APTEFF, 45, 1-283 (2014)
DOI: 10.2298/APT1445229R
UDC: 582.635.3+633.879.6:641.17
BIBLID: 1450-7188 (2014) 45, 229-237
Original scientific paper

CHARACTERIZATION OF *Morus* SPECIES IN RESPECT TO MICRO, MACRO, AND TOXIC ELEMENTS

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This study examines the mineral composition of the extracts of the fruits, leaves and roots of white mulberry (Morus alba L.) and black mulberry (Morus nigra L.) grown in Serbia. All extract samples of white and black mulberry were analyzed for the content of micro (B, Co, Cr, Cu, Fe, Li, Mn, Ni, Se, Sr, Zn), macro (Ca, Mg, Na), and toxic metals (Al, As, Cd, Hg, Pb) by inductively coupled plasma optical emission spectrometry (ICP-OES). The study revealed that parts of the plant had statistically significant impact on the levels of the examined elements among the two Morus species. All extracts contained high amounts of Ca, Mg, Na, B, Cu, Fe, Mn and Zn. The studies showed that in the most of extracts dominant macro element was Mg (591- 1942 μg/g of dry extract), while dominant microelements were Zn, B, Cu in all extracts, except for the black mulberry leaves, whose extract was most abundant in Fe (143 μg/g of dry extract). The highest content of micro and macro elements was detected in the extract of black mulberry leaves. This work contributes to the knowledge of the nutritional properties of Morus species. The obtained results may be useful in the evaluation of new dietary and food products.

KEY WORDS: mulberry, *Morus*, mineral composition, ICP-OES

INTRODUCTION

Plants and their products have always played a substantial role in human welfare, satisfying various essential needs ranging from food to medicines. Functional foods or the nutraceuticals of plant origin have gained in popularity and constitute a major share in health care market (1). The monitoring of metals in plant is of therapeutic and prophylactic importance (2). Deficiencies, excesses, or imbalances in the supply of inorganic elements from dietary sources can have an important deleterious influence on human health (3). Metals have an impact on human health in many ways. Some elements, such as Cu, Zn and Mn, are essential micronutrients with human intake requirements of no more than a few milligrams per day. In plants and/or animals, micro-nutrients such as Co,

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Cu and Mn are the elements necessary for maintaining the life processes (4, 5). Mulberry (*Morus* spp. L., *Moraceae*) has been domesticated over thousands of years ago and adapted to conditions of a wide area of tropical, subtropical and different temperature zones of Asia, Europe, North and South America, and Africa (6). *Morus* plant species has also started to gain an important position in the food industry due to the increasing findings of its health benefits such as reduced risk of certain types of cancer, coronary heart disease, stroke, high blood glucose, and ageing (6-8). Works carried out on certain selected species of this plant demonstrated bioactivities for which this plant has been used in folk medicine, e.g. mulberry fruit juice is used as a remedy for tumors of fauces, aptha, asthma, cold, cough, diarrhea, dyspepsia, edema, fever, headache, hypertension, and wounds. Mulberry leaves have been consumed in Asian countries as antihyperglycemic nutracetical foods for diabetic, and are a promising dietary source of antioxidants. In Japan, mulberry green tea has served as one of healthy drinks, in Thailand, mulberry tea (local name: Mon tea) claimed to be an antidiabetic drink (9, 10). Furthermore, studies of many species of the *Moraceae* family have demonstrated the presence of phenolic compounds and antioxidant activity (6, 7, 11). However, the potential health benefits and chemical characterization of *Morus* species grown in Serbia have not yet been studied in detail. The main objective of this study was the evaluation and provision of baseline information on the mineral composition and heavy metal contents of mulberry plants from Serbia.

EXPERIMENTAL

Chemicals

All chemicals and reagents were of analytical grade and were purchased from Sigma Chemical Co. (St Louis, MQ, USA), Aldrich Chemical Co. (Steinheim, Germany) and Carlo Erba (Italy).

