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Composition of the essential oil and of some extracts of the aerial parts of *Artemisia ludoviciana* var. *latiloba* Nutt

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Abstract

The essential oil of *Artemisia ludoviciana* is generally characterized by a high percentage of oxygenated monoterpenes (> 70%) such as camphor (~20%), borneol (~15%), and 1, 8-cineole (>10%). These three components seem very common in the essential oil obtained from plants collected in various part of the world, although percentages may be very different as we observed in our own samples. Microwave and CO₂ supercritical extracts were also studied. Several of the terpenes observed in the essential oil are also identified in these extracts with camphor and borneol as the main monoterpenes. Noticeable is the presence of irregular terpenes such as artemisia alcohol and its acetate, yomogi alcohol... particularly in the microwave extract. Several guaianolides, particularly leucomisnin, achillin, austricin, matricarin... as well as 4*H*-1-benzopyran-4-one derivatives (eupatilin, chrysosplenetin...) are present in the extracts. Finally, from the mass spectra and retention indices, 5-*n*-nonadecyl-, 5-*n*-heneicosyl- and 5-*n*-tricosyl-1,3-benzenediols (resorcinols) are proposed as new compounds.

Keywords: Essential oil, organic extracts, *Artemisia ludoviciana*, borneol, camphor, leucomisnin, achillin, 5-*n*-alkylresorcinol

1. Introduction

The characterization of essential oils and organic extracts obtained from several plants of the Asteraceae family finds an important place in scientific literature. In particular, the *Artemisia* genus has a good place. Numerous papers report several pharmacological properties pertaining to this genus: see for examples references [1, 2]. Essential oils of *Artemisia* sp. were largely studied for their antimicrobial and antioxidant activities [3]. Their artemisinin content, which is credited with various pharmacological including anti-malarial properties [4], is also the object of interest. This is for example the case of the well-known *Artemisia annua* [5]. Antifungal and hypoglycemic properties were studied for various organic extracts of flowers, leaves and roots of *Artemisia ludoviciana* [6, 7]. Antidiarrhoeal activity was tentatively linked to the presence of nonanal [8]. It is known that *Artemisia* plants may further contain various extractible molecules such that coumarins and flavonoids: see for example a review [9]. For commercial reason, this laboratory had the opportunity to investigate *A. ludoviciana*. The aim of this work is the analysis of the essential oil as well as organic extract of this plant.

2. Materials and methods

2.1. Plant Material

Fourteen kg of wild dried material of *A. ludoviciana* were collected in June 2014 and purchased from Prairie Edge, South Dakota, lot 167001. This material was manually sliced in thin pieces minimizing friction and kept at room temperature.

2.2. Extract Isolation

Steam distillation of 5.3 kg of this material for 5 h 25 min produced 15 mL of oil and 2.0 liters of hydrosol.

In a beaker, 50 g of plant were introduced with 350 mL of either anhydrous ethanol or acetone at room temperature. For ethanol sample, 800 watts microwave was set to 20% power during 3 minutes, spaced by 30 seconds ON/OFF extraction, and the manipulation was repeated two times. For acetone, microwave was set to 10% of power, during 3 minutes. This was repeated twice. Between each extraction, containers were cooled in ice water.

For supercritical CO₂ extractions, fresh material was crushed in 2 mm parts. Samples C and D

used 3.53 g and 1.26 g were used, respectively (Table 2). Temperature was set at 35 °C. Pressure and extraction time were 3000 and 4500 PSI during 1 and 2 hours, respectively. After recuperation of the extract, a quantity of 350 mL of ethanol/water, 95/5, was added for two hours to extract remaining organic compounds.

2.3. GC and GC/MS

Essential oils, hydrosols and organic extracts were analyzed in duplicate by GC on an Agilent 6890N gas chromatograph with an automatic injector and FID detector equipped with two columns: a polar Solgel-wax and a non-polar DB-5 fused silica capillary column (30 m × 0.25 mm × 0.25 µm). Detector and injection temperatures were 220 and 260 °C, respectively. The oils were also analyzed by GC/MS on an Agilent 5975C InertXL EI/CI triple axis detector mass spectrometer at 70 eV coupled to an Agilent 7890A GC equipped with the same columns as above. The temperature program for both GC/FID and GC/MS is from 40°C (2 min) to 210°C (33 min) at a rate of 2 °C/min. Helium was the carrier gas at a flow of 1.4 mL/min; inlet pressure: 104.5 kPa. Injection volume and split ratio: 3 µL and (50:1). Operating conditions for the MS: the carrier gas is He at a flow of 1.0 mL/min; inlet pressure 48.5 kPa; temperature of the source 280 °C and scan speed, 0.6 s between 40 and 450 amu. Identification of the components was done by comparison of their retention indices (RI) with normal even numbered n-alkanes ranging from C₈ to C₃₀ and by comparison of their mass spectra with literature data [10, 11, 12] and with our own data bases. Quantitative data were obtained electronically from GC-FID area percentages. Liquid

samples were stored in a room at -5 °C before GC or GC/MS analysis.

Leucomisin and hispidulin samples were obtained from Sigma and matricarin from Inter Bio Screen, <http://www.ibscreen.com/>.

