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Functional and Qualitative Metabolic Compounds in the Twigs of the Deciduous Mistletoe *Loranthus europaeus* Jacq.

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Abstract: In this study, a detailed phytochemical investigation of compounds in the twigs of the mistletoe *Loranthus europaeus* Jacq., which belongs to the Loranthaceae family, is presented. Specimens were collected from the mistletoe *L. europaeus* growing on oak trees in the mainland of Greece. The alliance of oaks and mistletoes became a symbol of knowledge and strength for many centuries. Although numerous compounds of aerial tissues of other mistletoes, e.g., *Viscum album*, have been published, few studies have been conducted to investigate the metabolic and physiological traits of the hemiparasitic, deciduous *Loranthus europaeus*. LC-HRMS-based analysis led to a detailed characterization of ethyl acetate and dichloromethane extracts of the twigs of *L. europaeus*, which, to the best of our knowledge, exhibit enhanced antioxidant potential. Hence, twenty-four and twenty-six compounds were tentatively identified from the ethyl acetate and dichloromethane twigs' extracts, respectively; these compounds belong to fatty acids, flavonoids, and flavonoid glycosides. Also, chlorophyll, soluble sugar, starch, and lipid contents in the twigs of *L. europaeus*, which have not hitherto been published, were investigated.

Keywords: mistletoe; hemiparasitic plant; chlorophyll; fatty acids; phenolics; starch; sugars; twigs

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1. Introduction

The mistletoe *Loranthus europaeus* Jacq. belongs to the Loranthaceae family; this mistletoe is noticeably chlorophyllous and deciduous. The native range of this hemiparasite plant, growing on host trees, is from South-Eastern Europe to W. Iran, E. Himalaya, and South Russia [1–3]. In the south of Europe, *Loranthus europaeus* grows on oaks (*Quercus* species) [4–6], being therefore widely known as the oak-mistletoe [7,8] (Figure 1), and occasionally on chestnuts and other species as host trees [9,10]. The alliance of oaks and mistletoes became a symbol of knowledge and strength for many centuries [11]: it was also aptly rendered in the word "Druid" (in Greek δρυΐδης i.e., the oak-knower), which is derived from the Greek word for an oak tree (δρύς) and the suffix-ίδης (-ides) [12]. Mistletoes on oaks were linked to interesting symbolism and healing status, and were highly prized by ancients, alchemists, and herbalists [13,14]. Mistletoes were found at spots where there had been ancient settlements [10,15]. The most powerful healing mistletoe species was supposed to be the so-called "golden bough"; this seemed to be linked to the mistletoe with the golden-yellow drupes [16] that grew especially on oaks and in regions of Europe where rituals and ceremonies of early cultures originated [17,18].

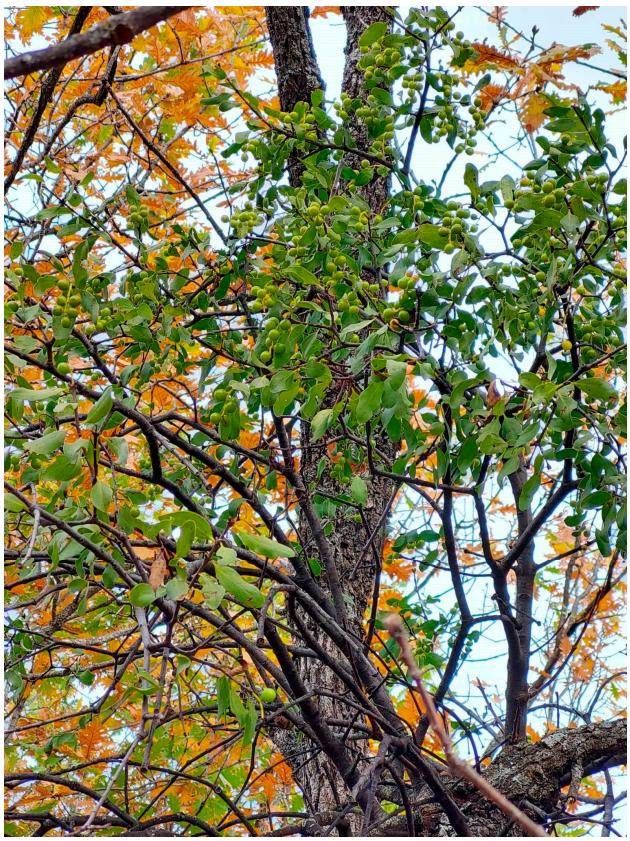

Figure 1. *Loranthus europaeus* growing on the host tree, the deciduous oak *Q. frainetto* (© A. Bampali).

