

The presence of amino acids in the plant raw material was confirmed by paper chromatography (PC) of the aqueous extract obtained from the roots, using Filtrak FN 7 chromatography paper, n-BuOH–HOAc–H_2O (1:4:2), and three-fold chromatography. Amino acids were detected using ninhydrin solution (0.1%) followed by heating in a drying cabinet up to 96 °C until spots of amino acids appeared[8].

Free protein-forming amino acids in the plant raw material were determined quantitatively after extraction of free amino acids from the plant raw material, and bound amino acids were determined after acid hydrolysis of the preparations, followed by HPLC analysis of the hydrolysates using pre-column derivatization by 9-fluorenylmethoxycarbonyl chloride (FMOC) and o-phthalaldehyde (OPA) and a fluorescence detector. Each analysis used five determinations.

Samples of plant raw material were prepared and analyzed as follows:

a) *Free amino acids:* 100 mg of powdered preparation was placed into a vial, treated with 2 ml of 1 N aqueous HCl solution, and held at 50 °C for 3 h in an ultrasonic bath.

b) *Total amino acids*: 100 mg of preparation was placed into a vial, treated with 2 ml of 6 N aqueous HCl solution, and placed into a thermostatic chamber at 110 °C for 24 h. Then, 0.5 ml of the centrifuged extract/hydrolysate was evaporated in a rotary vacuum evaporator, rinsed three times with distilled H_2O to remove HCl, re-suspended in 0.5 ml of distilled H_2O, and filtered through a 0.2 μm regenerated cellulose membrane. Amino acids were identified by comparing their retention times with a mixture of amino acid standards (Agilent 5061-3334). The contents of bound amino acids were determined by subtracting the contents of free amino acids from their total contents.

Chromatographic separation was performed on an Agilent 1200 liquid chromatograph (Agilent Technologies, USA) using a Zorbax AAA column (150 mm x 4.6 mm, 3 μm) and mobile phase A (Na_2HPO_4, 40 mm, pH 7.8) and B (AcCN–MeOH– H_2O, 45:45:10, v/v/v) in gradient mode at constant flow rate 1.5 ml/min. The column was thermostatted at 40 °C. The pre-column derivatization was carried out in automated programmed mode using FMOC (Agilent 5061-3337) and OPA (Agilent 5061-3335). Derivatized amino acids were detected using a fluorescence detector[8].

Determination of Sugars

Qualitative composition and quantitative content of sugars in plant material were determined by GC/MS based on the extraction of free sugars from plant material and full acid hydrolysis of herbal preparations to determine the total monosaccharide composition, followed by obtaining acetates of their aldonitrile derivatives and their analysis. Each analysis used five determinations.

Sample preparation and analysis of plant raw materials:

a) *free monosaccharides*: 0.5 g of plant material was placed in a vial and 5 ml of 80% ethanol was added. Extraction of free monosaccharides was performed in an ultrasonic bath at 80 °C for 4 h. Then, 2 ml of the extract was collected, evaporated to dryness, and re-suspended with 2 ml of an aqueous solution of the internal standard (2.5 mg per sample);

b*) monosaccharide composition after hydrolysis of plant raw material*: 5 ml of 2 M trifluoroacetic acid was added to 0.5 g of raw material; hydrolysis held at 110 °C for 6 h. Then, 2 ml of hydrolyzate was collected, evaporated, and washed with water to remove trifluoroacetic acid. The hydrolysate was then re-suspended with 2 ml of an aqueous solution of the internal standard (2.5 mg per sample).

Chromatographic separation was performed on the chromatograph Agilent 6890N/5973inert (Agilent technologies, USA) using capillary column HP-5ms (3mm×0.25mm×0.25μm, Agilent technologies, USA). Evaporator temperature was 250 °C, the interface temperature 280 °C. Separation was carried out in the programming mode of the temperature: initial temperature of 160 °C was maintained for 8 min, then raised with a gradient of 5 °C/min to 240 °C. The final temperature was held for 6 min. A sample of 1 μl was injected in a split flow mode 1:50. Detection was in the SCAN mode in a range 38-400 m/z. The flow rate of carrier gas through the column was 1.2 ml/min. Identification was carried by retention time of monosaccharide standards and by the library of mass spectra NIST 02. Quantitative analysis was carried out by adding a solution of internal standard to the test sample.

The following mixture of standard samples were used: monosaccharides: Rib, Rha, Ara, Xyl, Fuc, Man, Glu, Gal, Fru; disaccharides: Sucrose. For the internal standard solution Sorbitol was used[9].

