

Full Length Research Paper

Comparative study about antioxidant activities of *Viscum album* from different host trees, harvested in different seasons

Simona Ioana Vicaș^{1*}, Dumitrița Rugină², and Carmen Socaciu²

¹University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea; Romania.

²Department of Chemistry and Biochemistry, University of Agricultural Sciences and Veterinary Medicine, Cluj-Napoca; Romania.

Accepted 8 February, 2011

Viscum album L. is a medicinal plants used for many years as a remedy in the traditional medicine and in complementary cancer therapies. We investigated the influence of some host trees (*Acer campestre*, *Fraxinus excelsior*, *Populus nigra*, *Mallus domestica*, and *Robinia pseudoacacia*) and the seasons (May, July and December) on antioxidant activity of European mistletoe (*V. album*) using four different methods: (DPPH, ORAC, TEAC and Folin-Ciocalteu). Also, the influence of solvent (water and ethanol) was investigated. In general, antioxidant activity of mistletoe determined by DPPH and ORAC assay recorded the highest activity in the ethanolic extract, while the antioxidant activity of mistletoe extracts determined by TEAC and Folin-Ciocalteu assay recorded the highest activity in the aqueous extract. The promising mistletoe extracts recording with DPPH assay are VAF, hosted by *Fraxinus excelsior* followed by VAR, hosted by *R. pseudoacacia* (77.19% respectively, 76.6% DPPH scavenging effect). The values obtained from ORAC assay, in three different months (May, July and December), did not differ significantly, between all the ethanolic mistletoe extract investigated. The mistletoe hosted by *R. pseudoacacia* (VAR) has the highest level of scavenging cation-radicals ABTS^{•+} (1.7 ± 0.04 mM Trolox equivalents/g fresh matter). The values obtained for total phenolics in aqueous mistletoe extracts, harvesting in December decreased in the order: VAM (134.18 ± 0.01 mg GAE/ g fresh matter > VAF (122.08 ± mg GAE/ g fresh matter) > VAR (114.57 ± 0.004 mg GAE/ g fresh matter). The differences in the antioxidant activity between the leaves and stems of mistletoes harvested from different trees, and different seasons, can be attributed to environmental factors such as climate and temperature which can significantly affect the accumulation of the antioxidant components in the plant tissue.

Key words: *Viscum album*, antioxidant activity, DPPH, ORAC, TEAC, total phenolic.

INTRODUCTION

European mistletoe (*Viscum album* L.) is an evergreen, hemi-parasitic plant, normally found growing on a variety of trees, especially pine, poplar, apple trees, locus trees etc. Although there are many varieties of mistletoe, including the American (*Phorandendron serotinum* or *Phorandendron flavescens*), the European (*Viscum*

album L.), and the Korean (*V. album* L. coloratum), most investigative work has been done on European mistletoe. A number of biological effects, such as anticancer (Melzer et al., 2009), apoptosis-inducing (Büssing and Schietzel., 1999), antibacterial, antiviral, and immunomodulatory activities have been reported (Hajtó et al., 2005). In research paper Nwanjo (2007), investigated the role of aqueous *V. album* extract on hypoglycaemic and antioxidant potentials in streptozotocin (STZ) induced diabetic Wistar rats. This investigation shows that the aqueous extract of *V. album*

*Corresponding author. E-mail: svicas@uoradea.ro. Tel: +40 259 412550; Fax: +40 0259 416274.

leaves in addition to being hypoglycaemic seems to be effective for reducing oxidative stress and free radical-related diseases including diabetes.

Ofem et al. (2007), investigated the effect of the crude aqueous extract from *V. album* leaves on arterial blood pressure and heart rate in albino Wistar rats. The results of this study shown that the crude mistletoe extract significantly lowered the blood pressure but had no effect on the heart rate in normotensive rats. The antioxidant molecules found in mistletoe are represented by flavonoids (quercetin and quercetin methyl ethers, accumulated on the plant surface, occasionally also the flavonol kaempferol and its methyl derivatives, and rarely naringenin) (Haas et al., 2003) and phenolic acids, such as digallic and o-coumaric acid in the free or glycosilated forms (Luczkiewicz et al., 2001). The phytochemical profile of mistletoe depends on the host trees of this plant (Luczkiewicz et al., 2001). Mistletoe (*V. album*) is a hemiparasitic plant, that can biosynthesised their own compounds, but it can take some nutrients from the host trees. It has been suggested that pharmacologically active compounds may pass from the host trees to the parasitic plants (Büssing and Schietzel, 1999). So far, literature studies haven't reveal exactly what compounds are only biosynthesised by mistletoe and that could be taken from the host trees. Many plant extracts exhibit efficient antioxidant properties due their phytoconstituents, including phenolics (Aqil et al., 2006; Miliuskas et al., 2004). ÖnayUçar et al. (2006) investigated the antioxidant activity of methanol extract of mistletoe (*V. album*), using DPPH method, ferric thiocyanate method, and thiobarbituric acid method.

The authors were shown that the antioxidant activity of mistletoe extract depends on both the harvesting time and the host trees. Also, the antioxidant effects of *V. coloratum* that depends on the flavonoids present in the mistletoe (Leu et al., 2006; Yao et al., 2006; Shi et al., 2006) was intensively investigated. (Choudhary et al., 2010) isolated from the methanol extract of *V. album* six compounds, which were found to posses anti-glycation activity, whereas compounds: 3-(4-acetoxy-3,5-dimethoxy)-phenyl-2E-propenyl-β-D-glucopyranoside and 4',5-dimethoxy-7-hydroxy flavanone exhibited antioxidant activity. The objective of this study was to investigate the influence of the seasons (May, July, and December) on antioxidant activity of *V. album* samples originating from five different trees, located in North-West of Romania country. Also, we investigated the influence of the extraction solvent (water and ethanol) on antioxidant activity of mistletoe.