3. Results and discussion

The volatile components

The analysis of two samples **A** and **B** of the essential oil of the aerial parts of *Artemisia ludoviciana* appears in Table 1. 1,8-Cineole (~26 and 10.8%, in samples **A** and **B**, respectively), camphor (20.5 ± 0.5%) and borneol (15 ± 1%) were the main products. Depending on the analyzed sample, several products were reported in the 1-5% range in both cases, namely α-pinene, camphene, chrysanthenone, *p*-cymene, and terpinen-4-ol. In the same range linalool, *cis*- + *trans*-sabinene hydrate, *cis*- + *trans*-verbenol, and α-terpineol were also observed. The analysis of both samples shows qualitatively and quantitatively important differences. See, for example, the above-mentioned variation of the 1,8-cineole percentages in samples A and B. Various minor compounds are present in one sample and not observed in the other one and *vice-versa*. Although the majority of observed compounds are very common in essential oils, one has to mention some irregular terpenes such as yomogi alcohol, artemisia alcohol and its acetate, traces of davana ethers and the four artedouglasia oxides.

Table 1: Composition (%) of the essential oil of *A. ludoviciana*

Compounds	Ria ¹	Ria ²	Rip ³	Samples	
				A	B
Santolina triene [#]	917	906	1029	tr ⁴	
Tricyclene [#]	928	921	1001	0.1	
α-Thujene [#]	935	924	1022	0.4	
α-Pinene ^{#,*}	940	932	1016	2.0	0.3
Unidentified C ₁₀ H ₁₄ ?, U1 ⁵	952			0.1	
Camphene ^{#,*}	953	946	1057	3.4	0.6
Thuj-2,4(10)-diene [#]	958	953	1124	0.2	0.1
Benzaldehyde [#]	961	952	1515	0.1	tr
Sabinene ^{#,*}	976	969	1114	0.5	0.1
β-Pinene [#]	977	974	1098	0.9	0.2
1-Octen-3-ol	983	974	1443	1.3	0.6
3-Octanone	989	988	1256	tr	
Dehydro-1,8-cineole	989	988	1177	0.1	
Myrcene [*]	992	988	1160	0.1	
Hexanoic acid	997		1847		tr
Mesitylene [#]	998	994	1228		0.1
Yomogi alcohol	1000	999	1399	0.1	1.4
α-Phellandrene ^{#,*}	1000	1002	1157	tr	
Unidentified C ₁₀ H ₁₄ O?, U2	1016			0.2	
α-Terpinene	1017	1014	1169	0.5	0.2
<i>p</i> -Cymene ^{#,*}	1027	1020	1263	1.5	1.0
Limonene ^{#,*,6}	1034	1024	1187	0.2	tr
β-Phellandrene ⁶	1034	1025	1193	26.2	~0.1
1,8-Cineole ^{#,*}	1034	1026	1193		10.8
Benzeneacetaldehyde	1046	1036	1639	0.1	
<i>cis</i> -Arbusculone [#]	1062	1046	1436		tr
γ-Terpinene [#]	1068	1054	1237	1.0	0.6
Artemisia ketone	1072	1056	1344		0.2
<i>cis</i> -Sabinene hydrate [*]	1077	1065	1452	1.5	0.6
Octanol	1082	1063	1551	0.1	
Artemisia alcohol	1095	1080	1502	0.2	3.0
Terpinolene	1098	1086	1273	0.2	0.1

