



# Biochemical analysis of European mistletoe (*Viscum album* L.) foliage and fruit settled on Persian ironwood (*Parrotia persica* C. A. Mey.) and hornbeam (*Carpinus betulus* L.)

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## ABSTRACT

Mistletoe (*Viscum album* L.) from Santalaceae family is a hemiparasitic plant containing triterpene acids which widely was used as complementary remedy in cancer therapy for decades. Mistletoe is growing on various tree species as well as some endemic and native species of Hyrcanian forests of Iran such as *Parrotia persica* (Persian ironwood) and *Carpinus betulus* (Hornbeam). The aim of this work was to compare the amount of triterpene acids including oleanolic acid (OA), betulinic acid (BA) and ursolic acid (UA) in foliage (leaves and stems) and fruit of mistletoes settled on *P. persica* and *C. betulus* as hosts in autumn. In this study, mistletoe plant parts were collected from three bases of each host species in Daeiz forest of Hyrcanian forests of Iran. The samples were extracted with ethyl acetate and analyzed by HPLC. The results indicated that the host species had no significant effect on the amount of triterpene acids in the mistletoes. But the organ type (foliage and fruit), had significant effect on the BA content meanwhile it didn't show any significant effect on the OA. The highest level of BA was significantly detected in foliage of mistletoes on *P. persica* and *C. betulus* (1.23 and 0.97 mg/g dry weight) respectively. Independence of statistical analysis, the highest content of OA (8.52 mg/g dry weight) was observed in the foliage of *V. album* on *P. persica*. On the other hand, BA was not detected in mistletoe fruits on both host species. UA was not detected in any samples.

## 1. Introduction

Medicinal plants application coincides with the beginning of human life history (Sadeghi-Aliabadi et al., 2006). These plants have played a major role in the improvement and development of drugs and have a long history of treating cancer and tumors. Almost 60% of the effective drugs in cancer treatment come from natural resources such as plants, marine organisms, and microorganisms (Harvey, 2000; Piao et al., 2004; Büssing et al., 2005).

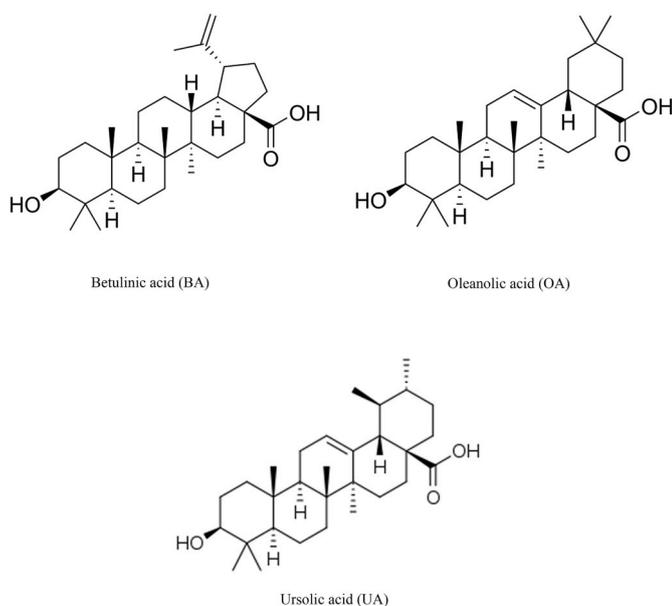
Mistletoe (*Viscum album* L.) belongs to the Santalaceae family (Gastauer et al., 2017). It is a hemiparasitic dioecious plant which contaminates different trees through development of its root like organ, haustoria, in the host xylem (Hosseini et al., 2007; Arruda et al., 2012; Mutlu et al., 2015; Park et al., 2019).

The viscous fruit is a small berry, which contains sticky and viscose glaze and is dispersed by birds (Zuber, 2004; Kołodziejek et al., 2013; Raftoyannis et al., 2015).