Determination of polysaccharides and lipophilic compounds

Lipophilic compounds

The content of lipophilic compound was determined using the Soxhlet method. Plant raw material first was defatted by exhaustive extraction with chloroform in the Soxhlet apparatus, and the lipophilic extract (LE) was received[10]. Each analysis used five determinations.

Fractioning polysaccharides

Isolation, purification and analysis of polysaccharides was performed by polysaccharide fractionation method. Plant raw material was dried and sequentially extracted with different solvents after obtaining the LE: 82% ethanol for alcohol soluble substances; purified water for water soluble polysaccharides (WSPS); a mix of 0.5% oxalic acid solution and 0.5% ammonium oxalate solution for pectin substances (PS); 7% sodium hydroxide solution for hemicellulose A (HC A) and hemicellulose B (HC B).

Each analysis used five determinations.

100 g of remaining raw material after extraction with 82% ethanol was mixed with purified water, at a ratio of plant raw material to extract 1:20. Extraction was performed twice at a constant temperature 30-35 °C for 3.5 hr with constant stirring. Extracts were combined and evaporated on a rotary heater to minimum volume under vacuum. WSPS were precipitated by fivefold volume of 96% alcohol. The precipitate was filtered and washed successively by hot 96% ethanol and acetone, dried in a drying oven to constant weight, and weighed. Plant material was dried after extraction.

The raw material after extraction of WSPS was used for obtaining PS. Extraction was done with hot mix of 0.5% oxalic acid solution and 0.5% ammonium oxalate solution in a ratio 1:1. Extraction was performed twice at a constant temperature 30-35 °C for 2 hours with constant stirring, with a ratio of plant raw material to extract 1:20.

The extracts were separated from the raw material, combined, concentrated, and precipitated by fivefold volume of 96% alcohol. Formed PS precipitate was filtered, washed successively with hot 96% ethanol and acetone, dried in a drying oven to constant weight, and weighed. Plant material was dried after extraction.

Hemicelluloses (HC) were obtained from the raw material that remained after PS was removed. Extraction was performed twice by 10% sodium hydroxide solution in the ratio of raw material to extract 1:5, at room temperature for 12 h. Alkaline extract was filtered, and the filtrate was acidified with glacial acetic acid to precipitation. The precipitate was filtered, dried to constant weight, and weighed. In this way, HC A was isolated. Twice the volume of 96% ethanol was added to the filtrate; in this case it formed a precipitate which was filtered, washed successively with hot 96% ethanol and acetone, dried in a drying oven to constant weight, and weighed. Thus, were obtained the fractions of HC B[11,12].

Isolation of starch

Starch was extracted from horse chestnuts, using alkaline steeping method as described by Sun et al. (2014) and Perez & Lares (2004), with slight modification. The endosperm was cut into pieces (2 cm^2), steeped in 0.25 % NaOH solution (w/v) in the ratio of 1:3 and stored at 4^o C for 24 h. The steeped endosperm, along with alkali, was ground in a laboratory grinder and filtered through a 100 mesh sieve and allowed to settle, then given 2–3 washings with distilled water. The slurry was again filtered through a 300 mesh sieve and the liquid was centrifuged at 3000 rpm for 15 min. The aqueous phase obtained upon centrifuging was discarded. The white starch layer was re-suspended in distilled water and centrifuged 2–3 times. The starch was then collected and dried in a hot air oven at 40 °C. Saponins, the bitter compounds present in horse chestnut, were removed by continuous washings of the starch[13]. The analysis used five determinations.

Determination of micro- and macroelements

To study the qualitative composition and quantitative content of macro and microelements in the skin and endosperm of horse chestnut seed, atomic absorption spectrophotometry with atomization in an air-acetylene flame was used. Analysis used five determinations.

Standard stock solutions with a concentration (1000 mg L^{-1}) of the individual metal element were used to prepare the requested concentrations by dilution using a 1% (v/v) nitric acid solution. The diluted standard solutions were used to build the calibration curves. Metal element standards were purchased from Sigma-Aldrich (St Louis, MO, USA). An analytical reagents grade of concentrated nitric acid (70%) and hydrogen peroxide (30%) were also purchased from Sigma- Aldrich (St Louis, MO, USA). In all the laboratory work, the glass and plastic containers were cleaned by soaking in 10% v/v HNO_3 for at least 24 h and rinsing with distilled water prior to use. All chemicals used were of analytical grade. Ultrapure deionized water, obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA), was used for preparing the solutions and for all dilutions.

