CHLOROPHYLL AND CAROTENOIDS PIGMENTS FROM MISTLETOE (*Viscum album*) LEAVES USING DIFFERENT SOLVENTS

Simona Ioana VICAŞ*, Vasile LASLO*, Stelian PANTEA*, Gheorghe Emil BANDICI*

*University of Oradea, Faculty of Environmental Protection, Oradea, Romania
Corresponding author: Simona Vicas, University of Oradea, Faculty of Environmental Protection, 26 Magheru, 410048 Oradea, Romania, phone: 0040259412550, fax: 0040259416274, e-mail: svicas@uoradea.ro

Abstract. European mistletoe *(Viscum album* L., family *Santalaceae*) is native from Europe and is an evergreen, perennial, hemiparasitic shrub that lives on a wide range of woody plants species. In the first part of this study we present the area, length and width of the leaves of five varieties of *Viscum album* subsp. *album* that are growing on five different host trees (*Acer campestre*, *Mallus domestica, Fraxinus excelsior, Populus nigra* and *Robinia pseudoacacia*), in order to observe if there are vegetative morphology changes within the same specie (*V. album*) due to host plant. In the second part, chlorophyll *a*, chlorophyll *b* and the total amounts of carotenoids in mistletoe extracts, that was harvesting on February were determined. The buffered aqueous 80% acetone, DMF and methanol were used as solvents. The contribution of these solvents to the extraction of assimilatory pigments and the time of extraction (24, 48 and 72 hours) was examined comparatively. We find, also, the difference between the mistletoe that are growing on different host trees. The highest level of total chlorophyll (*a* + *b*), after 48 hours of extraction, were observed in the case of VaM extract, in all the solvents used (21.92 mg/g fresh leaves, in the case of methanol; 20.45 mg/g fresh leaves, in the case of acetone and 16.00 mg/g fresh leaves in the case of DMF). The low concentration of pigments were recorded in the case of VaP extract (15.23 mg/g fresh leaves in methanol extract). It was observed that methanol is the best solvent for chlorophyll, while acetone is a better solvent for the carotenoids.

Keywords: chlorophyll *a* and *b*, carotene, spectrophotometric method, solvents, *Viscum album*

INTRODUCTION

Viscum is a genus of about 70-100 species of mistletoes, native from Europe, Africa, Asia, and Australia (from temperate and tropical regions) [29].

Traditionally, the genus *Viscum*, has been placed in its own family *Viscaceae*, but recent genetic research by APG II [31] system shows this family to be correctly placed within family *Santalaceae*.

Different species of *Viscum* are capable of parasitizing a large number of host species. From a review of literature, [3], identified 452 host species of *Viscum album* s.l. (96 genera and 44 families). For *V. album* subsp. *album* 190 hosts have been identified, for *V. album* subsp. *abieties* 10 hosts, and for *V. album* subsp. *austriacum* 16 hosts are known [29].

Viscul album s.l. is a hemi-parasitic plant that depends for water and mineral nutrition on its host but is able to produce carbohydrates by photosynthesis [8]. It contains all pigments, chlorophyll *a* and *b* as well as carotenoids that are necessary for photosynthesis [8]. The site of photosynthesis in plants is predominantly the green leaf. The chlorophylls of higher plants consist of chlorophyll *a* as the major pigment and chlorophyll *b* as an accessory pigments. Chlorophyll content as well as chlorophyll ratio can be modified by both internals factors and the environmental conditions. Plant parts, other than the leaf that can retain or develop chlorophyll, such as stems, branches, floral parts, fruits and some aerial roots do undergo photosynthesis.

Traditional methods for analysis of photosynthetic pigments employed spectroscopy and extinction coefficients that had been calculated for a range of solvents. For whole-leaf extracts these methods allowed for the accurate calculation of chlorophyll (chl) *a* and *b* concentration, but were limited to a pooling of the carotenoid pigments to give total carotenoid content [12].

Change in the concentration of leaf pigments (chlorophyll and carotenoids) and its relation are good indicators of perturbations in plants caused by environmental factors [10]. The ratio of chlorophyll *a* to *b* in land plants has been used widely as an indicator of response to shade and as an early indicator of senescence [6]. The ratio between chlorophyll and carotenoids has been much less widely used diagnostically, but this ratio is a sensitive indicator for distinction of natural full-term senescence and senescence due to environmental stress [7].