*Viscum album* L. contains various bioactive compounds such as lectins, viscotoxins, flavonoids, polysaccharides, alkaloids, terpenoids, tannins, phytosterol, phenolic acids, resins, saponin, and organic acids (Luczkiewicz et al., 2001; Vicaş et al., 2011; Wójciak-Kosior et al., 2017). Various researches have confirmed mistletoe therapeutic effects and its important role in the prevention and treatment of different diseases, so it is used as anticancer, antioxidant, anti-diabetes, blood pressure lowering, antibacterial, antiviral, apoptosis inducer, immune system stimulant, anti-inflammatory, anticonvulsants and cytotoxic (Orhan et al., 2005; Gupta et al., 2012; Park et al., 2019). Some medicinal effects of *V. album* is related to the presence of triterpene acids such as oleanolic acid (OA), betulinic acid (BA) and ursolic acid (UA) with the molecular formula  $C_{30}H_{48}O_3$  (Fig. 1) (Mulsow et al., 2016; Wójciak-Kosior et al., 2017). These compounds are effective in the treatment of acute lymphoblastic leukemia, myeloid leukemia (Delebinski et al., 2015) and induction of cell death in rat melanoma cells (Strüh et al., 2012). Also triterpene acids are known to exert

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**Fig. 1.** Chemical structures of the pentacyclic triterpenoids betulinic acid (BA), oleanolic acid (OA), and ursolic acid (UA).

apoptosis-inducing activity against different malignant cells (Urech et al., 2005) and have antioxidant properties which can suppress the free radicals produced during radiotherapy and chemotherapy (Bussing et al., 1994).

Mistletoe can contaminate a large number of tree species. The host species is an important factor for phytochemical properties and biological activities of *V. album* so that various studies have shown that the therapeutic effects and active ingredients of *V. album* can vary considerably depending on the host species (Vicaş et al., 2011; Orhan et al., 2014; Wójciak-Kosior et al., 2017). Since the production of biologically active substances is strongly depended on the vegetation period of plants (Barbasz et al., 2012) so the phenological stage is one of the key parameters for obtaining valuable plant materials (Wójciak-Kosior et al., 2017). Also various studies have shown that the quantity and quality of secondary metabolites can vary in different organs of plant (Figueiredo et al., 2008; McCreath and Delgoda, 2017). For example, in research on *Sambucus ebulus*, the highest level of flavonoids was observed in the leaves, flowers and fruits of this plant in comparison with the stem (Mazandarani et al., 2010). Also, in another study on the *Proveskia abrotanoides*, the soluble carbohydrates content was significantly different in various organs and phenological stages (Sabbagh et al., 2017). Since the medicinal activities of parasitic and hemiparasitic species are closely related to the ecological nature of the host species (Vicaş et al., 2011; Wójciak-Kosior et al., 2017), it is expected the quantity and quality of the bioactive compounds of *V. album* to be different depending on host species and organ type, therefore, the present study was conducted for the first time in order to recognize the effect of *Parrotia persica* and *Carpinus betulus* hosts on the quantity and quality of triterpene acids of *Viscum album* L. at the reproductive stage in Daeiz forest of Hyrcanian forests, Mazandaran province, Iran.

## 2. Material and methods

### 2.1. Plant material

*Viscum album* L. plants settled on *Parrotia persica* and *Carpinus betulus* in fruiting stage were collected from Daeiz forest of Hyrcanian forests in Mazandaran province of Iran in the autumn, 2017. In this region the average of annual rainfall is 668 mm and the annual temperature average is 17° Table 1. The sampling was performed from 3 bases of each

host species (3 replicates) (Fig. 2). The selected host trees were identical in terms of diameter, height and morphology. Also they were free of each stress, such as pests, diseases and rottenness in the trunk (Fig. 3).

### 2.2. Sample preparation

#### 2.2.1. Sample drying

The one, two and three year old foliage and fruit of the samples separately were freeze-dried in liquid nitrogen and then placed in a freeze-dryer (OPERON, OPR- FDU- 7012, Korea) for 72 h. The dried specimens of the foliage and fruit were pulverized completely by using a mill and a mortar and pestle, respectively.

#### 2.2.2. Triterpene acids extraction

Dried samples were extracted with ethyl acetate (W:V 1:25) in an ultrasonic water bath (ELMA, E120H, Germany) at the frequency of 35 kHz and 35 °C for 30 min. Then the extract centrifuged at 10,000 rpm for 10 min. Extraction steps were repeated three times with fresh solvent. The combined extracts were condensed in a rotary evaporator and stored at 4 °C until HPLC analysis. (Wójciak-Kosior et al., 2017).

