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Phytochemical Composition of Lichen *Parmotrema hypoleucinum* (J. Steiner) Hale from Algeria

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Citation: Kerboua, M.; Monia, A.A.; Samba, N.; Silva, L.; Raposo, C.; Díez, D.; Rodilla. J.M. Phytochemical Composition of Lichen *Parmotrema hypoleucinum* (J. Steiner) Hale from Algeria. *Molecules* **2022**, *27*, 5229. https://doi.org/10.3390/molecules27165229

Academic Editor: Carmen Formisano

Received: 22 June 2022
Accepted: 9 August 2022
Published: 16 August 2022

Abstract: In this work, we carried out studies of the chemical composition of hexane, chloroform and ethanol extracts from two samples of the lichen *Parmotrema hypoleucinum* collected in Algeria. Each sample of the lichen *P. hypoleucinum* was collected on two different supports: *Olea europaea* and *Quercus coccifera*. Hexane extracts were prepared, in Soxhlet; each hexane extract was fractionated by its solubility in methanol; the products soluble in methanol were separated (cold): 1-Hexane, 2-Hexane; and the products insoluble in methanol (cold): 1-Cires, 2-Cires. A diazomethane esterified sample of 1-Hexane, 2-Hexane, 1-Cires and 2-Cires was analyzed by GC-MS, and the components were identified as methyl esters. In the 1-Hexane and 2-Hexane fractions, the methyl esters of the predominant fatty acids in the lichen were identified: palmitic acid, linoleic acid, oleic acid and stearic acid; a hydrocarbon was also identified: 13-methyl-17-norkaur-15-ene and several derivatives of orsellinic acid. In the 1-Cires and 2-Cires fractions, the previous fatty acids were no longer observed, and only the derivatives of orsellinic acid were found. The analysis of the 1-Hexane, 2-Hexane fractions by HPLC-MS/MS allows us to identify different chemical components, and the most characteristic products of the lichen were identified, such as Atranol, Chloroatranol, Atranorin and Chloroatranorin. In the fractions of 1-Cires and 2-Cires, the HPLC-MS/MS analysis reveals that they are very similar in their chemical components; the characteristic products of this lichen in this fraction are Atranorin and Chloroatranorin. In the extracts of chloroform, 1-Chloroform and 2-Chloroform, the analysis carried out by HPLC-MS/MS shows small differences in their chemical composition at the level of secondary products; among the products to be highlighted for this work, we have chloroatranorin, the stictic acid, norstictic acid and other derivatives. In the analysis of the most polar extracts carried out in ethanol: 1-Ethanol and 2-Ethanol, HPLC-MS/MS analysis shows very similar chemical compositions in these two extracts with small differences. In these extracts, the following acids were identified as characteristic compounds of this lichen: constictic acid, stictic acid, substictic acid and methylstictic acid. In the HPLC–MS/MS analysis of all these extracts, alectoronic acid was not found.

Keywords: lichen; *Parmotrema hypoleucinum*; LC-MSD-Trap-XCT; phytochemical composition; norstictic acid and stictic acid

1. Introduction

Lichens live in symbiotic associations between fungi and algae and/or cyanobacteria, and in addition to these two symbiotic partners (photobiont and mycobiont) classically described, a third partner can also be integrated: epi and/or endophytic fungi as well as bacteriobiont or associated bacterial communities [1] which are important constituents of many of them. The production of various unique extracellular secondary metabolites known as lichen substances is the result of this symbiosis. The specific condition in which lichens live is the reason for the production of many metabolites that provide good protection against negative physical and biological influences. [2] The majority of lichens-forming fungi belong to Ascomycetes Lecanoromycetes [3]. Due to the vast genetic diversity and interactions with various environmental factors, lichens have unique profiles of primary and secondary metabolites (i.e., lichen substances) with interesting physiochemical properties. [4]

In this paper, we describe our studies on the chemical composition of the extracts of *Parmotrema Hypoleucinum*, Lecanorales Order, family Parmeliaceae, which, with 2765 species all over the world, is the largest family [5]

In Algeria, the Parmeliaceae family is very present compared to other families [6]; four *Parmotrema* have been identified so far: *P. perlatum*, *P. reticulatum*, *P. robustrum* and *P. hypoleucinum*. The last one is very common in the Mediterranean area [7]; it belongs to the Lecanorales Order and to the Parmeliaceae Family. It corresponds to a foliaceous lichen that can be up to 12 cm in diameter. In general, the lobes are very irregular, wide and raised, often forming tufts on small branches and can appear like curly lettuce. The upper side has a grayish appearance, and the lower side is largely white, which allows it to be easily distinguished from other similar species that have a dark back side. This species has black cilia and marginal Soralies.

Several activities of these lichen molecules, mainly those resulting from the polymalonate acetate pathway, are of interest for cosmetics: photoabsorbing, antioxidant and inducing melanogenesis. These properties have been studied for a still limited number of secondary metabolites (Mitrović, 2011) (Figure 1).

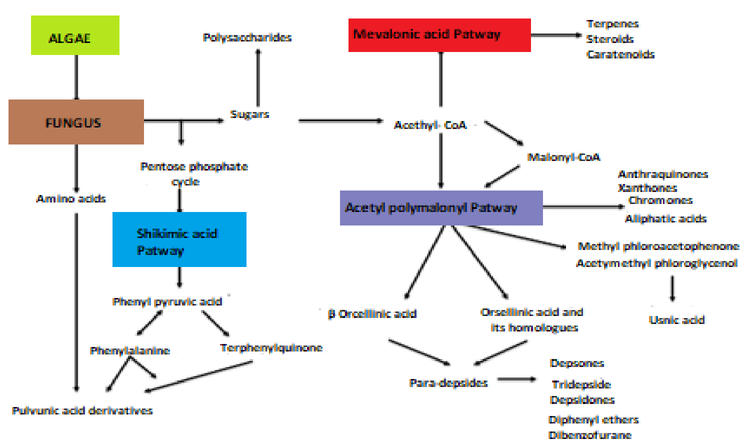


Figure 1. Biosynthetic pathways of lichen secondary metabolites (Elix, 1996; Stocker wörgötter, 2008).

The natural products isolated from different lichens (such as Usnic acid, Lobaric acid, Atranorin, Protolichesterinic acid and Salazinic acid) have good antibiotic activities against Gram-positive bacteria and are also active against pathogenic dermatophyte fungi [8]. Other products found in lichens, such as anthraquinones derivatives, bianthrones and hypericin, have an inhibitory action on the activity of viral enzymes, such as the integrase of HIV-1 and HSV-1 [9,10] and also on enzymes such as lipooxygenases, histidine decarboxylase and tyrosinase; other derivatives inhibit the biosynthesis of Leukotriene B_4 (LTB_4). [11–13]

Polyphenolic products isolated from lichens have limitations, low solubility and, above all, toxicity. Usnic acid is a polyphenolic compound very common in lichens that has good activity, among others, against microorganisms of the Mycobacterium genus. In the 1950s, Shibata and Miura [14] made modifications and derivatizations of the functional groups of usnic acid to carry out the structure–activity correlation study to enhance the biological activity profile.

Usnic and polyporic acids have a good growth inhibition activity of L1210 leukemic cells; in subsequent research [15,16], several derivatives of these acids have been prepared to enhance antitumor activity, but none of these derivatives have exceeded the activities presented by usnic and polyporic acids.

Kumar and Muller have prepared a series of analogs of barbatic, diffractaic and obtusatic acids isolated from lichens to evaluate the effects of inhibition of the biosynthesis of LTB_4 and as antiproliferative agents. Some of these derivatives show good potential as LTB_4 biosynthesis inhibitors [11,13].

Lichens can also have xanthones, that shown enzyme modulation that are therapeutic targets, such as protein kinase C [17], topoisomerase II [18,19], acetylcholinesterase [20] and monoamine oxidases [21]; antiretrovirals [22,23], antimalarials [24,25], antihypertensives [26], anti-inflammatory, cytotoxics [27] and antitumors [28,29]. For this reason, the use of the base skeleton of xanthone is justified to prepare derivatives with bioactive potential.

Many depsidones isolated from lichens and higher plants have important activities, including the inhibition of enzymatic activity [30], antimycobacterial, anti-inflammatory, analgesic, antitumor, cytotoxic and antiviral activity [31–33]. In the work published in 2015 by James C Lendemer and collaborators [34], they studied and delineated the *Parmotrema* species in eastern North America. Using morphological, chemical, reproductive and ecological characters, they define four species for this group: *P. hypoleucinum*, *P. hypotropum*, *P. perforatum* and *P. subrigidum*.

This group has found *P. hypoleucinum* and *P. subrigidum* to be momophyletic, the latter comprising two chemotypes that differ in the presence or absence of norstictic acid in addition to alectoronic acid.

Due to the pharmacological potential presented by compounds isolated in lichens, it was decided to study the chemical composition of the lichen *Parmotrema hypoleucinum* (J. Steiner) Hale, collected on two different supports in the area of Lac Tonga in Algeria.

2. Results and Discussion

Parmotrema hypoleucinum (J. Steiner) Hale is an epiphytic lichen collected in Algeria and studied in order to determine its metabolic composition and chemical fingerprint. *P hypoleucinum* (J. Steiner) Hale was collected in two different supports, the first one in Lac Tonga (Sector Brabtia) on *Olea europaea* and the second one in Lac Tonga (Sector Brabtia) on *Quercus coccifera*. The metabolic compositions of each lichen sample were studied by sequential extraction, first of all, with hexane in Soxhlet for low polarity products. The remaining vegetable mass was placed with chloroform at room temperature to obtain the chloroform extract for the products of intermediate polarity, and finally, the vegetable mass was extracted with ethanol at room temperature for the products with higher polarity.

The hexane extract of each sample was dissolved in hot methanol and allowed to cool slowly to obtain the products insoluble in cold methanol. In this way, the products insoluble in methanol were separated: **1-Cires** and **2-Cires**; remaining soluble: **1-Hexane** and **2-Hexane**. Initially, an aliquot of the samples **1-Hexane**, **2-Hexane, 1-Cires** and **2-Cires** were esterified with diazomethane to esterify the acid groups of the existing compounds.

These esterified samples were analyzed by GC-MS to identify compounds of lower polarity.

The different extracts obtained were:
From *P hypoleucinum* (J. Steiner) Hale on *Olea europaea*
1-Hexane, 1-Cires, 1-C 1-Ethanol
From *Parmotrema hypoleucinum* (J. Steiner) Hale on *Quercus coccifera*.
2-Hexane, 2-Cires, 2-Choroform, 2-Ethanol

Chemical analysis of the hexane extract soluble in MeOH esterified with diazometane, 1-Hexan and 2-Hexane of Parmotrema hypoleucinum collected from two different phorophytes by GC/MS

The **1-Hexane** and **2-Hexane** samples were esterified with diazomethane to esterify the existing acid groups to their methyl esters for GC-MS analysis, being the natural products of the acids indicated in Table 1 for **1-Hexane** sample and Table 2 for **2-Hexane** sample.

