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Original Article

DETERMINATION OF AMINO ACIDS AND SUGARS CONTENT IN *ANTENNARIA DIOICA* GAERTN

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ABSTRACT

Objective: The purpose of our study was to determine sugars and amino acids content of *Antennaria dioica* Gaertn. herb. In continuation of the investigation of biologically active substances from *Antennaria dioica* Gaertn., it advisable to study the qualitative composition and quantitative contents of sugars and amino acids from the herb of this plant.

Methods: The herb of *Antennaria dioica* Gaertn. was analyzed for the content of sugars by GC/MS. The amino acids were identified and quantified by HPLC method.

Results: The results of GC/MS analysis showed that in *Antennaria dioica* Gaertn. herb D-glucose had the highest content (7.16±0.09) mg/g, followed by D-fructose (5.27±0.06) mg/g and D-saccharose (6.72±0.08) mg/g. In the raw material a large amount of monosaccharides derivative–Myo-inositol was revealed, a content of which was (2.12±0.06) mg/g. We determined 17 bound and 16 free amino acids in the *Antennaria dioica* Gaertn. by HPLC method.

Conclusion: The contents of primary metabolites provide opportunities for creating medicine and food supplements. The results show that *Antennaria dioica* Gaertn. is a rich source of these important biologically active substances. The resulting data will be used with the further purpose to produce new drugs of natural origin.

Keywords: *Antennaria dioica* Gaertn, Polysaccharides, Monosaccharides, Amino acids, GC/MS, HPLC

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INTRODUCTION

The importance of medicinal plants did not reduce the annual increase in the number of synthetic medicines, which often model biologically active substances of plants and are their chemical equivalents. The most interesting are medicinal plants that have a long history of use and have been shown to possess low side effects and are well tolerated by patients, regardless of age [1].

The most representative plants for the treatment of diseases are plants of families *Asteraceae, Rosaceae, Fabaceae, Lamiaceae, Boraginaceae, Apiaceae, Poaceae*.

The *Asteraceae* family includes around 23,000 species in the world and is the largest family of flowering plants [2-4].

The diversity and heterogeneity of this family justify the great importance of its individual members, which are known and used from ancient times, not only as food sources or as spices, but also for medicinal purposes [5, 6].

Antennaria dioica Gaertn. is a perennial plant in the family *Asteraceae* [7]. It grows in almost throughout the whole of Ukraine on the wastelands and sandy or stony places [8].

Antennaria dioica Gaertn. traditionally used to treat biliary and respiratory ailments [9, 10], and also have astringent and hemostatic properties [9, 11]. Scientific sources give few data regarding the chemical composition of this species. Merili *et al.* [10] studied the extracts obtained from the flowers of *Antennaria dioica* Gaertn. and isolated chlorogenic and ursolic acids, apigenin-7-O-glucoside, and luteolin-7-O-glucoside. This is the only scientific study that describes the flavonoid composition of this species, and it does not establish a connection between the potential bioactivities of the species and the isolated compounds [2, 12].

However, there is no published information about some primary metabolites, such as polysaccharides and amino acids.

Thus, the aim of our research was to determine the content of amino acids and polysaccharides in aerial parts of *Antennaria dioica* Gaertn. collected from natural habitat.

MATERIALS AND METHODS

Plant materials

Aerial parts of the *Antennaria dioica* Gaertn. were collected in Western Ukraine, Vyzhnytsya district, Chernivtsi region (N 48 °13'23.2" E 25 °11'42.0"), during a mass flowering period in 2016. The raw material was authenticated by prof. Svitlana Marchyshyn (TNMU, Ternopil, Ukraine). A voucher specimen no. 189 is kept at the Department of Pharmacognosy and Medical Botany, TNMU, Ternopil, Ukraine.

Chemicals and standards

Standards of polysaccharides including L-rhamnose, D-mannose, D-galactose, D-ribose, D-arabinose, D-xylose, D-fructose, D-glucose, D-fucose, D-saccharose, D-sorbitol obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) were of analytical grade (>95 % purity) (fig. 1).