Fenchone	1098	1083	1374	tr	0.1
<i>Trans-Linalool</i> oxide (fur.)	1098	1084	1462	0.1	
<i>p</i> -Cymenene	1097	1089	1428		tr
<i>trans</i> -Sabinene hydrate *	1107	1098	1533	1.0	0.5
Linalool *	1112	1095	1542	4.1	1.5
α -Thujone	1116	1101	1394	0.3	0.4
Nonanal	1117	1100	1382	tr	0.4
1-Octen-3-yl acetate	1124	1110	1374	0.1	0.1
β -Thujone	1126	1112		tr	0.2
Isophorone #	1127	1118			0.3
<i>trans-p</i> -Mentha-2,8-dien-1-ol	1129	1119			0.2
Chrysanthenone #	1128	1124	1486	0.1	1.6
<i>cis-p</i> -Menth-2-en-1-ol	1130	1118	1547	0.1	0.1
α -Campholenal	1133	1122	1483	0.2	0.1
<i>trans</i> -Pinocarveol *	1144	1135	1627	0.5	
<i>cis</i> -Verbenol ⁵	1148	1137	1654	1.9	0.4
<i>trans</i> -Sabinol	1148	1137	1633		0.5
Camphor #, *	1148	1141	1487	20.3	20.8
<i>trans</i> -Verbenol	1151	1140	1572	0.1	1.6
Sabina ketone	1161	1154		0.1	
Pinocarvone *	1164	1160	1545	0.4	1.0
<i>cis</i> -Chrysanthenol	1164	1160	1647	0.4	0.1
Borneol #, *	1169	1165	1672	16.0	13.9
Artemisyl acetate	1170	1169	1667		1.6
Terpin-1-en-4-ol #, *	1179	1174	1580	2.3	1.4
Thuj-3-en-10-al	1182	1181		0.1	
<i>p</i> -Cymen-8-ol #	1185	1179	1829	0.1	0.1
α -Terpineol #, *	1189	1186	1672	1.3	1.1
Myrtenal *	1191	1195	1589	0.2	0.3
Myrtenol #, *	1193	1775			0.5
Verbenone	1201	1204	1662	0.7	0.2
<i>trans</i> -Piperitol #	1204	1207		0.2	0.2
<i>trans</i> -Carveol #, *	1218	1215	1811	0.2	0.4
Nor-davanone #	1225	1228			tr
Isobornyl formate ?	1226	1235		0.2	0.3
<i>cis</i> -Carveol	1231	1226	1852	0.1	0.1
Cuminaldehyde	1238	1238	1751	0.1	0.1
Carvone #	1243	1239	1700	tr	0.3
Piperitone #	1254	1249		0.1	0.1
<i>trans</i> -Chrysanthemum acetate	1263	1237			0.2
Chavicol	1264	1247			0.2
<i>cis</i> -Chrysanthenyl acetate	1266	1261	1589	0.1	0.1
2,4-Dimethylphenethyl alcohol	1274	1263 ⁷		tr	0.1
Bornyl acetate #	1292	1254	1560	0.5	2.2
Lavandulyl acetate	1296	1288			0.2
<i>trans</i> -Sabinyl acetate	1301	1289		0.1	0.6
Perillic alcohol	1304	1294	2000		0.2
Thymol	1304	1289	2171		0.1
Carvacrol	1311	1298	2205		0.1
Chrysanthenone epoxide	1319	1320 ⁸			0.4
Myrtenyl acetate	1328	1324			0.1
<i>p</i> -Mentha-1,4-dien-7-ol	1337	1325			0.1
<i>trans</i> -Carvyl acetate	1344	1339			0.1
α -Cubebene	1353	1345			0.2
α -Terpinyl acetate	1356	1346			0.4
Eugenol	1369	1356	2126	0.1	0.3
α -Copaene	1375	1374	1477	0.4	0.7
3-Methyltridecane ⁹	1375	1375	1371	0.1	
β -Bourbonene	1382	1387		0.1	0.3
β -Cubebene	1388	1387			0.1
β -Elemene	1390	1389			0.1
<i>trans</i> -Jasmone	1392	1390	1902		0.1
<i>cis</i> -threo-Davanafuran #	1400	1414			0.1
β -Caryophyllene #, *	1414	1417	1570	0.1	0.2
2,5-Dimethoxy- <i>p</i> -cymene	1423	1424			0.1
β -Copaene	1426	1430			0.1
α -Humulene *	1452	1452	1639	0.1	
Allo-aromadendrene	1461	1458	1612		0.2

Germacrene D *	1482	1484	1678	0.2	0.4
β -Selinene	1486	1481	1685	0.2	0.5
<i>ar</i> -Curcumene	1489	1479		tr	0.2
1,2,4-Trihydroxy- <i>p</i> -menthane	1490			0.1	
Davana ether 1 #	1493	1496	1872		0.1
α -Selinene	1497	1495 ⁸	1706		0.3
Davana ether 2 + 3	1498		1905		0.2
α -Muurolene	1504	1500		tr	0.1
Germacrene A	1506	1508			tr
Cubebol	1518	1514			0.1
γ -Cadinene	1518	1513			0.5
Artedouglasia oxide C	1526	1522			tr
δ -cadinene	1526	1522	1730	0.1	0.3
Davana ether 4	1536		1920		0.3
Artedouglasia oxide A	1536	1534			tr
Homoterpenyl methyl ketone	1543				0.2
Artedouglasia oxide D #	1557	1558			tr
<i>trans</i> -Nerolidol	1565	1561			0.1
Spathulenol	1573	1577		0.1	0.5
Artedouglasia oxide B	1573/81	1581			tr
Caryophyllene oxide ^{*,3}	1576	1582	1948	tr	0.4
<i>ar</i> -Turmerol ³	1576	1582	2176		0.1
Globulol	1577	1590		tr	
Humulene epoxide II	1601	1608			0.2
Unidentified, U3, isomer of U4 ?	1620			0.1	
Unidentified, U4, isomer of U3 ?	1622			0.1	
1- <i>epi</i> -Cubebol	1624	1627	2123		0.4
γ -Eudesmol	1631	1630	2154		0.4
τ -Cadinol	1637	1638	2134	tr	0.3
τ -Muurolol	1637	1640	2143	0.1	0.3
β -Eudesmol*	1643	1649			0.3
α -Cadinol	1652	1652	2199		0.4
α -Bisabolol	1690	1685	2202		0.1
Eudesm-7(11)-en-4-ol	1679	1700			0.1
Chamazulene	1730	1730			tr
(<i>Z</i>)-Ligustilide	1730	1734			tr
Hexadecanoic acid	1965	1959			0.7
Phytol	2115	2113 ⁸	2551		0.2
Monoterpenes				11.3	3.3
Oxygenated monoterpenes				81.6	73.7
Sesquiterpenes				1.2	4.3
Oxygenated sesquiterpenes				0.2	5.1
Unknown				0.4	-
Total (%)				94.7	86.4

#: Compounds previously reported in ref^[3] and :^[15]; 1: RI calculated on DB-5 column; 2: RI from ref unless otherwise indicated; 3: RI calculated on Solgel-wax column; 4: trace of compound identified by MS only, < 0.05%; 5: unidentified compound, see MS spectrum in Table 3; 6: The β -phellandrene peak is smaller than the limonene one on MS trace; 7: ref. ^[16]; 8: A.B. Tkachev, in "Study of volatile substances plants", Novosibirsk Institute of Organic Chemistry, 2008, pp. 969; 9: Compound included in the tetradecane reference.

