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Full Length Research Paper

Effect of horse chestnut tree genotype on production of fatty oil and fatty acids in seed cotyledons

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Horse chestnut (*Hippocastani semen*) seed is an important raw material for the pharmaceutical industry because it contains a series of biologically active substances: starch (30 to 40%), saponins (10%), fatty oil (5.5%), proteins (6%), cellulose (2%), reductive sugars (5.5%) and ash (1.3%). However, the variability of fatty oil content in horse chestnut seed (*H. oleum*) has been insufficiently investigated. This study was performed in populations of horse chestnut trees in towns of Serbia and in the plantation on Mt. Avala. The seeds were collected from 15 test trees of each locality. The content of fatty oil in the seed cotyledons was determined by extraction with petrol ether in the apparatus after Soxlet. Fatty acids were determined and identified in the fatty oil. The analysis was performed by the method of gas chromatography (by the gas chromatograph »Varian« model 1400 with flame ionising detector). The presence of ten fatty acids was determined: myristic, palmitic, palmitoleic, stearic, oleic, linoleic, arachic, linolenic, eicosenoic and erucic acids. Based on the results, it can be concluded that horse chestnut seed, according to fatty oil content, composition and representation of individual fatty acids, is a significant raw material for pharmaceutical and chemical industries.

Key words: *Aesculus hippocastanum* L., *Hippocastani semen*, special-purpose plantation, pharmaceutical industry.

INTRODUCTION

Horse chestnut, with its vegetative and generative organs, contains a series of biologically active substances, with variable quantitative ratios. Based on literature, the quality and quantity variability of these substances was investigated, special attention being focused on the seed, as a significant raw material in pharmaceutical industry (Bisler et al., 1986; Profumo et al., 1990; Shaw et al., 2005). The first results on the chemical composition of horse chestnut seed were reported in 1835 by Fremy (Stanković, 1995), who described numerous procedures of saponin isolation. The ingredients of horse chestnut seeds were dealt with in many papers, in which the data on the quantity of individual substances differ considerably. The Belgian pharmacopoeia lists the following values for the unshelled seed: starch (30 to 40%), saponins (10%), fatty oil (5.5%), proteins (6%), cellulose (2%), reductive sugars (5.5%) and ash (1.3%). Most of the so far published results range within these values.

Considering that previously, few papers were published on fatty oil from horse chestnut (*Hippocastani* o*leum*) seed, this paper presents results of the content of fatty oil in the seed of horse chestnuts grown in towns of Serbia and in the plantation of Mt. Avala. It also presents the results of the comparative quantitative analysis of fatty oil and fatty acids in horse chestnut seed cotyledons, aiming at the assessment of the effect of test tree genotype on their production.

MATERIALS AND METHODS

To study and analyse the scope of intraspecific variability of *Aesculus hippocastanum* L., 15 test trees were selected from each locality. Seed yield was monitored and the seeds were collected. This was followed by laboratory analysIs and the study of the contents of fatty oil and fatty acids.

The collected horse chestnut fruits were cleaned by separating the skinny seed coat and the cotyledons were released. They were dried naturally. The material was comminuted immediately prior to the analysis. Simultaneously, we also measured the moisture content (gravimetric method), and the results were gotten from

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Table 1. Population variability of fatty oil percentage in cotyledons of horse chestnut (*H. semen*) seed.

Locality	$\bar{x} \pm S\bar{x}$	$S \pm S_S$	$V \pm S_V$
Pančevo	6.9 ± 0.89	2.7 ± 0.63	39.1 ± 9.23
Avala	7.2 ± 0.11	0.4 ± 0.09	5.0 ± 1.12
Zemun	5.9 ± 0.34	1.1 ± 0.24	17.2 ± 4.05
Belgrade	7.4 ± 0.24	0.7 ± 0.17	9.8 ± 2.31

$\bar{x}$ - mean value; S - standard deviation; V - variation coefficient; ($S\bar{x}$; S_S; S_V) - mean errors for each of the calculated statistical parameters.

calculations of oven dried materials. All results represent mean values of at least three measurements.

In practice, two methods are used for producing oil from oil plants: By pressing and by extraction with a solvent. Pressing is applied only for oil plants with a high content of oil. The method applied to get oil from horse chestnut seed was extraction by petrol ether in a continuous extractor of the adequate size, type Soxlet.

After oil saponification, fatty acids were converted into methyl esters by methanolysis. The identification was performed by the method of gas chromatography (by the gas chromatograph »Varian« model 1400) with flame ionising detector. The stationary phase was 20% 3 – P – 720 (diethylene glycol succinate) on chromosorb W, AW (80 to 100 mesh) at the temperature of the block for sample injecting (220°C). The mobile phase was nitrogen (25 ml/min).

RESULTS AND DISCUSSION

Fatty oil (*H. oleum*), obtained by extraction of horse chestnut seed is yellow, with a specific mild smell and taste. At lower temperatures (in the refrigerator), it hardens partially. It dissolves in ether, petrol ether and acetone, and not soluble in alcohol (Kovačević, 2000). Fatty oil from horse chestnut seed has not been studied to a sufficient degree, although its utilisation value is considerable. In addition to healing the sunburned skin and spots caused by sunbathing, it is also used in soap manufacturing and for technical purposes.