MATERIALS AND METHODS

Plant material

Leaves and stems of *V. album* were harvested in December 2008, May and July 2009, from five different host trees located in North-West of Romania country (Borod-Gheghie region). The plant

materials were labeled accordingly with the host trees, thus:

Acer campestre (VAA), *Mallus domestica* (VAM), *Fraxinus excelsior* (VAF), *Populus nigra* (VAP) and *R. pseudoacacia* (VAR) for easy identification.

The mistletoe and host trees were identified by Dr. S. Pantea, University of Oradea and a voucher specimen (VA 101) of the mistletoe plants was deposited in the Herbarium of the Environmental Protection Faculty from University of Oradea.

Extraction methods

Aqueous and ethanol mistletoe extracts

Fresh leaves and stems (2 g) were homogenized with 10 ml distilled water, or with 10 ml 98% ethanol using an Ultra Turax homogenizator, for 1 min. This mixture was centrifuged (10 000 rpm, at 4°C, for 10 min) and the supernatants were filtered through filter paper. The filtrate was used for the antioxidant activity measurements and total phenolics content.

Determination of antioxidant activities

2,2-diphenyl-1-picrylhydrazyl (DPPH) method

The DPPH radical-scavenging activity was determined using the method proposed by Brand-Williams et al., (1995). The reaction was performed in 12 well-plate. A volume of 200 μl sample and 1.4 ml DPPH solution (80 μM) were added to each microplate well. The decrease in the absorbance of the resulting solution was monitored at 515 nm for 30 min. The percentage of scavenging effect of different extracts against DPPH radicals, was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = ((A_0 - A_s) \times 100) / A_0$$

Where, A_0 is absorbance of the blank, and A_s is absorbance of the samples at 515 nm.

Oxygen radical absorbance capacity (ORAC) method

The ORAC method measures antioxidant inhibition of peroxy radical-induced oxidations, and thus reflects classical radical chain breaking antioxidant activity by hydrogen atom transfer. The ORAC assay was performed essentially as described by Huang et al. (2002). A volume of 150 μl of working solution of sodium fluorescein (4×10^{-3} mM) was added to 25 μl samples, in a 12 well-microplate. The plate was allowed to equilibrate by incubating it for a minimum of 30 min in the Synergy™ HT Multi-Detection Microplate Reader (BioTek Instruments, Winooski, VT) at 37°C. Reaction was initiated by the addition of 25 μl of 2,2'-azobis(2-amidino-propane) dihydrochloride (AAPH) solution (153 mM) and the fluorescence was then monitored kinetically with data taken every minute, at 485 nm, 20 nm bandpass excitation filter, and a 528 nm, 20 nm bandpass emission filter.

ORAC values were calculated as described by Cao and Prior (1998). The area under the curve (AUC) and the Net AUC of the standards and samples were determined using Equations 1 and 2 respectively.

$$\text{AUC} = 0.5 + (R_2/R_1) + (R_3/R_1) + (R_4/R_1) + \dots + 0.5(R_n/R_1) \quad (1)$$

Where R_1 is fluorescence value at the initiation of reaction and R_n - fluorescence value after 30 min.

$$\text{Net AUC} = \text{AUC}_{\text{sample}} - \text{AUC}_{\text{blank}} \quad (2)$$

The standard curve was obtained by plotting the Net AUC of different Trolox concentrations against their concentration (6.25 to 100 μM). ORAC values of samples were then calculated automatically using Microsoft Excel to interpolate the sample's Net AUC values against the Trolox standard curve.

Trolox equivalents antioxidant capacity (TEAC) method

The TEAC is a spectrophotometric method, widely used for the assessment of antioxidant activity of various substances. This method measures the ability of compounds to scavenge the 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical cation in relation to Trolox. ABTS was dissolved in distilled water to a 7 mM concentration. ABTS^+ was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate and allowing the mixture to stand, in the dark, at room temperature for 12 to 16 h before use. ABTS stock solution was diluted with ethanol in order to obtain an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 17 μl of extract to 170 μl of diluted ABTS^+ , the interaction between the antioxidants and the ABTS^+ was monitored spectrophotometrically at 734 nm (Arnao et al., 2001). The results were expressed in mM Trolox equivalents/g fresh matter.

Total phenolics determined by the Folin-Ciocalteu method

Total phenolic content was determined by the Folin-Ciocalteu method (Singleton et al., 1999). Mistletoe extract (23 μl) was mixed with 1817 μl distilled water, 115 μl Folin-Ciocalteu reagent (dilution 1:10, v/v) and 345 μl of 15% Na_2CO_3 solution, and the mixture incubated at room temperature, in the dark, for 2 h. The absorbance was measured at 765 nm using a spectrophotometer (BioTek Synergy). The calibration curve was linear for the range of concentrations between 0.1 to 0.5 mg/ml gallic acid. The results were expressed in mg gallic acid equivalents (GAE)/g fresh matter).

Statistical analysis

All data were expressed as mean \pm standard deviation (SD) of three replications for each mistletoe extract tested. The data obtained from the antioxidant activity tests were analyzed statistically by the two-way ANOVA and Bonferroni post-test to compare each extract to VAA extract. The probability level of less than 0.05 was accepted as significant.