All standards of amino acids were of analytical grade (>99 % purity). The chemicals were obtained from Sigma-Aldrich, USA and were: L-aspartic acid, L-glutamic acid, L-serine, L-hystidine, Glycine, L-treonin, L-arginine, L-alanine, L-tyrosine, L-valine, L-methionine, L-phenylalanine, L-isoleucine, L-leucine, L-lysine, L-proline, L-cystine (fig. 2).

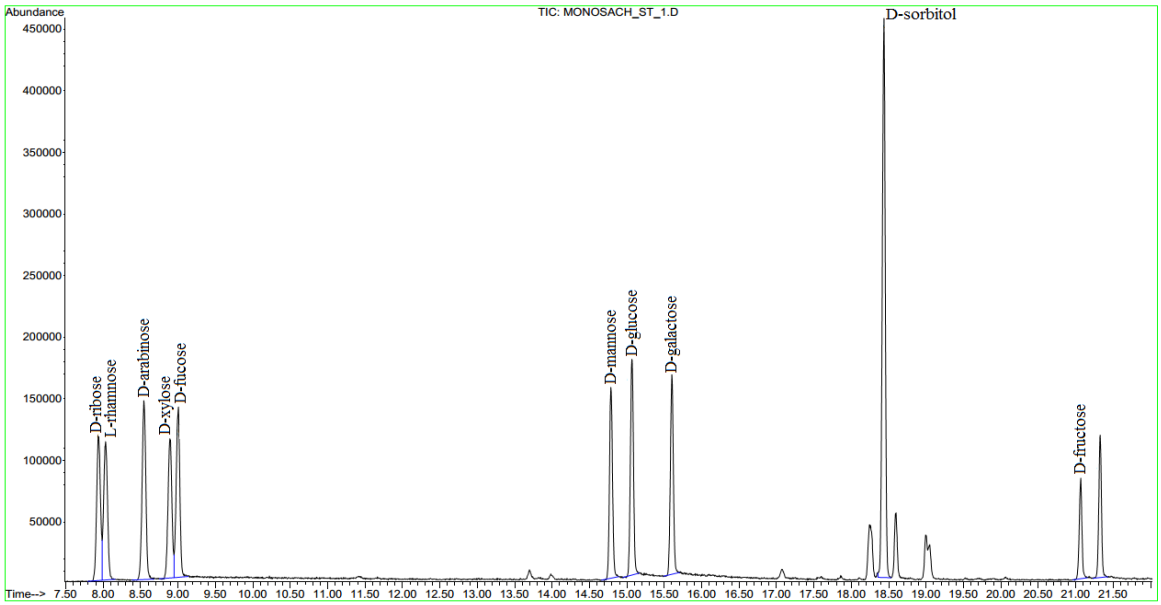

Fig. 1: GC/MS chromatogram of sugars standards

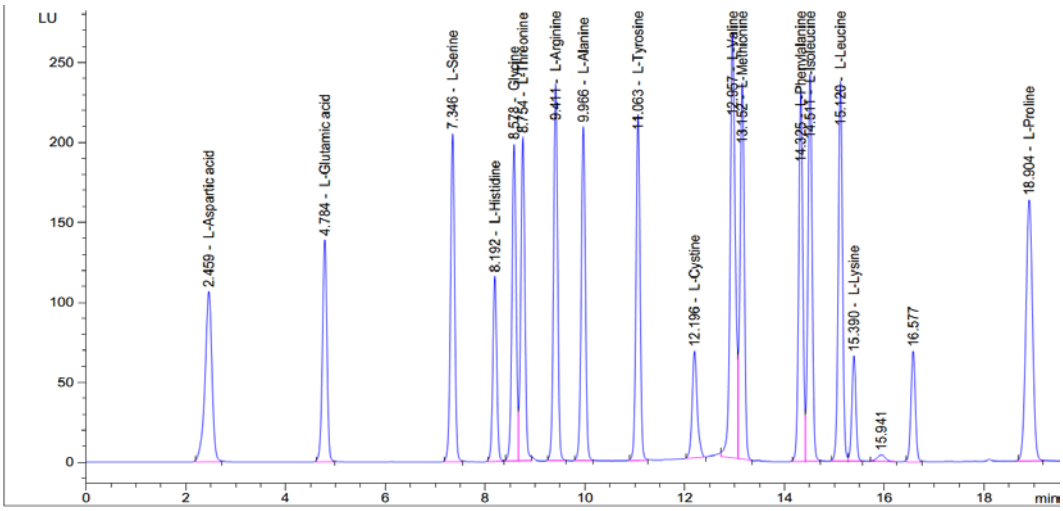

Fig. 2: HPLC chromatogram of amino acids standards

FMOC, OPA, HCl (ADAM) and HPLC-grade ACN were from Sigma (St. Louis, MO, USA). All other reagents were of the highest purity available.

Sample preparation, gas chromatography coupled with mass spectrometry (GC/MS) analysis and high-performance liquid chromatography (HPLC) method

GC/MS determination of sugars

GC/MS analysis of sugars was performed using gas chromatograph Agilent 6890N with mass detector 5973 inert (Agilent Technologies, USA). The initially set up oven temperature at 160 °C and held for 8 min, then at the rate of 5 °C/min raised to 240 °C and finally kept at this point for 6 min. The detection was performed in the SCAN mode at the width range of 38–400 m/z. Helium was used as the carrier gas at a constant flow rate of 1.2 ml/min.

For the determination of sugars 500 mg of powdered *Antennaria dioica* Gaertn. was added to 500 micrograms of methanol solution with sorbitol (internal standard). Extraction was performed in the ultrasonic bath at 80 °C for 4 h. Then, 2 ml of the extract were evaporated to dryness and 0.3 ml of derivatization reagent (hydroxylamine hydrochloride in pyridine/methanol (4:1 v/v)) was added. For acetylation of aldonitrile derivatives, 1 ml of acetic anhydride was subsequently added to the samples and incubated at 75 °C for 15 min. 2 ml of dichloroethane was added, and the excess removed by the double extraction with water and 1 M hydrochloric acid. Dichloroethane layer was dried and dissolved in the mixture of ethyl acetate/heptane (1:1 v/v).