Several solvents, such as acetone [14, 18], N,N-dimethylformamide (DMF) [4, 24], methanol [2, 21], ethanol [25] dimethylsulfoxide (DMSO) [23], have been used to extract chlorophyll pigments from a variety of plant tissues [7, 28].

Following a previous study of Vicaş et al. [27], regarding the extraction and quantification of the chlorophylian pigment from mistletoe harvested during summer, it was obtained a very small proportion of chlorophyll *a*/*b*. This sugests there is a high level of b chlorophyll. This kind of proportion suggests the possibility of a pollution effect of some kind (the small proportion of chlorophyll *a*/*b* represents a biochemical marker of pollution) [26]. It also could indicate that, during the summer, the mistletoe acts like a shade plant. In order to clear this aspect, we determined the *a* and *b* chlorophylls concentrations, respectively the *a*/*b* proportion of the mistletoe harvested during winter time, whilst it isn't shaded by the leaves of the host plant.

However, extraction and quantification of chlorophylls of European mistletoe (*Viscum album*) that are growing on different host trees, has never been mentioned thoroughly in any literature. Logan et al., [16] studied the photosynthetic characteristics of the aerial parasite eastern dwarf mistletoe (*Arceuthobium pusillum*) and they found that the chlorophyll contents (*a*+*b*) of eastern dwarf mistletoe were extremely low (0.12 μmol/g FW), the ratio of chlorophyll *a* to *b* was

3.63 ± 0.07, typical of plants acclimated to full sunlight [15], and the total carotenoids 714 ± 17 mmol/mol Chl.

Parasites are unusal plants, well adapted to their mode of life. They vary greatly in their dependence on their host plants. *Viscum* have chlorophyll but no roots and therefore depend on their hosts only for water and minerals [1].

As there are not available data in the literature for the extraction of the pigments from European mistletoe (*V. album*), we tested 3 different solvents: methanol, DMF (a solvent that is efficient when pigments concentration is low [20]) and acetone, which is the best solvent for the least polar carotenoids.

There can be observed lean vegetative morphology changing within the same species, due to environment changes and also due to host plant of the mistletoe. Thereby, in order to observe if there are vegetative morphology changes within the same specie (*V. album*) due to host plant, we determined the area, length and width of the leaves of mistletoe grown on different host trees. Mbagwu and Onuoha, [19], present the floral and vegetative characters of five *Viscum* variants found in Eastern Nigeria, that could be used for systematic characterization of the species under genus. The authors found more similarities than differences, it then means that the slight variations could be due to environmental influences and the nutrient of host trees.

MATERIALS AND METHODS

Site description and plant material

This study was conducted in February of 2010 on the North-West of Romania country (Borod – Gheghie region). The mean annual rainfall is 500-700 mm/an. The mean annual minimum and maximum temperatures were -22.3^{0}C and 35^{0}C, respectively (20-yr averages). The mean annual air temperature is 9.2 ^{0}C [30]. The area is opened to West, the frequent air mass is oriented to western circulation, transporting oceanic air, cold and humid. Multiannual average wind speed is 4.1 m/sec. The mistletoe samples have been harvested at the aproximate same height, the trees where from it has been hatvested having the same soil (brown soil) and climate conditions. The host trees were located in semi-shaded to sunny area.

Different variants of *V. album* plants were harvested from five different host trees and they were labeled according with the host trees, thus: *Acer campestre* (VaA), *Mallus domestica* (VaM), *Fraxinus excelsior* (VaF), *Populus nigra* (VaP) and *Robinia pseudoacacia* (VaR) for easy identification.

Full-sun exposed young leaves of mistletoe from horizontal branches (at about 2.5 m height from soil) of host trees were used for analysis. Leaves sample were collected in the field, immediately placed in plastic bags, and stored to -20^0 C until assayed.

Biometrics.

The area, length and width of the leaves were measured using an Area Meter AM 300 (ADC BioScientific Ltd.), a portable instrument, designed to find the area of leaves. Measurements are made optically using a simple scanning process. The leaves were measured for each of the variants, and the results are average of 20 determinations.

Quantification of plants green pigments and total carotene using different solvent.