In considering *Loranthus* Jacq. (Loranthaceae), there has been confusion in the nomenclature and the taxonomic circumscription has changed considerably over time in the literature [19]. Hence, within the context of scientific understanding, the mistletoe currently known as *Loranthus europaeus* has been cited as *Loranthus scurrula* (1762), *Scurrula parasitica* L. (1763), *Loranthus dioicus* Stokes (1812), *Viscum quernum* Rchb. (1831), and *Hyphear europaeum* (Jacq.) Danser (1929) [1]. In addition, it has been published that *Viscum aureum* was the mistletoe of the Druids [6], which was linked to the "golden bough" [20]. Finally, plant taxonomists at the Edinburgh International Botanical Congress of 1964, conserved, approved, and assigned the name *Loranthus* Jacq. (1762) to the species *Loranthus europaeus* Jacq. [21].

It is noteworthy that in the oldest known reports on mistletoes quoted by Theophrastus (about 372–287 B.C.), three different single-word names (in ancient Greek) had been used to describe mistletoes growing on host trees; the name "hyphear" (ὑφέαρ) corresponded to mistletoes grown on silver-fir and pine and so too the name "stelis" (στελίς), while the name "ixia" (ἰξία) corresponded to mistletoes grown on oaks, terebinth, and other trees [22–25]. John Sibthorp (1758–1796), the Professor of Botany at the University of Oxford who visited territories of Greece in the late 1780s and 1790s collecting and recording botanical specimens, noticed that the oaks were frequently infested with the mistletoe *Loranthus europaeus* Jacq. [26,27]; it is worth mentioning that Sibthorp regarded the deciduous mistletoe *Loranthus europaeus* Jacq. as the "true mistletoe of the ancients" [26,28].

The deciduous mistletoe *L. europaeus* possesses dull brown twigs and staminate (♂), pistillate (♀), and possibly bisexual sessile flowers [10,29] on spike inflorescences, and its immature fruits are greenish drupes in summer, while the mature fruits are yellow nearly globular drupes (6–9 mm in diameter) in winter [personal observation], with one seed within the viscin tissue.

Besides some published results linked to the antioxidant and antimicrobial properties of *L. europaeus* [27,30–32], and a few studies performed on the relationship between the hemi-

parasite *L. europaeus* and host trees [33–38], via a xylem- and/or phloem-tapping haustorium upon the branches of host trees, functional metabolic and physiological traits have not hitherto been published. Furthermore, it has been shown that *L. europaeus* amplifies drought stress in oak trees, affecting the metabolism and water relations of the host trees [39,40]. Due to the structural attachment of *L. europaeus* to the host trees, their phytoconstituents, and hence biological activities, are strongly dependent on the respective hosts [5,8,35,41,42].

In an earlier investigation, the antioxidant properties of the aerial parts of the neglected and poorly studied mistletoe *Loranthus europaeus* were evaluated [29] and elevated antioxidant potential was found in the twigs [29]. Thus, the interest of the current research was triggered by the functionality of *L. europaeus* twigs. The objective of this work was to study aspects of the metabolic profile (fatty acids and phenolics) in the twigs of the wild mistletoe *L. europaeus* grown on oaks and exposed to ambient conditions, which, according to the best of our knowledge, like the other aboveground plant parts of *L. europaeus*, have not hitherto been studied.

2. Results

2.1. Secondary Metabolites

In the twigs of *L. europaeus*, 24 and 26 secondary metabolites were tentatively identified in extracts from the ethyl acetate (Figure 2, Table 1) and the dichloromethane extract (Figure 3, Table 2), respectively; these metabolites belong to diverse chemical categories such as flavonoids, flavonoid glycosides, and fatty acids. The majority of the secondary metabolites were present in both of the above-mentioned extracts (Tables 1 and 2). The flavonoid/fatty acid ratio was higher for the ethyl acetate extract compared to the dichloromethane. Flavonoids such as catechin, epicatechin and procyanidine type B dimer, which may provide the antioxidant properties to *L. europaeus*, were only present in the ethyl acetate extract (Figure S1). In both the ethyl acetate and the dichloromethane extracts, flavonoid glycosides were detected, such as quercetin-3-*O*-pentoside, rhamnetin-3-*O*-pentoside, isorhamnetin-3-*O*-pentoside, and rhamnocitrin pentoside (Figure S2), which all bear a rhamnose sugar. Rhamnetin-3-*O*-pentoside and rhamnocitrin pentoside had previously been isolated from *L. europaeus* [9], while catechin and epicatechin have been found to be present in the fruit, stalks, and leaves of *L. europaeus* [32].