Identification was based on comparing their retention times with retention times of standards of the NIST 02 mass spectral library. Quantification was done using sorbitol (1 mg/ml) added to the sample [13-15].

HPLC determination of amino acids

The amino acids composition of *Antennaria dioica* Gaertn. herb is determined by HPLC method with a pre-column derivatization 9-fluorenylmethyl chloroformate (FMOC) and o-phthalaldehyde (OPA).

HPLC analysis of amino acids was performed using Agilent 1200 (Agilent Technologies, USA). Samples were analyzed on a column length Zorbax AAA–150 mm, inner diameter–4.6 mm, the diameter of sorbent grain 3 µ (Hypersil ODS (prepared by BST, Budapest,

Hungary)). Mobile phase A-40 mmol Na_2HPO_4, pH 7,8; mobile phase B–CH_3CN: CH_3OH: H_2O (45:45:10, v/v/v). Gradient separation mode with a constant flow rate of 1.5 ml/min. The temperature of the thermostat column is 40 °C.

The pre-column derivatization was performed in an automatic programmable mode using OPA reagent and FMOC reagent. Identification of derivatized amino acids was done by using a fluorescence detector [16, 17]. For the extraction of free amino acids to the 131 mg of powdered the raw material, placed in a vial, 0.1 mol/l aqueous solution of hydrochloric acid was added. The extraction was performed in the ultrasonic water bath at 50 °C for 3 h. Extraction of bound and free amino acids was carried out by adding 2 ml of an aqueous solution of 6 N hydrochloric acid to 136 mg of powdered the raw material. Hydrolysis was carried out for 24 h in a thermostat at 110 °C.