Table 1. Sample *Parmotrema hypoleucinum* (in *Olea europaea*), Hexane extract part soluble in MeOH esterified whit diazomethane, **1-Hexane.**

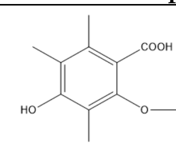
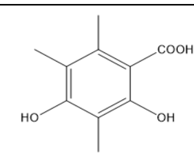
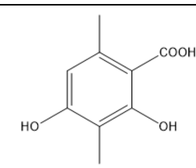
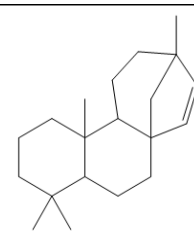
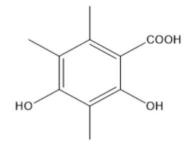
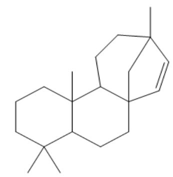
Nº	RT	Identified Product	Mass	%	Natural Compound, Structure
1	19:84	Methyl 4-hydroxy-2-methoxy-3,5,6-trimethylbenzoate	224	0.5	
2	20:00	Methyl 2,4-dihydroxy-3,5,6-trimethylbenzoate	210	62.4	
3	20:34	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	196	8.6	
3	22:36	Methylhexadecanoate	270	5.3	$C_{16}H_{32}O_2$ palmitic acid
4	23:53	13-methyl-17-norkaur-15-ene (hibaene)	272	10.1	
5	24:19	Methyl 9,12-octadecadienoate	294	4.2	$C_{18}H_{32}O_2$ linoleic acid
6	24:25	Methyl (*Z*)-9-octadecenoate	296	2.1	$C_{18}H_{34}O_2$ oleic acid
7	24:48	Methyloctadecanoate	298	3.6	$C_{18}H_{34}O_2$ stearic acid
8	25:39	Unidentified	288	0.8	Unidentified

Table 2. Sample *Parmotrema hypoleucinum* (in *Quercus coccifera*), Hexane extract part soluble in MeOH esterified whit diazometane, **2-Hexane.**

Nº	RT	Identified Product	Mass	%	Natural Compound, Structure
1	20:01	Methyl 2,4-dihydroxy-3,5,6-trimethylbenzoate	210	2.5	
2	22:55	Methylhexadecanoate	270	2.8	$C_{16}H_{32}O_2$ palmitic acid
3	23:54	13-Methyl-17-norkaur-15-ene Probably the natural product will be (-)-*ent*-Kauran-16α-ol	272	36.7	
4	24:19	Methyl (*Z*,*Z*)-9,12-octadecadienoate	294	1.2	$C_{18}H_{32}O_2$ linoleic acid
5	24:23	Methyl (*Z*)-9-octadecenoate	296	1.0	$C_{18}H_{34}O_2$ oleic acid
6	24:38	Methyl octadecanoate	298	1.4	$C_{18}H_{34}O_2$ stearic acid

The **1-Hexane** sample was analyzed by GC-MS, and eight products were identified, among them palmitic, linoleic, oleic and stearic acids and an unidentified compound. Figure 2 and Table 1. The esterified **2-Hexane** sample was also analyzed by GC-MS, identifying six products, palmitic, linoleic, oleic and stearic acid, a phenolic compound 2,4-dihydroxy-3,5,6-trimethylbenzoic acid and 13-methyl-17-norkaur-15-ene. Figure 3 and Table 2.

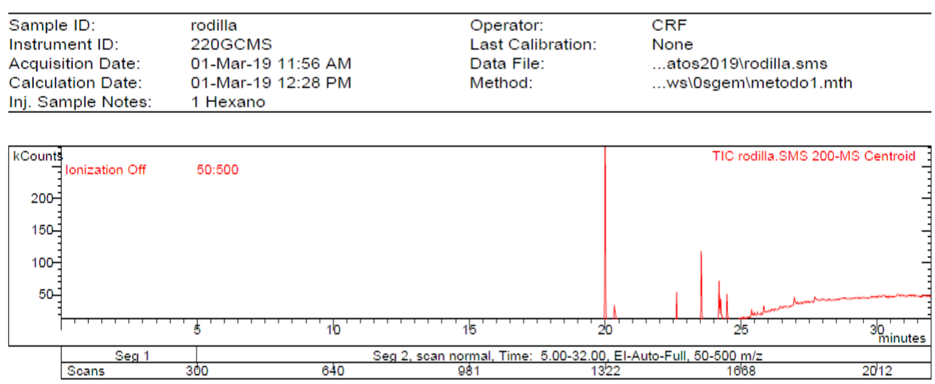


Figure 2. Chromatogram of Hexane extract part soluble in MeOH esterified with diazomethane, **1-Hexane.**

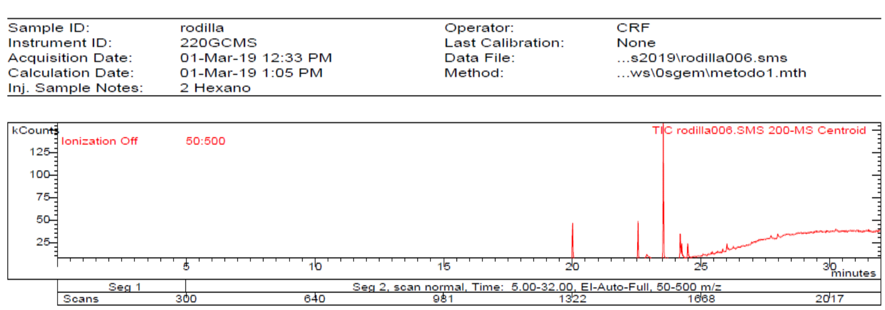


Figure 3. Chromatogram of Hexane extract part soluble in MeOH esterified whit diazomethane, **2-Hexane.**

The compound identified as Hibaene (13-methyl-17-norkaur-15-ene) is the product with a retention time of 23:53 in GC-MS; its mass spectrum shows the molecular ion at 272 and comes from the dehydration of alcohol (-)-*ent*-Kauran-16α-ol in the ionization source of the mass spectrometer. The natural product should be the alcohol (-)-*ent*-Kauran-16α-ol.

P hypoleucinum (J. Steiner) Hale, on *Quercus coccifera*, the **2-Hexane** sample, it has a lower number of components, and all were identified in the **1-Hexane** sample. The most important fatty acids in the extracts were identified as palmitic, linoleic, oleic and stearic acids. 2,4-Dihydroxy-3,5,6-trimethylbenzoic acid and (-)-*ent*-Kauran-16α-ol alcohol is also identified in the two samples, **1-Hexane** and **2-Hexane**. Only in the **1-Hexane** sample 4-hydroxy-2-methoxy-3,5,6-trimethylbenzoic acid and 2,4-dihydroxy-3,6-dimethylbenzoic acid are also identified.

For the fractions that have been obtained by crystallization from the crude hexane extract by solubilization in hot methanol, **1-Cires** and **2-Cires** are also esterified with diazomethane for GC-MS analysis.

In the **1-Cires** analysis, five products are found, with four being identified. Figure 4 and Table 3.

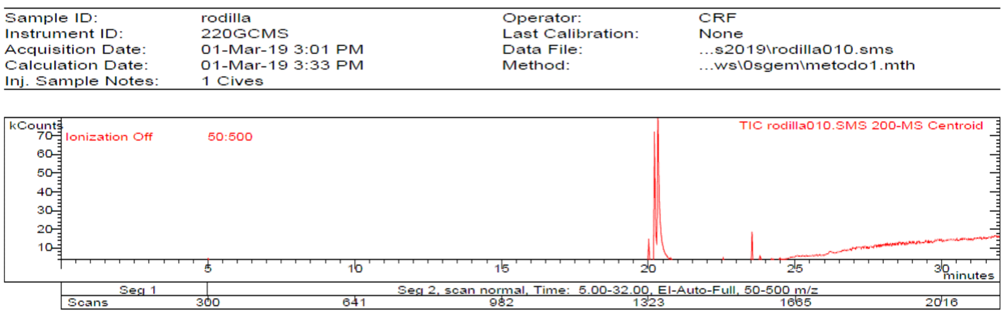
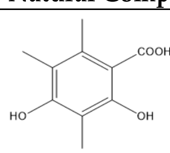
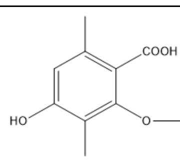
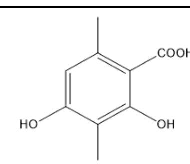
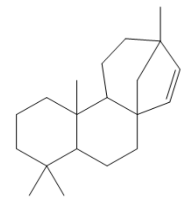


Figure 4. Chromatogram of Hexane extract part insoluble in MeOH esterified whit diazomethane, **1-Cires.**

Table 3. Sample *Parmotrema hypoleucinum* (in *Olea europaea*), Hexane extract part insoluble in MeOH esterified whit diazomethane, **1-Cires.**

Nº	RT	Identified Product	Mass	%	Natural Compound, Structure
1	20:02	Methyl 2,4-dihydroxy-3,5,6-trimethylbenzoate	210	1.1	
2	20:21	Methyl 4-hydroxy-2-methoxy-3,6-dimethylbenzoate	210	26.6	
3	20:33	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	196 $C_9H_{10}O_4$	38.9	
4	22:51	Unidentified	312	0.9	Unidentified

5	22:51	13-Methyl-17-nor-8*β*,13*β*-kaur-15-ene Probably the natural product will be (-)-*ent*-Kauran-16*α*-ol	272 $C_{20}H_{32}$ Hibaene	1.8	

In the **2-Cires** sample, three products have been identified from the methanol-insoluble part of the hexane extract of *P. hypoleucinium* (*Quercus coccifera*). Figure 5 and Table 4.

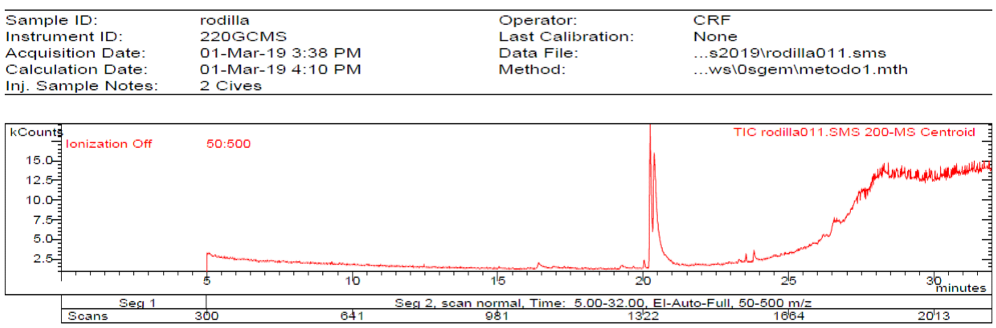
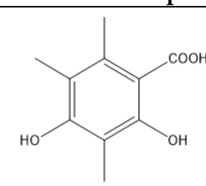
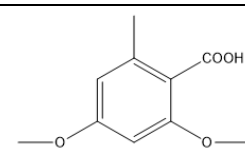
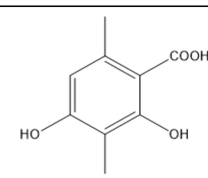


Figure 5. Chromatogram of Hexane extract part insoluble in MeOH esterified whit diazometane, **2-Cires.**

Table 4. Sample *Parmotrema hypoleucinum* (in *Quercus coccifera*), Hexane extract part insoluble in MeOH esterified whit diazomethane, **2-Cires.**

Nº	**RT**	**Identified Product**	**Mass**	**%**	**Natural Compound, Structure**
1	20:24	Methyl 2,4-dihydroxy-3,5,6-trimethylbenzoate	210	0.9	
2	20:35	Methyl 2,4-dimethoxy-6-methylbenzoate	210	41.4	
3	20:38	Methyl 2,4-dihydroxy-3,6-dimethylbenzoate	196	48.9	

In the **1-Cires** sample, there is a compound that could not be identified; this product does not appear in the **2-Cires** sample. The kaurene-skeletal alcohol is found in **1-Cires** and was not found in the **2-Cires** sample. Of the other three compounds, two were identified in **1-Cires** and **2-Cires**: 2,4-dihydroxy-3,5,6-trimetylbenzoic acid and 2,4-dihydroxy-3,6-dimethylbenzoic acid.

In the **1-Cires** sample, 4-hydroxy-2-methoxy-3,6-dimethylbenzoic acid is also identified, and this product does not appear in the 2-Cires sample; instead, the 2,4-dimethoxy-6-methylbenzoic acid appears in **2-Cires**.

In the HPLC—MS/MS analysis of the **1-Hexane** and **2-Hexane** fractions of *P. hypoleucinium*, 95 products were detected in the **1-Hexane** fraction, in which we proposed 78 structures (and 16 not identified). In total, 91 products were found in the **2-Hexane** fraction, of which we proposed 78 structures (and 13 no identified ones).

Figures 6 and 7 show the HPLC chromatograms that allowed this analysis and identification of the indicated compounds to be carried out; the complete result is shown in Table 5.