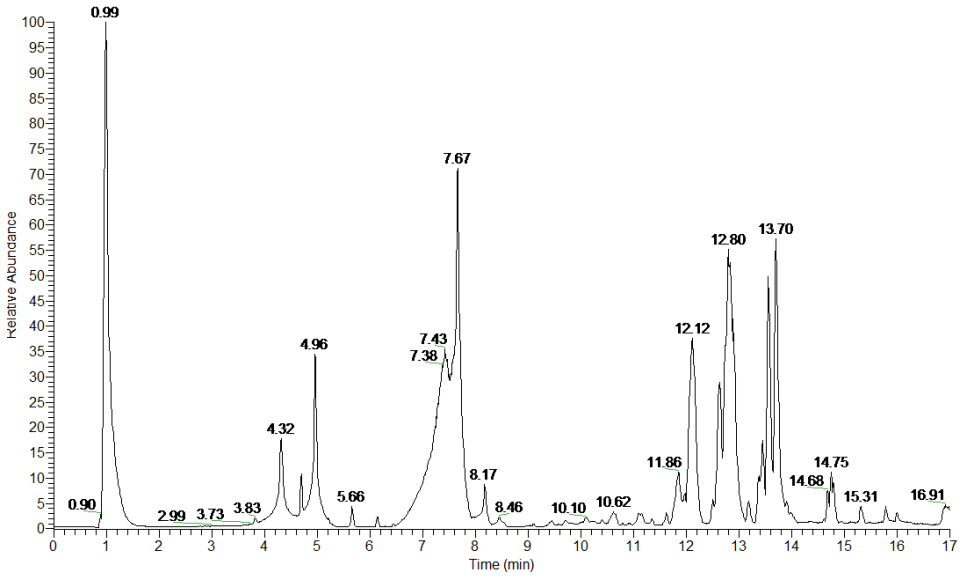

Figure 2. *Loranthus europaeus* twigs' EtOAc negative-base peak chromatogram.

Table 1. Compounds in the twigs of *Loranthus europaeus*: EtOAc negative mode; the intensity of detected fragments in the HRMS/MS spectra is given in parentheses.

Peak Number	Retention Time (min)	Proposed Compound	Molecular Formula, Elemental Composition	*m/z* Detected [M—H]	Delta Δ (ppm)	Ring and Double Bond Equivalent RDBeq.	HRMS/MS
1	4.32	Catechin [1]	$C_{15}H_{14}O_6$	289.0715	−0.904	9.5	245.0821 (100) 205.0505 (38) 179.0350 (15)
2	4.70	Procyanidin dimer type B isomer [1]	$C_{30}H_{26}O_{12}$	577.1346	−0.952	18.5	451.1031 (27) 425.0877 (100) 407.0772 (52) 289.0718 (20)
3	4.96	Epicatechin [1]	$C_{15}H_{14}O_6$	289.0714	−1.250	9.5	245.0818 (100) 205.0505 (38) 179.0350 (16)
4	5.66	Procyanidin dimer type B isomer [1]	$C_{30}H_{26}O_{12}$	577.1348	−0.605	18.5	451.1031 (29) 425.0876 (100) 407.0771 (53) 289.0717 (22)
5	6.15	Quercitrin (Quercetin-3-*O*-rhamnoside) [2]	$C_{21}H_{20}O_{11}$	447.0930	−0.636	12.5	301.0355 (100) 300.0278 (30)
6	7.43	Rhamnetin-3-*O*-pentoside [3]	$C_{22}H_{22}O_{11}$	461.1084	−1.159	12.5	315.0507 (100) 314.0431 (33)
7	7.67	Isorhamnetin-3-*O*-pentoside [3]	$C_{22}H_{22}O_{11}$	461.1083	−1.376	12.5	315.0506 (100) 314.0433 (33)
8	8.17	Rhamnocitrin pentoside	$C_{22}H_{22}O_{10}$	445.1137	−0.719	12.5	299.0560 (100)
9	8.46	Pinellic acid [4]	$C_{18}H_{34}O_5$	329.2332	−0.448	2.5	311.2228 (49) 229.1445 (91) 211.1340 (64) 171.1027 (100)
10	9.45	Hydroxy-oxo-octadecatrienoic acid isomer [5]	$C_{18}H_{28}O_4$	307.1915	−0.057	5.5	289.1807 (100) 260.9319 (41) 235.1910 (67) 185.1183 (39)
11	10.62	Hydroxy-oxo-octadecatrienoic acid isomer [5]	$C_{18}H_{28}O_4$	307.1915	−0.057	5.5	289.1808 (100) 260.9320 (8) 245.1910 (10)
12	11.11	Dihydroxyoctadecadienoic acid isomer [6]	$C_{18}H_{32}O_4$	311.2227	−0.266	3.5	293.2119 (100) 275.2015 (33) 201.1130 (41) 171.1025 (86)
13	11.34	Dihydroxyoctadecadienoic acid isomer [6]	$C_{18}H_{32}O_4$	311.2227	−0.266	3.5	293.2119 (100) 171.1025 (47)
14	11.63	Thapsic acid [7]	$C_{16}H_{30}O_4$	285.2073	0.587	2.5	267.1963 (66) 223.2065 (100)
15	11.86	Oxo-octadecadienoic acid isomer [8,9]	$C_{18}H_{30}O_3$	293.2122	−0.062	4.5	275.2015 (100) 235.1701 (11) 211.1338 (30) 183.1390 (14) 171.1026 (15)
16	11.97	Oxo-octadecadienoic acid isomer [8]	$C_{18}H_{30}O_3$	293.2121	−0.403	4.5	275.2016 (100) 195.1390 (59)
17	12.12	α-Licanic acid (Oxo-octadecatrienoic acid)	$C_{18}H_{28}O_3$	291.1962	−1.264	5.5	273.1858 (100) 247.2065 (81) 185.1182 (34)
18	12.63	Hydroxy-octadecadienoic acid [8]	$C_{18}H_{32}O_3$	295.2274	−1.586	3.5	277.2171 (100) 195.1389 (31) 171.1026 (55)