0.5 ml of centrifuged extract was evaporated on a rotary evaporator and then rinse three times with purified water to remove hydrochloric acid. The product obtained was resuspended in 0.5 ml water and filtered through membrane filters from regenerated cellulose with pores of 0.2 µm. Before entering the samples into the chromatographic column in the automatic program mode fluorescence derivative amino acids were obtained.

Identification of amino acids was done according to their retention time (with using standards as a reference) at 265 nm. The quantitative content of amino acids is calculated from the value of the of the peak area of the amino acids.

The amount of amino acids in µg/mg was calculated by the following equation:

$$X = \frac{C \times V}{m}$$

Where: C – concentration, obtained from the chromatogram by calculating the reference solution and the test solution;

V – the volume of solvent for extraction;

m – is a mass of plant material [18].

Statistical analysis

All the assays were carried out in five times. The results were expressed as mean values and standard deviation. Values were determined using Statistica v 10.0 (StatSoft I nc.) program. The level of significance was set at *p<0.05 for all statistical analyses.

RESULTS AND DISCUSSION

GC/MS chromatography analysis of sugars

The GC/MS method determined the qualitative composition and quantitative content of monosaccharides, their derivatives, and saccharose. The *Antennaria dioica* Gaertn. herb contains D-fructose, D-glucose, D-saccharose, L-rhamnose and Myo-inositol (fig. 3, table 1). The predominant ones were D-glucose (7.16±0.09) mg/g, D-fructose (5.27±0.06) mg/g and D-saccharose (6.72±0.08) mg/g. Among the monosaccharides, D-glucose dominates in the studied raw material. The basic function of glucose is to provide energy for physiological processes. Of all tissues and organs, mammalian, neurons and the brain have the supreme energy demand. The processes requiring mental effort are deteriorated if the concentration of this aldohexose reduces [19, 20]. In the *Antennaria dioica* Gaertn. herb, a large amount of monosaccharides derivative–Myo-inositol was revealed, a content of which was (2.12±0.06) mg/g. Myo-inositol decreases serum androgen concentrations, reduces circulating insulin, improves glucose tolerance and other metabolic values altered associated with insulin resistance in women affected by Polycystic ovary syndrome [21-23].

HPLC determination of amino acids

Table 2 presents the amino acids composition of the herb of *Antennaria dioica* Gaertn. The HPLC method we identified in the raw material sixteen free amino acids (fig. 4) and seventeen bound amino acids (fig. 5), of which nine were essential.