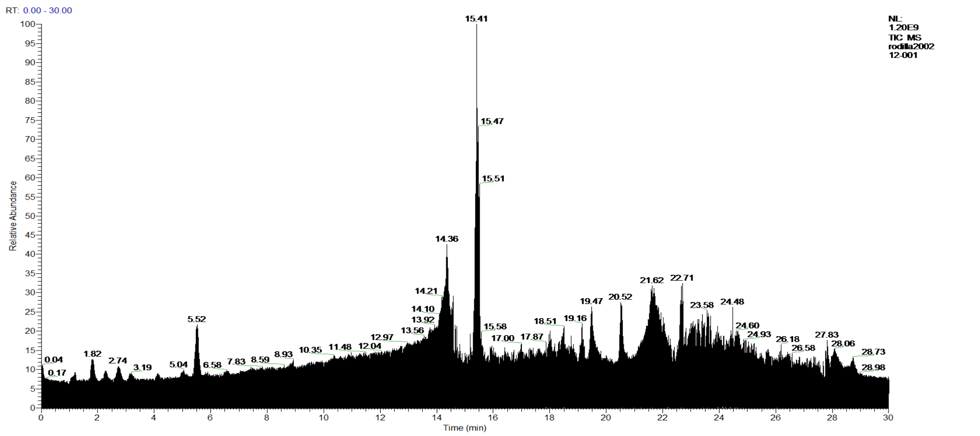


Figure 6. Chromatogram of Hexane extract part soluble in MeOH, **1-Hexane.**

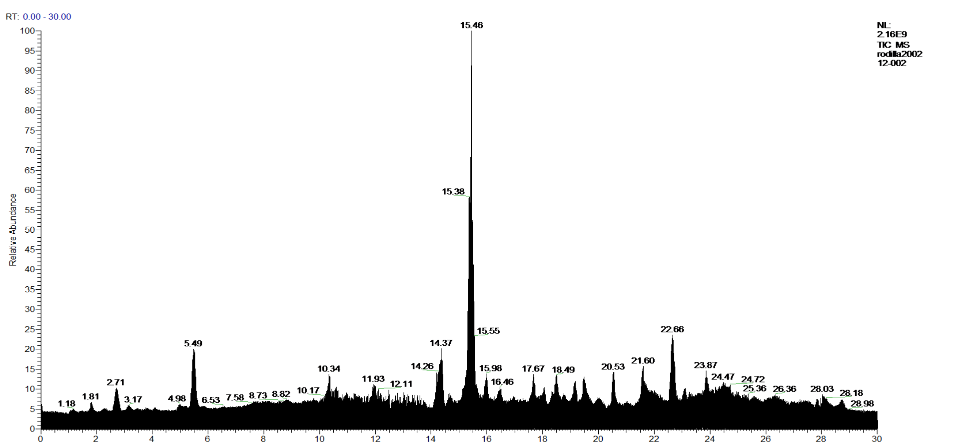
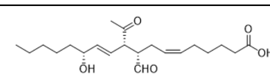
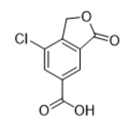
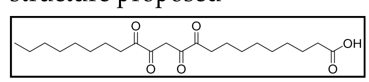
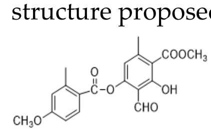


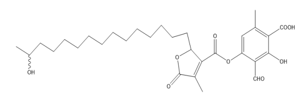
Figure 7. Chromatogram of Hexane extract part soluble in MeOH esterified whit diazomethane, **2-Hexane.**

Table 5. Samples *Parmotrema hypoleucinum* (in *Olea europea*), Hexane extract part soluble in MeOH **1-Hexane** and *Parmotrema hypoleucinum* (in *Quercus coccifera*), Hexane extract part soluble in MeOH, **2-Hexane**.

Nº	RT	[M-H]⁻	Mass Calc	Formula 1-Hexane	Formula 2-Hexane	Compounds
1	2.67	181.0502	182.0574	$C_9H_{10}O_4$	$C_9H_{10}O_4$	3-Methylorsellinic acid
2	2.72	187.0970	188.1043	$C_9H_{16}O_4$	$C_9H_{16}O_4$	3,5-Dimethoxyciclohexanecarboxilic acid
3	2.75	293.0669	294.0741	$C_{14}H_{14}O_7$	$C_{14}H_{14}O_7$	6-(Hydroxymethyl)-3,5-bis(methoxycarbonyl)-2,4-dimethylcyclohex-1-ene-1-carboxylic acid

4	3.08	243.1239	244.1311	$C_{12}H_{20}O_5$	$C_{12}H_{20}O_5$	3,5,6-Hydroxymethyl-2,4-dimethylcyclohex-1-ene-1-carboxylic acid
5	3.15	151.0393	152.0465	$C_8H_8O_3$	$C_8H_8O_3$	Atranol
6	3.20	225.1129	226.1201	$C_{12}H_{18}O_4$	$C_{12}H_{18}O_4$	5-Formyl-3-hydroxymethyl-2,4,6-trimethylcyclohex-1-ene-1-carboxylic acid
7	3.47	199.0973	200.1046	$C_{10}H_{16}O_4$	$C_{10}H_{16}O_4$	3,5-Dihydroxy-2,4,6-trimethylciclohexenecarboxilic acid
8	3.55	195.0660	196.0730	-	$C_{10}H_{12}O_4$	3,5-Dimethylorsellinic acid
9	3.58	149.0237	150.0310	$C_8H_6O_3$	$C_8H_6O_3$	4-Formylbenzoic acid
10	3.82	241.1081	242.1153	$C_{12}H_{18}O_5$	$C_{12}H_{18}O_5$	5-Formyl-3,6-dihydroxymethyl-2,4-dimethylcyclohex-1-enecarboxylic acid
11	4.10	201.1129	202.1202	$C_{10}H_{18}O_4$	$C_{10}H_{18}O_4$	2,4-Dihydroxy-3,5,6-trimethylcyclohexane-1-carboxylic acid
12	4.95	199.1337	200.1409	$C_{11}H_{20}O_3$	$C_{11}H_{20}O_3$	2-Hydroxy-10-undecenoic acid
13	5.00	185.0006	186.0079	$C_8H_7ClO_3$	$C_8H_7ClO_3$	Chloroatranol
14	5.10	213.1130	214.1203	$C_{11}H_{18}O_4$	$C_{11}H_{18}O_4$	2-Undecenedioic acid
15	5.21	169.0863	170.0936	$C_9H_{14}O_3$	$C_9H_{14}O_3$	4-Hydroxy-2,5-dimethylcyclohex-1-ene-1-carboxylic acid
16	5.49	163.0392	164.0470	$C_9H_8O_3$	$C_9H_8O_3$	*p*-Coumaric acid
17	5.60	209.1181	210.1253	$C_{12}H_{18}O_3$	$C_{12}H_{18}O_3$	6-(1-Oxopentyl)-1-cyclohexene-1-carboxylic acid
18	5.87	215.1286	216.1359	$C_{11}H_{20}O_4$	$C_{11}H_{20}O_4$	Undecanedioic acid
19	6.57	227.1288	228.1360	$C_{12}H_{20}O_4$	$C_{12}H_{20}O_4$	*trans*-Dodec-2-enedioic acid
20	7.44	282.2078	283.2150	$C_{16}H_{29}NO_3$	$C_{16}H_{29}NO_3$	N-Dodecanoyl-L-Homoserine lactone
21	7.67	329.2336	330.2252	-	$C_{18}H_{34}O_5$	9,10-Dihydroxy-8-oxo-12-octadecenoic acid
22	7.77	209.0817	210.0889	$C_{11}H_{14}O_4$	$C_{11}H_{14}O_4$	3,4-Dimethoxyhydrocinnamic acid
23	8.70	373.1294	374.1366	$C_{20}H_{22}O_7$	$C_{20}H_{22}O_7$	4-O-demethyldivaricatic acid
24	9.01	253.1809	254.1882	$C_{15}H_{26}O_3$	$C_{15}H_{26}O_3$	9-Hydroxy-10,12-pentadecadienoic acid
25	9.53	243.1601	244.1675	$C_{13}H_{24}O_4$	$C_{13}H_{24}O_4$	Tridecanedioic acid
26	10.13	359.1139	360.1211	$C_{19}H_{20}O_7$	-	Barbatic acid
27	10.30	375.1086	376.1159	$C_{19}H_{20}O_8$	$C_{19}H_{20}O_8$	8-Hydroxybarbatic acid
28	10.35	373.0931	374.1001	$C_{19}H_{18}O_8$	$C_{19}H_{18}O_8$	Baeomycesic acid
29	10.59	311.2230	312.2302	$C_{18}H_{32}O_4$	$C_{18}H_{32}O_4$	9Z-Octadecenedioic acid
30	10.77	351.2178	352.2256	$C_{20}H_{32}O_5$	$C_{20}H_{32}O_5$	 structure proposed
31	10.87	233.1547	234.1619	$C_{15}H_{22}O_2$	$C_{15}H_{22}O_2$	Fukinanolide
32	10.89	323.2230	324.2303	$C_{19}H_{32}O_4$	$C_{19}H_{32}O_4$	*allo*-Protolichestrinic acid
33	10.91	411.0045	412.0118	$C_{18}H_{14}Cl_2O_7$	$C_{18}H_{14}Cl_2O_7$	Leoidin
34	10.91	396.9888	397.9961	$C_{17}H_{12}Cl_2O_7$	$C_{17}H_{12}Cl_2O_7$	Lecideoidin
35	11.49	403.1399	404.1473	$C_{21}H_{24}O_8$	$C_{21}H_{24}O_8$	4′-*O*-demethylsekikaic acid
36	12.28	313.2388	314.2266	-	$C_{18}H_{34}O_4$	Octadecanedioic acid
37	13.00	269.2124	270.2195	$C_{16}H_{30}O_3$	$C_{16}H_{30}O_3$	2-Oxopalmitic acid
38	13.04	389.1245	390.1315	$C_{20}H_{22}O_8$	$C_{20}H_{22}O_8$	8-Hydroxydiffractaic acid
39	13.34	293.2124	294.2202	$C_{18}H_{30}O_3$	$C_{18}H_{30}O_3$	2-Hydroxylinolenic acid
40	13.58	291.1968	292.2041	$C_{18}H_{28}O_3$	$C_{18}H_{28}O_3$	α-Licanic acid
41	14.38	295.2279	296.2351	$C_{18}H_{32}O_3$	$C_{18}H_{32}O_3$	2-Hydroxylinoleic acid isomer
42	14.39	455.1711	456.1783	$C_{25}H_{28}O_8$	$C_{25}H_{28}O_8$	Glomelliferonic acid

