biological effects, such as anticancer, antimycobacterial, apoptosis – inducing, antiviral, and immunomodulatory activities have been reported [9].

The chemical profile of mistletoe depends of the host trees, which this plant grow[11]. The main bioactive compounds that are found in mistletoe are: lectins, viscotoxin [16], oligo- and polysaccharides [7], alkaloids [14], flavonoids [8] and phenolic acids **[**11**]**.

Although it is know that there are morphological and, especially, phytochemical differences in mistletoes occupying different species of host trees, the aspect of mistletoe's taxonomy is not quite clear [13].

Literature is scarce regarding to the antioxidant effects of European mistletoe (*Viscum album*). But, in the last years, the antioxidant effects of *Viscum coloratum* was intensive investigated [10, 19, 17], effects that depends the flavonoids present in the mistletoe.

It has been suggested that pharmacologically active compounds may pass from the host trees to the parasitic plants [5]. One of the most important problems for researchers working on parasitic plants concerns the nature of the biological connections established between the host and the parasite. The influence of the host tree may play a very significant part in the assessment of the mistletoe as a plant raw material.

The aim of this study was to determine *in vitro* antioxidant activity and the total phenolics of the aqueous extract of leaves and stems of *V. album*, from five different host trees, harvested in December 2008, via FRAP assay, and to compare the results, depending on the host trees.

MATERIAL AND METHOD

Different variants of V. album plants were harvested in December 2008, from five different host trees located in North-West of Romania country. They were labeled according with the host trees, thus: Acer campestre (VAJ), Mallus domestica (VAM), Fraxinus excelsior (VAF), Populus nigra (VAP) and Robinia pseudoacacia (VAS) for easy identification.

Preparation of aqueous extract of Viscum album

Different V.album plants were harvested in May and July 2007 from five different host trees: *Acer campestre* (VAJ), *Mallus domestica* (VAM), *Fraxinus excelsior* (VAF), *Populus nigra* (VAP) and *Robinia pseudoacacia* (VAS) located in North-West region of Romania. Fresh leaves and stems (10g) were homogenizated with 50 ml distillated water, using a blender commercial, for 1 minute. This mixture was centrifugated (10 000 rpm, at 4^0C, for 10 minutes) and the supernatants were filtered through a filter paper. The filtrate was used for the measurements of antioxidant activity and phenol content.

Antioxidant activity determinations - FRAP assay

The ferric reducing antioxidant power (FRAP) assay was used to determine both hydrophilic and lipophilic antioxidant activities. The assay was determined according to the method of [4] with some modifications. The FRAP assay depends upon the ferric tripyridyltriazine (Fe(III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe(II)-TPTZ) by a reductant at low pH. The stock solutions included: 300 mM acetate buffer; 250 mg $Fe_2(SO4)_3 \cdot H2O$ dissolved in 50 ml distillated water; 150 mg TPTZ and 150 µl HCl, dissolved in 50 ml distillated water. The working solution (FRAP solution) was freshly

prepared by mixing 50 ml acetate buffer, 5 ml $Fe_2(SO4)_3 \cdot H_2O$ solution and 5 ml TPTZ solution. Mistletoe extracts (100 µl) were allow to react with 500 ml FRAP solution and 2 ml distillated water, for 1 hour in dark conditions. Readings of the colored product (ferrous tripyridyltriazine complex) were then taken at 595 nm. The standard curve was linear, between 5 and 100 mg/l vitamin C. Results were expressed in mg/l vitamin C equivalents/ g fresh weight. Adequate dilution was needed if the FRAP value measured was over the liniar range of the standard curve.

Antioxidant determination

Total phenolic content was determined by the Folin-Ciocalteu method. This method combined 100 µl mistletoe extract, 2000 µl distillated water and 200 µl Folin-Ciocalteu reagent; then mixed well using a Vortex. The mixture was allowed to react for 3 minutes, and then 1 ml of 15% Na_2CO_3 solution was then added and mixed well. The samples were incubated at room temperature, in the dark for 2 hours. The absorbance was taken at 750 nm using a spectrophotometer. The standard curve was linear, between 0.1-0.5 mg/ml gallic acid. The results were expressed in gallic acid equivalents (GAE; mg/g fresh weight). Adequate dilution was needed if the absorbance value measured was over the linear range of the standard curve.

RESULTS AND DISCUSSIONS

Many plant extracts exhibit efficient antioxidant properties due their phytoconstituents, including phenolics [2, 12].

The studies about the chemical composition of mistletoe showed that this hemiparasitic plant contain flavonoid, specially, quercetin, kaempferol and its methyl ethers, and naringenin [8]. Also, phenolic acids were identified in the mistletoe (caffeic acid, sinapic acid, ferrulic acid, protecatechic acid, syringic acid, vanillie acid, anisic acid and gentisic acid) [6].