RESULTS AND DISCUSSION

Antioxidant activity

Phenolic compounds have attracted the interest of many researchers because they are powerful antioxidants and can protect the human body from oxidative stress. The antioxidant activity of phenolics is mainly due their redox properties. Antioxidant properties of aqueous and ethanol extracts from leaves and stems of mistletoe were determined by three methods. Their ability to deactivate stable DPPH radical, to inhibit peroxy radical and deactivation of cation radicals ABTS^+ .

The present study inquired a variety of *in vitro* tests, based on the capacity to scavenge free radicals. On the basis of the chemical reactions involved, major

antioxidant capacity can be divided into two categories:

i) hydrogen atom transfer (HAT) and ii) single-electron transfer (SET) reaction.

HAT-based procedures measure the classical ability of an antioxidant to quench free radicals by hydrogen donation (ORAC method). SET-based method detect the ability of a potential antioxidant to transfer one electron to reduce a species, including metals, carbonyls, and radicals (TEAC method).

DPPH inhibition by mistletoe extracts

The comparative antioxidant activity of *V. album* leaves and stems hosted by different host trees, in three different periods (May, July and December) evaluated by DPPH method is presented in Table 1. In general, our results showed that the DPPH scavenging effect (%) was higher in May and July, comparative with December, where some aqueous extracts lose their antioxidant activity. For example, the highest DPPH scavenging effect (%) was recorded in the case of mistletoe growing on *R. pseudoacacia* (VAR) (11.49 ± 0.04 , 10.97 ± 0.01 and 2.72 ± 0.01 in July, May and in December, respectively). Also, aqueous stem extracts exhibited antioxidant activity, but its activity was lower than in leaves. Regarding to the ethanol extracts of mistletoe, we recorded the DPPH scavenging effect (%) in all three months, both in leaves and stems. Results showed that the DPPH scavenging effect (%) varied between 77.19 ± 0.00 in the case of mistletoe harvesting from *Fraxinus excelsior* in July to 40.17 ± 0.03 in the same case but harvesting in December. The DPPH scavenging effect (%) of stem ethanol extracts was lower than in leaves, but the differences were not so pronounced as in the case of aqueous extract.

Similar results were obtained by ÖnayUçar et al., 2006, who investigated antioxidant activity of methanol extract of *V. album* grown on different host trees that are harvested in July. Their results showed that mistletoe hosted by *R. pseudoacacia* exhibited 73.44% inhibition of DPPH, and mistletoe hosted by *A. campestre* presented 59.52% inhibition of DPPH. The slight modification between our results and theirs can be assigned to the solvent used for extraction and also, to environmental factors. Sharma and Bhat (2010) showed that the absorbance profiles of DPPH were highest in buffered methanol solution, followed by methanol and ethanol solutions. Higher absorbance in methanol solution implies better sensitivity vis-à-vis ethanol solution of DPPH. Roman et al. (2009) investigated the efficiency of ultrafiltration process on the antioxidant activity of aqueous extract of *V. album*. The values obtained by the DPPH assay ranges between 66.2 and 88.2% DPPH inhibition for mistletoe concentrated extract. The

Table 1. DPPH scavenging effect (%) of aqueous (A) versus ethanol (B) extracts of mistletoe leaves and stems harvested in December 2008, May and July 2009, from five different host trees.

Mistletoe samples	DPPH scavenging effect (%)					
	Aqueous extract			Ethanol extract		
	May	July	December	May	July	December
	Leaves					
VAA	5.51 ± 0.00	6.64 ± 0.00	0.00	49.87 ± 0.02	50.47 ± 0.00	56.93 ± 0.01
VAF	7.29 ± 0.02***	7.83 ± 0.01***	0.74 ± 0.00***	49.02 ± 0.00***	77.19 ± 0.00***	40.17 ± 0.03***
VAP	2.49 ± 0.00***	6.98 ± 0.01***	0.00ns	52.17 ± 0.00***	55.06 ± 0.00***	44.00 ± 0.01***
VAM	5.71 ± 0.01***	2.22 ± 0.01***	2.55 ± 0.00***	60.51 ± 0.03***	59.06 ± 0.00***	44.77 ± 0.00***
VAR	10.97 ± 0.01***	11.49 ± 0.04***	2.72 ± 0.01***	41.62 ± 0.00***	63.91 ± 0.00***	76.60 ± 0.02***
	Stems					
VAA	4.94 ± 0.03	6.64 ± 0.00	0.00	48.48 ± 0.03	42.04 ± 0.00	40.85 ± 0.00
VAF	6.34 ± 0.02***	0.00***	0.50 ± 0.00**	34.04 ± 0.00***	37.19 ± 0.02***	39.32 ± 0.00***
VAP	0.88 ± 0.00***	6.72 ± 0.00ns	0.00ns	50.38 ± 0.02***	40.68 ± 0.00***	43.57 ± 0.00***
VAM	3.57 ± 0.00***	0.00***	0.00ns	59.27 ± 0.00***	53.61 ± 0.00***	34.8 ± 0.00***
VAR	10.07 ± 0.01***	2.56 ± 0.02***	2.31 ± 0.01***	31.91 ± 0.00***	61.70 ± 0.01***	69.8 ± 0.00***

The data were expressed as means ± standard deviation (n=3) and evaluated by two-way ANOVA and Bonferroni post-test to compare each extracts to VAA extract. Differences were considered to be statistically significant if ** p < 0.001, *** p < 0.0001, ^{ns} – no significant.

correlation coefficient between data of DPPH inhibition and total protein content was 0.94, confirming that viscolectins have a great contribution to the radical scavenging activity, besides phenolic compounds from *Viscum* extract.