Table 1. *Cont.*

Peak Number	Retention Time (min)	Proposed Compound	Molecular Formula, Elemental Composition	*m/z* Detected [M—H]	Delta Δ (ppm)	Ring and Double Bond Equivalent RDBeq.	HRMS/MS
19	12.80	Oxo-octadecadienoic acid isomer [10]	$C_{18}H_{30}O_3$	293.2118	−1.426	4.5	275.2017 (27) 249.2223 (100) 197.1183 (27) 185.1283 (76)
20	13.45	Ricinoleic acid [11]	$C_{18}H_{34}O_3$	297.2432	−1.070	2.5	279.2327 (100) 171.1026 (19) 155.1076 (68)
21	13.56	Hydroxy-octadecadienoic acid isomer [12]	$C_{18}H_{32}O_3$	295.2276	−0.908	3.5	251.2379 (100) 185.1183 (38) 151.1128 (85)
22	13.70	Hydroxy-octadecadienoic acid isomer [12]	$C_{18}H_{32}O_3$	295.2276	−0.908	3.5	277.2171 (31) 251.2327 (100) 155.1440 (78)
23	14.64	Hydroxyeicosadie-noic acid	$C_{20}H_{36}O_3$	323.2593	0.408	3.5	305.2488 (22) 279.2692 (40) 179.1441 (51)
24	14.75	Unknown compound	$C_{26}H_{40}O_6$	447.2745	−1.369	7.5	-

References: [1] [43], [2] [44], [3] [45,46], [4] [47], [5] [48], [6] [49], [7] [50], [8] [51], [9] [52], [10] [53], [11] [54], [12] [55].

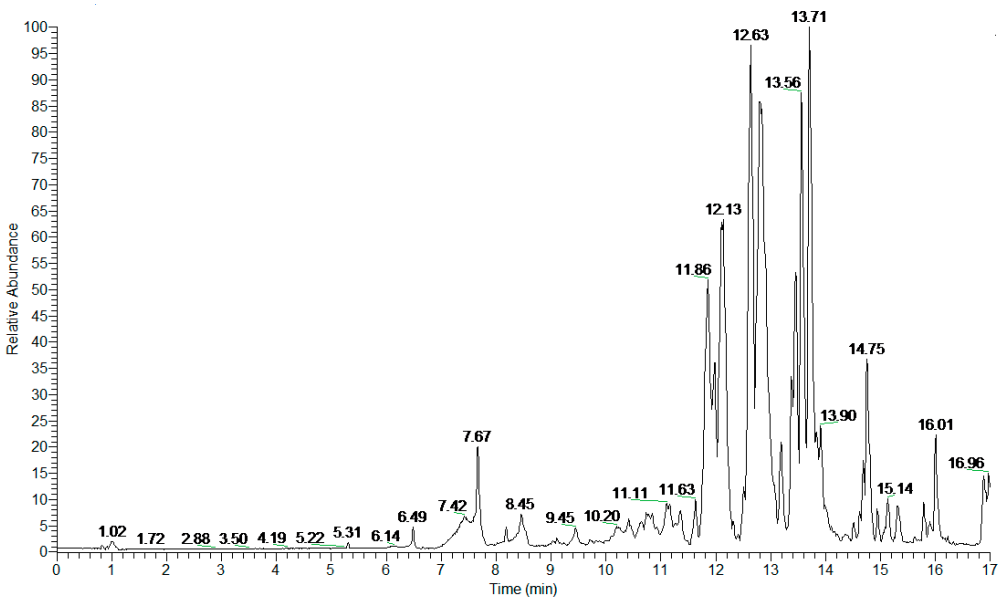

Figure 3. *Loranthus europaeus* twigs' DCM negative-base peak chromatogram.

Table 2. Compounds in the twigs of *Loranthus europaeus*: DCM negative mode; the intensity of detected fragments in the HRMS/MS spectra is given in parentheses.

Peak Number	Retention Time (min)	Proposed Compound	Molecular Formula, Elemental Composition	*m/z* Detected [M—H]	Delta, Δ (ppm)	Ring and Double Bond Equivalent RDBeq.	HRMS/MS
1	6.49	Azelaic acid [13]	$C_9H_{16}O_4$	187.0975	−0.044	2.5	125.0970 (100)
2	7.42	Rhamnetin-3-*O*-pentoside [3]	$C_{22}H_{22}O_{11}$	461.1084	−1.159	12.5	315.0506 (100) 314.0429 (34)
3	7.67	Isorhamnetin-3-*O*-pentoside [3]	$C_{22}H_{22}O_{11}$	461.1082	−1.29	12.5	315.0505 (100) 314.0430 (34)