The relevant chemical reaction of the FRAP method involves a single electron reaction between Fe $(TPTZ)_2$(III) and a single electron donor ArOH (flavonoid, for example) according to the following reaction:

$$Fe\ (TPTZ)_2\ (III) \ + \ ArOH \ \rightarrow Fe\ (TPTZ)_2\ (II) \ + \ ArOH^{+\cdot}$$

Antioxidants are strong reducing agents and this is principally based on the redox properties of their hydroxyl groups and the structural relationships between different parts of their chemical structure [15].

The FRAP values for investigated aqueous extracts of fresh leaves and stems from *V. album* are shown in *tab 1*.

We noticed differences between the antioxidant activity of mistletoe extract from leaves and stems. Among the selected plants, crude aqueous extract of *V. album* leaves that grown on *Mallus domestica* (apple trees) (VAM) exhibits the highest antioxidant activity (0.52 mg/l vitamin C equivalent/g of fresh leaves), while the lowest antioxidant activity was recorded in the case of VAJ, that grown on *Acer campestre* (0.23 mg/l vitamin C equivalent/g of fresh leaves).

Table 1

The antioxidant potential, expressed in mg/l vitamin C equivalent/g leaves, as determined by FRAP assay) of *V. album* fresh leaves and stems from differen host trees, harvested in December

Samples	Antioxidant activity (mg/l vitamin C equivalent/g leaves)*	
	Leaves	Stems
VAJ	0.23 ± 0.03	0.54 ± 0.20
VAM	0.52 ± 0.05	0.90 ± 0.40
VAF	0.31 ± 0.10	0.70 ± 0.10
VAP	0.38 ± 0.07	0.73 ± 0.11
VAS	0.41 ± 0.20	0.81 ± 0.42

*Data are presented as mean ± SD (n=3)

Similar results were obtained in the case of antioxidant activity from *V. album* stems. Also, the highest activity was recorded in the case of VAM (0.90 mg/l vitamin C equivalent/g of fresh stem).

It was observed that in mistletoe extracts the antioxidant activity was exceptionally high in its stem than its leaves.The FRAP values were 2 times higher in stem than leaves. This is in concordance with another studies [1, 3].

Total phenolic content were expressed as mg GAE/g fresh weight, using the following equation based on the calibration curve (*fig.1a*): y = 0,0079x – 0,0604, R^2 = 0,9979, where x was the absorbance and y was the gallic acid equivalent (mg/g).

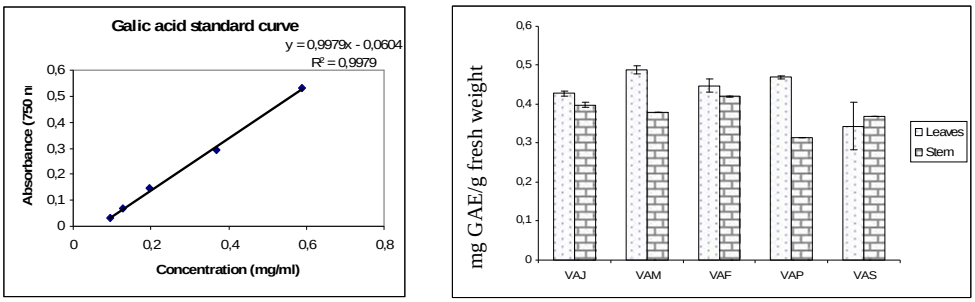

Figure 1 **a The standard curve of galic acid 0-0.8 mg/ml); b The content of total phenolics (mg GAE/g) from mistletoe growing on different host trees, as determinated by Folin-Ciocalteu method**

There was no recorded variation of the total phenolics in mistletoe extracts. The results are shown in *fig.1a* . Among leaves, the highest found in VAM (0.489 ± 0.01 GAE/FW) and the lowest in VAS (0.344 ± 0.06 GAE/FW). The amount of total phenolic content of leaves from mistletoe extracts under investigation can be arranged in descending order: VAM > VAP > VAF > VAJ > VAS. Stem of mistletoe extracts contain aproximativelly the same amount of phenolics, comparative with the leaves. The highest was found in VAF (0.42 ± 0.002

GAE/FW) and lowest in VAP (0.314 ± 0.001 GAE/FW), the descending order being: VAF > VAJ > VAM > VAS > VAP.

Our recent study [18] showed that the aqueous of *V. album* extracts, have antioxidant activity (determined by TEAC, ORAC and DPPH assay) and can be considered as good source for medicinal applications. Among the leaf sample aqueous extracts of *V. album*, those of VAS, following the VAM appear as the most promising sources of powerful antioxidants. The influence of the host tree and the harvesting time, may play a very significant role in the elaboration of specific antioxidants and becomes important parameter in the assessment of the mistletoe as an appropriate raw material for phytopharmaceutical formulas.