Other research paper (Oluwaseun and Ganiyu, 2008) investigated the antioxidant properties of methanol extracts of *V. album* isolated from cocoa and cashew trees in South Western part of Nigeria. The scavenging ability of each methanol extract against DPPH followed a dose-dependent pattern (0 to 10 mg/ml). The free radical scavenging ability of the *V. album* extract from cocoa tree performs better than that from cashew tree, that is in agreement with total phenol content (182 mg/100 g and 160 mg/100 g respectively).

ORAC method

The results obtained by ORAC assay are shown in Figure 1. In the aqueous leaves, the values obtained varied between 9.64 ± 0.097 mM Trolox equivalents/g fresh matter for the VAM extracts that are harvesting in December to 0.17 ± 0.66 mM Trolox equivalents/g fresh matter for the mistletoe that are growing on *A. campestris* (VAA) and harvesting in May. Also, the results obtained in the case of aqueous stem extracts are similar with the results obtained in the case of leaves extracts, and the ORAC values varied between 9.13 ± 0.85 mM Trolox equivalents/g fresh matter for the VAM extracts that are harvesting in December to 0.37 ± 0.89 mM Trolox equivalents/g fresh matter for the VAA extracts,

harvesting in May. Ethanol mistletoe extracts exhibit the highest inhibition of peroxy radical-induced fluorescein oxidation in ORAC assay.

The highest value was recorded in the case of leaves VAP extract, harvesting in July (10.73 ± 1.90 mM Trolox equivalents/g fresh matter) and VAP extract, harvesting in May (10.42 ± 3.83 mM Trolox equivalents/g fresh matter). In general, antioxidant activity of leaves and stem mistletoe ethanolic extracts did not differ significantly. Our results showed that the ethanol extract exhibit highest ability to inhibit peroxy radical. These results are in concordance with the data obtained by Al-Duais et al. (2009) in which is reported that the raw leaves ethanol has 103.3 ± 2.5 mmol/100 g Trolox equivalents and water extract of *Cyphostemma digitatum* (Vitaceae) has 16.7 mmol/100 g Trolox equivalents.

TEAC method

Based upon the conducted research, it has been found that all mistletoe extract (aqueous or ethanol, leaf or stem) from three different seasons (May, July and December) have the ability of scavenging cation-radicals ABTS⁺ (Figure 2). The highest level of scavenging radicals provided water extract but the lowest deactivation level had ethanol extract. The leaves of aqueous mistletoe extract that are growing on *R. pseudoacacia* (VAR) recorded the highest TEAC value (1.7 ± 0.04 mM Trolox equivalents/g fresh matter) while for the ethanol extracts the highest level of scavenging cation-radicals ABTS⁺ was recorded for leaves from VAP