For extraction of chlorophylls (*a* and *b*), 50 mg fresh weight of leaves were homogenizated with 5 ml different solvents [buffered aqueous 80% acetone, DMF (*N,N* –dimethylformamide) and methanol] using SilentCrusher M instruments (Heidolph). Then, the samples were centrifugate at 12000 rpm, for 10 minutes, at 4^{0}C. The supernatants were separated and the content of green pigments was determined by measured the absorbance at 663.6 nm, 663.8 nm, and 665.2 nm for chlorophyll *a*, 646.6 nm, 646.8 nm and 652.0 nm for chlorophyll *b* using UV-visible mini-1240- Schimatzu spectrophotometer, at 24, 48 and 72 hours.

The results obtained for quantification of green pigments are average of 3 determinations and the data obtained after the spectrophotometrycal determination, was mathematically processed [23] (Table 1), and re-expressed as milligrams of chlorophyll per gram of tissue fresh weight (mg/g) [9,17]. The equations are different regarding the solvents used.

For the determination of total carotene, we used the following formulas depending the solvents used (Table 2).

Table 1. Simultaneous equations for the determination of Chlorophyll *a* and *b* concentrations (µg/ml) [23].

Solvent	Equations for chlorophyll concentrations (µg/ml)
In buffered aqueous 80% acetone	[Chl *a*]= 12.25 ·Abs.$^{663.6}$-2.55·Abs.$^{646.6}$ [Chl *b*]= 20.31 ·Abs.$^{646.6}$-4.91·Abs.$^{663.6}$ [Chl *a*+*b*]= 17.76 ·Abs.$^{646.6}$+7.34·Abs.$^{663.6}$
In DMF	[Chl *a*]= 12.00 ·Abs.$^{663.8}$-3.11·Abs.$^{646.8}$ [Chl *b*]= 20.78 ·Abs.$^{646.8}$-4.88·Abs.$^{663.8}$ [Chl *a*+*b*]= 17.67 ·Abs.$^{646.8}$+7.12·Abs.$^{663.8}$
In Methanol	[Chl *a*]= 16.29 ·Abs.$^{665.2}$-8.54·Abs.$^{652.0}$ [Chl *b*]= 30.66 ·Abs.$^{652.0}$-13.58·Abs.$^{665.2}$ [Chl *a*+*b*]= 22.12 ·Abs.$^{652.0}$+2.71·Abs.$^{665.2}$

*Where Abs.^{x} represent the absorbance at x nm

Statistical analysis

Each spectrophotometric determination of different extracts was done three times from the same extract. One way -ANOVA, Tukey's Multiple Comparison Test was used to test any difference in chlorophyll *a* and *b*, and also carotene pigments, from leaves of mistletoe, and also, to establish the sustainable solvents for the extraction of pigments.

Table 2. The equations for the determination of total carotene (mg/g fresh leaves) in three different solvents (buffered aqueous 80% acetone, DMF and Methanol).

Solvent	Equations for total carotene (mg/g leaves)	Reference
In buffered aqueous 80% acetone	Total carotene = 1000*Abs.^{470}-2.270 ·[Chl *a*]-81.4·[Chl *b*]/227	[11]
In DMF	Total carotene = 1000 Abs.^{470} –0.89·[Chl *a*]-52.02[Chl *b*]/ 245	[20]
In Methanol	Total carotene = 1000*Abs.^{470}-2.860 ·[Chl *a*]-129.2·[Chl *b*]/245	[11]

*Where Abs.^{x} represent the absorbance at x nm

RESULTS

The results of biometrical investigations (area, length and width of the leaves) are showed in Table 3. No significant difference relationship among the area and width of all investigated variants of mistletoe were observed. The only significant difference, are in the length of leaves. For example, we found the significant difference between the leaves of VaF vs. VaM, VaM vs. VaA, VaM vs. VaP, and VaM vs. VaR. The biggest length of leaves are recorded in the case of mistletoe that are grown on *M. domestica* (VaM) (75.58 ± 6.09 mm) and the small length leave are recorded in the case of mistletoe that are grown on *R. pseudoacacia* (VaR) (52.98 ± 9.05 mm). The area of *V. album* s.l. varies from 643.56 to 864.78 mm^2, whereas the width of leaf varies from 13.27 to 16.85 mm. The ratio between length and width ranging from 3.11 for VaR to 5.68 for VaM.

Table 3. The biometric investigations of *Viscum album* s.l. leaves that are growing on five different trees.