At least three papers have reported a short list of compounds detected in the essential oil of the aerial parts of *Artemisia ludoviciana* collected in North America. Borneol (40%), and camphor (35%) are the main compounds observed in the steam distillation product of the plant collected in Monterrey, Mexico^[13]. One unspecified phellandrene (4%) and α -pinene are also noticeable compounds. Camphor (44.9%), *p*- α -dimethylbenzyl alcohol (15.5%), and linalool (14.9%) are the main compounds reported in the essential oil of one of the above-mentioned paper^[8]. A third paper on the analysis of the essential oil shows the presence of high amounts of irregular terpenes in *A. ludoviciana* var. *latiloba*^[14]. They are: artemisia acetate (62.5%), yomogi alcohol (11.1%), and artemisia alcohol (6.5%). Other compounds include 1,8-cineole (5.1%), camphene (3.5%), linalool (2.3%), and seven other compounds, each in concentrations lesser than 1%. In each of these studies oxygenated monoterpenes are the most important compounds.

A more complete analysis was published in 2008 on oil hydrodistilled from plant collected in Alberta, Western Canada^[3]. It shows a list of 45 compounds. The major ones were: 1,8-cineole (22.0%), camphor (15.9%), and davanone (11.5%). Half a dozen of compounds had concentration located in the 1 to 4% range. At least two Davana ether isomers reached a total of 3.9%. α -Pinene (1.6%) and camphene (2.3%) were the main monoterpenes. Filifolone (1.8%), chrysanthenone (1.3%), myrtenol, and nor-davanone (each 1.0%) were also significant components.

More recently, thirty compounds were identified in an essential oil sample collected in Serbia^[15]. Borneol (40.8%), 1,8-cineole (23.9%), and camphor (8.5%) were the main components. Camphene (3.3%) and germacrene D (2.0%) were the main hydrocarbon terpenes. Terpinen-4-ol (2.2%) and α -terpineol (1.8%) were the main noticeable oxygenated monoterpenes. Again, oxygenated monoterpenes were quantitatively the most important components. Several of the

reported compounds in Table 1 were observed in these two last papers.

About one third of the 145 components reported in the composition of the essential oils are observed in the microwave extract. These amount to ca. 26% of the total analyzed components. Heavier compounds, around 45%, are also observed in the analyzed mixture (see below). Taking into account the percentages of non-volatile components present in this extract the distribution of volatile components is similar to what is observed in the essential oils. The irregular terpenes, yomogi and artemisia alcohols, and artedouglasia oxides, have higher percentages in the microwave extracts.

Thus, it appears that a more complete study is needed to get an insight on the possible importance of either the origin of the plant or the presence of chemotypes. One have to mention the presence of 4-hydroxy-4-methyl-2-pentanone (diacetone): a dimer compound formed *in situ* from the solvent itself. The presence of rather rare compounds in essential oil, must also be confirmed. 2,4-Dimethylphenethyl alcohol was observed in the essential oils of *A. caerulea* subsp. *densiflora* from Sardinia [16], while homoterpenyl methyl ketone is known to be the result of the air oxidation of monoterpenes such as limonene [17] and α -terpineol [18].

3.1 Non-volatile compounds

The composition of various extracts of the aerial parts of *A. ludoviciana* are included in Table 2. About forty non-volatile compounds, with retention indices higher than 2000, are quantitatively important and account for 60% of the reported compounds (43.6% / 70%) in the microwave extract (Fig. 1). Several guaianolides such as achillin, austricin, parishin B and parishin C, and maybe artecanin as well as traces of jaceosidin and jaceidin were observed. Achillin is the most important one: it accounts for 19% of the reported compounds. It is followed by austricin (desacetylmaticarin) (4.8%) and matricarin (2.5%). Four benzopyran-4-one (flavonoids) derivatives, namely chrysosplenetin, eupatilin, hispidulin, and pectolarigenin (each less than 1%), were also identified as well as the triterpenes α - (1.7%) and β -amyryn (0.4%). Several components were only identified by their mass spectra and as such demand a more rigorous identification although in several cases, their RI values measured on the non-polar column agree with published values (Footnote, Table 2). For example, the observed spectrum of chrysosplenetin is quasi super-imposable to that given in the NIST database [19]. The MS given for 3,5-dihydroxy-6, 7, 3', 4'-tetramethoxyflavone extracted in Chile from *A. copa* Phil. is very similar [20].