An AAS instrument (Perkin Elmer AAnalyst 700 model AAS) with deuterium background corrector was used for the determination of Fe, Si, P, Al, Mn, Mg, Pb, Ni, Mo, Ca, Cu, Zn, Na, K, Sr, Co, Cd, As, and Hg. Pb, Cd, and Ni were determined by HGA graphite furnace using high purity argon, while other measurements were carried out in an air/acetylene flame. The operating parameters for working elements were set according to the recommendations of the manufacturer.

Procedure for drying ash

One gram of sample was transferred into a porcelain crucible. The muffle furnace temperature was gradually increased from room temperature to 450℃ in 1 h. The sample was re-dried for 1 h in the oven, cooled, and reweighed. The steps were repeated at 1 h drying intervals until the differences in the variations in the released water were less than 0.05%. The obtained sample was ashed for about 8 h until a gray or white ash residue was obtained. The residue was dissolved in 5 ml of HNO_3 (25% v/v) and, if necessary, the mixture was heated slowly to dissolve the residue. Then the mixture was heated up using an electric hot plate at 150 °C until evaporated to near dryness. The residue was filtered through Whatman filter paper and transferred into a volumetric flask and filled to 25 ml with 3% HNO_3. The control experiment was also prepared in the same way.

Analytical procedure

AAS is a widely used technique for determining a large number of metals. In AAS, an aqueous sample containing the metal analyte of interest is aspirated into an air-acetylene flame, causing evaporation of the solvent as well as vaporization of the free metal atoms. Fe, Si, P, Al, Mn, Mg, Pb, Ni, Mo, Ca, Cu, Zn, Na, K, Sr, Co, Cd, As, and Hg in samples was analyzed using AAS equipped with flame and graphite furnace. A graphite furnace was used for the determination of trace and ultra-trace concentrations (Pb, Ni, Mo, Co, Cd, As, Hg). The operational conditions used to operate AAS instrument were as recommended by the manufacturer. Data were rounded off properly based on the value of standard deviation from measurement conducted in triplicate[6,14].

Table 1: Quantitative content of identified amino acids in endosperm and skin of seeds from *A. hippocastanum* L., μg/mg.

№ п/п	Aminoacid	Retention time	Free		Bound		Total	
			endosperm	skin	endosperm	skin	endosperm	skin
1.	Asp	2.464	-	0.002288±0.00025	6.56±0.28	2.98±0.05	6.56±0.05	2.98±0.58
2.	Glu	4.808	1.62±0.07	0.002683±0.0002	11.26±0.99	1.61±0.58	12.88±2.83	1.61±0.44
3.	Ser	7.332	0.32±0.02	0.001513±0.00038	2.61±0.06	1.91±0.44	2.94±0.34	1.92±0.39
4.	His*	8.254	0.11±0.02	0.00256±0.00028	0.87±0.07	2.54±0.29	0.98±0.05	2.54±0.68
5.	Gly	8.554	0.25±0.05	0.001262±0.00031	2.61±0.17	1.02±0.77	2.86±0.38	1.02±0.49
6.	Thr*	8.731	0.3±0.05	0.001463±0.0003	2.09±0.19	2.54±0.05	2.38±0.22	2.54±0.21
7.	Arg*	9.352	0.76±0.05	0.003815±0.00019	3.27±0.05	0.86±0.05	4.04±0.75	0.86±0.28
8.	Ala	9.941	0.59±0.08	0.001287±0.00029	2.39±0.05	1.09±0.29	2.98±0.55	1.09±0.54
9.	Tyr	11.033	0.14±0.03	0.001344±0.00072	0.68±0.03	0.29±0.06	0.82±0.48	0.29±0.05
10.	Cys	12.162	0.51±0.06	0.004722±0.00056	8.24±0.58	3.44±0.55	8.85±0.61	3.44±0.05
11.	Val*	12.980	0.32±0.01	0.001014±0.00037	2.15±0.08	0.99±0.01	2.47±0.51	0.99±0.04
12.	Met*	13.507	-	0.000468±0.00032	-	0.05±0.03	-	0.05±0.03
13.	Phe*	14.320	0.38±0.01	0.010514±0.00068	2.28±0.15	0.96±0.21	2.65±0.82	0.96±0.05
14.	Ile*	14.506	0.19±0.01	0.00087±0.00029	2.34±0.85	1.13±0.21	2.52±0.59	1.13±0.21
15.	Leu*	15.109	0.29±0.03	-	3.54±0.52	0.56±0.08	3.83±0.12	0.56±0.04
16.	Lys*	15.369	0.27±0.03	0.000866±0.00077	2.64±0.47	1.95±0.09	2.91±0.23	1.95±0.45
17.	Pro	18.864	0.2±0.03	0.000918±0.00032	2.3±0.29	0.96±0.49	2.50±0.28	0.96±0.09
The sum of essential amino acids			2.62	0.02157	19,16	11.56	21.78	11.58
The sum of nonessential amino acids			3.63	0.016017	36.76	13.29	40.39	13.31
The sum of amino acids			6.25	0.037587	55.92	24.85	62.17	24.89

Note: * - essential amino acid.