Plant material and sample preparation

The experiments were carried out on different parts (fruits, leaves and root) of two wild mulberry trees, growing in the same location, near Novi Sad, Serbia. The plant species were identified by Goran Anačkov, Faculty of Natural Sciences, University of Novi Sad, Serbia. Voucher specimens for material (*Morus alba* L. N^{o} 2-1794, Kać, UTM 34TDR211, 25.06.2010. and *Morus nigra* L. N^{o} 2-1753, Novi Sad, Rimski šančevi, UTM 34TDR2 01, 23.06.2010.) were confirmed and deposited at the Herbarium of the Department of Biology and Ecology (BUNS Herbarium) Faculty of Natural Sciences, University of Novi Sad, Serbia. Samples were taken under the same conditions. Each material (fruits, leaves and roots) for the analysis and preparation of extracts was taken on the same day from both trees, during the year 2010. Samples of the mulberry fruits were stored in polyethylene bags at -20^{o}C (up to 1 month) until the analysis. Prior to extraction, the samples of mulberry fruits were ground in a blender. The samples of mulberry leaves and roots were dried naturally (in the shade, on draft) during one month. Immediately

after drying the samples of leaves and roots were processed and before the extraction they were also ground in a blender. Particle size was determined using sieve sets (Erweka, Germany). The mean particles were: 0.307 mm for *M. nigra* leaves, 0.309 mm for *M. alba* leaves, 0.764 mm for *M. nigra* roots and 0.712 mm for *M. alba* roots. The samples (100 g) were extracted with 70% ethanol, at temperature of 30°C, while liquid-solid ratio was 15 mL/g (solvent volume per g of material). The extraction process was carried out in a shaker (3015 GFL, Germany) during 24 hours. Extractions under the same conditions were repeated three times. After filtration, the liquid extracts were joined, and 50 mL were used to determine the extraction yield. The solvent was removed on a rotary vacuum evaporator (Devarot, Elektromedicina, Ljubljana, Slovenia) and dried at 60°C until constant mass. All dry extracts were stored in glass bottles at -4°C for prevention from oxidative damage until analysis.

Microwave digestion

The digestion was performed on an Advanced Microwave Digestion System (ETHOS 1, Milestone, Italy) using HPR-1000/10S high pressure segmented rotor. The pressure-resistant PTFE vessels (volume 100 mL), consisted of the fluoropolymer liner. Before use, the PTFE vessels were acid cleaned and rinsed with deionized water. This type of vessel permitted a maximum temperature of 240ºC and a maximum pressure of 100 bar. Maximally 10 PTFE vessels could be simultaneously mounted on the rotor. The internal temperature was monitored only with one vessel equipped with a sensor unit, and this vessel had a sensor-protecting tube that was in direct contact with the digested solution, differing from the other common PTFE vessels. In the digestion, about 1 g of mulberry extract precisely weighed was mixed in each clean vessel with a mixture of 8 mL HNO_3 (65%, Carlo Erba, Italy) and 1 mL H_2O_2 (30%, Carlo Erba, Italy), and then microwave-heated for 40 min. The temperature was controlled with a predetermined power program. The temperature was gradually raised to 200ºC in the first 20 min, and to a peak temperature of 200ºC in the next 20 min, and cooled down rapidly. After cooling and without filtration, the solution was diluted to a fixed volume (25 mL).

Determination of mineral contents

The contents of the elements in the solution samples were determined by inductively coupled plasma optical emission spectrometry (ICP-OES). The ICP-OES measurement was performed using a Thermo Scientific iCAP 6500 Duo ICP (Thermo Fisher Scientific, Cambridge, United Kingdom) spectrometer equipped with RACID86 Charge Injector Device (CID) detector, concentric type nebulizer, quartz torch, quartz injector and cyclonic spray chamber. The optical system purged with argon and the Echelle polychromator were thermostated at 38ºC. The instrumental conditions were optimized to obtain sufficient sensitivity and precision. The instrumental operating conditions for were: Radio frequency power (RF), 1150 W, plasma view: axial, nebulizer: standard glass concentric (without for HG-ICP-OES), spray chamber: standard glass concentric (without for HG-ICP-OES), ceramic centre tube: 2 mm, purge gas: argon, nebulizer argon flow: 0.70 L/min, auxiliary argon flow: 0.5 L/min, coolant argon flow: 12 L/min, sample flush time:

40 s, analysis pump rate: 50 rpm, integration times: low (166-230 nm) 15 s, high (230-847 nm) 5 s, analysis mode: speed, software: iTEVA. As and Hg in the plasma were initiated into the form of their gaseous hydrides using an integrated unit for hydride generation (HG-ICP-OES). For hydride generation use was made of $NaBH_4$ (Merck, Germany) 0.5% (w/v), stabilized in 0.12 M NaOH and 6 M HCl solution (arsenic free, Merck, Germany). Because of the lack of the white and black mulberry standard reference materials the accuracy of the analytical method was evaluated using the NIST (National Institute of Standard and Technology, Gaithersburg, MD 20899, USA) standard reference material (SRM): SRM 1573a (tomato leaves). The SRM was prepared for analysis in the same way as the samples. The ICP-OES and HG-ICP-OES measurements for each digested sample were carried out in five replicates.