The content of fatty oil in horse chestnut seed is 2 to 7%. The results of study analysis were complexly and statistically processed, and all major statistic parameters were determined (limit of variability, standard deviations, variation coefficient and their errors) at the population (Table 1) and individual levels (Table 2). The amount of fatty oil in the analysed samples ranged between 4.9 and 10.4%. At the population level, the seed from the tree row in Belgrade had the highest content of fatty oil in cotyledons (7.4 ± 0.24%) and the seed from Zemun had the lowest content of fatty oil in cotyledons of horse chestnut seed (5.9 ± 0.34%).

At the individual level, the analysis of fatty oil content in cotyledons of horse chestnut seed shows that tree number 6 from Pančevo has the highest amount of this secondary metabolite (10.4 ± 0.31%). In comparison with

Table 2. Individual variability of fatty oil percentages in horse chestnut (*H. semen*) seed cotyledons.

Locality	Tree	$\bar{x} \pm S\bar{x}$	$S \pm S_S$	$V \pm S_V$
Pančevo	4	4.9 ± 0.03	0.1 ± 0.02	2.1 ± 0.49
	6	10.4 ± 0.31	0.9 ± 0.22	8.9 ± 2.11
	9	5.3 ± 0.10	0.3 ± 0.07	5.8 ± 1.37
Avala	19	7.4 ± 0.12	0.4 ± 0.08	5.1 ± 1.20
	23	7.1 ± 0.14	0.4 ± 0.10	6.1 ± 0.14
	28	7.2 ± 0.24	0.7 ± 0.17	10.2 ± 2.41
	32	5.1 ± 0.27	0.8 ± 0.19	15.7 ± 3.69
Zemun	37	7.3 ± 0.12	0.4 ± 0.08	5.0 ± 1.18
	41	5.7 ± 0.14	0.4 ± 0.10	7.6 ± 1.80
	47	7.4 ± 0.06	0.2 ± 0.04	2.2 ± 0.53
Belgrade	48	6.4 ± 0.07	0.2 ± 0.05	3.4 ± 0.81
	49	8.2 ± 0.11	0.3 ± 0.08	4.1 ± 0.97

$\bar{x}$ - mean value; S - standard deviation; V - variation coefficient; ($S\bar{x}$; S_S; S_V) - mean errors for each of the calculated statistical parameters.

literature data, the study results surpass the values reported at the level of this species, according to which, the yield of fatty oil from horse chestnut seed is 4 to 6% (Lemajić et al., 1986; Lukić, 1993; Ocokoljić et al., 1997). The lowest amount of fatty oil was measured in the tree number 4 at the same locality (4.9 ± 0.03%).

The difference in the content of fatty oil was also observed at the population level, but more significant differences were measured between selected trees at some localities (Zemun and Pančevo). Based on the study results, trees number 6, 19, 47 and 49 can be set aside as the trees with a high content of fatty oil. Based on the comparative analysis of the values of variation coefficients, and also based on the results of the analysis of variance (Table 3), it can be concluded that the content of this secondary metabolite in horse chestnut seed is genetically conditioned.

Fatty acids were determined and identified in the fatty oil extracted from horse chestnut seed cotyledons (at the population level). We determined the presence of ten fatty acids. They are myristic, palmitic, palmitoleic, stearic, oleic, linoleic, arachic, linolenic, eicosenoic and erucic acids. Unsaturated fatty acids are dominant in fatty oil (GC/FID; 87 to 92%), and the most represented acids are oleic and linoleic acids (Table 4). The study results agree with literature data on fatty acids in fatty oil in the seed at the level of the species and with the highest percentage of oleic and linoleic acids.

Horse chestnut (*A. hippocastanum* L.) is an Arctotertiary endemic of the southern part of the Balkan Peninsula (with range in Greece, Albania, Macedonia and Bulgaria). This species is often grown in the Europe. It has a special role in forest communities and in town cul-

Table 3. F- values of the analysis of variance of fatty oil amounts.

A (locality)	B (tree)	A x B (interaction)
12.10470*	27.87528**	24.87812**

Table 4. Statistic parameters of fatty acid percentage in total lipids of horse chestnut (*Hippocastani semen*) seed cotyledons.