### 2.3. HPLC analysis

Waters 2695 HPLC (USA0) instrument with a photodiode array detector of PDA Waters 996 (USA) at 210 nm was used for determination and quantification of triterpene acids. The chromatography column was Eurospher 100-5 C<sub>18</sub> and 25 × 4.6 mm. The mobile phase in the form of a gradient consisted of acetonitrile and distilled water with 1 mL/min flow rate. The levels of triterpene acids were plotted at the four concentrations of 50, 100, 150 and 250 µg/mL based on the area under the curve of the standard compounds (Sigma products).

### 2.4. Statistical analysis

The statistical analysis was performed using SPSS 23.0 software. This experiment was conducted by Two-way ANOVA based on a completely randomized design (CRD) with three replications. The Kolmogorov-Smirnov test was used for normal distribution of residuals and homogeneity of data variances was investigated using the Levene test. Also treatment means were compared with Duncan's test at the 5% probability level.

## 3. Results

The results of the analysis of variance showed that the host species had no significant effect on the amount of OA and BA in *V. album* (Table 2). According to the results, the type of organ (foliage and fruit) had significant effect on the BA content at 1% probability level, while it did not show significant effect on OA. Also, the interaction between host and organ had no significant effect on the content of studied compounds (Table 2). It was noticeable that UA was not detected in any mistletoes on both host species. The highest amount of BA (1.23 and 0.97 mg/g dry weight) was found in the foliage of *V. album* grown on *P. persica* and *C. betulus* respectively and also it was not shown in the fruit of this plant based on both host species. Although the organ type did not have significant effect on the content of OA, but the highest amount of this compound was detected in the foliage of *V. album* settled on *P. persica* and *C. betulus* (8.52 and 8.20 mg/g dry weight) respectively (Fig. 4). According to Table 3, *V. album* settled on *P. persica* contained the highest level of total OA (8.18 mg/g dry weight), total BA (0.62 mg/g dry weight), and the highest total amount of OA + BA (8.79 mg/g dry weight), but in terms of the ratio of OA/BA, it was in the second rank. Also in the comparison between OA and BA content in the mistletoes on the both host species was observed the OA was the main triterpene acid in these plants (Table 3). According to the results of the ANOVA test, the most important factor (among the investigated factors) on the amount of