RESULTS AND DISCUSSION

Amino acids

PC identified these free amino acids in the endosperm and skin of *A. hippocastanum:* Ser, Val, Glu, Arg, and Ala (violet); His, Tyr, and Phe (gray-violet); Asp (blue-violet); and Pro (yellow)[15].

HPLC identified 15 free amino acids in the endosperm of *A. hippocastanum* and 15 in the skin (Table 1).

Free Glu was present in endosperm in the greatest amount (1.62 μg/mg). The amounts of the other amino acids were less. Free amino acids in endosperm of *A. hippocastanum* included seven essential amino acids (His, Thr, Val, Phe, Ile, Leu, and Lys) in addition to Arg, which is considered conditionally essential because it is extremely necessary for young people[8,16]. Asp and Met were not found.

Free amino acids in *A. hippocastanum* skin were detected in trace amounts. Among them were eight essential amino acids His, Thr, Val, Met, Phe, Ile, Lys, and Arg. Leu was not found.

Sixteen bound amino acids were identified in *A. hippocastanum* endosperm. The quantitative contents of all amino acids showed a tendency to increase significantly after hydrolysis. The contents of bound Glu (11.26±0.99 μg/mg), Asp (6.56±0.28μg/mg), and Cys (8.24±0.58 μg/mg) were the greatest. However, Asp was found only in bound form. The content of bound Glu increased compared with the other acids from 1.62±0.07 to 11.26±0.99 μg/mg; the content of bound Cys increased from 0.51±0.06 to 8.24±0.58 μg/mg.

Seventeen bound amino acids were identified in the skin of *A. hippocastanum.* The quantitative contents of all amino acids also showed a tendency to increase

Table 2: Free monosaccharides and sum of monosaccharides in endosperm and skin of seeds from *Aesculus hippocastanum* L., mg/kg.

Standard	Retention time	Endosperm		Skin	
		Free	Sum	Free	Sum
Rha	8.0442	-	0.66±0.04	-	-
Ara	7.976	-	3.53±0.08	0.09±0.02	24.97±0.93
Xyl	8.627	-	-	-	4.12±0.29
Fuc	9.005	-	0.65±0.02	-	-
Man	14.334	-	-	-	6.63±0.77
Glu	14.628	1.84±0.06	158.35±1.57	0.06±0.02	21.64±0.85
Gal	15.163	-	8.03±0.09	-	20.29±1.06
Sorbitol	18.07	i/s	i/s	i/s	i/s
Sucrose	34.041	31.94±0.78	-	-	-
Total		33.78	171.22	0.15	77.65

Table 3: Content of polysaccharides and lipophilic compounds in *A. hippocastanum* seeds.

Fraction	Yield, %	
	skin	endosperm
LE	3.3±0.66	18.80±1.06
WSPS	0.56±0.04	3.23±0.36
PS	15.0±1.33	1.5±0.39
HC A	6.7±0.83	4.2±1.01
HC B	3.8±0.72	1.7±0.33
Starch	-	54.5±2.75

significantly after hydrolysis, but compared with contents in the endosperm they were found in lower amounts. The concentrations of bound Asp (2.98±0.05 μg/mg), His (2.54±0.29 μg/mg), Thr (2.54±0.05 μg/mg), and Cys (3.44±0.55 μg/mg) were the greatest. Several amino acids that were found in trace quantities in the free state occurred in greater quantities in the bound form. These were Ile (1.13±0.21 g/mg), Lys (1.95±0.09 μg/mg), and Pro (0.96±0.49 μg/mg). The quantitative content of one amino acid that was not identified in the free state but was found in the bound form was 0.56 μg/mg (Leu). Bound amino acids from *A. hippocastanum* seed skin included nine essential amino acids (His, Thr, Arg, Val, Met, Phe, Ile, Leu, and Lys).