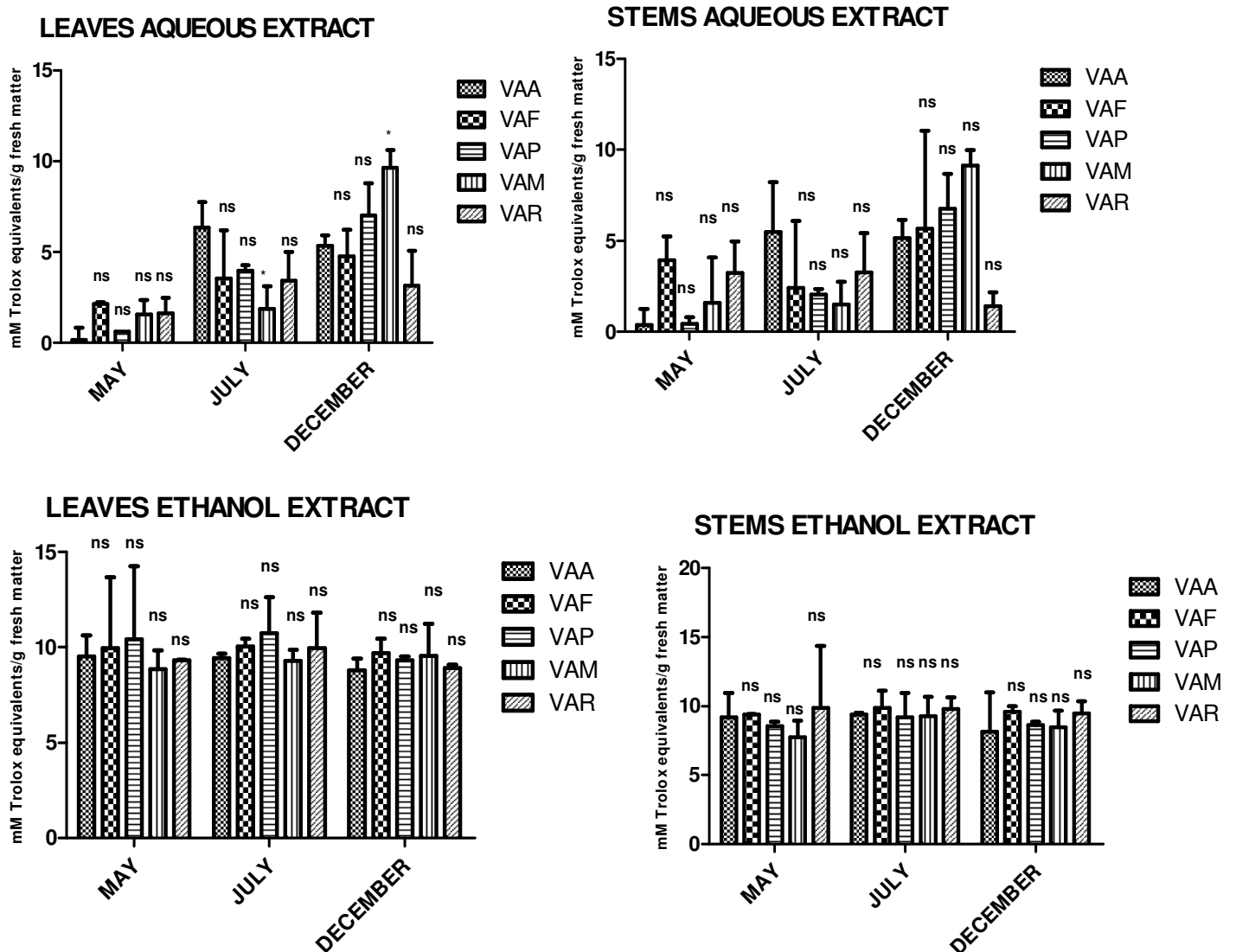


Figure 1. The antioxidant capacity (as determined by ORAC assay) of fresh leaves and stems from mistletoe extracts (in water or ethanol) expressed in mM Trolox equivalents/g fresh matter, harvesting in three different period. The data were expressed as means \pm standard deviation ($n=3$) and evaluated by two-way ANOVA and Bonferroni post-test to compare each extracts to VAA extract. Differences were considered to be statistically significant if * $p < 0.05$. ^{ns} – no significant.

and VAF (0.96 ± 0.005 , and 0.95 ± 0.106 mM Trolox equivalents/g fresh matter respectively) harvesting in July. We may suppose that water extract had the highest antioxidant activity because it contain many bioactive compounds that have ability to scavenging cation-radicals $ABTS^+$, comparing with ethanol extract.

Total phenolics evaluation by the Folin Ciocalteu method

Total phenolics' content was determined and expressed as mg gallic acid echivalent/g fresh matter. In Figure 3 we presented comparatively the total phenolic content from

leaves of mistletoe, in three different periods (May, July and December). Generally, the content of total phenolic were higher in aqueous extract comparative with ethanol extract. In aqueous leaves extract, the highest phenolic content was found in VAR (209.51 ± 0.01 mg GAE/ g fresh matter), harvesting in May, while the lowest value was 83.93 ± 0.001 mg GAE/ g fresh matter for VAF, harvesting in July. The mistletoe stem extracts contained lower levels of phenolics, comparing with leaves, in both solvents. In ethanolic extract, the highest phenolic content was found in VAM (58.97 ± 0.009 mg GAE/ g fresh matter), harvesting in December, followed by VAA extract (51.96 ± 0.006 mg GAE/ g fresh matter). In a recent research paper (Vicaş et al., 2009) it was shown