PARAMETER[1]	VaA	VaM	VaF	VaP	VaR
Area (mm^2)	727.89±21.02 ns	831.11±17.23 ns	643.56±16.69 ns	790.44±27.98 ns	864.78±32.85 ns
Length (mm)	56.46±4.186 *	75.58±6.903 *, **	57.06±4.501 *	59.94±1.989 ns	52.98±9.050 **
Width (mm)	15.43±1.801 ns	13.3±1.015 ns	13.27±1.401 ns	16.63±2.12 ns	16.85±4.494 ns
Ratio (Length/ Width)	3.66	5.68	4.29	3.60	3.11

ANOVA, Tukey's Multiple Comparison Test. * significant (VaF vs. VaM; VaM vs VaA. VaM vs. VaP). ** distinctly significant (VaM vs. VaR); ns – no significant
[1]mean value ± standard deviation

Table 4 contains the mean ± standard deviation of the average levels of chlorophyll *a* and *b* concentrations (mg/g fresh leaves), from leaves of *Viscul album* s.l.that are growing on five different host trees using three different solvents: buffered aqueous 80% acetone, methanol and DMF. Studying the content of photosynthetic pigments from mistletoe leaves, we observed the difference between the variants used in this study. The highest level of total chlorophyll (*a* + *b*), after 48 hours of extraction, were observed in the case of VaM extract, in all the solvents used (21.92 mg/g fresh leaves, in the case of methanol; 20.45 mg/g fresh leaves, in the case of acetone and 16.00 mg/g fresh leaves in the case of DMF). The low concentration of pigments were recorded in the case of VaP extract (for example, 15,23 mg/g fresh leaves in methanol extract comparative with 6.71 mg/g fresh leaves in DMF).

The ratio of chlorophyll *a* to chlorophyll *b* in the chloroplast of higher plants is usually between 2.5 and 3.5 [5]. It is known that the chlorophyll *a* to *b* ratio is higher in high-light growth conditions than in low-light growth conditions [13]. The samples were collect on February, when the leaves of host trees don't cover the mistletoe plant. We observed that the ratio of chlorophyll *a* to chlorophyll *b* decrease with the time extraction increase (for example, 3.20; 3.11; 3.06 for the 24, 48 respectively 72 hours, in the case of VaM methanol extract). In time, the chlorophyll *a* is degraded, and may be this is the explanation of decreased of this ratio.

The statistical correlation between the level of total chlorophyll (*a*+*b*) (mg/g fresh leaves) of all variants used in this study (VaA, VaR, VaP, VaF, VaM) and solvents used (acetone, methanol and DMF) are summarized in Table 5.

Also, the solvent play an important role in the extraction of these pigments. From the data obtained, we observed that the highest level of total chlorophyll was obtained after 48 hours of extraction. To investigate the extraction efficiency of various organic solvents, three commonly used solvents - methanol, acetone and DMF - were selected to extract the chlorophyll and carotene pigments from fresh leaves of mistletoe grown on five different host trees. The content of leaves total chlorophyll extracted with methanol is higher that using the other two solvent, and for the extraction of carotene the best solvent was acetone (Table 4).

Total carotene (mg/g fresh leaves) from the mistletoe leaves, in three organic solvents are summarized in Table 6.

The highest level of total carotene, were observed in the case of acetone VaM extract, (4.26±0.14 mg/g fresh leaves), after 48 hours of extraction, while the concentration of the same pigments were aproximatelly 2 times less in the case of metanolVaM extract (1.86 ± 0.26 mg/g fresh leaves).

Table 4. The average ± standard deviation of the average levels of chlorophyll *a* and *b* concentrations (mg/g fresh leaves), total chlorophyll (*a* + *b*) and ratio *a*/*b* from leaves of *Viscum album* s.l. that are growing on five different host trees using three different solvents: buffered aqueous 80% acetone, methanol and DMF.