Sugars

The results of identification of monosaccharides by GC/MS are in Table 2. Free sugars in the endosperm of *Aesculus hippocastanum* seeds consist of the monosaccharide Glu and the disaccharide Sucrose. Sucrose is present in a higher amount. Free sugars in the skin consist of Glu and Ara.

Rha, Ara, Fuc, Glu, and Gal were found in a composition of monosaccharides in the endosperm of *A. hippocastanum* seeds by GC/MS method after hydrolysis. The content of Glu increased after hydrolysis. Rha, Ara, Fuc, and Gal were found only after hydrolysis (Table 2). Ara, Xyl, Man, Glu, and Gal were identified as a part of the sum of sugars from skin of *A. hippocastanum* seeds. Also, the amount of Glu and Ara increased significantly after hydrolysis, compared to its amount as a free sugar. Xyl, Man, and Gal were found only after hydrolysis.

We can distinguish specific sugars for the endosperm of seeds, which are not present in the skin of seeds: Rha, Fuc, and Sucrose. The specific sugars in the skin of seeds are Xyl and Man. The amount of Glu is higher in the endosperm than in the skin, both in free form and after hydrolysis. The amount of Ara and Gal in skins exceeds its content in endosperms, both in free form and after hydrolysis.

Polysaccharides

Polysaccharide contents are listed in a Table 3. Skin and endosperm have different contents of polysaccharides and lipophilic compounds. The yields of LE and WSPS in endosperm vastly exceeds those in skin, but the content of PS, HC A, and HC B is higher in skin. Starch, WSPS, and fats are an energy source and are needed for complex physiological and biochemical processes during seed's stratification and germination. Accordingly, the higher content of these compounds is more important in the endosperm than in the skin of seeds.

Micro- and macroelements

Our research identified 19 macro- and microelements (Table 4). Elemental composition showed the following pattern in the content of macroelements in the skin: K> Ca> Mg, Si > P > Na. Microelements accumulated in this way: Fe> Al> Zn> Sr> Mn> Cu> Ni> Mo. In the endosperm metals were found in very small amounts; they showed this pattern in their contents: macroelements – Ca>P>Mg>Si>K>Na; microelements – Cu>Fe, Al> Ni, Mo>Mn, Zn, Sr.

Heavy metals were found in very small quantities: Co <0.03 mg/100g; Pb <0.03 mg/100g; Cd <0.01 mg/100g; As <0.01 mg/100g; Hg <0.01 mg/100g. The seeds do not accumulate toxic metals, and this enables their use as medicinal plant material. This absence of toxic metals is also important because of anthropogenic factors, pollution, and quality control methods for raw materials.

CONCLUSIONS

The results showed big differences between primary metabolites and content of elements in endosperm and skin of *A. hippocastanum*. It depends on the functions that skin and endosperm have in the plant. The qualitative composition and quantitative content of amino acids were studied by PC and HPLC method. 15 free amino acids were identified in the endosperm and skin of *A. hippocastanum*. 16 bound amino acids were identified in *A. hippocastanum* endosperm. 17 bound amino acids were identified in *A. hippocastanum* skin. The GC/MS

Table 4: Content of minerals in endosperm and skin of *A. hippocastanum* seeds.

Content	Sample	
mg/100g	Skin	Endosperm
Fe	5.0±0.21	0.03±0.002
Si	64±1.08	3.0±0.27
P	24±0.97	15±0.98
Al	4.0±0.72	0.03±0.002
Mn	0.48±0.03	<0.01
Mg	64±2.2	8,7±0,74
Ni	0.016±0.004	<0.03
Mo	<0.03	<0.03
Ca	255±3.25	15±1.12
Cu	0.16±0.03	0.058±0.0019
Zn	1.6±0.3	<0.01
Na	16±1.6	<0.1
K	720±3.8	<1.0
Sr	0.8±0.07	<0.01

Pb <0.03; Co <0.03; Cd <0.01; As <0.01; Hg <0.01.

method showed that *A. hippocastanum* skin and endosperm contain 2 free sugars and 5 after hydrolysis. We can also distinguish specific sugars in the endosperm which are not present in the skin: Rha, Fuc, and Sucrose. Specific sugars in the skin are Xyl and Man. Endosperms accumulate starch, WSPS, and lipophilic compounds. PS and HC are concentrated in skin. The qualitative composition and quantitative content of 19 macro and microelements in skin and endosperm of seeds of *A. hippocastanum* were studied by atomic emission spectrophotometry. The skins accumulate macro- and microelements. Seeds do not accumulate toxic metals, and this enables their use as medicinal plant material. The contents of primary metabolites provide opportunities for creating medicine and food supplements.

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