CONCLUSIONS

The results of this study show that the aqueous of *V. album* extracts have antioxidant activity and can be considered as good source of phenolic compounds. Among of aqueous extracts from *V. album*, the misteltoe stem from *Mallus domestica* (VAM), appears to be the most potent regarding its ferric reducing ability. We also reported for the first time the significant antioxidant potential of different plant parts of mistletoe (stems versus leaves), stems being more concentrated in antioxidants, being more protected from sun irradiation than leaves. The influence of the host tree may play a very significant role in the elaboration of specific antioxidants and becomes important parameter in the assessment of the mistletoe as a plant raw material for phytopharmaceutical formulas.

Further studies will attempt to identify and characterize the antioxidant biomarkers responsible for these properties (e.g. phenolics and flavonoids).

BIBLIOGRAPHY

1. Adedapo, A.A., Jimoh, F.O., Afolayan A.J., Masika P.J., 2009 - *Antioxidant Properties of the methanol extracts of the leaves and stems of Celtis africana*, Rec. Nat.Prod., 3 (1), p. 23-31.
2. Aqil, F., Ahmad, I., Mehmood, Z., 2006 - *Antioxidant and free radical scavenging properties of twelve traditionally used Indian medicinal plants*, Turk J. Biol., 30, p.177-183.
3. Banerjee, D., Chakrabarti, S., Hazra, A.K., Banerjee, S.M., Ray, J., Mukherjee, B., 2008 - *Antioxidant activity and total phenolics of some mangroves in Sundarbans*, Afr. J. Biotechnol., 7 (6), p.805-810.
4. Benzie, I.F., Strain, J. J., 1996 - *The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay,* Anal. Biochem, 239, p. 70-76.
5. 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, p. 23-28.
6. Büssing A., 2000 - *Mistletoe. The genus Viscum*, Harwood Academic Publishers, p.39.
7. Edlund, U., Henzel, A., Frose, D., Pfuller, U., Scheffler A., 2000 - *Polysaccharides from fresh Viscum album L berry extract and their interaction with Viscum album agglutin I*, Arzneimittelforschung 50, p. 645-651.
8. Haas K., Bauer, M., Wollenweber, E. 2003 - *Cuticular waxes and flavonol aglycones of mistletoes*, Z. Naturforsch, 58c, p. 464-470.

9. Hajto, T., Hostanska, K., Berki, T., Palinkas, L., Boldizsar, F., Nemeth 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, p. 59–67.
10. Leu, Y.L., Hwang, T.L., Chung, Y.M., Hong, P.Y., 2006 - *The inhibition of superoxide anion generation in human neutrophils by Viscum coloratum*, Chem. Pharm. Bull, 54, p. 1063-1066.
11. Luczkiewicz, M., Cisowski, W., Kaiser P., 2001 - *Comparative analysis of phenolic acids in mistletoe plants from various hosts,* Acta Pol Pharm. 58, p. 373-379.
12. Miliauskas, G, Venskutonis, P.R., vanBeek, T.A., 2004 - *Screening of radical scavenging activity of some medicinal and aromatic plant extracts*, Food Chem. 85, p. 231-237.
13. Ochocka, J.R., Piotrowski A., 2002 - *Biologically active compounds from European mistletoe (Viscum album L.).* Can. J. Plant Pathol. 24, p. 21-28.
14. Peng, H.Y., Zhang, Y.H., Han, Y. Wang, M. 2005 - *Studies on the anticancer effects of total alkaloid from Viscum coloratum.* Zhongguo Yhong Yao Za Zhi 30, p. 381-387.
15. Rice, Evans, C., Miller, N.J., Paganga, G., 1996 - *Structure-antioxidant activity relationships of flavonoids and phenolic acids,* Free Radic. Biol. Med., 20, p. 933-956.
16. Romagnoli, S., Ugolini, R., Fogolari, F., Schaller, G., Urech, K., Giannattasio, M., Ragona, L., Molinari, H., 2000 - *NMR structural determination of viscotoxin A3 from Viscum album* L. Biochem J. 350, p. 569-577.
17. Shi, Z.M., Feng, P., Jiang, D.Q., Wang, X.J., 2006 - *Mistletoe alkali inhibits peroxidation in rat liver and kidney,* World J. Castroenterol, 12, p. 4052-4055.
18. Vicaş, S., Rugina, D., Socaciu C., 2008 - *Antioxidant activities of Viscum album's leaves from various host trees,* Bulletin UASVM, Agriculture Cluj-Napoca. 65 (1), p. 327-332.
19. Yao, H., Liao, Z.X., Wu, Q., Lei, G.Q., Liu, Z.J., Chen, D.F., Chen, J.K., Zhou, T.S. 2006 - *Antioxidative flavanone glycosides from the branches and leaves of Viscum coloratum*, Chem. Pharm. Bull, 54, p.133-135.