43	14.40	295.2278	296.2352	-	$C_{18}H_{32}O_3$	18-Hydroxylinoleic acid
44	14.68	321.2437	322.2509	$C_{20}H_{34}O_3$	$C_{20}H_{34}O_3$	Hydroxyeicosatrienoic acid
45	14.95	305.2125	306.2197	$C_{19}H_{30}O_3$	$C_{19}H_{30}O_3$	14-Oxo-7,10,12-nonadecatrienoic acid
46	15.19	297.2436	298.2508	$C_{18}H_{34}O_3$	$C_{18}H_{34}O_3$	9-Oxooctadecanoic acid
47	15.25	295.2280	296.2351	$C_{18}H_{32}O_3$	$C_{18}H_{32}O_3$	Coriolic acid
48	15.36	297.2435	298.2508	$C_{18}H_{34}O_3$	$C_{18}H_{34}O_3$	Ricinoleic acid
49	15.42	373.0925	374.0999	$C_{19}H_{18}O_8$	$C_{19}H_{18}O_8$	**Atranorin**
50	15.43	177.0187	178.0259	$C_9H_6O_4$	$C_9H_6O_4$	6,7-Dihydroxycoumarin
51	15.44	277.2536	278.2610	$C_{19}H_{34}O$	$C_{19}H_{34}O$	7,10,12-nonadecatrien-1-ol
52	15.46	365.2330	366.2403	$C_{21}H_{34}O_5$	$C_{21}H_{34}O_5$	Muronic acid
53	15.70	277.2538	278.2612	$C_{18}H_{30}O_2$	$C_{18}H_{30}O_2$	Linolenic acid
54	15.98	367.2488	368.2562	$C_{21}H_{36}O_5$	$C_{21}H_{36}O_5$	Murolic acid
55	16.16	311.2594	312.2667	$C_{19}H_{36}O_3$	$C_{19}H_{36}O_3$	Lichesterylic acid
56	16.29	461.2550	462.2619	$C_{26}H_{38}O_7$	$C_{26}H_{38}O_7$	Unidentified
57	16.37	471.3481	472.3553	$C_{30}H_{48}O_4$	$C_{30}H_{48}O_4$	Unidentified
58	16.45	381.2282	382.2356	$C_{21}H_{34}O_6$	$C_{21}H_{34}O_6$	Praesorediosic acid
59	16.47	210.9834	211.9873	$C_9H_5ClO_4$	$C_9H_5ClO_4$	 7-Chloro-3-oxo-1,3-dihydroisobenzofuran-5- carboxylic acid.
60	16.47	381.2285	382.2355	$C_{21}H_{34}O_6$	$C_{21}H_{34}O_6$	19-Acetoxy-protolichesterinic acid
61	16.51	407.0540	408.0611	$C_{19}H_{17}ClO_8$	$C_{19}H_{17}ClO_8$	**Chloroatranorin**
62	16.90	421.9285	422.9363	$C_{16}H_{10}Cl_4O_5$	$C_{16}H_{10}Cl_4O_5$	Diploicin
63	16.97	201.1493	202.1564	$C_{11}H_{22}O_3$	$C_{11}H_{22}O_3$	11-Hydroxyundecanoic acid
64	16.97	385.2960	386.3032	$C_{22}H_{42}O_5$	$C_{22}H_{42}O_5$	Unidentified
65	17.59	387.2544	388.2616	$C_{24}H_{36}O_4$	$C_{24}H_{36}O_4$	Unidentified
66	17.68	389.1242	390.1314	$C_{20}H_{22}O_8$	$C_{20}H_{22}O_8$	Leprolomin
67	17.69	395.2442	396.2520	$C_{22}H_{36}O_6$	-	structure proposed 
68	17.80	357.0983	358.1057	$C_{19}H_{18}O_7$	$C_{19}H_{18}O_7$	structure proposed: 
69	17.97	449.3276	450.3354	$C_{35}H_{38}O_7$	$C_{35}H_{38}O_7$	Unidentified
70	18.04	253.2173	254.2244	$C_{16}H_{30}O_2$	$C_{16}H_{30}O_2$	Palmitoleic acid
71	18.33	241.2172	242.2245	$C_{15}H_{30}O_2$	$C_{15}H_{30}O_2$	Pentadecanoic acid
72	18.35	455.3531	456.3605	$C_{30}H_{48}O_3$	$C_{30}H_{48}O_3$	Ursolic acid
73	18.50	279.2330	280.2403	$C_{18}H_{32}O_2$	$C_{18}H_{32}O_2$	Linoleic acid
74	18.66	299.2595	300.2667	$C_{18}H_{36}O_3$	$C_{18}H_{36}O_3$	2-Hydroxyoctadecanoic acid
75	18.78	279.2332	280.2403	$C_{18}H_{32}O_2$	$C_{18}H_{32}O_2$	Linoleic acid isomer, *cis,trans*
76	19.03	497.2548	498.2626	$C_{29}H_{38}O_7$	-	Superpicrolichenic acid
77	19.16	255.2329	256.2401	$C_{16}H_{32}O_2$	$C_{16}H_{32}O_2$	Palmitic acid
78	19.49	281.2485	282.2559	$C_{18}H_{34}O_2$	$C_{18}H_{34}O_2$	Oleic acid
79	19.62	269.2488	270.2561	$C_{17}H_{34}O_2$	$C_{17}H_{34}O_2$	15-Methylhexadecanoic acid
80	19.89	269.2488	270.2561	$C_{17}H_{34}O_2$	$C_{17}H_{34}O_2$	Heptadecanoic acid
81	19.99	483.3481	484.3553	$C_{31}H_{48}O_4$	$C_{31}H_{48}O_4$	Unidentified

82	20.07	327.2543	328.2616	$C_{20}H_{40}O_3$	$C_{20}H_{40}O_3$	2-Hydroxyeicosanoic acid
83	20.17	473.2548	474.2626	$C_{27}H_{38}O_7$	$C_{27}H_{38}O_7$	Unidentified
84	20.54	283.2643	284.2716	$C_{18}H_{36}O_2$	$C_{18}H_{36}O_2$	Stearic acid (octadecanoic acid)
85	20.74	309.2801	310.2875	$C_{20}H_{38}O_2$	$C_{20}H_{38}O_2$	Eicosenoic acid
86	21.02	441.3377	442.3448	$C_{29}H_{46}O_3$	$C_{29}H_{46}O_3$	Unidentified
87	21.14	297.2801	298.2873	$C_{19}H_{38}O_2$	$C_{19}H_{38}O_2$	Nonadecanoic acid
88	21.70	311.2957	312.3029	$C_{20}H_{40}O_2$	$C_{20}H_{40}O_2$	(Eicosanoic acid) Arachidic acid
89	22.02	597.4164	598.4242	$C_{37}H_{58}O_6$	-	Unidentified
90	22.02	669.4739	670.4817	$C_{41}H_{66}O_7$	-	Unidentified
91	22.02	545.2758	546.2836	$C_{30}H_{42}O_9$	$C_{30}H_{42}O_9$	Structure proposed and confirmed by the ions 
92	22.52	579.2368	580.2446	$C_{36}H_{36}O_7$	-	Unidentified
93	22.66	753.4057	754.4135	$C_{37}H_{62}O_{14}$	$C_{37}H_{62}O_{14}$	Unidentified
94	22.68	637.4841	638.4908	$C_{41}H_{66}O_5$	$C_{41}H_{66}O_5$	Unidentified
95	22.69	751.4779	752.4857	$C_{45}H_{38}O_9$	$C_{45}H_{38}O_9$	Unidentified
96	23.78	603.3334	604.3412	$C_{37}H_{48}O_7$	$C_{37}H_{48}O_7$	Unidentified
97	23.81	633.3798	634.3876	$C_{39}H_{54}O_7$	-	Unidentified
98	24.12	467.4109	468.4187	$C_{30}H_{56}O_4$	-	12-Triacontenedioic acid
99	24.51	605.3483	606.3561	$C_{37}H_{50}O_7$	$C_{37}H_{50}O_7$	Unidentified

The most characteristic products of the **1-Hexane** and **2-Hexane** extracts of the lichens in the two samples are 3-methylorsellinic acid, atranol, 4-formylbenzoic acid, chloroatranol, *p*-coumaric acid, atranorin and chloroatranorin.

In the fatty acid profile, we find the following acids common to the two samples: 2-hydroxy-10-undecenoic, 2-undecenedioic, undecanedioic, trans-2-dodecenedioic, 9-hydroxy-10,12-pentadecadienoic, tridecanedioic, 9Z-octadecenedioic, 10-acyl-9-formyl-13-hydroxyoctadeca-6,11-dienoic, octadecanedioic, 2-oxopalmitic, nonadecanoic, arachidic and 12-triacontenedioic acids.

Among the acids that have a cyclohexanecarboxylic base, the following derivatives were also found, as described in the publication of the lichen *Physcia mediterranea* Nimis [35], the 3,5-dimethoxycyclohexanecarboxylic acids, 6-(hydroxymethyl)-3,5-bis(methoxycarbonyl)-2,4-dimethylcyclohex-1-ene-1-carboxylic, 3,5,6-hydroxymethyl-2,4-dimethylcyclohex-1-ene-1-carboxylic, 5-formyl-3-hydroxymethyl-2,4,6-trimethylcyclohex-1-ene-1-carboxylic, 3,5-dihydroxy-2,4,6-trimethylcyclohex-1-ene-1-carboxylic, 5-formyl-3,6-dihydroxymethyl-2,4-dimethylcyclohex-1-ene-1-carboxylic, 2,4-dihydroxy-3,5,6-trimethylcyclohexane-1-carboxylic, 4-hydroxy-2,5-dimethylcyclohex-1-ene-1-carboxylic, 6-(1-oxopentyl)-cyclohex-1-ene-1-carboxylic acids.

Lactones were also identified: *N*-dodecanoyl-*L*-homoserine lactone and fukinanolide, other derivatives such as leoidin, lecideoidin, an alcohol such as 7,10,12-nonadecatrien-1-ol and a triterpenic acid identified as ursolic acid (found in the two extracts).

In the comparison of the characteristic polyphenolic compounds of the lichens in these two samples, 20 compounds were identified: 4-O-demethyldivaricatic, barbatic, 8-hydroxybarbatic, baeomycesic, allo-protolichesterinic, 4′-O-demethylsekikaic acids, 8-hydroxydiffractaic, glomelliferonic occurs in *Xanthoparmelia subincerta* [36], muronic is detected in *P. praesorediosum* [37], murolic, lichesterylic [35], praesorediosic, 19-acetoxyprotolichesterinic, diploicin, leprolomin, methyl 3-formyl-2-hydroxy-4-((4-methoxy-2-methylbenzoyl)oxy)-6-methylbenzoate, superpicrolichenic and 3-formyl-2-hydroxy-4-((2-(14-hydroxypentadecyl)-4-methyl-5-oxo-2,5-dihydrofuran-3-carbonyl)oxy)-6-methylbenzoic acids.

In the **1-Hexane** sample, the following compounds were not identified: 3,5-dimethylorselinic acid, 9,10-dihydroxy-8-oxo-12-octadecenoic acid, octadecanedioic acid and 18-hydroxylinoleic acid, which were found in the **2-Hexane** sample. The following compounds were not identified in the **2-Hexane** sample: barbatic acid, tetraoxodocosanoic acid and superpicrolichenic acid; these products were identified in the **1-Hexane** sample too. In the 1-Hexane sample, there are 17 products that could not be identified and in the **2-Hexane** 13.

In the analysis of the **1-Cires** and **2-Cires** fractions, obtained by precipitation of the initial hexane extract by HPLC-MS/MS, it has been possible to detect in **1-Cires** 56 products, of which 11 products were not identified. In **2-Cires**, 53 products were detected, and 13 products could not be identified. For the acids and diacids identified in **1-Cires** there are 23 products, and in **2-Cires**, we have 27 products. As benzoic acids or derivatives we have p-coumaric acid and 6,7-dihydroxycoumarin. Among the polyphenolic compounds and esters, **1-Cires** and **2-Cires** were identified: *allo*-protolichestrinic acid, atranorin, 7-chloro-3-oxo-1,3-dihydroisobenzofuran-5-carboxylic acid, chloroatranorin, 8-hydroxydiffractaic acid and 19-acetoxylichestrinic acid.

The chromatograms and the analysis of the **1-Cires** and **2-Cires** compounds are shown in Figures 8 and 9 and Table 6.

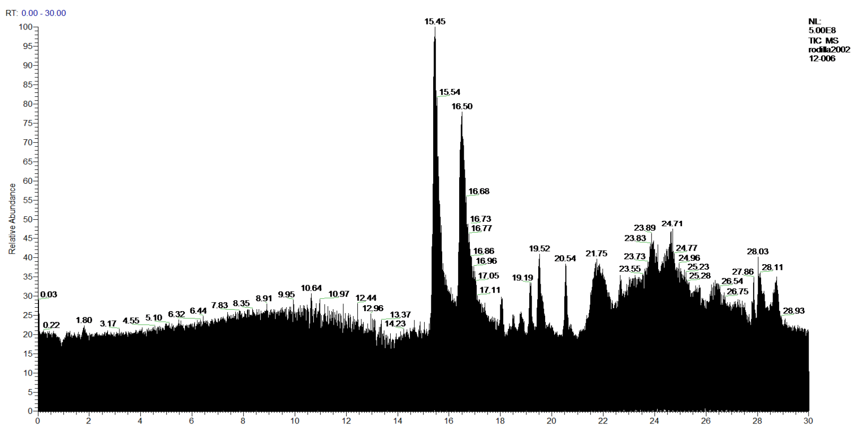


Figure 8. Chromatogram of Hexane extract part insoluble in MeOH, **1-Cires.**

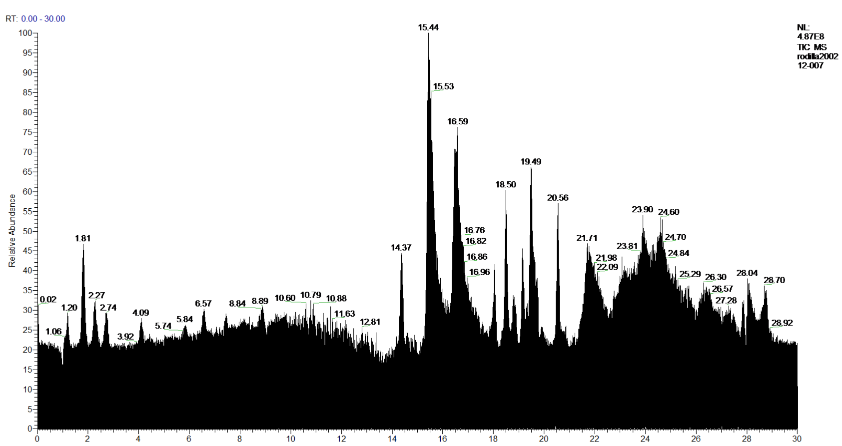
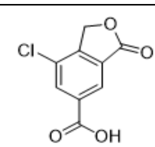


Figure 9. Chromatogram of Hexane extract part insoluble in MeOH, **2-Cires.**

Table 6. Samples *Parmotrema hypoleucinum* (in *Olea europea*), Hexane extract part insoluble in MeOH, **1-Cires** and *Parmotrema hypoleucinum* (in *Quercus coccifera*), Hexane extract part insoluble in MeOH, **2-Cires**.