Table 2. *Cont.*

Peak Number	Retention Time (min)	Proposed Compound	Molecular Formula, Elemental Composition	*m*/*z* Detected [M—H]	Delta, Δ (ppm)	Ring and Double Bond Equivalent RDBeq.	HRMS/MS
4	8.18	Rhamnocitrin pentoside	$C_{22}H_{22}O_{10}$	445.1135	−1.168	12.5	299.0560 (100)
5	8.48	Pinellic acid [4]	$C_{18}H_{34}O_5$	329.2332	−0.448	2.5	311.2224 (49) 229.1442 (91) 211.1337 (64) 171.1025 (100)
6	9.45	Hydroxy-oxo-octadecatrienoic acid isomer [5]	$C_{18}H_{28}O_4$	307.1915	0.057	5.5	289.1806 (100) 260.9318 (37) 235.1337 (73) 185.1182 (43)
7	10.20	Linolenic acid 13-hydroperoxide [14]	$C_{18}H_{30}O_4$	309.2070	−0.429	4.5	291.1965 (100)
8	10.75	Dihydroxyoctadecadienoic acid isomer [6]	$C_{18}H_{32}O_4$	311.2227	−0.266	3.5	293.2120 (100) 275.2016 (6) 201.1131 (10)
9	11.11	Dihydroxyoctadecadienoic acid isomer [6]	$C_{18}H_{32}O_4$	311.2227	−0.266	3.5	293.2119 (100) 275.2015 (32) 201.1130 (35) 171.1025 (75)
10	11.34	Dihydroxyoctadecadienoic acid isomer [6]	$C_{18}H_{32}O_4$	311.2227	−0.266	3.5	293.2119 (100) 275.2015 (22) 171.1025 (45)
11	11.63	Thapsic acid [7]	$C_{16}H_{30}O_4$	285.2075	0.061	2.5	267.1966 (67) 223.2067 (100)
12	11.86	Oxo-octadecadienoic acid isomer [8,9]	$C_{18}H_{30}O_3$	293.2118	−1.426	4.5	275.2015 (100) 235.1702 (14) 211.1319 (28) 183.1390 (13) 171.1026 (15)
13	11.95	Oxo-octadecadienoic acid isomer [8]	$C_{18}H_{30}O_3$	293.2118	−1.426	4.5	275.2013 (100) 211.1338 (19) 195.1389 (37)
14	12.12	α-Licanic acid (Oxo-octadecatrienoic acid)	$C_{18}H_{28}O_3$	291.1964	−0.577	5.5	273.1859 (99) 247.2066 (100) 219.1390 (29) 185.1182 (24)
15	12.63	Hydroxy-octadecadienoic acid isomer [8]	$C_{18}H_{32}O_3$	295.2276	−0.908	3.5	277.2170 (100) 195.1389 (41) 171.1026 (47)
16	12.80	Oxo-octadecadienoic acid isomer [10]	$C_{18}H_{30}O_3$	293.2118	−1.085	4.5	275.2015 (26) 249.2222 (100) 185.1183 (52)
17	13.17	Oxo-octadecadienoic acid isomer [10]	$C_{18}H_{30}O_3$	293.2121	−0.403	4.5	275.2015 (11) 249.2223 (12) 197.1182 (35) 185.1182 (100)
18	13.45	Ricinoleic acid [11]	$C_{18}H_{34}O_3$	297.2431	−1.407	2.5	-
19	13.56	Hydroxy-octadecadienoic acid isomer [12]	$C_{18}H_{32}O_3$	295.2277	−0.569	3.5	251.2378 (100) 151.1127 (77) 141.1284 (43) 125.0907 (55)
20	13.71	Hydroxy-octadecadienoic acid isomer [12]	$C_{18}H_{32}O_3$	295.2276	−0.908	3.5	277.2170 (30) 251.2376 (100) 155.1439 (77)
21	13.90	Hydroxy-octadecadienoic acid isomer [12]	$C_{18}H_{32}O_3$	295.2276	−0.908	3.5	251.2376 (100) 155.1440 (54) 151.1127 (29)

Table 2. *Cont.*

Peak Number	Retention Time (min)	Proposed Compound	Molecular Formula, Elemental Composition	*m/z* Detected [M—H]	Delta, Δ (ppm)	Ring and Double Bond Equivalent RDBeq.	HRMS/MS
22	14.68	Hydroxyeicosadienoic acid	$C_{20}H_{36}O_3$	323.2589	−0.830	3.5	305.2486 (27) 279.2693 (52) 179.1442 (62)
23	14.75	Unknown compound	$C_{26}H_{40}O_6$	447.2745	−1.592	7.5	-
24	15.14	Linolenic acid [15]	$C_{18}H_{30}O_2$	277.2173	−0.013	4.5	275.2015 (21) 259.2066 (30) 233.2273 (100)
25	15.69	Unknown compound	$C_{22}H_{42}O_4$	369.3007	−0.902	2.5	351.2901 (33) 307.3004 (100)
26	16.00	Linoleic acid [15]	$C_{18}H_{32}O_2$	279.2325	−1.624	3.5	279.2329 (80) 261.2219 (100)

References: [3] [45,46], [4] [47], [5] [48], [6] [49], [7] [50], [8] [51], [9] [52], [10] [53], [11] [54], [12] [55], [13] [56], [14] [57], [15] [58,59].