Time extraction	24 hours				48 hours				72 hours			
Pigments / Sample	Clorofila *a*	Clorofila *b*	Clorofila *a+b*	Clorofila *a/b*	Clorofila *a*	Clorofila *b*	Clorofila *a+b*	Clorofila *a/b*	Clorofila *a*	Clorofila *b*	Clorofila *a+b*	Clorofila *a/b*
ACETONE												
VaA	11.07±0.05	3.13±1.05	14.20	**3.54**	12.07±1.30	4.50±2.45	16.57	**2.68**	11.61±1.02	4.13±1.23	15.74	**2.81**
VaM	13.95±1.05	4.35±2.03	18.30	**3.21**	14.80±0.11	5.65±0.9	20.45	**2.62**	14.84±0.98	5.42±2.09	20.27	**2.74**
VaF	9.83±2.60	2.53±2.11	12.36	**3.88**	10.54±0.26	3.87±0.22	14.41	**2.72**	10.64±0.09	3.85±1.11	14.49	**2.76**
VaP	5.99±0,11	1.84±0.16	7.83	**3.25**	7.10±2.54	3.12±0.11	10.23	**2.27**	6.87±1.11	2.66±1.09	9.54	**2.58**
VaR	6.60±1.23	2.18±0.10	8.77	**3.03**	6.92±1.11	2.33±0.23	9.25	**2.97**	6.90±2.44	2.14±1.09	9.04	**3.22**
METHANOL												
VaA	14.64±0.5	4.86±0.23	19.51	**3.01**	14.60±0.67	4.82±2,56	19.42	**3.03**	14.33±0.65	4.73±0,11	19.06	**3.03**
VaM	11.88±0.95	3.71±0.56	15.59	**3.20**	12.23±0.09	3.94±0.78	16.17	**3.11**	12.27±1.11	4.01±0.23	16.28	**3.06**
VaF	12.79±0.60	4.10±0.81	16.89	**3.12**	13.08±0.78	4.20±0.07	17.28	**3.12**	13.10±0.25	4.24±0.56	17.34	**3.09**
VaP	11.35±0.65	3.65±0.27	15.00	**3.11**	11.63±1.98	3.60±0.72	15.23	**3.23**	11.56±1.99	3.68±2.65	15.25	**3.14**
VaR	7.27±0.76	2.36±0.78	9.63	**3.08**	7.30±2.45	2.28±.87	9.58	**3.20**	7.21±2.11	2.35±0.11	9.56	**3.06**
DMF												
VaA	12.82±0.45	4.03±0.96	16.85	**3.19**	12.41±0.11	4.02±0.78	16.43	**3.09**	11.97±0.11	4.15±2.34	16.12	**2.88**
VaM	12.55±0.12	3.77±2.50	16.32	**3.33**	12.23±0.78	3.77±0.66	16.00	**3.25**	12.02±0.23	3.92±2.78	15.94	**3.07**
VaF	9.06±0.67	2.89±0.10	11.95	**3.14**	8.97±0.23	2.93±0.98	11.89	**3.07**	8.90±0.09	3.06±1.22	11.96	**2.91**
VaP	5.16±0.50	1.59±1.59	6.75	**3.24**	5.02±0.45	1.69±2.09	6.71	**2.97**	5.03±0.23	1.92±0.09	6.95	**2.62**
VaR	7.60±0.66	2.12±2.01	9.72	**3.59**	7.36±0.56	2.28±1.89	9.64	**3.23**	7.27±2.01	2.49±0.98	9.76	**2.92**

Table 5. Statistical correlation[1] between the level of total chlorophyll (*a+b*) of all variants used in these study (VaA, VaR, VaP, VaF, VaM) and solvents used (acetone, methanol and DMF).

Samples	Samples	VaM			VaF			VaP			VaR			VaA		
	Slovent[2]	DMF	ME	AC	DMF	ME	AC	DMF	ME	AC	DMF	ME	AC	DMF	ME	AC
VaA	**AC**	***	**	***	***	***	***	***	***	***	***	***	***	***	***	-
	ME	***	***	*	***	***	***	***	***	***	***	***	***	***	-	N/A
	DMF	ns	*	***	***	ns	***	***	ns	***	***	***	***	-	N/A	N/A
VaR	**AC**	***	***	***	***	***	***	***	***	ns	ns	ns	-			
	ME	***	***	***	***	***	***	***	***	***	ns	-	N/A			
	DMF	***	***	***	***	***	***	***	***	***	-	N/A	N/A			
VaP	**AC**	***	***	***	***	***	***	*	***	-						
	ME	ns	ns	***	***	ns	***	***	-	N/A						
	DMF	***	***	***	***	***	***	-	N/A	N/A						
VaF	**AC**	***	***	***	ns	***	-									
	ME	ns	**	**	***	-	N/A									
	DMF	***	***	***	-	N/A	N/A									
VaM	**AC**	***	***	-												
	ME	ns	-	N/A												
	DMF	-	N/A	N/A												

[1]ANOVA, Tukey's Multiple Comparison Test. * significant,** distinctly significant;*** very significant; ns – no significant; [2]AC- acetone; ME-methanol; DMF- N,N-dimethylformamide

Table 6. The average ± standard deviation of the average levels of total carotene (mg/g fresh leaves) in the mistletoe leaves using for extraction three organic solvents.