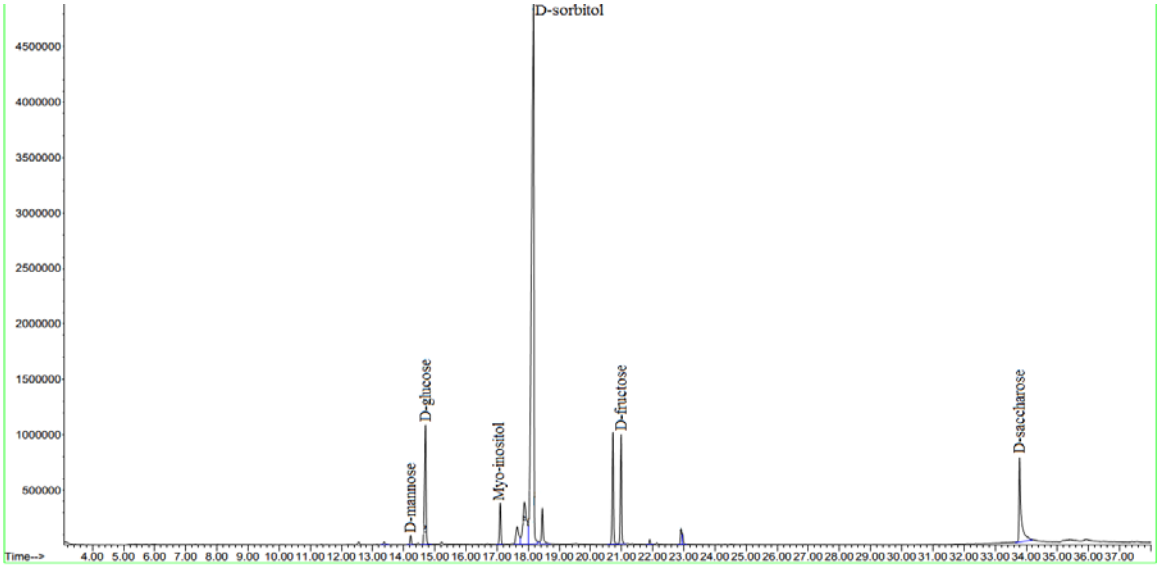

Fig. 3: GC/MS chromatogram of sugars, their derivatives, and saccharose of *Antennaria dioica* gaertn. herb

Table 1: The content of free monosaccharides, their derivatives, and saccharose of *Antennaria dioica* gaertn herb

RT, min	Carbohydrate	Content in the plant material, (mg/g) $\bar{x} \pm \Delta \bar{x}$, n=5, P<0.05
14.22	D-mannose	0.48±0.02
14.78	D-glucose	7.16±0.09
17.10	Myo-inositol	2.12±0.07
18.17	D-sorbitol	internal standard
20.99	D-fructose	5.27±0.06
33.79	D-saccharose	6.72±0.08