2.2. Other Components

The chlorophyll content of the twigs (Table 3) of the mistletoe *L. europaeus* was estimated at 0.50 mg g^{-1}, which was substantially lower than that of its leaves and higher than that of its fruits (unpublished data). Also, relatively low values of carotenoids were detected (0.23 mg g^{-1}) in the twigs. The soluble sugar content in the twigs (Table 3) of the mistletoe *L. europaeus* geophyte was 142.13 mg g^{-1} in October, when the visible leaf development ceased. The starch content of the twigs was 33.62 mg g^{-1}.

Table 3. Chlorophyll, carotenoid, lipid, sugar, and starch content in the twigs of *L. europaeus*.

Substance	Twigs' Content
Chlorophyll a + b	0.50 ± 0.45 mg g^{-1}
Carotenoids	0.23 ± 0.02 mg g^{-1}
Soluble sugars	142.13 ± 2.32 mg g^{-1}
Starch	33.62 ± 0.89 mg g^{-1}

3. Discussion

The results revealed the presence of numerous components in the extracts from the twigs of *Loranthus europaeus*, such as fatty acids and flavonoids (Tables 1 and 2). The EtOAc extracts of *L. europaeus* twigs (Table 1) revealed the presence of catechin and epicatechin. It is noteworthy that the concentrations of both catechin and epicatechin were found to be significantly higher in the twigs in comparison with the leaves and fruits of *L. europaeus* [32]. The main biological benefits of these compounds are the adipogenic activity, the differentiation of human bone marrow mesenchymal stem cells into adipocytes, and the inhibition of oxidative stress and neuro-inflammation [60–63]. The investigated procyanidins B, a subclass of flavonoids, are frequently occurring in plants [64]. Quercitrin, which was found in the twigs of *L. europaeus*, is a naturally available flavonoid with antioxidant properties [65,66]; recently, quercitrin was also found in the leaves of *L. europaeus* [67].

In the DCM extracts of *L. europaeus* twigs (Table 2), the presence of azelaic acid was revealed; this is a naturally occurring and nontoxic C_9 dicarboxylic acid, which has anti-inflammatory properties and an antimicrobial effect on aerobic and anaerobic microorganisms [68,69]. Also, the twigs of *L. europaeus* accumulate medium-chain, unsaturated fatty acids, such as licanic (18:5), linolenic (18:3), and linoleic (18:2) acids; such compounds enhance the antioxidant properties in plant tissues and twigs of different species [70–73]. Unsaturated fatty acids such as linoleic and α-linolenic acids play crucial roles in plant physiology and comprise important economic traits of oil crops. These simple compounds also display significant functional roles in plants and are deeply associated with both abiotic

and biotic stresses. Apart from being intrinsic antioxidants, unsaturated fatty acids have emerged as general defenders against abiotic stresses such as cold, heat, drought, and salt. It has been shown that α-linolenic acid (ALA) accumulates in plant membranes, leading to an increase in membrane fluidity and resistance to membrane rigidification caused by chilling [73,74]. Their biosynthesis takes place in plastids, used by the plant in various ways, including as components of organelles and endoplasmic reticulum membrane phospholipids, storage lipids, or extracellular waxes [75]. Linoleic acid affects plant cell membranes, while linolenic acid is a component of the thylakoid membranes of chloroplasts and is most probably linked to the chlorophyll content of the twigs ([76] and references therein). Furthermore, twigs' photosynthesis may help in bridging the energy and carbon gap during the period between total defoliation and re-foliation [77], as well as in contributing to new organ development [78,79].

Also, numerous compounds have been found in both EtOAc and DCM twig extracts (Tables 1 and 2); these compounds, according to the retention time, are rhamnetin pentoside, pinellic acid, octadecatrienoic acid, dienoic acid, thapsic acid, octadecadienoic acid with a hydroxy substituent, licanic acid, ricinoleic acid, and hydroxyeicosadienoic acid. Actually, both twig extracts (i.e., EtOAc and DCM) were rich in fatty acids including the unsaturated fatty acids, i.e., linolenic; linoleic (precursors of oxylipins); and oxylipins such as hydroxy-octadecadienoic acid, keto-octadecadienoic acid, hydroxydiecanoic acid (oxoODE), hydroxyeicosadienoic adic, hydroxy-oxo-octadecatrienoic acid, trihydroxy-octadecenoic acid (TriHOME) (pinellic acid), and oxooctadecatrienoic acid (licanic acid). Certain identified lipophilic signaling molecules descend from oxidation of polysaturated fatty acids (oxylipins definition) [80]. One of the key roles of oxylipins is the response to environmental abiotic stresses, such as drought and salinity [81].