Sample	Total carotene (mg/g fresh leaves)								
	ACETONE			METHANOL			DMF		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
VaA	3.60±0.06	3.67±0.22	3.63±0.12	2.46±0.11	2.57±0.09	2.53±0.09	2.73±0.23	2.49±0.11	2.36±0.11
VaM	4.13±0.14	4.26±0.14	4.11±0.11	1.92±0.34	1.86±0.26	1.97±0,05	2.73±0.11	2.60±0.34	2.47±0.25
VaF	3.28±0.12	3.51±0.20	3.47±0.23	2.18±0.26	2.19±0.23	2.26±0.12	2.03±0.11	1.99±0.11	1.87±0,15
VaP	2.12±0.12	2.33±0.19	2.35±0.34	2.56±0.11	2.59±0.08	2.68±0.16	1.40±0.16	1.34±0.08	1.22±0.13
VaR	2.36±0.30	2.69±0.40	2.68±0.04	1.74±0.23	1.64±0.12	1.72±0.23	2.39±0.15	2.19±0.17	2.02±0.08

DISCUSSION

The observation on the vegetative morphology of *Viscum album* s.l., that are growing on five different host trees (Table 3), shown significantly differences (VaF vs. VaM; VaM vs VaA. VaM vs. VaP) and distinctly significant differences (VaM vs. VaR) only, on the length of leaves. The observation made by Mbagwu and Onuoha, [19] on the vegetative and floral morphology of the five *Viscum* variants showed an interesting taxonomic relationships among the investigated variants. Their results regarding to vegetative morphology shown that the leaf length varies from 7.78 – 15.09 cm, whereas the leaf width varies from 4.04 -10.24 cm. Since the authors found that are more similarities than differences in the vegetative and floral morphology, it then means that the slight variations could be due to environmental influences and the nutrient of the host plant. The same conclusion reached the Nwachukwu and Mbagwu [22].

The ratio of chlorophyll *a* to chlorophyll *b* in the chloroplast of leaves from *V. album* s.l. (Table 4) harvesting in February, depends on the solvents used, extraction time and vary between 2.27 to 3.88. This values are consistent with literature data for higher plants [5]. In an another study [24], the ratio *a*/*b* of mistletoe extract harvesting in July was very low (0.60). The content of chlorophyll *b* was increase comparative with *a*, and this lead to lower ratio chlorophyll *a/b*. The lowest ratio was recorded in the case of VaF extract from leaves. In July, the leaves of host trees cover the mistletoe plants, and hereby, mistletoe plant has low access to the light. Chlorophyll *b* differs from chlorophyll *a* only in one of the functional groups bonded to the porphyrin [5]. It is an accessory pigments and acts indirectly in photosynthesis by transferring the light it absorbs to chlorophyll *a*. The reduced chlorophyll *a*/*b* ratio is due to increase levels of chlorophyll *b*, a typical shade adaptation that permits more trapping of photons that are then transferred to chlorophyll *a*.

The solvents play an important role in the extraction of total carotene from the leaves of mistletoe, also (Table 6). Our results showed that acetone is a better solvent for the least polar carotenoids, and this results is similary with the study of Dunn et al. [12]. Their results show that the least polar pigments, particularly β-carotene, are inefficiently extracted by aqueous methanol or acetone in a single extraction.

In conclusion, we found more similarities than differences regarding to vegetative morphology, it means that the slight variations could be due to the nutrient of the host plant. The following solvents were used for the extraction of plants green pigments and total carotene from leaves of mistletoe: acetone, methanol, dimethylformamid. Our results show, that the extraction efficiency of green pigments (chlorophyll *a* and *b*) was higher when using methanol, comparative with other two solvents. Also, the extraction time is important, in our study the best time was obtained after 48 hours extraction. In the case of the extraction of carotene pigments, the best solvent was acetone. That, the selection of the solvent to be used in the studies in connection with green pigments and carotene according to the species will be more useful. The composition and contents of photosynthetic pigments appear to be important for the determination of physiological characteristics of different varieties of *V. album* s.l.

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