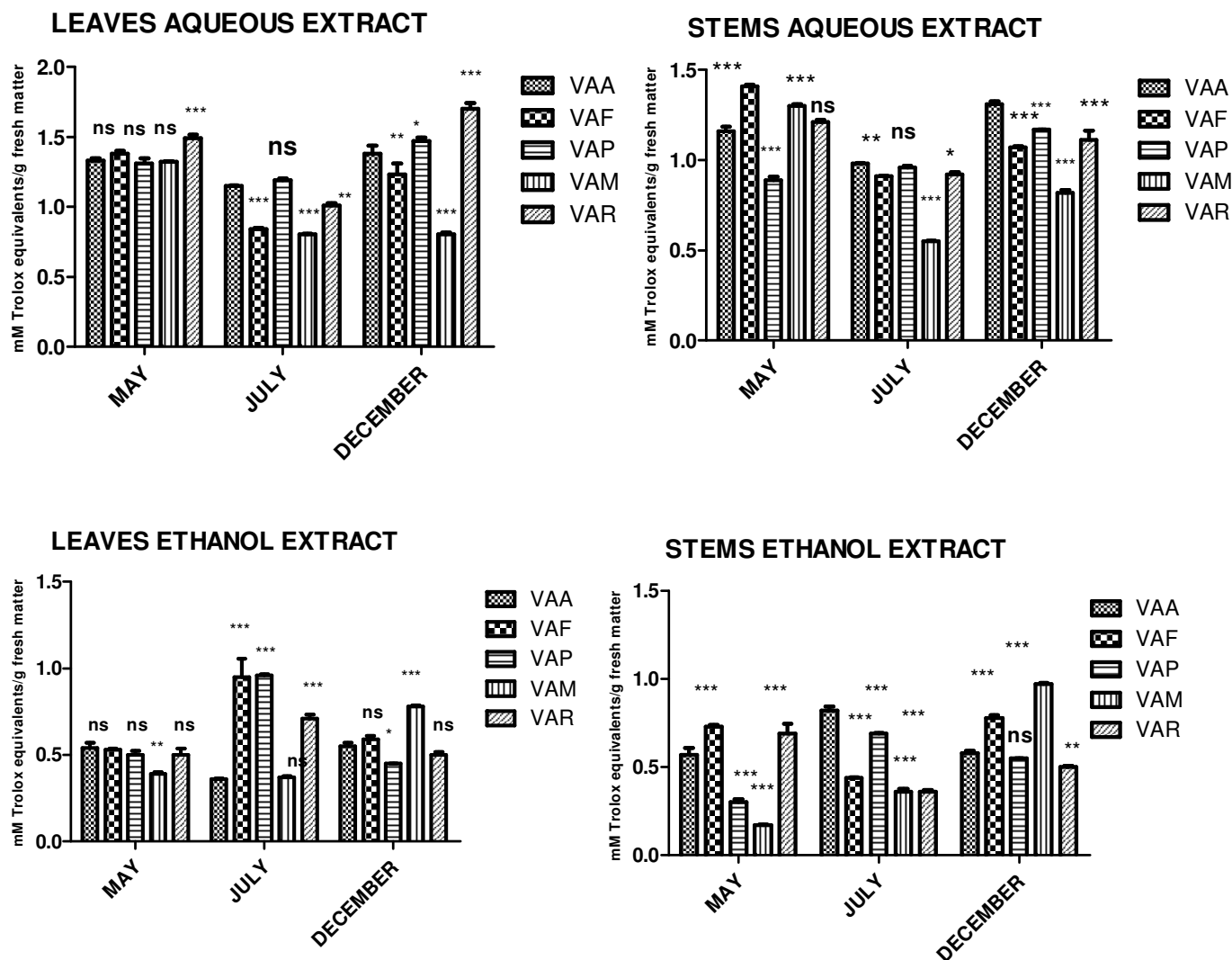


Figure 2. The antioxidant potential (as determined by TEAC assay) of fresh leaves and stems from mistletoe extracts (water and ethanol) expressed in mM Trolox equivalents/g fresh matter, harvesting in three different period. The data were expressed as means \pm standard deviation ($n = 3$) and evaluated by two-way ANOVA and Bonferroni post-test to compare each extracts to VAA extract. Differences were considered to be statistically significant if * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, ^{ns} – no significant.

that methanolic extracts of *V. album*, harvested in December 2008, were rich in phenolics, potential antioxidants, with ferric reducing ability. In that case, mistletoe leaves, originating from *A. campestre* (VAA), followed by VAM and VAF, showed higher concentration of phenolics. These results can be explained by the influence of harvesting time on the chemical composition and antioxidant activity.

There are many research studies that have established a correlation between the total phenol content of plants and their antioxidant properties (Kılıçgün and Altınar, 2010; Song et al., 2010; Tosun et al., 2009; Alali et al., 2007). The antioxidant activity of the *V. album* extract from *R. pseudoacacia* has the highest, especially in the case of DPPH method ($11.49 \pm 0.04\%$ for VAR leaves aqueous extracts harvesting in July; $76.60 \pm 0.02\%$ for

VAR leaves ethanol extracts harvesting in December) and TEAC assay (11.49 ± 0.04 mM Trolox equivalents/g fresh matter, 76.60 ± 0.02 mM Trolox equivalents/g fresh matter for VAR leaves aqueous extracts harvesting in May and December respectively) and this is also in agreement with total phenolic content.

Conclusion

The influence of the host tree of *V. album*, and also the harvesting time, may have key-role in the phenolic composition of mistletoe leaves or stems, and also, in their antioxidant activity. As it was observed by other authors (Cao and Prior, 1998) the values obtained for antioxidant capacity of extracts highly depends on the