Statistical Analysis

Statistical analysis was carried out using the SPSS statistical package program. The elements content in plant samples were shown as mean values ± SD (standard deviation) for five replicates. One-way analysis of variance (ANOVA) was used to assess whether the elements concentration varied significantly among the plant species and plant tissues ($P = 0.05$). Mean values were compared by Tukey's test. Data were considered statistically significantly different when $p \leq P$

RESULTS AND DISCUSSION

The results for the contents of macro and micro elements in the extracts of different parts of white and black mulberry including fruit, leaves and root, are shown in Table 1. The studies showed that the dominant macro element in the most of extract was Mg. Its highest content was found in the extract of the *Morus nigra* leaves (1942±4 μg/g of dry extract). A second most present macronutrient in the extracts of leaves of both *Morus* species was Ca; with the highest detected amount in the extract of *M. nigra* leaves (1548±3 μg/g of dry extract). The same results were also obtained in the case of both investigated roots: a most dominant macronutrient was Mg, followed by Ca. The Na contents were significantly lower in comparison to that of Ca and Mg, irrespective of the variety or plant part, and varied between 187 and 440 μg/g of dry extract.

In fruit extract of both *Morus* species one of the most dominant micronutrient metal was Zn, and its content (32.5 μg/g) was detected in the extract of *M. alba* leaves. B was a dominant micronutrient in two extracts – white mulberry leaves and roots. The Fe was a dominant micronutrient in the leaves extracts of black mulberry (143 μg/g), with the amount of a few times higher in comparison to all other investigated extracts. In the extracts of black mulberry roots a dominant micronutrient was Cu (22.9 μg/g), and it was the highest content that was detected in our investigation. The highest amounts of Mn and Ni were detected also in the extracts of the black mulberry roots.

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Table 1. Contents of macro, micro and toxic elements in different parts of the white and black mulberry

Element	*White mulberry* Content (μg/ g of dry extract)			*Black mulberry* Content (μg/ g of dry extract)		
	Fruits	**Leaves**	**Roots**	**Fruits**	**Leaves**	**Roots**
Macroelement						
Ca	660±6	1399 ±10	450±2	873±8	1548±3	674±1
Mg	591±3	1641±2	652±4	769±1	1942±4	1269±1
Na	226±1	264±2	216±0	187±1	293±0	440±3
Microelement						
B	7.25±0.0	40.4±0	24.1±0	8.9±0.0	23.3±0	17.2±0
Co	< 0.016	< 0.016	0.14±0.01	< 0.016	< 0.016	0.44±0.01
Cr	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002	< 0.002
Cu	10.5±0	17.4±0	10.0±0	11.9±0	19.5±0	22.9±0
Fe	6.35±0.1	2.63±0.0	8.97±0.1	5.45±0.1	143±0	18.3±0
Li	0.14±0.00	2.43±0.0	0.69±0.00	0.13±0.00	0.77±0.01	2.06±0.0
Mn	2.56±0.0	4.22±0.0	4.59±0.0	6.02±0.0	9.80±0.0	19.3±0
Ni	1.25±0.0	5.90±0.0	1.73±0.0	1.42±0.0	4.81±0.0	14.2±0
Se	< 0.003	0.10±0.03	0.04±0.01	< 0.003	< 0.003	0.09±0.04
Sr	2.18±0.0	2.77±0.0	1.15±0.1	2.34±0.0	3.01±0.0	1.68±0.0
Zn	17.7±0	32.5±0	3.36±0.03	10.7±0	31.9±0	13.7±0
Toxic element						
Al	3.52 ±0.0	1.93±0.1	7.30±0.1	3.95±0.0	2.23±0.0	17.8±0
As	0.04±0.01	0.08±0.05	0.08±0.01	0.04±0.03	0.13±0.02	0.13±0.01
Cd	< 0.0007	< 0.0007	< 0.0007	< 0.0007	0.01±0.00	< 0.0007
Hg	< 0.003	< 0.003	0.66±0.01	< 0.003	0.11±0.00	< 0.003
Pb	0.86±0.02	< 0.014	< 0.014	< 0.014	0.26±0.02	0.12±0.01