In the twigs of *L. europaeus,* elevated soluble sugar and starch content is accumulated in comparison with that in tissues of oak trees not infected with the mistletoe [82–84]. *L. europaeus*' capacity to assimilate carbon in the twigs may depend on the water-use efficiency of the mistletoe, which in turn relies on the abiotic stress the host tree is subjected to. Further work is required to illustrate traits contributing to the functionality of the aerial tissues of the mistletoe *L. europaeus* grown on oak trees.

4. Materials and Methods

4.1. Research Site

The study was conducted with twigs (Figure 4) of the hemiparasite plant *Loranthus europaeus* Jacq., developed on oak trees (*Quercus frainetto* Ten.) grown in the wild (Figure 1), in central Greece (39.388180° N, 21.643281° E, 487.8 m a.s.l.) [85]. Values of mean monthly precipitation and temperature, obtained from a meteorological enclosure provided by the National Observatory of weather conditions in Greece, are presented in Figure 5.

Figure 4. Twigs of *Loranthus europaeus* bearing immature drupes (**left**), harvested mature leaves and immature drupes (**right**), during October 2022 (© A. Bampali).

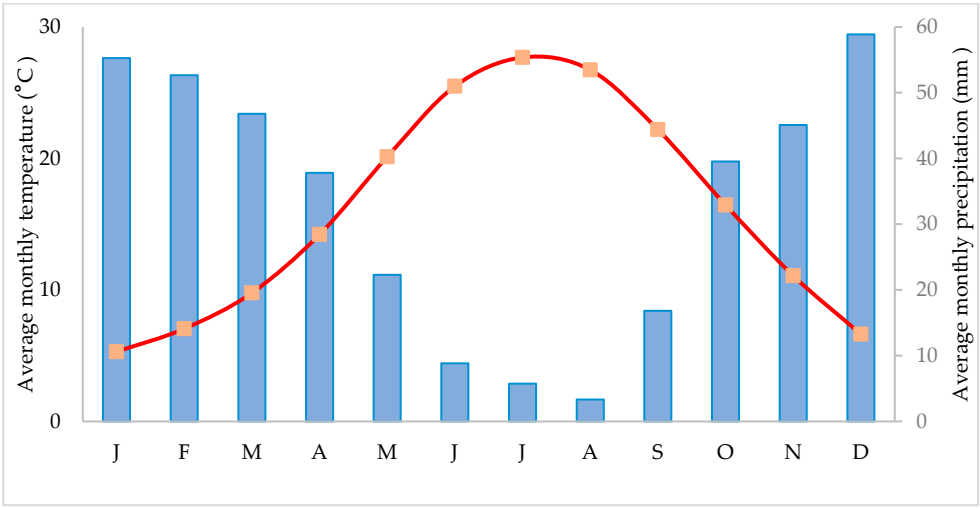

Figure 5. Multiannual ombrothermic diagram for the study site from January 2000 to December 2022; the order of months is from January (J) to December (D). Mean monthly precipitation is indicated by blue bars, mean monthly temperature by pink closed squares and the red line.

4.2. Collection of Specimens

In October 2022, randomly selected samples of haustoria, leaves, stems, current-year twigs, and drupes were collected from *Quercus frainetto*, surrounded by evergreen and deciduous Mediterranean species; these specimens were transferred to the laboratory. The twig samples were dried in a Gallenkamp incubator (Model IH-150) at 70 °C. The dried samples were powdered, using a MFC mill (Janke and Kunkel GMBH & Co., Staufen im Breisgau, Germany), and stored in tightly sealed containers.

Loranthus europaeus loses its leaves late in the autumn concomitantly with the abscission of the leaves of its host tree (Figure 1). The botanical authentication of the collected plant material was identified by the staff at the Section of Systematic Botany (National and Kapodistrian University of Athens) and voucher specimens were deposited at the herbarium of the Department of Biology of the National and Kapodistrian University of Athens [29].

4.3. Extraction of Plant Material for HRMS Analysis

Pulverized dried aerial twigs of *L. europaeus* (5 g) were extracted by ultrasonication with 40 mL of ethyl acetate (EtOAc) in two cycles of 20 min each at 30 °C. The obtained extract was filtered and evaporated to dryness using a vacuum evaporator (Buchi, Switzerland) to yield 166 mg of a dark green paste (3.32% *w*/*w* yield). The same procedure was followed with 40 mL dichloromethane (DCM) in two cycles of 20 min each at 30 °C. The obtained extract was filtered and evaporated to dryness under vacuum to yield 102 mg of a greenish paste (2.04% *w*/*w* yield).

4.4. LC-HRMS-Based Analysis and Dereplication

Chromatographic and spectrometric tools were used for dereplication. Orbitrap in full scan and MS/MS level offer high resolution and accuracy for the high confidence compound identification. Proposed Elemental Composition (EC), Ring Double Bond equivalents (RDBeq.) values, detection, and *m*/*z* measurements accuracy as expressed by Δm (*m*/*z* theoretical—experimental), and fragmentation patterns in tandem MS spectra were applied. The proposed EC was considered only if Δm was <5 ppm. All the peaks were examined according to databases and literature searches for putative identification compounds. Due to the fact that there are limited studies concerning the phytochemical composition of *Loranthus europaeus*' twigs, the focus of our study turned to the characterization of their ethyl acetate and dichloromethane extract via UPLC-HRMS and HRMS/MS analysis.