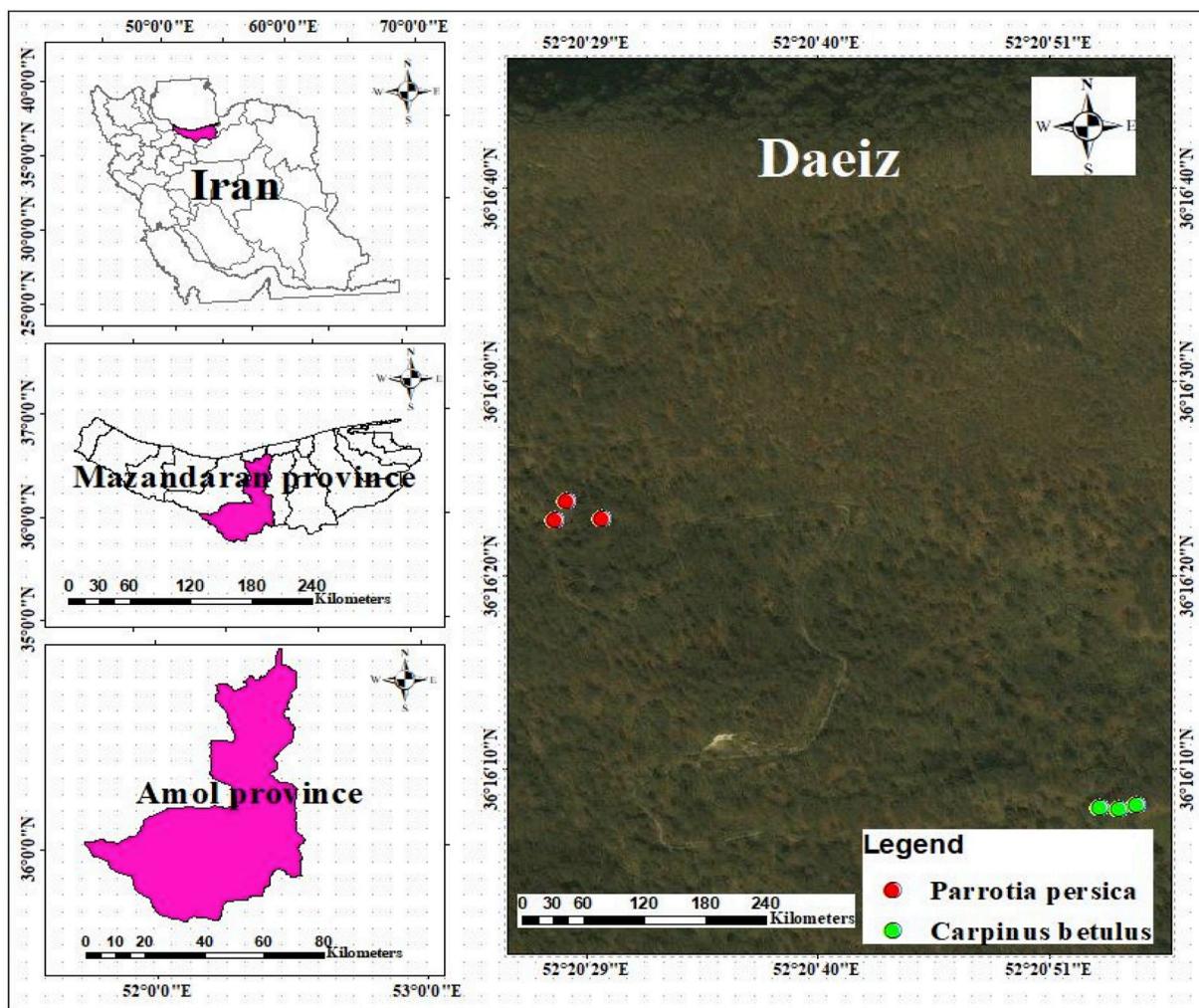


Fig. 2. Geographical location of the region and the *Parrotia persica* and *Carpinus betulus* trees.



Fig. 3. A) *Viscum album* L. in the fruiting stage; B) Sampling of *Viscum album* L.

**Table 2**  
Analysis of variance of OA and BA values in the foliage and fruit of *V. album* settled on the *P. persica* and *C. betulus*.

Sources of variation	DF	Mean square	
		BA	OA
Plant organ	1	3.64**	5.66 <sup>ns</sup>
Host	1	0.05 <sup>ns</sup>	3.04 <sup>ns</sup>
Host × Plant organ	1	0.05 <sup>ns</sup>	1.42 <sup>ns</sup>
Error	8	0.01 <sup>ns</sup>	1.80 <sup>ns</sup>

**Table 1**  
The characteristics of host and collection place of *Viscum album* L.

Sample no.	Host	Family	Site in Iran	Replicate	Coordinates	
					Longitude	Latitude
1	<i>Parrotia persica</i> C.A.Mey.	Hamamelidaceae	Daeiz forest	1	52°20' 34'	36° 16' 30''
				2	52°20' 34''	36° 16' 22''
				3	52°20' 31'''	36° 16' 22'''
2	<i>Carpinus betulus</i> L.	Corylaceae	Daeiz forest	1	52°20' 31''	36° 16' 8''
				2	52°20' 31''	36° 16' 7'''
				3	52°20' 31'''	36° 16' 7'''

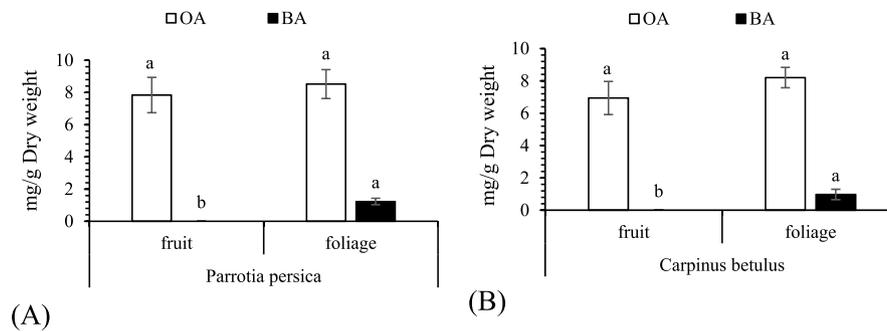


Fig. 4. The content of OA and BA in the foliage and fruit of *Viscum album* L. settled on (A) *P. persica* (B) *C. betulus*.

Table 3

Total OA, total BA, OA/BA ratio and total OA + BA content in mistletoes on *P. persica* and *C. betulus* (mg/g $\pm$ SD).