Table 2. Composition (mg) of various extracts of *A. ludoviciana*

	Compounds	RIa ¹	Micro-wave extract (%)	Supercritical CO ₂ extracts		C ₂ H ₅ OH/H ₂ O (95/5) extracts	
				3000 psi	4500 psi		
1	4-Hydroxy-4-methyl-2-pentanone ¹⁰	838	1.25				
2	Sabinene ^{#,*}	976	tr ⁴	tr			
3	1-Octen-3-ol	983	0.1	tr		0.1	0.1
4	Dehydro-1,8-cineole	989		0.03			
5	hexanoic acid	997	tr				
6	α -Terpinene	1017	tr				
7	<i>p</i> -Cymene ^{#,*}	1027	tr				0.01
8	Limonene ^{#,*,5}	1034	tr				tr
9	1,8-Cineole ^{#,*,5}	1034	3.5		0.02	0.08	0.18
10	Lavender ketone	1040	0.1			tr	
11	γ -Terpinene [#]	1068	tr				tr
12	<i>cis</i> -Sabinene hydrate [*]	1077	0.7	0.03	0.02	0.04	0.02
13	Artemisia alcohol	1095				tr	
14	Fenchone	1098	tr				
15	<i>trans</i> -Sabinene hydrate [*]	1107	0.7	0.03	0.03	0.04	0.01
16	Linalool [*]	1112	0.2	0.03	0.03	0.04	0.02
17	α -Thujone	1116	0.8				
18	β -Thujone	1126	0.2				
19	Chrysanthenone [#]	1128	tr				
20	<i>cis-p</i> -Menth-2-en-1-ol	1130	tr				
21	α -Campholenal	1133	tr			tr	
22	<i>trans</i> -Pinocarveol [*]	1144		0.01	0.01	0.01	
23	<i>cis</i> -Verbenol ⁵	1148	0.3				
24	Camphor ^{#,*,5}	1148	4.5	0.14	0.10	0.20	0.14
25	<i>trans</i> -Verbenol ⁵	1151		0.05	0.05	0.05	
26	Sabina ketone	1161	0.1				
27	Pinocarvone ^{*,5}	1164	0.3	0.01		0.01	tr
28	<i>cis</i> -Chrysanthenol ⁵	1164		0.02	0.02	0.03	tr
29	Borneol ^{#,*}	1169	5.8	0.28	0.25	0.28	0.10
30	Terpin-1-en-4-ol ^{#,*}	1179	0.3	0.01	0.01	0.01	0.01
31	<i>p</i> -Cymen-8-ol [#]	1185	0.1				
32	α -Terpineol ^{#,*}	1189	0.2	0.03	0.02	0.03	0.01
33	Hodiendiol	1191	0.7	0.06	0.06	0.07	0.02

34	Verbenone	1201	0.4	0.02	0.09	0.02	0.01
35	<i>trans</i> -Piperitol #	1204	tr			tr	
36	<i>trans</i> -Carveol #,*	1218	0.1	0.01		0.01	
37	exo-2-Hydroxycineole	1223				tr	
38	Piperitone #	1254	tr	tr	tr	tr	
39	Linalyl acetate	1263	0.1				
40	2,4-Dimethylphenethyl alcohol	1274	tr	tr	tr	tr	
41	2,6-Dimethyl-1,7-octadiene-3,6-diol	1283	0.9	0.09	0.08	0.10	0.03
42	Bornyl acetate #	1292	0.3			0.01	tr
43	Carvacrol	1311	0.1				
44	Eugenol	1369	0.1			0.01	
45	α -Copaene ⁵	1375	0.1	0.01	0.01	0.01	
46	3-Methyltridecane ^{5,7}	1375	tr	tr	tr	tr	tr
47	Geranyl acetate	1386	0.1				
48	β -Cubebene	1388	0.1				
49	β -Elemene	1390	0.1				
50	β -Caryophyllene #,*	1414	tr				
51	Germacrene D *	1482		0.01	0.01	0.01	
52	β -Selinene	1486	0.1				
53	1,2,4-Trihydroxy- <i>p</i> -menthane	1490	1.5				
54	Homoterpenyl methyl ketone	1543	0.2				
55	Spathulenol	1573		0.01	tr	0.01	
56	τ -Cadinol ⁵	1637		0.02	tr	tr	
57	τ -Muurolool ⁵	1637			0.01	0.01	
58	β -Eudesmol*	1643	0.8				
59	α -Cadinol	1652				0.01	
60	Chamazulene	1730	tr				
61	(<i>Z</i>)-Ligustilide	1730			0.01		0.01
62	Hexahydrofarnesyl acetone	1844		tr	0.02		
63	Hexadecanoic acid	1965	1.3	0.03	0.01	0.02	0.01
64	Ethyl hexadecanoate	1995	0.1				
65	Unidentified U5 could be 2 isomers	2087	2.0	0.07	0.06	0.10	
66	Unidentified U6	2092	0.3	-	-	-	
67	Unidentified U7, isomer of U5?	2099	0.9			0.1	
68	Unidentified U8	2103		0.05		0.15	
69	Unidentified U9	2111		0.10	0.01	0.05	
70	Phytol	2115	tr	tr	tr	tr	0.01
71	Unidentified U10	2129	1.3	0.07	0.07	0.12	
72	Leucomisin	2150	0.7	0.17	0.14	0.16	
73	Unidentified. U11	2166	1.0			0.07	0.01
74	Unidentified U12	2185	0.3	0.02	0.02	0.03	
75	Achillin ¹¹	2191	19.3	0.15	0.14	0.15	
76	Parishin B	2240	0.5				
77	Parishin C	2291	1.4				
78	Anhydroaustricin	2305	1.2				
79	Unidentified U13	2350	0.2				
80	Unidentified U14, isomer of U16?	2355	1.8				
81	Austricin	2360	4.8	0.04	0.04	0.07	
82	Matricarin	2391	2.5	0.04			
83	Unidentified U15	2394	0.7			0.04	
84	Unidentified U16, isomer of U14?	2408				0.05	
85	Nonacosane	2893		0.17	0.13		
86	Pectolarigenin ¹¹	~3030	0.1				
87	Hispidulin ¹¹	~3090	0.1				
88	Untriacontane	3092		0.24	0.17		
89	Jaceosidin = 4'-Demethyleupatilin	~3120	tr				
90	α -Tocopherol = Vitamin E ¹¹	3137	tr				
91	Jaceidin	3143	tr				
92	Eupatilin	3157	0.7				
93	Chrysofenetin?	3196	0.4				
94	5- <i>n</i> -Nonadecyl-1,3-benzenediol ¹²	3236	tr				

