SHORT REPORT

A C G publications

Rec. Nat. Prod. 6:4 (2012) 394-397

records of natural products

Antioxidant Potential of the Aerial Tissues of the Mistletoe *Loranthus europaeus* Jacq.

Alexandra Katsarou[1], Sophia Rhizopoulou[2 *] and Panagiotis Kefalas[3]

[1] *University of Athens, Department of Dermatology, Faculty of Medicine, Hospital A. Sygros, 5 Dragoumi st., Athens 16121, Greece*

[2] *University of Athens, Department of Botany, Faculty of Biology, Panepistimiopolis, Athens, 15784, Greece*

[3] *Mediterranean Agronomic Institute of Chania, P.O. Box 85, Chania 73100, Crete, Greece*

(Received January 23, 2011; Revised March 17, 2011; Accepted June 5, 2012)

Abstract: The aim of the study was to evaluate the antioxidant activity of extracts of aerial tissues (i.e. flowers, leaves, stems, twigs and berries) of the mistletoe *Loranthus europaeus* that grow on oak trees in a natural forest, in the mainland of Greece. Total phenolic content and antioxidant potential of aerial issues of *L. europaeus* was evaluated by the Folin-Ciocalteu method, the Ferric reducing antioxidant power assay, the free radical DPPH˙ scavenging and the Co(II)/EDTA induced luminol plateau chemiluminescence assay. Extracts of twigs and stems of *L. europaeus* exhibited higher antioxidant activity in comparison to that of fruits, leaves and flowers.

Keywords: Antioxidant activity; berries; flowers; fruits; leaves; *Loranthus europaeus*; phenolics; stems; twigs.

1. Plant Source

Loranthus europaeus Jacq. (Loranthaceae) is hemiparasitic mistletoe of South-Eastern Europe, Anatolia and South Russia [1, 2]. *L. europaeus* has a similar branching pattern to the evergreen mistletoe *Viscum album* L., but it is deciduous, yellow-berried mistletoe, with dull brown twigs, with flowers located in stipitate influoresences and respectively berries [3, 4]. *L. europaeus* grows mostly on branches of *Quercus* species [5, 6] and occasionally of chestnuts [7] as host trees.

The alliance of oaks and mistletoes became a symbol of knowledge and strength, and it was aptly rendered in the word “Druid” (i.e. the oak-knower), which is derived from the Greek word for oak (δρυς) and the suffix-ίδης [8, 9]. Mistletoes on oaks had a symbolism and a healing status that is very interesting, because both species were highly prized by ancient people, alchemists and herbalists [10, 11]. Mistletoes were found at spots where there had been ancient settlements and the most powerful healing mistletoe species supposed to be the so called “golden bough”, i.e. golden-yellow-berried mistletoe [12] that grew especially on oaks and in regions of Europe where the tribes originated [13].

* Corresponding author: E-Mail: srhizop@biol.uoa.gr, Fax: 0030 210 7274702

The article was published by Academy of Chemistry of Globe Publications
www.acgpubs.org/RNP © Published 7/20/2012 EISSN: 1307-6167

Although, the term mistletoe was originally applied to the evergreen, native species of Europe *Viscum album* [14], it broadly describes several hemiparasitic shrubs that grow attached to and within the branches of trees [15, 16, 17]. *L. europaeus* thrives on xylem sap diverted from hosts through direct xylem connections, with longitudinal strands, while there is not phloem link and exchange of photosynthates, thus its mineral nutrition is connected with the host tree [18, 19]. Mistletoes were recently recognized as ecological keystone species, in forests and woodlands worldwide [20, 21].

Tissues of mistletoes have been used for healing in traditional medicine and the most frequently studied species for antioxidants is the white-berried, evergreen *V. album* [22, 23, 24, 25], presumably because of its wide-ranging geographical distribution. In contrast, to the best of our knowledge the antioxidant potential of aerial tissues of *Loranthus europaeus* has not been hitherto reported.

An investigation was initiated to evaluate the antioxidant properties of the neglected and poorly studied mistletoe *Loranthus europaeus*. Branches of *Loranthus europaeus* were pickled from old, deciduous oak tress, *Quercus pubescens* Willd., growing in the mainland of Greece (Natura GR2450002, E 22°15′, N 38°39′), early in May of 2009 and 2010. The plant material was identified by Dr. Theophanis Contsantinidis of the University of Athens, Department of Ecology and Systematics, Athens, Greece. Voucher specimens of the investigated species have been deposited at the herbarium of the Department of Botany, University of Athens.

2. Previous Studies

In an early study [7], three flavonoids have been isolated from leaves of *L. europaeus* (i.e. 7-methyl kaempferol, rharnnocitrin-3-0-rhamnoside and 7-methyl-quercetin derivatives). In another work related with flavonoids and terpenoids isolated from of *L. europaeus*, the tissues used for the crude extract were not mentioned [26].