Nº	**RT**	**[M-H]⁻**	**MW Calc**	**Formula 1-Cires**	**Formula 2-Cires**	**Compounds**
1	2.67	146.9397	147.9475	$C_4H_4O_6$	-	Dihydroxyfumaric acid
2	0.04	190.9281	191.9359	$C_5H_4O_8$	-	Methanetetracarboxylic acid
3	1.80	116.9276	117.9354	$C_4H_6O_4$	-	Butendioic acid
4	2.19	112.9845	113.9932	$C_4H_2O_4$	$C_4H_2O_4$	2,3-Dioxobuten-1,4-dial
5	2.72	187.0971	188.1049	$C_9H_{16}O_4$	$C_9H_{16}O_4$	3,5-Dimethoxyciclohexanecarboxilic acid
6	4.12	201.1129	202.1207	$C_{10}H_{18}O_4$	$C_{10}H_{18}O_4$	2,4-Dihydroxy-3,5,6-trimethylcyclo-hexane-1-carboxylic acid
7	5.51	163.0395	164.0473	$C_9H_8O_3$	-	*p*-Coumaric acid
8	5.85	268.1919	269.1997	$C_{12}H_{28}O_6$	$C_{12}H_{28}O_6$	Unidentified
9	6.57	227.1286	228.1364	-	$C_{12}H_{20}O_4$	*trans*-Dodec-2-enedioic acid
10	7.46	282.2077	283.2155	$C_{16}H_{29}NO_3$	$C_{16}H_{29}NO_3$	*N*-Dodecanoyl-*L*-homoserine lactone
11	8.09	174.9556	175.9634	$C_5H_4O_7$	-	2-Hydroxy-3,4-dioxopentanedioc acid
12	8.15	293.1762	294.1840	$C_{17}H_{26}O_4$	-	gingerol
13	8.39	323.2230	324.2308	-	$C_{19}H_{32}O_4$	allo-protolichestrinic acid
14	9.50	350.2337	351.2415	$C_{17}H_{34}O_7$	$C_{17}H_{34}O_7$	Xylitollaurate
15	10.88	233.1546	234.1624	-	$C_{15}H_{22}O_2$	Fukinanolide
16	11.43	334.2389	335.2467	$C_{17}H_{34}O_6$	$C_{17}H_{34}O_6$	Unidentified
17	11.75	311.2231	312.2309	$C_{18}H_{32}O_4$	$C_{18}H_{32}O_4$	9Z-octadecenedioic acid
18	13.16	293.2126	294.2204	-	$C_{18}H_{30}O_3$	2-Hydroxylinolenic acid
19	13.95	319.2280	320.2358	-	$C_{20}H_{32}O_3$	5-Hydroxyeicosatetraenoic acid
20	14.37	295.2280	296.2358	$C_{18}H_{32}O_3$	$C_{18}H_{32}O_3$	18-Hydroxylinoleic acid
21	14.67	321.2436	322.2514	$C_{20}H_{34}O_3$	-	Hydroxyeicosatrienoic acid
22	14.90	346.2390	347.2468	$C_{18}H_{34}O_6$	$C_{18}H_{34}O_6$	9,10,14-trihydroxy-12-oxooctadecanoic acid
23	14.98	297.2434	298.2512	$C_{18}H_{34}O_3$	$C_{18}H_{34}O_3$	9-oxooctadecanoic acid
24	15.36	177.0186	178.0264	$C_9H_6O_4$	$C_9H_6O_4$	6,7-Dihydroxycoumarin
25	15.44	373.0929	374.1007	$C_{19}H_{18}O_8$	$C_{19}H_{18}O_8$	**Atranorin**
26	16.42	210.9801	211.9879	$C_9H_5O_4Cl$	$C_9H_5O_4Cl$	 7-chloro-3-oxo-1,3-dihydroisobenzofu-ran-5- carboxylic acid.
27	16.47	407.0539	408.0617	$C_{19}H_{17}O_8Cl$	$C_{19}H_{17}O_8Cl$	**Chloroatranorin**
28	17.57	277.2175	278.2203	-	$C_{18}H_{30}O_2$	Octadeca-9,12,15-trienoic acid
29	17.65	389.1246	390.1324	$C_{20}H_{22}O_8$	-	8-Hydroxydiffractaic acid,
30	17.77	265.1480	266.1558	$C_{15}H_{22}O_4$	$C_{15}H_{22}O_4$	(4*E*,6*E*,9*E*)-Pentadeca-4,6,9-trienedioic acid
31	18.03	253.2331	254.2249	$C_{16}H_{30}O_2$	$C_{16}H_{30}O_2$	Palmitoleic acid
32	18.28	402.3016	403.3094	-	$C_{22}H_{42}O_6$	Unidentified
33	18.34	241.2173	242.2251	$C_{15}H_{30}O_2$	$C_{15}H_{30}O_2$	Pentadecanoic acid
34	18.35	455.3534	456.3612	$C_{30}H_{48}O_3$	-	Oleanolic acid
35	18.50	279.0936	280.2409	$C_{18}H_{32}O_2$	$C_{18}H_{32}O_2$	Linoleic acid

36	18.66	489.3375	490.3453	-	$C_{26}H_{50}O_8$	Unidentified
37	18.86	403.2645	404.2723	-	$C_{28}H_{36}O_2$	Unidentified
38	18.91	267.2331	268.2409	$C_{17}H_{32}O_2$	$C_{17}H_{32}O_2$	*cis*-9-Heptadecenoic acid
39	19.15	255.2329	256.2407	$C_{16}H_{32}O_2$	$C_{16}H_{32}O_2$	Palmitic acid
40	19.35	459.3271	460.3349	$C_{25}H_{48}O_7$	$C_{25}H_{48}O_7$	Unidentified
41	19.51	281.2487	282.2565	$C_{18}H_{34}O_2$	$C_{18}H_{34}O_2$	Oleic acid
42	19.75	459.3272	460.3350	$C_{25}H_{48}O_7$	-	Methyl glucose isostearate
43	19.89	269.2488	270.2566	$C_{17}H_{34}O_2$	$C_{17}H_{34}O_2$	Heptadecanoic acid
44	19.99	307.2645	308.2723	$C_{20}H_{36}O_2$	$C_{20}H_{36}O_2$	11,14-Eicosadienoic acid
45	20.07	457.3722	458.3800	$C_{27}H_{54}O_5$	$C_{27}H_{54}O_5$	Unidentified
46	20.18	295.2645	296.2723	$C_{19}H_{36}O_2$	$C_{19}H_{36}O_2$	10*E*-nonadecenoic acid
47	20.54	283.2643	284.2721	$C_{18}H_{36}O_2$	$C_{18}H_{36}O_2$	Stearic acid (Octadecanoic acid)
48	20.82	309.2800	310.2878	$C_{20}H_{38}O_2$	$C_{20}H_{38}O_2$	Eicosenoic acid
49	20.86	505.3326	506.3404	-	$C_{26}H_{50}O_9$	Unidentified
50	21.05	457.3722	458.3800	$C_{27}H_{54}O_5$	-	Unidentified
51	21.71	311.2957	312.3035	-	$C_{20}H_{40}O_2$	(Eicosanoic acid) arachidic acid
52	22.62	297.1532	298.1610	$C_{12}H_{26}O_8$	$C_{12}H_{26}O_8$	Unidentified
53	22.67	637.4836	638.4914	$C_{24}H_{60}O_{12}N_7$	-	Unidentified
54	22.77	339.3268	340.3346	$C_{22}H_{44}O_2$	$C_{22}H_{44}O_2$	Docosanoic acid
55	23.08	309.1743	310.1821	$C_{17}H_{26}O_5$	$C_{17}H_{26}O_5$	Portentol
56	23.58	353.2003	354.2081	$C_{19}H_{30}O_6$	$C_{19}H_{30}O_6$	Unidentified
57	23.81	311.1689	312.1767	$C_{13}H_{30}O_8$	$C_{13}H_{30}O_8$	Unidentified
58	23.89	367.3579	368.3657	$C_{24}H_{48}O_2$	$C_{24}H_{48}O_2$	Lignoceric acid
59	24.01	397.2266	398.2344	$C_{21}H_{34}O_7$	$C_{21}H_{34}O_7$	Stephanol
60	24.60	293.1793	294.1871	$C_{17}H_{26}O_4$	$C_{17}H_{26}O_4$	Heptadecatrienedioic acid
61	24.94	325.1844	326.1922	$C_{14}H_{30}O_8$	$C_{14}H_{30}O_8$	Unidentified
62	25.33	395.3895	396.3973	$C_{26}H_{52}O_2$	-	Hexacosanoic acid or cerotic acid
63	25.89	337.2055	338.2133	$C_{19}H_{30}O_5$	$C_{19}H_{30}O_5$	6-Oxononadeca-8,11-dienedioic acid
64	26.10	339.2000	340.2078	$C_{15}H_{32}O_8$	$C_{15}H_{32}O_8$	Unidentified
65	26.24	381.2317	382.2395	$C_{21}H_{34}O_6$	$C_{21}H_{34}O_6$	19-Acetoxylichesterinic acid
66	26.75	425.2581	426.2659	$C_{23}H_{38}O_7$	-	Asebotoxin I
67	27.53	321.2106	322.2184	$C_{19}H_{30}O_4$	$C_{19}H_{30}O_4$	Nonadecatrienedioic Acid
68	27.85	304.9143	305.9221	Noformula	-	Unidentified

In the work carried out in 2016, in a general analysis of the chemical relationship in the group of *Parmotrema perforatum* (Parmeliaceae, Ascomycota), each sorediate species is descended from an apotheciate species with the same secondary chemicals [38]. The lichen *Parmotrema hypoleucinum* is derived from the ancestor *Parmotrema perforatum* represented by its secondary metabolites in stictic, constictic and norstictic acids. These acids have not been extracted in the Hexane extract, and they do not exist in the part insoluble in methanol, **1-Cires** and **2-Cires** nor in the part soluble in methanol: **1-Hexane** and **2-Hexane**.

The products identified in the **1-Chloroform** and **2-Chloroform** extracts are shown in the chromatograms in Figures 10 and 11 and the results of the identification of the compounds in Table 7.