The liquid chromatography analysis of the extracts was performed on an Acquity H-Class UPLC System (Waters, Milford, MA, USA). Thermo Velos Pro Orbitrap Elite hybrid mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with a heated ESI (HESI) source was used for the detection. Separation was conducted on a Supelco C18 column (100 × 2.1 mm, 2 µm) with a gradient containing water with 0.1% (*v*/*v*) formic acid (A) and ACN (B). Elution initiated at 5% B, which was maintained for 1 min and increased to 100% B up to 15 min. These conditions were maintained for 2 min and then returned to the initial conditions in 0.5 min for a 2.5 min re-equilibration (20 min in total). The column was kept at 40 °C and the flow rate was set to 0.3 mL min^{-1}. For the HESI source, heater and capillary temperatures were set to 350 °C and the source voltage was 2.7 kV. Rf lens level was tuned at 45%; 10 µL of each extract at 250 µg mL^{-1} was injected into the system. HRMS data were acquired in the negative mode in the full scan *m*/*z* range of 113–2000 with a resolution of 60,000. Data-dependent acquisition was simultaneously performed using one dependent scan event, with a CID value of 35% and a mass resolution of 30,000. As sheath gas (45 au) and auxiliary gas (15 au), Nitrogen was used. Spectral acquisition was performed using the XCalibur software version 2.2. Compounds were identified based on their mass spectra, their retention times, their mass fragmentation patterns, and data from the literature.

For the preparation of the dichloromethane extract sample, 2.5 mg were dissolved in ethyl acetate, filtered through PTFE 0.22 µm/13 mm filter, evaporated to dryness, and redissolved in ACN LC-MS to reach the concentration of 250 µg mL^{-1}. The 2.5mg ethyl acetate extract samples were directly diluted in ACN LC-MS until 250 µg mL^{-1}.

4.5. Soluble Sugar and Starch Content

In order to extract soluble sugars from the twigs of *L. europaeus*, the plant tissues were dried in a dryer at 70 °C and finely powdered in a grinding mill. These powdered samples were then placed in 10 mL of 80% ethanol (*v*/*v*) and agitated using a shaker. The resulting extracts were filtered using Whatman #2 filter paper. The concentration of soluble sugars was determined through colorimetric analysis, employing a modified phenol–sulfuric acid method [86,87] at a wavelength of 490 nm, using a spectrophotometer (Novaspec III+ Spectrophotometer; Biochrom, Cambridge, UK). After the extraction of sugars, the residue was used for starch determination. The anthrone method [87] was utilized to measure the starch content in the residue; for the colorimetric analysis, a spectrophotometer (Novaspec III+ Spectrophotometer; Biochrom, Cambridge, UK) was employed at a wavelength of 490 nm. D-glucose from Serva (Heidelberg, Germany) was used to prepare aqueous solutions for the standard curve. The results obtained from the analysis are expressed as mg g^{-1} d.w. (dry weight).

4.6. Chlorophyll Content

The total chlorophyll (Chl) content was spectrophotometrically determined in dried twig samples according to a modified acetone method [88]. Chlorophyll was extracted from 0.05 g dried, ground leaf samples homogenized with 10 mL acetone (80% *v*/*v*) using porcelain pestle and mortar and filtered through Whatman #2 filter paper to become fully transparent [89]. The chlorophyll content was measured in aliquots of the leaf extracts using a Novaspec II (Pharmacia, Biotech, Cambridge, UK) spectrophotometer; the absorbance readings of five replicates have been used for the calculations [90]. The values of total chlorophyll content (a + b) in the leaf tissue are expressed as mg g^{-1} of dry weight (d.w.) and are means of five replicates ± standard deviation.

5. Conclusions

Although numerous compounds of the evergreen mistletoe *Viscum album* have been intensively studied (reaching the drug Iscador, for treatments against cancer), research work on the photosynthetic, deciduous mistletoe *Loranthus europaeus* is still very limited and linked to either rare botanic information, or a few ethnomedicinal practices. In this

study, a qualitative metabolic profiling investigation of the twigs of *Loranthus europaeus*, extracted with ethyl acetate (24 compounds) and dichloromethane (26 compounds), is reported. Also, total chlorophyll, soluble sugars, starch, and lipids have been identified in the twigs of *L. europaeus*. However, further research is required to fully exploit metabolic traits during its life-cycle and medicinal properties.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/stresses4010002/s1, Figure S1: Twigs of *Loranthus europaeus*: Proposed fragmentation pattern for epicatechin/catechin;. Figure S2: Twigs of *Loranthus europaeus*: Proposed fragmentation pattern for rhamnetin-3-*O*-pentoside.

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