Fatty acid	$\bar{x} \pm S\bar{x}$	$S \pm S_S$	$V \pm S_V$
Pančevo			
Palmitic	7.6 ± 0.24	0.7 ± 0.16	9.4 ± 2.19
Palmitoleic	0.3 ± 0.02	0.1 ± 0.02	19.8 ± 4.60
Stearic	3.5 ± 0.18	0.5 ± 0.13	15.6 ± 3.63
Oleic	46.2 ± 1.07	3.2 ± 0.75	6.9 ± 1.62
Linoleic	30.6 ± 0.41	1.2 ± 0.28	4.0 ± 0.93
Arachic	0.6 ± 0.04	0.1 ± 0.03	20.4 ± 4.75
Linolenic	2.5 ± 0.15	0.5 ± 0.11	18.6 ± 4.33
Eicosenoic	5.4 ± 0.24	0.7 ± 0.17	13.2 ± 3.07
Erucic	3.1 ± 0.46	1.4 ± 0.32	45.1 ± 10.50
Avala			
Palmitic	6.5 ± 0.05	0.2 ± 0.04	2.4 ± 0.57
Palmitoleic	0.3 ± 0.02	0.1 ± 0.02	27.4 ± 6.38
Stearic	2.8 ± 0.43	1.3 ± 0.30	46.5 ± 10.82
Oleic	52.4 ± 0.41	1.2 ± 0.29	2.4 ± 0.55
Linoleic	29.1 ± 0.88	2.6 ± 0.61	9.1 ± 2.11
Arachic	0.4 ± 0.04	0.1 ± 0.02	27.6 ± 6.43
Linolenic	1.6 ± 0.13	0.4 ± 0.09	24.3 ± 5.64
(1)	(2)	(3)	(4)
Eicosenoic	4.4 ± 0.40	1.2 ± 0.28	27.7 ± 6.45
Erucic	2.5 ± 0.43	1.3 ± 0.30	50.8 ± 11.80
Zemun			
Palmitic	6.8 ± 0.41	1.2 ± 0.28	18.1 ± 4.21
Palmitoleic	0.3 ± 0..3	0.1 ± 0.23	37.6 ± 8.74
Stearic	2.3 ± 0.20	0.6 ± 0.04	26.1 ± 6.06
Oleic	44.7 ± 0.56	1.7 ± 0.40	3.7 ± 0.87
Linoleic	34.9 ± 1.72	5.2 ± 1.19	14.8 ± 3.43
Arachic	0.6 ± 0.08	0.2 ± 0.05	39.4 ± 9.17
Linolenic	2.4 ± 0.27	0.8 ± 0.19	34.5 ± 8.03
Eicosenoic	5.1 ± 0.30	0.9 ± 0.21	17.6 ± 4.10
Erucic	2.9 ± 0.48	1.4 ± 0.33	49.1 ± 11.45
Belgrade			
Palmitic	6.5 ± 0.21	0.6 ± 0.15	9.8 ± 2.28
Palmitoleic	0.3 ± 0.04	0.1 ± 0.03	50.7 ± 11.80
Stearic	2.8 ± 0.21	0.6 ± 0.14	22.1 ± 5.14
Oleic	51.7 ± 1.36	4.1 ± 0.95	7.9 ± 1.83
Linoleic	26.9 ± 0.89	2.7 ± 0.62	9.9 ± 2.32
Arachic	0.6 ± 0.05	0.1 ± 0.04	24.8 ± 5.76
Linolenic	1.9 ± 0.11	0.3 ± 0.07	16.8 ± 3.91
Eicosenoic	6.2 ± 0.24	0.7 ± 0.17	11.8 ± 2.74
Erucic	2.9 ± 0.54	1.6 ± 0.38	55.4 ± 12.87

$\bar{x}$ - mean value; S - standard deviation; V - variation coefficient; ($S\bar{x}$; S_S; S_V) - mean errors for each of the calculated statistical parameters specter of its products significance for intensive establishment of special purpose plantation: Exploitation of vegetative and generative organs as raw material for pharamaceutical and chemistry industries and also, woody mass as raw material for industry of remodel wood (Ocokoljić, 2006).

The results of this study were gotten from four years investigation of populations of horse chestnut trees in towns of Serbia and in the plantation of Mt. Avala. During the analysis, we got introduced biological heterogeneous and genetic potential of this species as significant elements for the establishment of special-purpose plantations. The aspect of the content of major secondary metabolites (fatty oil and fatty acids) was studied with the aim of selecting horse chestnut mother tree from different localities in Serbia.

Conclusions

Woody species, as complex plant organisms, are the result of hereditary (genetic) and environmental factors. In this respect, the aim of species selection has to be multidisciplinary, because the characters of properties depend on the interaction of genetic, biochemical, physiological and morphoanatomical systems. In such researches, the starting point is the variability of quantitative properties, as one of the basis for the improvement of woody species.

Based on the results, it can be concluded that the study of horse chestnut seed should be intensified, because according to the content of fatty oil, the composition and representation of individual fatty acids, represents a significant base for pharmaceutical and chemical Industry. The content of fatty oil is relatively low in the seed of test trees, but as horse chestnut starts bearing seed early and abundantly, its seed can easily be collected in large amounts.

With the selection of superior genotypes and by their reproduction, new varieties can be synthesised. As a result of their high degree of general adaptivity, especially as it is manifested in urban and periurban zones, they will supply proportionally abundant seed. In this sense, the selected genotypes should be used for the more intensive establishment of special-purpose plantations for the production of horse chestnut seed as a raw material for pharmaceutical and chemistry industries.

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