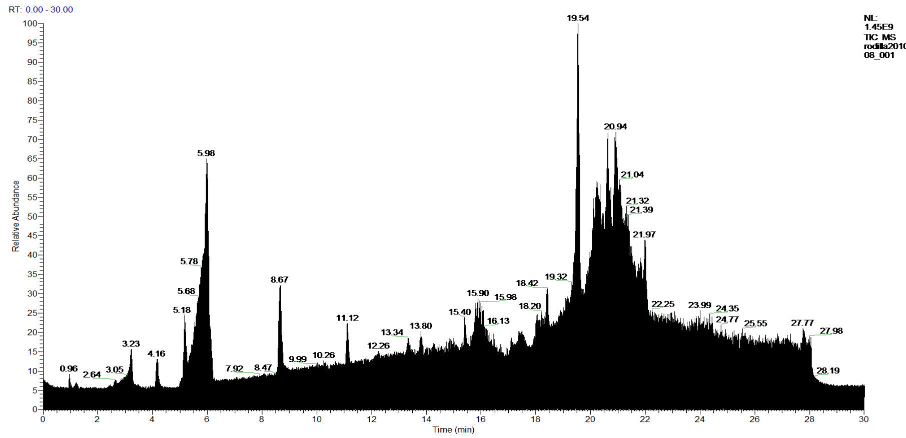


Figure 10. Chromatogram of **1-Chloroform** extract from *P. hypoleucinum*.

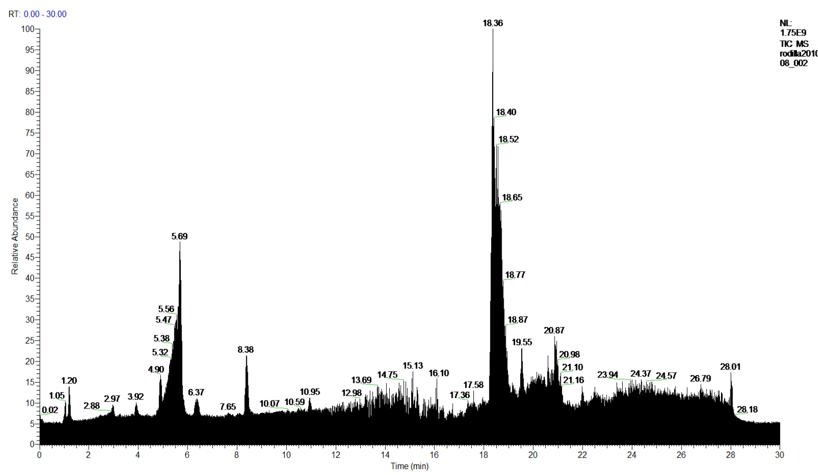


Figure 11. Chromatogram of **2-Chloroform** extract from *P. hypoleucinum*.

Table 7. Samples *Parmotrema hypoleucinum* (in *Olea europea*), Chloroform extract, **1-Chloroform** and *Parmotrema hypoleucinum* (in *Quercus coccifera*), Chloroform extract, **2-Chloroform**.

Nº	**RT**	**[M-H]⁻**	**Mass Calc**	**Formula**	**Formula**	**Compounds**
				1-Chloroform	**2-Chloroform**	
1	0.98	174.9557	175.9635	$C_5H_4O_7$	-	2-Hydroxy-3,4-dioxopentanedioic acid
2	1.01	145.0975	146.1053	-	$C_7H_{14}O_3$	2-Hydroxyheptanoic acid
3	1.05	112.9845	113.9923	-	$C_4H_2O_4$	Acetylenedicarboxylic acid (Squaric acid)
4	1.12	182.9882	183.9960	-	$C_7H_4O_6$	Chelidonic acid
5	1.14	341.1091	342.1169	-	$C_{12}H_{22}O_{11}$	Sucrose
6	1.52	215.0097	216.0175	$C_8H_8O_7$	$C_8H_8O_7$	D-diacetyltartaric anhydride
7	1.74	433.0778	434.0856	$C_{20}H_{18}O_{11}$	$C_{20}H_{18}O_{11}$	Avicularin
8	1.76	403.0675	404.0753	$C_{19}H_{16}O_{10}$	$C_{19}H_{16}O_{10}$	Euxanthic acid
9	1.92	417.0468	418.0546	$C_{19}H_{14}O_{11}$	-	Shoyuflavone C
10	2.41	433.0780	434.0858	$C_{20}H_{18}O_{11}$	$C_{20}H_{18}O_{11}$	Morin 3-alpha-L-arabinopyranoside
11	2.57	401.0518	402.0596	$C_{19}H_{14}O_{10}$	$C_{19}H_{14}O_{10}$	Shoyuflavone B
12	2.64	403.0672	404.0750	$C_{19}H_{16}O_{10}$	$C_{19}H_{16}O_{10}$	Euroxanthone B
13	3.15	447.0934	448.1012	$C_{21}H_{20}O_{11}$	$C_{21}H_{20}O_{11}$	Quercitrin
14	3.23	401.0515	402.0593	$C_{19}H_{14}O_{10}$	$C_{19}H_{14}O_{10}$	**Constictic acid**

15	3.38	182.9882	183.9960	$C_8H_5O_3Cl$	$C_8H_5O_3Cl$	3-Chloro-4-formylbenzoic acid
16	3.48	187.0972	188.1050	$C_9H_{16}O_4$	-	Azelaic acid
17	3.63	371.0412	372.0490	$C_{18}H_{12}O_9$	-	**Norstictic acid**
18	3.90	313.0722	314.0800	-	$C_{17}H_{14}O_6$	Cirsimaritin
19	3.91	442.1145	443.1223	$C_{18}H_{29}O_8Cl_2$	-	Unidentified
20	3.99	373.0567	374.0645	-	$C_{18}H_{14}O_9$	Menegazziaic acid
21	4.02	399.0361	400.0439	$C_{19}H_{12}O_{10}$	-	Kynapcin-28
22	4.13	357.0617	358.0695	$C_{18}H_{14}O_8$	$C_{18}H_{14}O_8$	Succinyldisalicylic acid
23	4.24	401.0515	402.0593	$C_{19}H_{14}O_{10}$	-	Siphulellic acid
24	4.27	373.0568	374.0646	$C_{18}H_{14}O_9$	-	Protocetraric acid
25	5.03	417.0830	418.0908	$C_{20}H_{18}O_{10}$	$C_{20}H_{18}O_{10}$	Conphysodalic acid
26	5.09	385.0568	386.0646	$C_{19}H_{14}O_9$	$C_{19}H_{14}O_9$	**Stictic acid**
27	5.18	387.0721	388.0799	$C_{19}H_{16}O_9$	$C_{19}H_{16}O_9$	Cryptostictic acid
28	6.02	385.0565	386.0643	$C_{19}H_{14}O_9$	$C_{19}H_{14}O_9$	3,3′-Carbonylbis [6-(methoxycar-bonyl)-benzoic acid]
29	6.14	431.0984	432.1062	$C_{21}H_{20}O_{10}$	$C_{21}H_{20}O_{10}$	Genistein 7-glucoside (Genistin)
30	6.32	209.0849	210.0927	-	$C_{11}H_{14}O_4$	Sinapyl alcohol
31	7.63	309.1017	310.1095	-	$C_{15}H_{18}O_7$	1-O-cis-cinnamoyl-β-D-glucopyranose
32	8.11	373.0568	374.0646	-	$C_{18}H_{14}O_9$	Menegazziaic acid isomer
33	8.67	371.0408	372.0486	$C_{18}H_{12}O_9$	$C_{18}H_{12}O_9$	**Substictic acid**
34	9.54	293.1763	294.1841	$C_{17}H_{26}O_4$	$C_{17}H_{26}O_4$	Nordihydrocapsiate
35	10.26	771.1205	772.1283	$C_{38}H_{28}O_{18}$	$C_{38}H_{28}O_{18}$	fucofuroeckol A hepta-acetate
36	10.61	426.9681	427.9759	-	$C_{16}H_{12}O_{14}$	Unidentified
37	11.11	475.3278	476.3356	$C_{25}H_{48}O_8$	$C_{25}H_{48}O_8$	Tetrahydroxypentacosanedioic acid
38	12.21	345.0982	346.1060	$C_{18}H_{18}O_7$	-	Isooptusatic acid (or 3′-Methylevernic acid)
39	13.12	265.1482	266.1560	$C_{15}H_{22}O_4$	$C_{15}H_{22}O_4$	EthyI 4-O-methylolivetolcarboxylate
40	13.34	467.0985	468.1063	$C_{24}H_{20}O_{10}$	$C_{24}H_{20}O_{10}$	Gyrophoric acid
41	13.36	317.0670	318.0748	$C_{16}H_{14}O_7$	-	Lecanoric acid
42	13.80	503.3593	504.3671	$C_{27}H_{52}O_8$	$C_{27}H_{52}O_8$	Tetraglyceryl monooleate
43	14.47	517.3745	518.3823	$C_{28}H_{54}O_8$	-	13-beta-D-glucosyloxy)docosanoic acid
44	14.83	359.0776	360.0854	-	$C_{18}H_{16}O_8$	Ramalinaic acid
45	15.40	365.2334	366.2412	$C_{21}H_{34}O_5$	$C_{21}H_{34}O_5$	Muronic acid
46	15.87	265.1479	266.1557	$C_{15}H_{22}O_4$	$C_{15}H_{22}O_4$	Ivambrin
47	16.02	367.2491	368.2569	-	$C_{21}H_{36}O_5$	Constipatic acid or Protoconstipatic acid
48	16.28	297.1532	298.1610	$C_{12}H_{26}O_8$	$C_{12}H_{26}O_8$	Unidentified
49	17.14	177.0187	178.0265	$C_9H_6O_4$	$C_9H_6O_4$	6,7-dihydroxycoumarin
50	17.37	309.1743	310.1821	$C_{17}H_{26}O_5$	$C_{17}H_{26}O_5$	Portentol
51	18.01	311.1690	312.1768	$C_{13}H_{28}O_8$	$C_{13}H_{28}O_8$	heptahydroxytridecanol
52	18.03	407.0541	408.0619	$C_{19}H_{17}O_8Cl$	$C_{19}H_{17}O_8Cl$	**Chloroatranorin**
53	18.05	210.9801	211.9879	$C_7H_{10}O_3Cl_2$	$C_7H_{10}O_3Cl_2$	2-Methoxy-3,4-dichloro-6-methyltetra-hydropyran-5-one
54	18.32	353.2004	354.2082	$C_{19}H_{30}O_6$	$C_{19}H_{30}O_6$	tetraoxononadecanoic acid
55	18.36	421.2265	422.2343	-	$C_{23}H_{34}O_7$	Sarmentologenin
56	18.85	397.2268	398.2346	$C_{21}H_{34}O_7$	-	Stephanol
57	19.13	387.2544	388.2622	-	$C_{24}H_{36}O_4$	Dehydrodeoxycholic acid
58	19.16	441.2530	442.2608	$C_{23}H_{38}O_8$	-	Asebotoxin IV

59	19.32	325.1846	326.1924	$C_{21}H_{26}O_3$	-	Linderanolide
60	19.55	253.2172	254.2250	$C_{16}H_{30}O_2$	$C_{16}H_{30}O_2$	palmitoleic acid (9-cis-hexadecenoic acid)
61	19.64	255.2331	256.2409	$C_{16}H_{32}O_2$	-	palmitic acid
62	19.74	293.1796	294.1874	-	$C_{17}H_{26}O_4$	Gingerol
63	19.92	279.2332	280.2410	-	$C_{18}H_{32}O_2$	Linoleic acid
64	20.35	267.2332	268.241	$C_{17}H_{32}O_2$	$C_{17}H_{32}O_2$	2-Heptadecenoic acid
65	20.51	283.2645	284.2723	$C_{18}H_{36}O_2$	$C_{18}H_{36}O_2$	Stearic acid
66	20.88	281.2488	282.2566	$C_{18}H_{34}O_2$	$C_{18}H_{34}O_2$	Oleic acid
67	22.25	565.3784	566.3862	$C_{32}H_{54}O_8$	-	Unidentified
68	23.08	679.4650	680.4728	$C_{35}H_{68}O_{12}$	$C_{35}H_{68}O_{12}$	Unidentified
69	23.11	761.5977	762.6055	$C_{35}H_{68}O_{12}$	$C_{35}H_{68}O_{12}$	Unidentified
70	23.14	395.3897	396.3975	-	$C_{26}H_{52}O_2$	Hexacosanoic acid
71	23.84	337.2057	338.2135	$C_{19}H_{30}O_5$	$C_{19}H_{30}O_5$	Idebenone
72	23.91	367.3583	368.3661	$C_{24}H_{48}O_2$	-	Lignoceric acid (tetracosanoic acid)
73	24.13	637.4844	638.4922	-	$C_{34}H_{70}O_{10}$	Unidentified
74	24.25	639.3973	640.4051	$C_{31}H_{60}O_{13}$	-	Unidentified
75	24.44	381.3741	382.3819	$C_{25}H_{50}O_2$	-	Pentacosanoic acid
76	24.56	339.2000	340.2078	$C_{15}H_{32}O_8$	$C_{15}H_{32}O_8$	Heptahydroxypentadecanol
77	24.60	679.4650	680.4728	$C_{35}H_{68}O_{12}$	$C_{35}H_{68}O_{12}$	Unidentified
78	24.80	535.3132	536.3210	$C_{26}H_{48}O_{11}$	$C_{26}H_{48}O_{11}$	Unidentified

In the analysis of **1-Chloroform** and **2-Chloroform** extracts, the acids of the secondary metabolites that define the Sorediate species are identified for *Parmotrema hypoleucinum*; these acids are as follows. Constictic acid is also detected in *Parmotrema tinctorum*; Norstictic acid, according to mycologia 2015, this compound appeared to be present in variable concentrations throughout the thallus. Often the medulla of a lobe tested negative while the medulla adjacent to the apothecia tested positive or vice versa. Stictic acid and other derivatives identified as Substictic acid, there are depsidone were detected in *Parmotrema tinctorum, P. grayanum*, also in *P. robustum* and *P. andinum* [39].