95	γ -Sitosterol ¹¹	3293	0.2				
96	β -Amyrin ¹¹	3313	0.4	-	-		0.05
97	Unidentified, MS similar to the #102 ¹²	3340	tr				
98	α -Amyrin ¹¹	3370	1.7	0.04	0.04	0.05	0.02
99	Tetracontatriene	3392			0.02	0.02	
100	α -Tocopherol acetate	~3400	0.1				
101	5- <i>n</i> -Heneicosyl-1,3-benzenediol ¹²	~3400					
102	Unidentified : see Fig. 3	>3440	tr				
103	5- <i>n</i> -Tricosyl-1,3-benzenediol ¹²	>3450	tr				
104	Unidentified U17	>3450	0.2				
105	Unidentified U18	>3500	0.3				
	Monoterpenes		0.3	-	-	-	0.01
	Oxygenated monoterpenes		20.3	0.85	0.79	1.14	0.65
	Sesquiterpenes		0.4	0.02	0.02	0.02	0.01
	Oxygenated sesquiterpenes		2.2	0.06	0.05	0.05	0.03
	Retention time >2000		43.5	1.16	0.84	1.16	0.09
	Total		70.1%	2.09	1.70	2.37	0.78
	Measured compounds (mg)			2.57	2.15	2.78	1.36
	Analyzed compounds (mg)			7.3	7.8	16.9	15.0
	Amount of fresh material (g)			3.53	1.26		

#, *, 1-9: See footnotes. Table 1; ¹⁰: artefact: condensation product from acetone; ¹¹: Retention indices on the a-polar column DB-5 from literature: β -amyrin: 3337 and α -amyrin: 3376 ^[42]; achillin: 2206, Vitamin E: 3149, hispidulin: 3151, pectolinarigenin: 3039, and γ -sitosterol 3351 ^[43]; ¹²: see text.

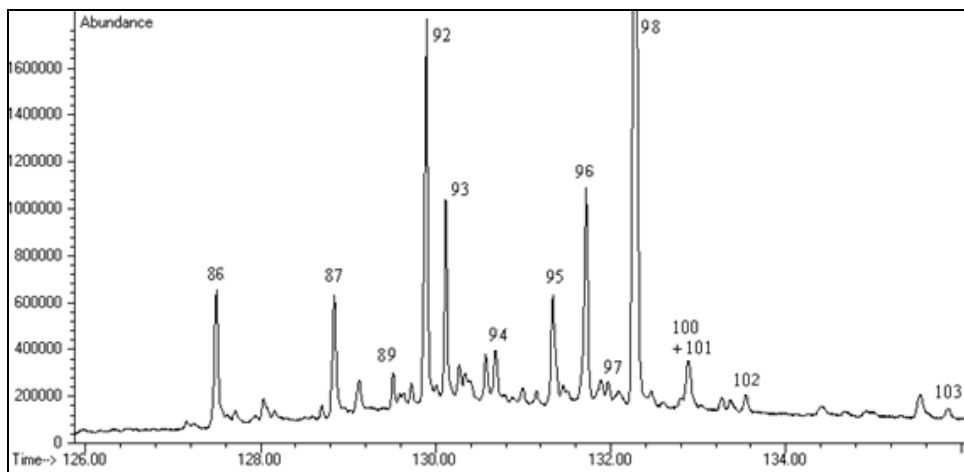


Fig 1: Partial FID chromatogram on the non-polar column of the microwave extract, Table 2. Figures correspond to those of the first column of this table.

The two last columns in Table 2 give some insights on the composition of extracts obtained with supercritical CO₂. In these experiments, the measured compounds account for about 30% of the analyzed mixture. Thus, the majority of the extracted molecules were not seen by GC capillary methods. Borneol and camphor are important components as it is the

case in the microwave experiments as well as several unidentified compounds (Table 3). Numerous supercritical fluid extraction studies and patents are reported in literature for the *Artemisia* genus especially in view of the extraction of artemisinin, an antimalarial drug, in *Artemisia annua*.