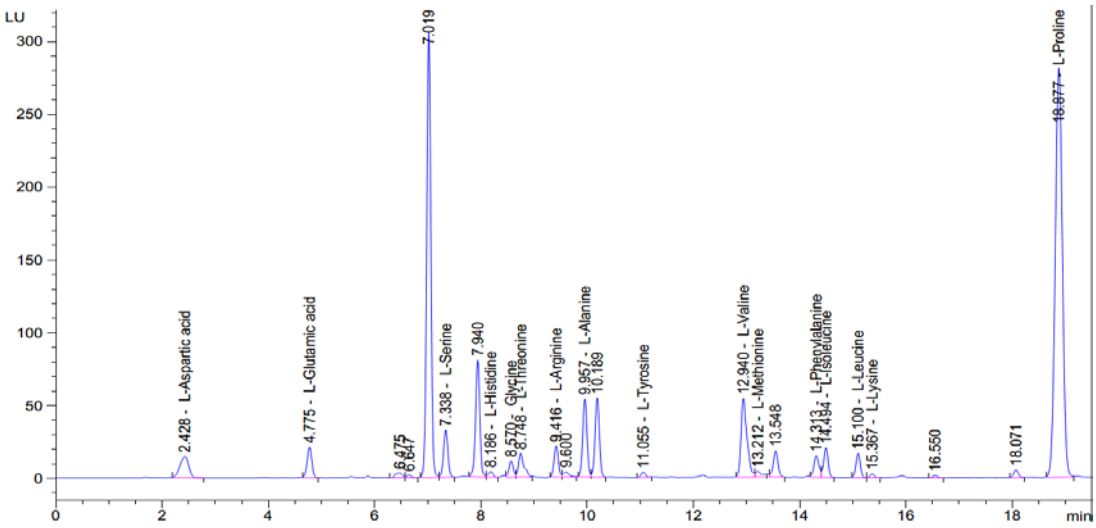

Fig. 4: HPLC chromatogram of free amino acids of *Antennaria dioica* gaertn

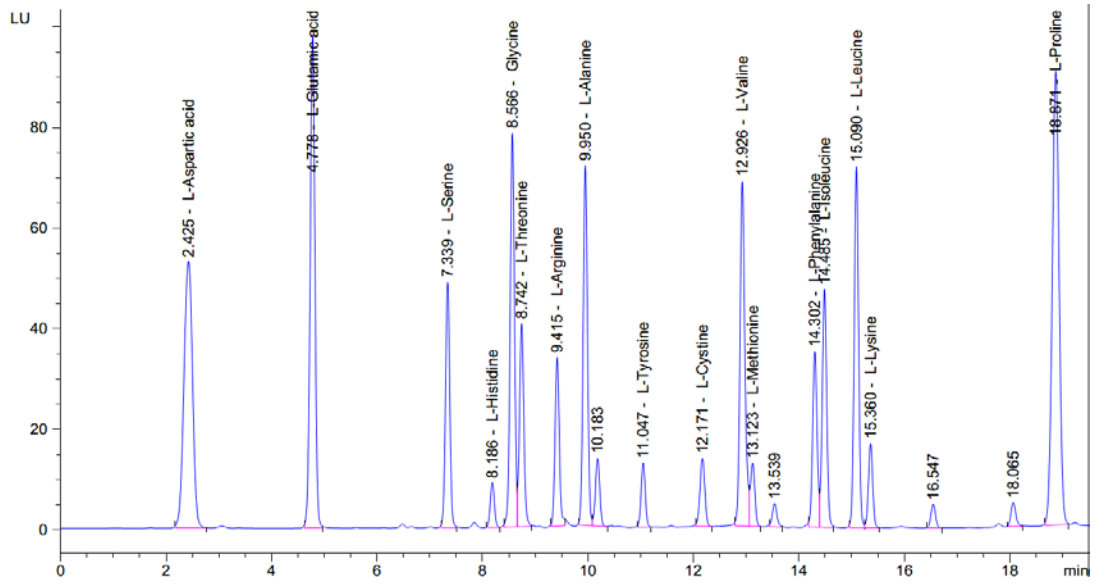

Fig. 5: HPLC chromatogram of bound amino acids of *Antennaria dioica* gaertn

Table 2: The content of the amino acid composition *Antennaria dioica* gaertn herb

The name of the amino acid	The content of the amino acid, µg/mg $\bar{x}\pm\Delta\bar{x}$, n=5, P<0.05	
	Free	Bound
L-aspartic acid (Asp)	0.33±0.02	5.38±0.12
L-glutamic acid (Glu)	0.34±0.03	7.38±0.20
L-serine (Ser)	0.25±0.02	1.56±0.09
L-hystidine (His)*	0.12±0.01	1.05±0.08
Glycine (Gly)	0.06±0.01	2.10±0.11
L-treonin (Thr)*	0.19±0.01	1.57±0.07
L-arginine (Arg)*	0.24±0.03	1.59±0.06
L-alanine (Ala)	0.34±0.03	1.86±0.15
L-tyrosine (Tyr)	0.05±0.01	0.72±0.05
L-cystine (Cys)	0	3.29±0.09
L-valine (Val)*	0.40±0.04	1.62±0.11
L-methionine (Met)*	0.06±0.01	0.52±0.07
L-phenylalanine (Phe)*	0.19±0.01	1.69±0.08
L-isoleucine (Ile)*	0.17±0.01	1.74±0.09
L-leucine (Leu)*	0.14±0.02	2.74±0.13
L-lysine (Lys)*	0.11±0.01	3.31±0.16
L-proline (Pro)	3.06±0.07	1.52±0.07

Note: * -essential amino acid.

The dominant components of amino acids from *Antennaria dioica* Gaertn. with respect to total content were L-proline, L-aspartic acid, L-glutamic acid, L-alanine, and L-valine. Free L-proline was present of the *Antennaria dioica* Gaertn. in the greatest amount (3.06±0.07) μg/mg. Proline is a proteinogenic amino acid with an exceptional conformational rigidity, and is essential for primary metabolism [24, 25]. Valine support the balance of branched chain amino acids, whereas alanine is involved on hepatic autophagy, transamination and gluconeogenesis [26-28]. The amounts of the other amino acids were less. Free amino acids in the herb of *Antennaria dioica* Gaertn. included 9 essential amino acids (His, Thr, Val, Met, Phe, Ile, Leu, and Lys) in addition to L-arginine, which is considered conditionally essential because it is extremely necessary for young people [29-31]. L-cystine was not found.