Host bases	Total OA (mg/g dry weight)	Total BA (mg/g dry weight)	OA/BA ratio	Total of OA + BA (mg/g dry weight)
<i>P. persica</i>	8.18 $\pm$ 1.66	0.62 $\pm$ 0.10	13.28 $\pm$ 0.55	8.80 $\pm$ 1.76
<i>C. betulus</i>	7.17 $\pm$ 0.58	0.49 $\pm$ 0.02	14.74 $\pm$ 1.2	7.66 $\pm$ 0.58

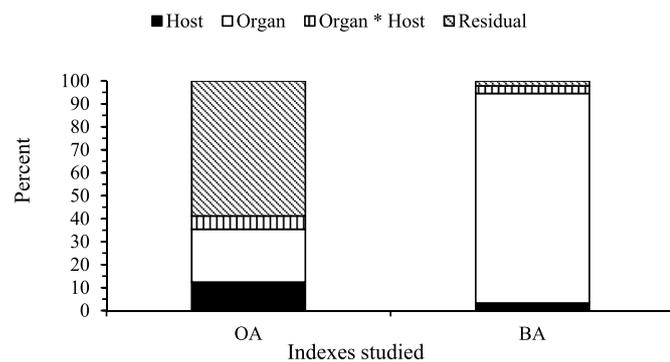


Fig. 5. Percentage of each independent factor including host tree, organ (foliage and fruit) and interaction of host \* organ on the studied indexes.

OA and BA was the organ type (foliage and fruit) (Fig. 5).

#### 4. Discussion

Mistletoe is a hemiparasitic plant whose metabolites depend on the host's growth period (Wójciak-Kosior et al., 2017). Umucalilar et al. (2007) in study on *V. album* concluded that its dry material, crude protein, crude ash and some mineral elements like copper and zinc can significantly be affected by different seasons and host trees. The present study revealed that the OA content was not affected by the organ type and the tested deciduous host trees. However, in contrast to this compound, BA showed a significant difference between the foliage and fruit of mistletoes on the both *P. persica* and *C. betulus* host trees and also was not affected by the host species. In line with this study, Schaller et al. (1998) found that host trees did not have significant effect on visco-toxins quality of *V. album* different subspecies but the total visco-toxins were dependent to host species. Also in another study on *V. album* subsp. *coloratum* grown on 12 different host trees, was reported that there was not notable variation in constituents of isolated flavonoid glycosides. However, some variation was detected in the content of two flavonoid glycosides compounds (Fukunaga et al., 1989).

It seems that the fruit of the studied mistletoes has trend to produce OA, because this compound was the only triterpene acid found in the

mistletoes fruit on studied host species. In line with the results obtained in this study, Tomczyk and Gudej (2003), found that the amount of phenolic compounds varies in different organs, leaves and flowers, of *Ficaria verna*. In the present study, the highest amount of BA was found in the foliage of *V. album* based on *P. persica* and its amount was affected by the type of organ. Also, OA was the main triterpene acid in extracts of both organs. In accordance with our results, Fukunaga et al. (1989) in study on *V. album* subsp. *coloratum* grown on 12 different host trees observed that the leaves consist more of the flavonoid glycosides than the twigs in three kinds of host species. Also, Jorjani et al. (2018) reported the significant difference between the aerial and underground organs of *Chelidonium majus* in terms of secondary metabolites content such as alkaloids, total phenol, and anthocyanins and primary metabolites (soluble sugars and proline) in different phenological stages. Therefore, it can be concluded that the type of organ is one of the important factors affecting the quantity and quality of triterpene acids in *V. album*. In this study the ratio of OA/BA was more than 10 times, which is consistent with the results of Wójciak-Kosior et al. (2017).

#### 5. Conclusion

Due to the valuable medicinal properties of the two triterpene acids OA and BA, the content and ratio of them in *V. album* based on different hosts is very important. This research for the first time revealed that in the fruiting phenological stage, the foliage of *V. album* based on *P. persica* and *C. betulus* contains both triterpene acids and it is the best organ for obtaining the highest quantity and quality of these compounds. Also OA was the dominant triterpene acid in the foliage and fruit of this plant at both host species. So, due to the production of valuable triterpene acids such as OA and BA in *V. album*, this plant can be used as a valuable drug in the pharmaceutical industry.

#### Declaration of competing interest

The authors declare that they have no conflict of interest.

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