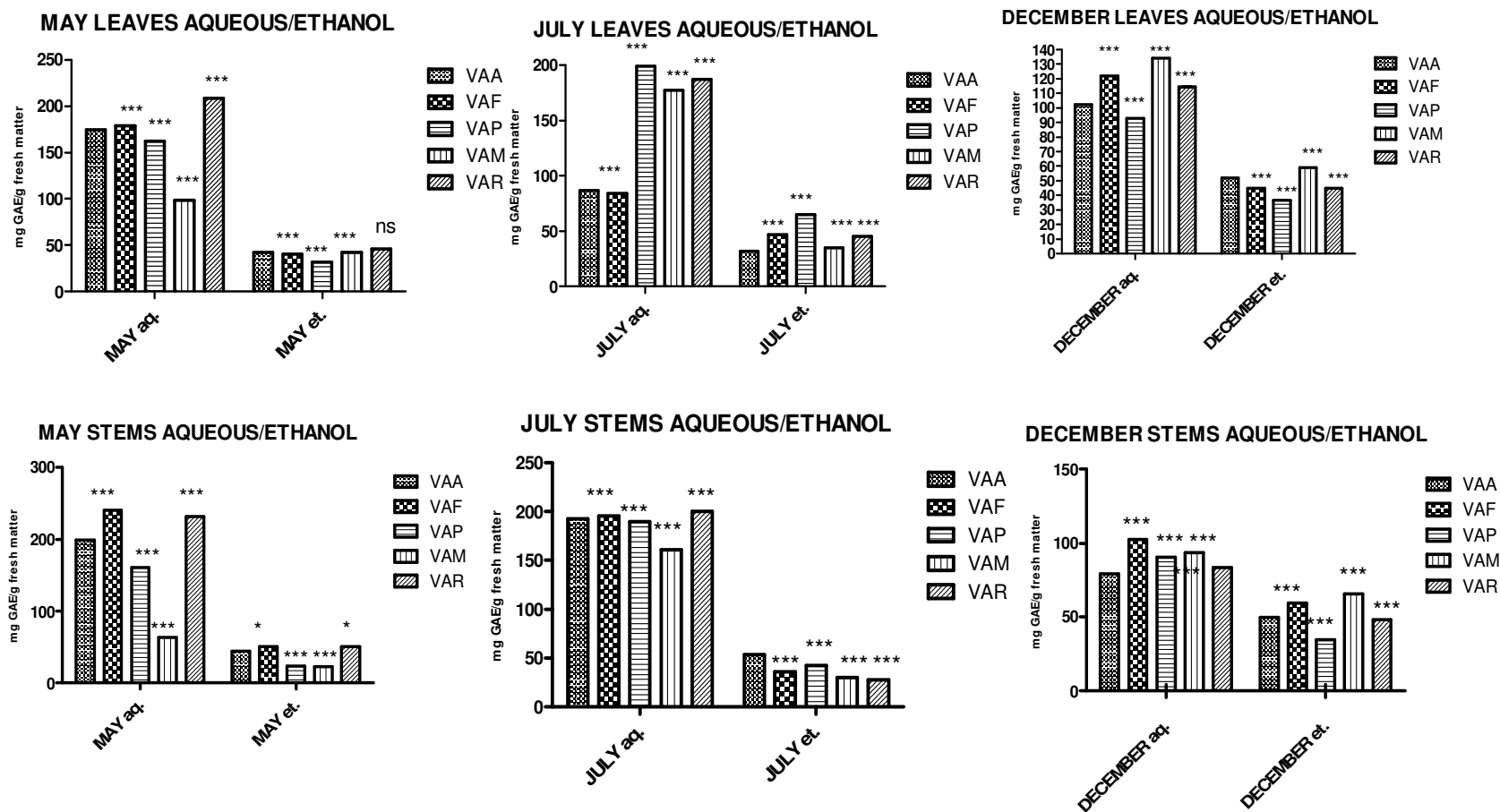


Figure 3. Comparative total phenolic content of *V. album* leaves and stems (aqueous or ethanol extracts) expressed in mg GAE/ g fresh matter, from three different seasons (May, July and December). The data were expressed as means ± standard deviation (n₁ = 3) and evaluated by two-way ANOVA and Bonferroni post-test to compare each extracts to VAA extract. Differences were considered to be statistically significant if * p < 0.05, *** p < 0.001, ^{ns}- no significant.

methodology used, without evident correlations between the values obtained by the different antioxidant procedures. The antioxidant potential is reflected by a more complex synergy of active molecules, not only phenolics. In general, anti-

oxidant activity of mistletoe determined by DPPH and ORAC assay recorded the highest activity in the ethanolic extract comparative with aqueous extracts. The promising mistletoe extracts recording with DPPH assay are VAF, hosted by

Fraxinus excelsior followed by VAR, hosted by *R. pseudoacacia* (77.19 and 76.6% respectively of DPPH scavenging effect). The values obtained from ORAC assay, in May, July and December did not differ significantly, when the ethanolic

extract was investigated. On the other hand, antioxidant activity of mistletoe extracts determined by TEAC and Folin-Ciocalteu assay recorded the highest activity in the aqueous extract comparative with the ethanol extracts. The mistletoe hosted by *R. pseudoacacia* (VAR) has the highest level of scavenging cation-radicals ABTS⁺ (1.7 ± 0.04 mM Trolox equivalents/g fresh matter).

In this study, the mistletoe hosted by *R. pseudoacacia* (VAR), harvested in May, proved to be the richest in total phenols (209.04 ± 0.001 mg GAE/ g fresh matter). In July, the highest level of total phenolic of aqueous mistletoe extracts were recorded in the case of VAP (199.03 ± 0.007 mg GAE/g fresh matter) followed by VAR (186.93 ± 0.003 mg GAE/ g fresh matter). The values obtained for total phenolics in aqueous mistletoe extracts, harvested in December decreased in the order:

VAM (134.18 ± 0.01 mg GAE/ g fresh matter) > VAF (122.08 ± mg GAE/g fresh matter) > VAR (114.57 ± 0.004 mg GAE/g fresh matter).

The differences in the antioxidant activity between the leaves and stems of mistletoes harvested from different trees, and different seasons, can be attributed to environmental factors such as climate and temperature which can significantly affect the accumulation of the antioxidant components in the plant tissue.

ACKNOWLEDGEMENTS

This work was supported by CNCSIS-UEFISCSU, project number 1120, PN II – IDEI 696/2008, Romania.