Seventeen bound amino acids were identified in the herb of *Antennaria dioica* Gaertn. The quantitative contents of all amino acids showed a tendency to increase significantly after hydrolysis. The contents of bound L-glutamic acid (7.38±0.20) μg/mg, L-aspartic acid (5.38±0.12) μg/mg, L-cystine (3.29±0.09) μg/mg and L-lysine (3.31±0.16) μg/mg were the greatest (table 2). Aspartic acid function is essential for pyrimidine, asparagine, purine and inositol synthesis. Glutamic acid take part of the synthesis of glutathione [26, 28]. However, L-cystine was found only in bound form.

CONCLUSION

We determined the primary metabolites in the *Antennaria dioica* Gaertn. herb. Using the GC/MS method, we identified the monosaccharides, their derivatives, and saccharose. The main compounds identified in the herb of *Antennaria dioica* Gaertn. were D-glucose, D-saccharose, and Myo-inositol. The amino acids composition of *Antennaria dioica* Gaertn. herb was determined by the HPLC method. We determined sixteen free and seventeen bound amino acids which were identified in the raw material. The content of bound L-glutamic acid increased compared with the other acids from (0.34±0.03) μg/mg to (7.38±0.20) μg/mg; the content of bound L-aspartic acid increased from (0.33±0.02) μg/mg to (5.38±0.12) μg/mg. The contents of primary metabolites provide opportunities for creating medicine and food supplements. The results show that *Antennaria dioica* Gaertn. is a rich source of these important biologically active substances.