Table 3: Mass spectra of unidentified compounds

Name	Ria ¹	m/z (Intensity)	See in Table
U1	1016	81(100), 41 ~ 69 ~ 79(70), 150(54), 135(40), 80(32), 107(28), 91(26)...	1
U2	1620	111(100), 93(55), 109(32), 55(24)...	1
U3	1622	111(100), 93(55), 109(32), 55(24)...	1
U4	2087	43(100), 94(80), 112(66), 111(56), 163(40), 166(33)...	2
U5	2092	156(100), 228(98), 141(84), 112(62), 111(55), 163(36), 166(30)...	2
U6	2099	43(100), 94(76), 112(64), 111(52), 163(38), 166(32)...	2
U7	2103	43(100), 163(50), 111(42), 98(32), 94(30), 97(28)...	2
U8	2111	43(100), 111(44), 123(32), 98(28), 124(27), 98(28), 260(24)...	2
U9	2129	43(100), 94(72), 163(60), 111(52), 112(48)...	2
U10	2166	43(100), 124(48), 123(46), 111(44), 166(38), 217(30), 98(28)...	2
U11	2185	151(100), 109(90), 262(70), 69(50), 122(48), 41(47), 91(46), 55(36)...	2

U12	2350	43(100), 217(30), 53(10), 97(10)... 262(4).	2
U13	2355	111(100), 43(30), 53(10), 95(10)... ³	2
U14	2394	43(100), 242(72), 302(66), 91(60), 227(52), 199(48), 136(40)...	2
U15	2408	111(100), 43(30), 95(9)... 260(1) ³	2
U16	>3400	246(100), 247(95), 217(18), 173(18), 248(18), 91(13), 105(8)...	2
U17	>3500	247(100), 246(47), 173(17), 248(16), 91(12)...	2

¹: Retention index on DB-5 column; ²: U2 and U3, U4, U6, and U9, U8 and U10 could be isomers; ³: U13 and U15 could be either arteminin or canin A or B.

The variety of structures as well as the number of geometrical and, more particularly, optical isomers, precludes a definitive identification since it is only based on mass spectra and the occurrence of the same molecule in other *Artemisia* species. This is particularly true for canin (also named chrysanthemin A), a compound identified in the leaves of *A. frigida* [21]. There are many unidentified compounds: see Table 3 for their

mass spectra. Some spectra are quite similar. One may expect the presence of isomers. The observed composition is a little bit surprising in the sense that, generally, such compounds are analyzed through the use of HPLC in conjunction with various detectors such as UV-visible spectroscopy or mass spectrometry, and rarely by capillary GC [22].