3. Present Study

Samples of immature berries, flowers, leaves, twigs and stems of *L. europaeus* were treated in the laboratory as described by previous investigators [26]. Pure antioxidants including gallic acid, caffeic acid and quercetin were tested at various concentrations; all chemicals and reagents used in this study were analytical grade and purchased from Sigma, Merck, Aldrich and Fluka. The total content of phenols was determined, in ethyl acetate (EtOAc) and methanol (MeOH) extracts according to the Folin-Ciocalteu method [27, 28] and the results were expressed as gallic acid equivalents (Table 1). The total phenolic content varied among the aerial tissues of *L. europaeus*. It ranged from 61 and 94 mg of gallic acid/ g of tissue measured in flowers to 320 mg measured in twigs; the TP content ranging in the order twigs stems fruits leaves flowers (Table 1).

DPPH free radical scavenging activity (% DPPH inhibition) and antiradical activity by ferric reducing power (FRAP) assays were performed as it has been described by other investigators [27] and the results are expressed as ascorbic acid equivalents (AAE). The values obtained by DPPH assay varied from the highest (92%) DPPH inhibition for the twigs to the lowest (27 %) for the flowers (Table 1); the DPPH (%) inhibition was ranging in the order: twigs stems leaves fruits flowers. The same order was detected with the FRAP assay, with the exception of leaves and flowers.

The antioxidant activity of grounded samples of all the aerial tissues was also determined using a highly sensitive, luminol chemiluminescence method [29, 30]. Values of IC_{50} of the extracts of *Loranthus europaeus* ranged from 24 for the fruits to 65 for the twigs. The results obtained from chemiluminescence, expressed in quercetin equivalents for each extract are shown in Table 1. the twigs extract was found to be 200 times more active than that of flowers and fruits. High correlation coefficients were obtained for all samples. The results might be useful for evaluating antioxidant efficiency, regarding the increased interest for antioxidants derived from natural resources; natural products are truly products of human knowledge [10, 31, 32].

According to the best of our knowledge this is the first report on the antioxidant potential of all the aerial tissues of *Loranthus europaeus*. Further studies on the chemical composition of a larger number of samples from different locations and throughout the year is required to identify the active metabolites and the determination of the antioxidant profile of the tissues of the mistletoe *Loranthus europaeus*.

Table 1. Total phenolic content (TPs) and antioxidant activity of MeOH and EtOAc extraxts of aerial tissues of *Loranthus europaeus*.

Samples	TPs (mg galic acid g^{-1} tissue)[a]	FRAP (mM AAE) [b]	DPPH (%)	IC_{50}[c], Correlation coefficient
MeOH extracts				
Fruits (berries)	226±4.3	37.99±0.2	86±0.5	$36 \pm 0.9 \times 10^{-2}$, 0.98
Flowers	94±3.3	22.87±0.4	79±0.9	$57 \pm 0.4 \times 10^{-1}$, 0.98
Leaves	158±1.5	14.47±0.3	86±0.2	$24 \pm 0.2 \times 10^{-2}$, 0.99
Twigs	320±9.0	57.33±0.5	92±0.8	$65 \pm 0.5 \times 10^{-4}$, 0.99
Stems	197±6.8	42.62±0.2	88±0.4	$62 \pm 0.5 \times 10^{-4}$, 0.98
EtOAc extracts				
Fruits (berries)	222±5.3	27.79±0.7	47±0.5	$24 \pm 0.5 \times 10^{-1}$, 0.96
Flowers	61±2.8	11.20±0.6	38±0.2	$46 \pm 0.3 \times 10^{-1}$, 0.97
Leaves	161±7.2	28.27±0.2	86±0.4	$46 \pm 0.8 \times 10^{-2}$, 0.98
Twigs	313±8.0	40.10±0.6	92±0.7	$49 \pm 0.5 \times 10^{-4}$, 0.97
Stems	246±7.4	31.35±0.6	90±0.6	$45 \pm 0.8 \times 10^{-4}$, 0.99

Data presented here are means of three samples ±S.D., at 95% confidence intervals, and with [a] gallic acid equivalents, [b] ascorbic acid equivalents and [c] quercetin equivalents.

References

[1] S. Balle, J. E. Dandy, J. S. L. Gilmour, R. E. Holltum, W. T. Stearn and D. Thoday (1960). *Loranthus*, *Taxon* **9**, 208-210.

[2] S. Snogerup and B. Snogerup (1997). *Loranthus* L. In: Flora Hellenica, *ed*. A. Strid and K. Tan, Koeltz, Königstein, vol. 1., p. 67.

[3] D. Zuber (2004). Biological flora of central Europe: *Viscum album* L., *Flora* **199**, 181-203.

[4] J. Briggs (2009). *Loranthus europaeus*, new to Britain, and notes on *Viscum album*, both new to Kew Gardens, *Haustorium* **56**, 2-3.

[5] G. Glatzel (1983). Mineral nutrition and water relations of hemiparasitic mistletoes: a question of partitioning. Experiments with *Loranthus europaeus* on *Quercus petraea* and *Quercus robur*. *Oecologia,* **56**, 193-201.

[6] E.-D. Schulze, N. C. Turner and G. Glatzel (1984). Carbon, water and nutrient relations of two mistletoes and their hosts: a hypothesis, *Plant Cell Environ.* **7**, 239-299.