Values are given as mean ± SD (n=5)

The obtained results indicate the possible health benefits of mulberry extracts. Mg plays a vital role in a wide range of biochemical and physiological processes, particularly those involving energy metabolism and utilization. The Dietary Reference Intake (DRI) established by the Institute of Medicine, USA, for Mg is 320 mg for female and 400-420 mg for adult male. On the other hand, Fe is an essential component of the myoglobin in muscles, cytochromes, and other enzymes, including the antioxidant enzyme catalase. The Mg and Fe levels in our investigated extracts are adequate. The importance of these elements cannot be overemphasized since vast variety of enzymes requires them as cofactors (12). Further, Li is yet another element with beneficial pharmacological properties (13). The highest amount of Li in the investigated extracts was detected for the leaf extract of white mulberry (2.43 μg/g). The DRI for Mn is 1.8 mg per day (for female) and 2.3 mg per day (for male) (14). It activates several enzymes that protect cells from free radicals attack, regulate glucose homeostasis, and Ca mobilization. The highest content of Mn (19.32 μg/g) was found in the extract of the black mulberry roots. Several papers have previously been publi-

shed on the composition of micro-, macro and toxic elements in various mulberry species. Certain results of mineral contents of mulberry fruits show differences when compared to the literature data. In comparing to the *Morus* species from (11) other regions and other berries the highest content of Ca and Mg has been detected in black mulberry grown in Serbia. Also, the *Morus* fruits extracts from Serbia have higher Cu, Mn and Li contents compared to the *Morus* fruits and other berries extracts from Turkey. (15). The Mg, Na, B, Cu, Li and Zn contents in the mulberry leaves and roots from Serbia were higher than those in the plants examined by Bhat et al. (16) and Ozcan (15). The reported differences might be due to the different growth conditions, genetic factors, geographical variations in the level of soil fertility, efficiency of mineral uptake, and analytical procedures employed.

Also, Table 1 presents the results for the contents of toxic metals. The toxic heavy metals Cd, Hg and Pb in all analyzed tissues, irrespective of the mulberry variety, were present at very low or levels too low to be detected by the analytical technique used in this study. A low content of toxic elements in the tested mulberry extracts is appropriate in terms of use. The nature of soil is considered one of the most important factors that affect the heavy metal content in food plants, probably since it is the binding and retention site for the toxicants (4). The low contents of toxic metals can be related to the unpolluted environment, where the examined plants were grown. Cd and Pb are best known for their toxic properties (13), and the low levels of these toxic elements make the investigated materials attractive for their wider use.

One-way analysis of variance indicates that the plant part and plant variety exhibited significant influence on the levels of the examined elements in mulberry (Table 2). The contents of Ca, Na and Mg were significantly higher in black mulberry than in white mulberry ($p \leq 0.05$, Sig=1). Furthermore, the content of macro elements differed signifycantly depending on plant part as well. An exception was the case of Na, where the contents were not statistically different in leaves, fruit and root ($p > 0.05$, Sig=0). The levels of Fe, Cu, Mn and Ni were significantly lower in white compared to black mulberry. A statistically significantly higher content of B was found in white mulberry in comparison to black mulberry. Contents of the determined elements in leaves, fruit and root differed between the plant parts. Also, the results indicate that there were significant differences in the Al and As concentrations in two types of mulberry samples. Significant differences between the contents of Al, As and Hg in the different plant parts were noticed as well. Therefore, the degree of accumulation of those metals varied between species in a part-specific manner. However, no significant differences were detected for Cd and Pb in all analyzed cases.