REFERENCE

- Alali FQ, Tawaha K, El-Elimat T, Syouf M, El-Fayad M, Abulaila K, Nielsen SJ, Wheaton WD, Falkinham JO 3rd, Oberlies NH (2007). Antioxidant activity and total phenolic content of aqueous and methanolic extracts of Jordanian plants: an ICBG project. *Nat. Prod. Res.*, 21(12):1121-1231.
- Al-Duais M, Müller L, Böhm V, Jetschke G (2009). Antioxidant capacity and total phenolics of *Cyphostemma digitatum* before and after processing: use different assays. *Eur. Food Res. Technol.*, 228: 813-821.
- Aqil F, Ahmad I, Mehmood Z (2006). Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants. *Turk J. Biol.*, pp. 177-183.
- Arnao MB, Cano A, Alcolea JF, Acosta M (2001). Estimation of free radical quenching activity of leaf pigment extracts. *Phytochem. Anal.*, 12: 138-143.
- Brand-Williams W, Cuvelier ME, Berset C (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und -Technologie/Food Sci. Technol.*, 28: 25-30.
- Büssing A, Schietzel M (1999). Apoptosis-inducing properties of *Viscum album* L. extracts from different host trees, correlate with their content of toxic mistletoe lectins. *Anticancer Res.*, 19: 23-28.
- Cao G, Prior RL (1998). Comparison of different analytical methods for assessing total antioxidant capacity of human serum. *Clin. Chem.*, 44: 1309-1315.
- Choudhary MI, Maher S, Begum A, Abbaskhan A, Ali S, Khan A, Shafique-ur-Rehman, Atta-ur-Rahman A (2010). Characterization and Antiglycation Activity of Phenolic Constituents from *Viscum album* (European Mistletoe). *Chem. Pharm. Bull.*, 58(7): 980-982.
- Haas K, Bauer M, Wollenweber E (2003). Cuticular waxes and flavonol aglycones of mistletoes. *Z. Naturforsch.*, 58c: 464-470.
- Hajtó T, Hostanska K, Berki T, Pálincás L, Boldizsár F, Németh P (2005). Oncopharmacological perspectives of a plant lectin (*Viscum album* Agglutinin-I): overview of recent results from *in vitro* experiments and *in vivo* animal models, and their possible relevance for clinical applications. *Evid Based Complement. Alternat. Med.*, 2: 59-67.
- Huang D, Ou B, Hampsch-Woodill M, Flanagan JA, Prior RL (2002). High-throughput assay of oxygen radical absorbance capacity (ORAC) using a multichannel liquid handling system coupled with a microplate fluorescence reader in 96-well format. *J. Agric Food Chem.*, 50(16): 4437-44.
- Kiliçgün H, Altiner D (2010). Correlation between antioxidant effect mechanisms and polyphenol content of *Rosa canina*. *Pharmacogn. Mag.*, 6(23): 238-241.
- Leu YL, Hwang TL, Chung YM, Hong PY (2006). The inhibition of superoxide anion generation in human neutrophils by *Viscum coloratum*. *Chem. Pharm. Bull.*, 54: 1063-1066.
- Luczkiewicz M, Cisowski W, Kaiser P, Ochocka R, Piotrowski A (2001). Comparative analysis of phenolic acids in mistletoe plants from various hosts. *Acta Poloniae Pharmaceutica-Drug Res.*, 58(5): 373-379.
- Melzer J, Iten F, Hostanska K, Saller R (2009). Efficacy and safety of mistletoe preparations (*Viscum album*) for patients with cancer diseases. A systematic review. *Forsch Komplementmed*, 16(4): 217-226.
- Miliauskas G, Venskutonis PR, vanBeek TA (2004). Screening of radical scavenging activity of some medicinal and aromatic plant extracts. *Food Chem.*, 85: 231-237.
- Nwanjo HU (2007). Free Radicals Scavenging Potential of the Aqueous Extract of *Viscum album* (Mistletoe) Leaves In Diabetic Wistar Rats Hepatocytes. *Internet J. Nutr. Wellness.*, 3: 2.
- Ofem OE, Eno AE, Imoru J, Nkanu E, Unoh F, Ibu JO (2007). Effect of crude aqueous leaf extract of *Viscum album* (mistletoe) in hypertensive rats. *Indian J. Pharmacol.*, 39: 15-19.
- Oluwaseun AA, Ganiyu O (2008). Antioxidant properties of methanolic extracts of mistletoes (*Viscum album*) from cocoa and cashew trees in Nigeria. *Afr. J. Biotechnol.*, 7(17): 3138-3142.
- ÖnayUçar E, Karagöz A, Arda N (2006). Antioxidant activity of *Viscum album* ssp. *album*. *Fitoterapia*, 77: 556-560.
- Roman GP, Neagu E, Radu GL (2009). Antiradical activities of *Salvia officinalis* and *Viscum album* L. Extracts concentrated by ultrafiltration process. *Acta Sci. Pol. Technol. Aliment.*, 8(3): 47-58.
- Sharma OP, Bhat TK (2010). DPPH antioxidant assay revisited. *Food Chem.*, 113: 1202-1205.
- Shi ZM, Feng P, Jiang DQ, Wang XJ (2006). Mistletoe alkali inhibits peroxidation in rat liver and kidney. *World J. Gastroenterol.*, 12: 4052-4055.
- Singleton VL, Orthofer R, Lamuela-Raventós RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Meth. Enzymol.*, 299: 152-178.
- Song FL, Gan RY, Zhang Y, Xiao Q, Kuang L, Li HB. (2010). Total phenolic contents and antioxidant capacities of selected chinese medicinal plants. *Intl. J. Mol. Sci.*, 11(6): 2362-2372.
- Tosun M, Ercisli S, Sengul M, Ozer H, Polat T, Ozturk E. (2009). Antioxidant properties and total phenolic content of eight *Salvia* species from Turkey. *Biol Res.*, 42(2): 175-181
- Vicaş S, Prokisch J, Rugină D, Socaciu C (2009). Hydrophilic and Lipophilic Antioxidant Activities of Mistletoe (*Viscum album*) as determined by FRAP method. *Not. Bot. Hort. Agrobot. Cluj*, 37(2): 112-116.
- Yao H, Liao ZX, Wu Q, Lei GQ, Liu ZJ, Chen DF, Chen JK, Zhou TS (2006). Antioxidative flavanone glycosides from the branches and leaves of *Viscum coloratum*. *Chem. Pharm. Bull.*, 54: 133-135.