AUTHORS CONTRIBUTIONS

All the authors have contributed equally

CONFLICTS OF INTERESTS

The authors declare no conflict of interest

REFERENCES

1. Stoiko L, Kurylo Khr. Development of optimal technology of alcohol extract *Centaurium erythraea* Rafn. herb. Arch Balkan Med Union 2018;53:523-8.
2. Babota M, Mocan A, Vlase L, Cris O, Ielciu I, Gheldiu AM, *et al.* Phytochemical analysis, antioxidant and antimicrobial activities of *Helichrysum arenarium* (L.) moench. and *Antennaria dioica* (L.) gaertn. flowers. Molecules 2018;23:409.
3. Erbar C, Leins P. Diversity of styles and mechanisms of secondary pollen presentation in basal asteraceae-new insights in phylogeny and function. Flora Morphol Distrib Funct Ecol Plants 2015;217:109-30.
4. Bohm BA, Stuessy TF. Flavonoids of the sunflower family (*Asteraceae*) springer-verlag wien; New York, USA: Introduction to the Sunflower Family; 2001. p. 1.
5. Garcia Herrera P, Sanchez Mata MC, Camara M, Fernandez Ruiz V, Diez Marques C, Molina M, *et al.* Nutrient composition of six wild edible mediterranean *Asteraceae* plants of dietary interest. J Food Compos Anal 2014;34:163–70.
6. Bessada SMF, Barreira JCM, Oliveira MBPP. *Asteraceae* species with most prominent bioactivity and their potential applications. A Rev Ind Crops Prod 2015;76:604–15.
7. Bayer RJ. Antennaria. Flora of North America Editorial Committee. New York: Oxford University Press; 2006. p. 388.
8. Marchyshyn S, Basaraba R, Berdey T. Investigation of phenolic compounds of *Antennaria dioica* (L.) Gaertn. herb. Pharma Innov J 2017;6:09-11.
9. Semeniv DV, Belik GV. The experience of *Antennaria dioica* application in folk medicine and prospects of this plant use for creation of new phytohemostatics. Ukr Biopharm J 2016;6:37-41.
10. Mericli AH. Constituents of *Antennaria dioica*. J Nat Prod 1983;46:941.
11. Coady Y, Boylan F. Ethnopharmacology in Ireland: an overview. Braz J Pharmacogn 2014;24:197-205.
12. Abad MJ, Bedoya LM, Bermejo P. Fighting multidrug resistance with herbal extracts, essential oils and their components. Academic Press: San Diego, USA; 2013. p. 205-19.
13. Husak L, Dakhym I, Marchyshyn S, Nakonechna S. Determination of sugars and fructans content in *Stachys sieboldii*. Int J Green Pharm 2018;12:70-4.
14. Chen Y, Xie MY, Wang YX, Nie SP, Li C. Analysis of the monosaccharide composition of purified polysaccharides in ganoderma atrum by capillary gas chromatography. J Phytochem Anal 2009;20:503-10.
15. Kozachok S, Marchyshyn S, Ostapchuk A, Zavyalova L. Monosaccharide composition of *Herniaria glabra* L. and *Herniaria polygama.* J Gay Curr Issues Pharm Med Sci 2016;29:142-4.
16. Jámbor A, Molnar Perl I. Quantitation of amino acids in plasma by high performance liquid chromatography: simultaneous deproteinization and derivatization with 9-fluorenylmethyloxycarbonyl chloride. J Chromatogr A 2009; 1216:6218-23.
17. Hanczko R, Jambor A, Perl A, Molnar Perl I. Advances in the ophthalaldehyde derivatizations: comeback to the o-phthalaldehydeethanethiol reagent. J Chromatogr A 2007;1163:25-42.
18. Jámbor A, Molnar Perl I. Amino acid analysis by high-performance liquid chromatography after derivatization with 9-fluorenylmethyloxycarbonyl chloride literature overview and further study. J Chromatogr A 2009;1216:3064-77.
19. Stoiko L, Dakhym I, Pokotylo O, Marchyshyn S. Polysaccharides in *Centaurium erythraea* Rafn. Int J Res Ayurveda Pharm 2017;8:252-5.
20. Mergenthaler P, Lindauer U, Dienel GA, Meisel A. Sugar for the brain: the role of glucose in physiological and pathological brain function. Tren Neur 2013;36:587-97.
21. Costantino D, Minozzi G, Minozzi F, Guaraldi C. Metabolic and hormonal effects of myo-inositol in women with polycystic ovary syndrome: a double-blind trial. Eur Rev Med Pharmacol Sci 2009;13:105-10.
22. Stanford Kr I, Takahashi H, So K, Alves Wagner AB, Prince NB, Lehnig AC, *et al.* Maternal exercise improves glucose tolerance in female offspring. Diabetes 2017;66:2124-36.
23. Yoshino J, Conte C, Fontana L, Mittendorfer B, Imai Sh, Schechtman KB, *et al.* Resveratrol supplementation does not improve metabolic function in non-obese women with normal glucose tolerance. Cell Metab 2012;16:658-64.
24. Szabados L, Savoure A. Proline: a multifunctional amino acid. Trends Plant Sci 2009;15:89-97.
25. Verbruggen N, Hermans C. Proline accumulation in plants: a review. Amino Acids 2008;35:753-59.
26. Moran Palacio EF, Tortoledo Ortiz O, Yanez Farias GA, Zamora Alvarez LA, Stephens Camacho NA, Sonanez Organis JG, *et al.* Determination of amino acids in medicinal plants from southern sonora, mexico. Trop J Pharm Res 2014;13:601-6.
27. Wu G. Amino acids: metabolism, functions, and nutrition. Amino Acids 2009;37:1-17.
28. Rhoads MJ, Wu G. Glutamine, arginine, and leucine signaling in the intestine. Amino Acids 2009;37:111-22.
29. Karpyuk UV, Kislichenko VS, Gureva IG. HPLC determination of free and bound amino acids in *Bryonia alba*. Chem Nat Comp 2015;51:399-400.
30. Karpiuk UV, Abudayeh ZHM, Kyslychenko VS, Yemelianova OI. Isolation and identification of some primary metabolites, micro-and macroelements of *Aesculus hippocastanum* L. seeds. Intern J Pharm Phytochem Res 2017;9:108-13.
31. Radovick S, Margaret H, MacGillivray. Pediatric endocrinology: a practical clinical guide, Second Edition Contemporary Endocrinology. 2d ed. Springer Science and Business Media; 2013. p. 624.