Table 2. The results of statistical analysis of micro, macro and toxic elements (ANOVA, Tukey's Test)

Element		Ca		Mg		Na		B		Co	
Factor		p	Sig	p	Sig	p	Sig	p	Sig	p	Sig
Species	W/B	<0.001	1	<0.001	1	0.030	1	0.003	1	0.016	1
Parts	L/F	<0.001	1	<0.001	1	0.156	0	<0.001	1	0.978	0
	F/R	<0.001	1	0.001	1	0.013	1	<0.001	1	<0.001	1
	L/R	<0.001	1	<0.001	1	0.385	0	0.001	1	<0.001	1
Element		Cr		Cu		Fe		Li		Mn	
Species	W/B	<0.001	1	0.002	1	0.012	1	0.763	0	<0.001	1
Parts	L/F	0.007	1	0.002	1	0.017	1	0.007	1	0.241	0
	F/R	0.424	0	0.021	1	0.929	0	0.021	1	<0.001	1
	L/R	0.075	0	0.518	0	0.035	1	0.846	0	0.021	1
Element		Ni		Se		Sr		Zn		Al	
Species	W/B	0.034	1	0.568	0	<0.001	1	0.650	0	0.011	1
Parts	L/F	0.147	0	0.132	0	<0.001	1	<0.001	1	0.552	0
	F/R	0.013	1	0.028	1	<0.001	1	0.072	0	<0.001	1
	L/R	0.417	0	0.671	0	<0.001	1	<0.001	1	<0.001	1
Element		As		Cd		Hg		Pb			
Species	W/B	0.032	1	<0.001	1	0.072	0	0.195	0		
Parts	L/F	0.002	1	0.204	0	0.638	0	0.925	0		
	F/R	0.004	1	0.743	0	0.012	1	0.791	0		
	L/R	0.968	0	0.057	0	0.069	0	0.958	0		

W - white mulberry, B - black mulberry, L - leaves, F - fruit, R - root; p (P=0.05) – significance level
Sig equals 1 indicates that the means difference is significant at the 0.05 level, Sig equals 0 indicates that the means difference is not significant at the 0.05 level.

CONCLUSIONS

This work contributes to the knowledge of the physiology and nutritional properties of *Morus* species. This is the first report on the levels of micro and macronutrient and the toxic heavy metals in different parts of *Morus* extracts. The study revealed that parts of the plant had statistically significant impact on the levels of the examined elements among the two *Morus* species. The highest mineral contents were those of B, Ca, Cu, Fe, Mg, Mn, Na and Zn in both *M. nigra* and *M. alba* extracts. A particularly interesting result is the Fe content in the extract of *M. nigra*, and this can be the basis for its testing in the future. The knowledge of the mineral contents, as condiments is of great interest. Further research is needed to highlight practical applications for the better utilization of mulberry extracts.

Acknowledgement

Financial support of this work by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Project No. TR 31013) is gratefully acknowledged.

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КАРАКТЕРИЗАЦИЈА *Morus* ВРСТА У ОДНОСУ НА МИКРО, МАКРО И ТОКСИЧНЕ ЕЛЕМЕНТЕ

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Испитивање је базирано на одређивању минералног састава екстраката *Morus alba* L. (бели дуд) и *Morus nigra* L . (црни дуд) укључујући плод, лист и корен са територије Србије. У свим анализираним екстрактима одређен је садржај микро (B, Co, Cr, Cu, Fe, Li, Mn, Ni, Se, Sr, Zn), макро (Ca, Mg, Na) и токсичних елемената (Al, As, Cd, Hg, Pb) применом оптичке емисионе спектроскопије са индуктивно куплованом плазмом (ICP-OES). Испитивање је показало да постоји статистички значајна корелација између делова биљке Morus врсте и садржаја испитиваних елемената. У свим екстрактима је доказан висок садржај елемената: Ca, Mg, Na, B, Cu, Fe, Mn и Zn, респективно. Доминантан макро елемент у већем броју екстраката је Mg (591 - 1942 μг/г сувог екстракта). Што се тиче микроелемената, у свим испитиваним екстрактима у већем садржају су заступљени Zn, B и Cu. У узорку *M. nigra* посебно се издваја Fe по свом садржају (143 μг/г сувог екстракта) у односу на остале присутне елементе. Од свих испитиваних узорака највиши садржај микро и макро елемената је детектован у екстрактима листа *M. Nigra*. Овај рад доприноси познавању нутритивних својстава *Morus* врста. Добијени резултати могу бити корисни у креирању нових дијететских и прехрамбених производа.

Кључне речи: дуд, *Morus*, минерални састав, ICP-OES

Received: 15 July 2014.
Accepted: 24 September 2014.