In sample **1-Chloroform**, 60 products were detected, of which 52 products were identified, and 8 products were not identified, and in the sample **2-Chloroform**, 59 compounds were detected, of which 52 products were identified and 7 unidentified.

As in the previous analyses, fatty acids, hydroxy acids, oxo acids, some xanthones and flavones have been found, in addition to menegazziaic, siphullelic, protocetraric, conphysodalic, cryptostictic, menegazziaic isomer, gyrophoric, lecanoric and muronic acids among others.

In the extracts of the more polar products made with ethanol, in the **1-Ethanol** sample 57 products were found, of which 54 products were identified, and 3 compounds were not identified. Figure 12 and Table 8. In the sample **2-Ethanol** analyzed, 54 compounds were detected, of which 52 products were identified, and 2 compounds were not identified. Figure 13 and Table 8.

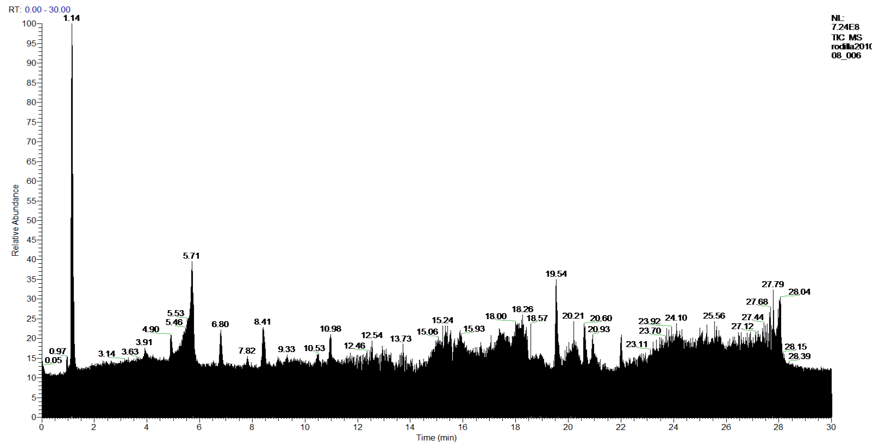


Figure 12. Chromatogram of **1-Ethanol** extract from *P. hypoleucinum*.

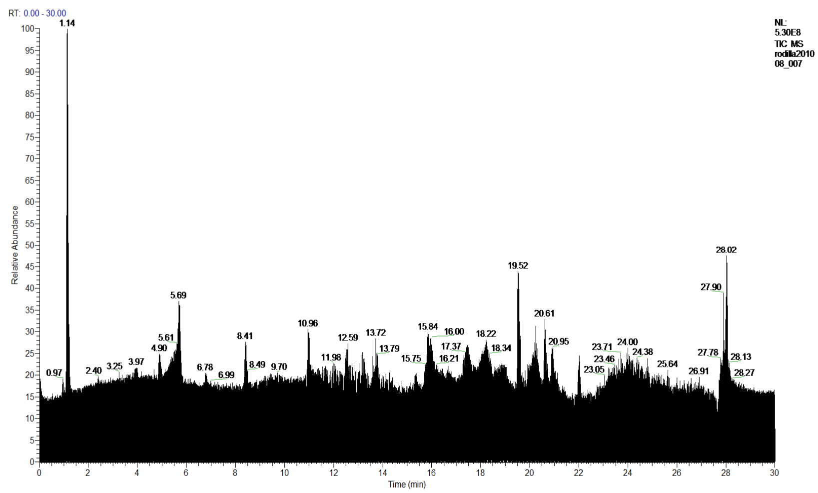


Figure 13. Chromatogram of **2-Ethanol** extract from *P. hypoleucinum*.

Table 8. Samples *Parmotrema hypoleucinum* (in *Olea europea*), Ethanol extract, **1-Ethanol** and *Parmotrema hypoleucinum* (in *Quercus coccifera*), Ethanol extract, **2-Ethanol**.

Nº	RT	$[M-H]^-$	MW Calc	Formula 1-Ethanol	Formula 2-Ethanol	Compounds
1	0.98	174.9556	175.9634	$C_5H_4O_7$	$C_5H_4O_7$	2-Hydroxy-3,4-dioxopentanedioic acid
2	1.05	112.9845	113.9923	$C_4H_2O_4$	$C_4H_2O_4$	Acetylenedicarboxylic acid (Squaric acid)
3	1.15	311.1156	312.1234	$C_{15}H_{20}O_7$	$C_{15}H_{20}O_7$	Neoanisatin
4	1.19	151.0603	152.0681	$C_5H_{12}O_5$	$C_5H_{12}O_5$	Arabitol
5	1.82	182.9882	183.9960	$C_7H_4O_6$	$C_7H_4O_6$	Chelidonic acid
6	2.99	401.0517	402.0595	$C_{19}H_{14}O_{10}$	$C_{19}H_{14}O_{10}$	**Constictic acid**
7	3.95	357.0617	358.0695	$C_{18}H_{14}O_8$	$C_{18}H_{14}O_8$	Hyposalazinic acid, Psoromic acid or Virensic acid
8	4.17	519.1147	520.1225	$C_{24}H_{24}O_{13}$	-	Eujambolin
9	4.91	387.0722	388.0800	$C_{19}H_{16}O_9$	$C_{19}H_{16}O_9$	Cryptostictic acid
10	5.06	385.0568	386.0646	$C_{19}H_{14}O_9$	$C_{19}H_{14}O_9$	**Stictic acid**
11	5.19	417.0830	418.0908	$C_{20}H_{18}O_{10}$	$C_{20}H_{18}O_{10}$	Juglanin
12	5.71	373.0566	374.0644	$C_{18}H_{14}O_9$	$C_{18}H_{14}O_9$	Protocetraric acid

13	5.88	431.0985	432.1063	$C_{21}H_{20}O_{10}$	-	Genistin
14	6.51	328.0597	329.0675	$C_{20}H_{11}O_4N$	-	Unidentified
15	6.80	163.0393	164.0471	$C_9H_8O_3$	$C_9H_8O_3$	Coumaric acid
16	7.25	359.0775	360.0853	-	$C_{18}H_{16}O_8$	Conhypoprotocetraric acid, [40]
17	7.83	399.0723	400.0801	$C_{20}H_{16}O_9$	-	**Methylstictic acid**
18	8.44	371.0408	372.0486	$C_{18}H_{12}O_9$	$C_{18}H_{12}O_9$	**Substictic acid**
19	8.98	209.0453	210.0531	$C_{10}H_{10}O_5$	-	5,6-Dihydroxy-7-methoxy-4-methyl-2-benzofuran-1(3H)-one
20	9.24	293.1763	294.1841	$C_{17}H_{26}O_4$	$C_{17}H_{26}O_4$	(+)-[6]-Gingerol
21	9.33	413.0881	414.0959	$C_{21}H_{18}O_9$	$C_{21}H_{18}O_9$	Vesuvianic acid
22	9.84	461.3123	462.3201	-	$C_{24}H_{46}O_8$	Unidentified
23	10.28	389.2911	390.2989	-	$C_{21}H_{42}O_6$	9,10,12,13-tetrahydroxyheneicosanoic acid
24	10.49	243.0065	244.0143	$C_{10}H_9O_5Cl$	-	(4-Chloro-2-formyl-6-methoxyphenoxy)acetic Acid
25	10.97	475.3276	476.3354	$C_{25}H_{48}O_8$	$C_{25}H_{48}O_8$	Tetrahydroxypentacosanedioic acid
26	11.38	265.1481	266.1559	$C_{15}H_{22}O_4$	$C_{15}H_{22}O_4$	EthyI 4-O-methylolivetolcarboxylate
27	11.42	403.3068	404.3146	$C_{22}H_{44}O_6$	$C_{22}H_{44}O_6$	9,10,12,13-Tetrahydroxydocosanoic acid
28	11.62	447.3331	448.3409	-	$C_{24}H_{48}O_7$	D-Glucitol monostearate
29	11.96	489.3434	490.3512	$C_{26}H_{50}O_8$	$C_{26}H_{50}O_8$	Icosanedioic acid bis(2,3-dihydroxypropyl) ester
30	12.05	345.0982	346.1060	$C_{18}H_{18}O_7$	$C_{18}H_{18}O_7$	Isooptusatic acid (or 3′-Methylevernic acid)
31	12.54	417.3224	418.33.02	$C_{23}H_{46}O_6$	$C_{23}H_{46}O_6$	Heptadecyl D-glucoside
32	13.20	343.0823	344.0901	$C_{18}H_{16}O_7$	$C_{18}H_{16}O_7$	Usnic acid
33	13.57	431.3379	432.3457	$C_{24}H_{48}O_6$	$C_{24}H_{48}O_6$	6-Ethyl-6-n-pentyl-pentadecan -4,5,7,8,15-pentol-I5-acetate
34	13.73	503.3593	504.3671	$C_{27}H_{52}O_8$	$C_{27}H_{52}O_8$	Tetraglyceryl monooleate
35	14.25	309.1746	310.1824	$C_{17}H_{26}O_5$	$C_{17}H_{26}O_5$	Portentol
36	14.38	293.2126	294.2204	$C_{18}H_{30}O_3$	$C_{18}H_{30}O_3$	17-Hydroxylinolenic acid
37	14.40	517.3750	518.3828	$C_{28}H_{54}O_8$	$C_{28}H_{54}O_8$	13-(beta-D-Glucosyloxy)docosanoic acid
38	15.34	365.2335	366.2413	$C_{21}H_{34}O_5$	$C_{21}H_{34}O_5$	Muronic acid
39	16.68	297.2438	298.2516	$C_{18}H_{34}O_3$	$C_{18}H_{34}O_3$	Ricinoleic acid
40	18.00	311.1691	312.1769	$C_{13}H_{28}O_8$	$C_{13}H_{28}O_8$	Heptahydroxytridecanol
41	18.20	353.2005	354.2083	$C_{19}H_{30}O_6$	$C_{19}H_{30}O_6$	Tetraoxononadecanoic acid
42	18.71	397.2267	398.2345	$C_{21}H_{34}O_7$	$C_{21}H_{34}O_7$	Stephanol
43	18.93	421.2269	422.2347	$C_{23}H_{34}O_7$	$C_{23}H_{34}O_7$	Sarmentologenin
44	19.09	441.2531	442.2609	$C_{23}H_{38}O_8$	$C_{23}H_{38}O_8$	Asebotoxin IV
45	19.41	485.2796	486.2874	$C_{25}H_{42}O_9$	$C_{25}H_{42}O_9$	2-(7Z,10Z,13Z)-hexadecatrienoyl-3-(β-D-galactosyl)-sn-glycerol
46	19.54	253.2173	254.2251	$C_{16}H_{30}O_2$	$C_{16}H_{30}O_2$	palmitoleic acid (9-cis-hexadecenoic acid)
47	19.83	241.2172	242.2250	-	$C_{15}H_{30}O_2$	Pentadecanoic acid
48	19.93	279.2332	280.2410	$C_{18}H_{32}O_2$	$C_{18}H_{32}O_2$	Linoleic acid
49	20.03	293.1795	294.1873	$C_{17}H_{26}O_4$	$C_{17}H_{26}O_4$	Gingerol
50	20.50	325.1844	326.1922	$C_{21}H_{26}O_3$	$C_{21}H_{26}O_3$	Linderanolide
51	20.63	255.2329	256.2407	$C_{16}H_{32}O_2$	$C_{16}H_{32}O_2$	Palmitic acid