[7] E. Harvala, J. Exner and H. Becker (1984). Flavonoids of *Loranthus europaeus*, *J. Nat. Prod.* **47**, 1054-1055.

[8] S. Rhizopoulou (2004). Symbolic plant(s) of the Olympic Games, *J. Exp. Bot.* **55**, 1601-1606.

[9] R. K. Barnhart and S. Steinmetz (2008). Chambers dictionary of etymology. Chambers Harrap Publishers Ltd., Edinburgh.

[10] J. M. Riddle (2002). History as a tool in identifying 'new' old drugs, *Adv. Exp. Med. Biol.* **505**, 89-94.

[11] M. De Cleene and C. Lejeune (2003). In: Compendium of symbolic land ritual plants in Europe, *ed*. M. De Cleene and C. Lejeune, Man and Culture Publishers, Ghent, pp. 411-425.

[12] W. R. Paton (1911). The golden bough, *Class. Rev*. **25**, 205.

[13] A. H. Krappe (1923). The myth of Balder: a study in comparative mythology, *Folklore* **34**, 184-215.

[14] J. D. Box (2000). Mistletoe *Viscum album* L. (Loranthaceae) on oaks in Britain, *Watsonia* **23**, 237-256.

[15] B. A. Barlow (1987). Mistletoes, *Biologist.* **34**, 261-269.

[16] M. Popp and A. Richter (1998). Ecophysiology of xylem-tapping mistletoes, *Prog. Bot.* **59**, 659-674.

[17] R. Vidal-Russell and D. L Nickrent (2008). Evolutionary relationships in the showy mistletoe family (Loranthaceae), *Am. J. Bot*. **95**, 1015-1029.

[18] H. Shen, W. Ye, L. Hong, H. Huang, Z. Wang, X. Deng, Q. Yang and Z. Xu (2006). Progress in parasitic plant biology: host selection and nutrient transfer, *Plant Biol.* **8**, 175-185.
[19] M. C. Press and G. K. Phoenix (2005) Impacts of parasitic plants on natural communities, *New Phytol.* **166**, 737-751.
[20] G. Glatzel and B. W. Geils (2009). Mistletoe ecophysiolgy: host-parasite interactions, *Botany* **87**, 10-15.
[21] D. M. Watson (2009). Parasitic plants as facilitators: more Dryad than Dracula? *J. Ecol.* **79**, 1151-1159.
[22] A. Büssing (2000). Biological and pharmacological properties of *Viscum album*. In: Mistletoe the genus *Viscum*, *ed.* A. Büssing, Harwood Academic Publishers, Amsterdam, pp. 123-182.
[23] E. Önay-Uçar, A. Karagöz and N. Arda (2006). Antioxidant activity of *Viscum album* ssp. *album*, *Fitoterapia* **77**, 556-560.
[24] J. Eggenschwiler, A. Patrignani, U. Wagner, H. Rehrauer, R. Schlapbach, L. Rist, M.H. Ramos and A. Viviani (2006). Gene expression profiles of different breast cancer cells compared with their responsiveness to fermented mistletoe (*Viscum album* L.) extracts Iscador from oak (*Quercus*), pine (*Pinus*), white fir (*Abies*) and apple tree (*Malus*) *in vitro*, *Arzneimittelforschung.* **56**, 483-496.
[25] S.I. Vicaş, D. Rugină and C. Socaciu (2008). Antioxidant activities of *Viscum album*'s leaves from various host tress, *Bull. UASVM.* **65**, 327-332.
[26] M. Cholakova, V. Christov, D. Dimitrova, L. Evstatieva, R. Alexandrova and E. Nikolova (2002). Flavonoid and terpenoid isolated from *Loranthus europaeus* with stimulatory effect on lymphocyte proliferation, *Exp. Pathol. Parasit.* **91**, 45-48.
[27] A. Guri, P. Kefalas and V. Roussis (2006). Antioxidant potential of six pine species, *Phytother. Res.* **120**, 263-266.
[28] A. Arnous, D. P. Makris and P. Kefalas (2002). Correlation of pigment and flavanol content with antioxidant properties in selected aged regional wines from Greece, *J. Food Compos. Anal.* **15**, 655-665.
[29] I. Parejo, C. Petrakis and P. Kefalas (2000). A transition metal enhanced luminol chemiluminescence in the presence of a chelator, *J. Pharm. Toxic. Meth.* **43**, 183-190.
[30] I. Parejo, C. Codina, C. Petrakis and P. Kefalas (2000). Evaluation of scavenging activity assessed by Co(II)/EDTA-induced luminol chemiluminescence and DPPH (2,2-diphenyl-1-picrylhtdrazyl) free radical assay, *J. Pharm. Toxic. Meth.* **44**, 507-512.
[31] E. J. Buenz, D. J. Schnepple, B. A. Bauer, P. L. Elkin, J. M. Riddle and T. J. Motley (2004). Techniques: bioprospecting historical herbal texts by hunting for new leads in old tomes, *Trends Pharmacol. Sci*. **25,** 494-498.
[32] S. Rhizopoulou and A. Katsarou (2008). The plant material of medicine, *Adv. Nat. Appl. Sci.* **2**, 94-98.