52	20.89	281.2488	282.2566	$C_{18}H_{34}O_2$	$C_{18}H_{34}O_2$	Oleic acid
53	21.18	325.1846	326.1924	-	$C_{14}H_{30}O_8$	Hexahydroxytetradecan-1-ol
54	21.38	591.2616	592.2694	$C_{34}H_{40}O_9$	-	Scortechinone F
55	22.01	283.2645	284.2723	$C_{18}H_{36}O_2$	$C_{18}H_{36}O_2$	Stearic acid
56	22.21	761.5975	762.6053	$C_{46}H_{82}O_8$	$C_{46}H_{82}O_8$	Unidentified
57	22.92	373.0932	374.1010	$C_{19}H_{18}O_8$	-	Baeomycesic acid
58	22.94	535.3136	536.3214	$C_{26}H_{48}O_{11}$	-	Unidentified
59	23.31	311.2958	312.3036	$C_{20}H_{40}O_2$	$C_{20}H_{40}O_2$	Arachidic acid
60	23.54	337.2057	338.2135	$C_{19}H_{30}O_5$	$C_{19}H_{30}O_5$	Idebenone
61	24.82	339.2000	340.2078	$C_{15}H_{32}O_8$	$C_{15}H_{32}O_8$	Heptahydroxypentadecanol
62	26.80	367.3584	368.3662	$C_{24}H_{48}O_2$	$C_{24}H_{48}O_2$	Tetracosanoic acid
63	27.03	381.2319	382.2397	$C_{21}H_{34}O_6$	$C_{21}H_{34}O_6$	Praesorediosic acid or Protopraesorediosic acid [41–43]

Among the compounds identified in the 1-Ethanol and 2-Ethanol samples were also found the acids that define this lichen *Parmotrema hypoleucinum*, the Constictic and Stictic acids and a derivative such as methylstictic acid.

The products analyzed by GC-MS were identified by their mass spectra and compared with the mass spectra of the NIST and Wiley databases.

3. Materials and Methods

3.1. Lichen Material

Parmotrema hypoleucinum is a foliose epiphytic lichen, which was collected on *Quercus coccifera* and on *Olea europea* at Lake Tonga (Sector Brabtia), at an altitude of 2.20 m above sea level, coordinate 36°51′38″ N; 08°28′46″ E in June 2017. The area of lake tonga is 2600 ha communicating with the sea through the artificial channel of the Messida.

This station is located in the national park of el kala (80,000 ha) Figure 14, classified as a biosphere reserve by UNESCO in 1990, located in the extreme northeast of Algeria.

Figure 14. Location of El Kala National Park (P.N.E.K., 2010).

Parmotrema hypoleucinum (J. Steiner) Hale was identified by Professor Monia Ali Ahmed lichenologist and research director of the Pathology of Ecosystems team at the University of Badji-Mokhtar, Annaba, Algeria. This sample has been deposited in Badji-Mokhtar University, Annaba, code AAM-2.

3.2. HPLC Orbitrap

3.2.1. Sample Preparation

Hexane extraction was carried out for each lichen sample; for *Parmotrema hypoleucinium* (J. Steiner), Hale was made from 40 g of powder material for the two samples. The extraction was carried out in Soxhlet apparatus with hot n-Hexane for 24 h; after this time, the solvent was evaporated to obtain the *Parmotrema hypoleucinium* (*Olea europaea*) n-Hexane extract 0.66 g, which represents (1.65%) and *Parmotrema hypoleucinium* (*Quercus coccifera*)) n-Hexane extract 0.27 g, which represent (0.68%). The n-Hexane extracts are then dissolved in hot methanol and allowed to cool to room temperature so that insoluble products crystallize. With this treatment, the methanol insoluble part (cires) and the cold methanol soluble part (Hexane) are obtained for each n-Hexane extract. For *P hypoleucinium,* the part insoluble in methanol produced **1-Cires** of 0.379 g and **2-Cires** of 0.058 g; the part soluble in methanol produced **1-Hexane** of 0.281 g and **2-Hexane** of 0.219 g.

The vegetable mass recovered and dried from the extractions with Hexane was placed to extract with chloroform at room temperature for 5 days. After this time, the chloroform extract was filtered, and the solvent was evaporated, obtaining the **1-Chloroform** extract with a weight of 0.226 g, which represents (5.65%) and the **2-Chloroform** extract with a weight of 0.196 g, which represents (4.90%).

After being extracted the vegetable mass with chloroform was placed with ethanol for 5 days to prepare the Ethanol extracts. Evaporation of the solvent gave the ethanol extracts: for **1-Ethanol,** a mass of 2385 g was recovered, which represented 5.96%, and for the **2-Ethanol** extract, it presented a mass of 2.417 g, which represented 6.04%.

3.2.2. Instruments

For the GCMS analysis, an Agilent MS220 mass spectrometer coupled to a 7890A GC was used.

HPLC analyses were carried out on an orbitrap Thermo q-Exactive mass spectrometer coupled to a Vanquish HPLC.

3.2.3. GCMS Parameters

The oven temperature was initially set to 50 °C, held for 5 min and then a ramp of 30 °C/min was applied up to 270 °C that was held for 5 additional mins. A VF-5 ms columns was used, with a length of 30 m, inner diameter 0.25 mm and layer width of 0.25 micron.

MS spectra were acquired in EI mode with a mass range from 50 uma to 600 uma.

3.2.4. LC Parameters

For the HPLC separation, a Kinetex XB-C18 (Phenomenex) with a particle size of 2.6 microns, 100 mm in length and a diameter of 2.1 mm was used as column. As solvent A, water with 0.1% of formic acid was used, and as solvent B, acetonitrile was chosen. The column flow war 0.200 mL/min. The following gradient was used (in Table 9):

Table 9. Solvents used, % of solvent **A** and % of solvent **B**.

Time (min)	%A	%B
0	50	50
20	0	100
25	0	100
26	50	50

Total analysis time was set to 30 min.

3.2.5. MS Parameters

For the ionization electrospray in negative mode was used, with the following parameters: Electrospray voltage −3.8kV, Sheath gas 30, Aux gas 10, drying gas temperature 310 ºC. Capillary temp. 320 and S-lens value of 55.0.

The acquisition was performed in a mass range from 100 to 1000 uma, and an auto MS2 program was used with a fragmentation voltage of 30.

4. Conclusions

Due to the biological importance of lichens isolated natural products, studies of the chemical composition of two samples of the lichen *Parmotrema hypoleucinum*, collected on two different supports: Olea europaea and Quercus coccifera, in Algeria, were carried out. For each sample, the extracts of hot *n*-Hexane, Chloroform at room temperature and Ethanol at room temperature were carried out.

The *n*-Hexane extract of each sample is dissolved in hot methanol and allowed to cool slowly so that the products insoluble in methanol precipitate. The parts soluble in methanol are obtained by filtration, the solvent is evaporated, and they are designated as **1-Hexane** and **2-Hexane** fractions for each sample, respectively. The product insoluble in methanol, washed and dried, are designated as the **1-Cires** and **2-Cires** fractions, respectively. An aliquot sample of the fractions: **1-Hexane**, **2-Hexane**, **1-Cires** and **2-Cires**, were esterified with diazomethane to produce the methyl esters of the existing acids.

Esterified samples of **1-Hexane** and **2-Hexane** were analyzed by GC-MS to identify components of lower polarity. In both samples, the methyl esters of 2,4-dihydroxy-3,5,6-trimethylbenzoic acids, palmitic acid, linoleic acid, oleic acid, stearic acid and the hydrocarbon 13-methyl-17-norkaur-15-ene (Probably the natural product will be (-)-ent-kauran-16α-ol). 4-Hydroxy-2-methoxy-3,5,6-trimethylbenzoic and 2,4-dihydroxy-3,6-dimethylbenzoic acid methyl esters and a product that does not it has been possible to identify mass 288; these products were not found in the esterified sample of **2-Hexane**.

The **1-Cires** and **2-Cires** esterified samples were analyzed by GC-MS to separate and identify the components of lower polarity. In the analysis carried out, 2,4-dihydroxy-3,5,6-trimethylbenzoic and 2,4-dihydroxy-3,6-dimethylbenzoic acids were found as existing products in the two samples. In the **2-Cires** esterified sample, 2,4-dimethoxy-6-methylbenzoic acid, which does not exist in the esterified **1-Cires** fraction, was also identified in this analysis. In the esterified fraction **1-Cires**, the analysis also identified 4-hydroxy-2-methoxy-3,6-dimethylbenzoic acid, the hydrocarbon 13-methyl-17-norkaur-15-ene, and a product that was not identified with a mass of 312, which were not found in the esterified sample of **2-Cires**.

The analysis, identification and comparison of the components of the original fractions of **1-Hexane** and **2-Hexane** by HPLC-MS/MS indicate that they are very similar and show almost no differences, except for some very minor components; in these fractions of low polarity, the most characteristic components *P hypoleucinum* will be: Atranol, Chloroatranol, Atranorin and Chloroatranorin; a triterpenic acid identified as Ursolic acid has also been found in both samples.

The analysis of the original **1-Cires** and **2-Cires** fractions by HPLC-MS/MS shows that they are very similar, presenting some small differences in some minor components. The predominant components identified are the fatty acids indicated in the Tables in addition to the predominant product Atranorin and Chloroatranorin, which will be characteristic for this lichen.

In the analysis carried out by HPLC-MS/MS of the original extracts of **1-Chloroform** and **2-Chloroform** of the two samples of *P hypoleucinum* and in the comparison of their components, small differences were found in some secondary compounds. In this analysis, the following acids can be considered as characteristic products of the lichen: Consticic acid, Norstictic acid (this acid was only found in the **1-Chloroform** extract), Stictic acid, Substictic acid and Chloroatranorin.

The HPLC-MS/MS analysis of the original extracts of **1-Ethanol** and **2-Ethanol** did not suggest great differences, and most of their components were identified; the following compounds were found as more representative of the lichen: Constictic acid, Stictic acid, Substictic acid; Methylstictic acid was only identified in the extract of 1-Ethanol.

In this work, a more complete study of the components of the different extracts made from *P. hypoleucinum* was carried out due to the importance of the biological activities. In the most important identified products, in general, their biological activities were referred to derivatives of orsellinic acid, atranol, chloroatranol, atranorin, chloroatranorin, stictic acid and norstictic acid.

For these components, their antioxidant activities, apoptotic effects, cytotoxic, antimicrobial and antitumor activity are already known.

Author Contributions: Conceptualization, J.M.R. and A.A.M.; methodology, J.M.R., A.A.M., L.S. and D.D.; software, C.R. and M.K.; validation, L.S., D.D. and J.M.R.; formal analysis, C.R., N.S. and M.K.; investigation, A.A.M., J.M.R., L.S., N.S. and M.K.; writing-original draft preparation, J.M.R., A.A.M., L.S. and M.K.; writing-review and editing, J.M.R., A.A.M., and D.D.; funding acquisition, DGRSDT. All authors have read and agreed to the published version of the manuscript.

Funding: DGRSDT: Badji Mokhtar University, Algeira. We thank the Ministry of Higher Education and Scientific Research (MESRS) and the General Directorate of Scientific Research and Technological Development (DGRSDT) of Algeria for the financial support of this study. The authors thank to FibEnTech-UBI, which is financed by National Funds from Fundação para a Ciência e a Tecnologia (FCT) and Project UIDB/00195/2020 (financing by the FCT).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

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