Table 4: Sesquiterpene lactones observed in organic extracts of *A. ludoviciana*

Common names CAS N°	Ref. for MS identification	Guaianolides	Synonyms	Other names
Achillin 5956-04-7 See leucomisin	19	(3 <i>R</i> ,3 <i>aS</i> ,9 <i>aS</i> ,9 <i>bS</i>)-3,3 <i>a</i> ,4,5,9 <i>a</i> ,9 <i>b</i> -Hexahydro-3,6,9-trimethylazuleno[4,5- <i>b</i>]furan-2,7-dione		(11 <i>R</i>)-6 <i>α</i> -Hydroxy-2-oxoguaia-1(10),3-dien-12-oic acid, γ -lactone
Arteminin 29431-84-3	19, 21	(3 <i>aS</i> ,6 <i>R</i> ,6 <i>aS</i> ,7 <i>aR</i> ,7 <i>bS</i> ,8 <i>aR</i> ,8 <i>bS</i> ,8 <i>cS</i>)-Octahydro-6-hydroxy-6,8 <i>a</i> -dimethyl-3-methylene-4 <i>H</i> -bisoxireno[1,8 <i>a</i> :2,3]-azuleno[4,5- <i>b</i>]furan-2(3 <i>H</i>)-one	Chrysartemin B	
Austricin 10180-88-8	44, 45	(3 <i>S</i> ,3 <i>aR</i> ,4 <i>S</i> ,9 <i>aS</i> ,9 <i>bR</i>)-3,3 <i>a</i> ,4,5,9 <i>a</i> ,9 <i>b</i> -Hexahydro-4-hydroxy-3,6,9-trimethylazuleno[4,5- <i>b</i>]furan-2,7-dione	8-Deacetylmaticarin	(11 <i>S</i>)-6 <i>α</i> ,8 <i>α</i> -Dihydroxy-2-oxoguaia-1(10),3-dien-12-oic acid, 12,6-lactone
Anhydroaustricin 35945-79-0 or Anhydrogrossmizin 35345-78-9	21	(3 <i>R</i> ,3 <i>aR</i> ,9 <i>aS</i>)-3,3 <i>a</i> ,9,9 <i>a</i> -Tetrahydro-3,5,8-trimethylazuleno[6,5- <i>b</i>]furan-2,7-dione (3 <i>S</i> , 3 <i>aR</i> ,9 <i>aR</i>)-isomer		
Canin 24959-84-0	19, 21	(3 <i>aS</i> ,6 <i>R</i> ,6 <i>aR</i> ,7 <i>aS</i> ,7 <i>bR</i> ,8 <i>aS</i> ,8 <i>bS</i> ,8 <i>cS</i>)-Octahydro-6-hydroxy-6,8 <i>a</i> -dimethyl-3-methylene-4 <i>H</i> -bisoxireno[1,8 <i>a</i> :2,3]-azuleno[4,5- <i>b</i>]furan-2(3 <i>H</i>)-one	Chrysanthemin A	1,2 <i>α</i> :3 <i>α</i> ,4-Diepoxy-6 <i>α</i> ,10-dihydroxyguaia-11(13)-en-12-oic acid, γ -lactone
Leucomisin 17946-87-1 See achillin	Our lab., 19, 44, 45,	(3 <i>S</i> ,3 <i>aS</i> ,9 <i>aS</i> ,9 <i>bS</i>)-3,3 <i>a</i> ,4,5,9 <i>a</i> ,9 <i>b</i> -Hexahydro-3,6,9-trimethylazuleno[4,5- <i>b</i>]furan-2,7-dione	Desacetoxymaticarin, leucodin	(11 <i>S</i>)-6 <i>α</i> -Hydroxy-2-oxoguaia-1(10),3-dien-12-oic acid, γ -lactone
Maticarin 5989-43-5	Our lab., 44	(3 <i>S</i> ,3 <i>aR</i> ,4 <i>S</i> ,9 <i>aS</i> ,9 <i>bR</i>)-4-(Acetyloxy)-3,3 <i>a</i> ,4,5,9 <i>a</i> ,9 <i>b</i> -hexahydro-3,6,9-trimethylazuleno[4,5- <i>b</i>]furan-2,7-dione	Austricin acetate	
Parishin B 23554-79-2	44	(α ,5 <i>R</i>)-1,5,6,7-Tetrahydro- α ,3,8-trimethyl-1-oxo-5-azuleneacetic acid		(11 <i>S</i>)-2-Oxoguaia-1(10),3,5-trien-12-oic acid
Parishin C 74636-03-6	44	(3 <i>R</i> ,3 <i>aS</i> ,9 <i>aR</i> ,9 <i>bS</i>)-3,3 <i>a</i> ,4,5,9 <i>a</i> ,9 <i>b</i> -Hexahydro-9 <i>a</i> -hydroxy-3,6,9-trimethylazuleno[4,5- <i>b</i>]furan-2,7-dione		
Others				
Chrysosplenetin 603-56-5	19	5-Hydroxy-2-(4-hydroxy-3-methoxyphenyl)-3,6,7-trimethoxy-4 <i>H</i> -1-benzopyran-4-one	3,6,7,3'-Tetra-O-methylquercetagenin	4',5'-Dihydroxy-3,3',6,7-tetramethoxyflavone
Eupatilin 22368-21-4	19, 26, 47	2-(3,4-Dimethoxyphenyl)-5,7-dihydroxy-6-methoxy-4 <i>H</i> -1-benzopyran-4-one	Stillen	5,7-Dihydroxy-3',4',6-trimethoxyflavone
Hispidulin 1447-88-7	Own lab., 47	5,7-Dihydroxy-2-(4-hydroxyphenyl)-6-methoxy-4 <i>H</i> -benzopyran-4-one	6-Methoxyapigenin	4',5,7-Trihydroxy-6-methoxyflavone
Jaceosidin 18085-97-7	19	5,7-Dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-6-methoxy-4 <i>H</i> -1-benzopyran-4-one	4'-Demethyleupatilin, Jaseocidin	3',6-Dimethoxy-4',5,7-trihydroxyflavone
Jaceidin 10173-01-0	19	5,7-Dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-3,6-dimethoxy-4 <i>H</i> -1-benzopyran-4-one	Quercetagenin 3,3',6-trimethyl ether	4',5,7-Trihydroxy-3,3',6-trimethoxyflavone
Pectolarigenin 520-12-7	19, 31, 47	5,7-Dihydroxy-6-methoxy-2-(4-methoxyphenyl)-4 <i>H</i> -benzopyran-4-one		5,7-Dihydroxy-4',6-dimethoxyflavone
β -Amyrin 559-70-6	20, 34	Olean-12-en-3 β -ol		
α -Amyrin 638-95-9	34	Urs-12-en-3 β -ol		

In literature, several of these non-volatile compounds were observed in various extracts obtained from the aerial parts of *A. ludoviciana*. Borneol (6.2%), spathulenol (3.1%) as well as derivatives of caryophyllene (5.1%) were the main terpenes observed in a methanol/chloroform extract [5]. Achillin (see Table 4 for systematic names) was observed in light petrol extraction of *A. ludoviciana* at a concentration of 0.24% from dried material [13]. Achillin, artemisin and parishin C, two other guaianolides, were identified in a chloroform extract from *A. ludoviciana* var. *ludoviciana* [23]. Other eudesmanolides and germacranolides not observed in this study were identified in *A. ludoviciana* ssp. *albula* and ssp. *mexicana*, [23, 24]. All other non-volatile compounds identified in Table 2 were observed in organic extracts of various *Artemisia* species. Austricin was an important compound isolated from *A. klotzchiana*: 506 mg in 500 g of plant material [13]. Matricarin was identified in an ethanol extract of *A. leucodes* collected in the Chemkent Province, Kazakh SSR [25]. Anhydroaustricin, the fourth most important guaianolide in this study as well small amount of eupatilin were observed in *A. albida* collected in Kazakhstan [26, 27]. Parishin B and parishin C were identified in an ethanol extract of *A. absinthium* (common wormwood) in the Tashkent province [28].

The ethanol/water extracts of *A. judaica* and *A. vestita* [29, 30] contain pectolarigenin and hispidulin. Among other flavone derivatives, pectolarigenin was also identified in *A. glabella* from Kazakhstan [31] and from *A. mongolica* from the Tibet region [32]. The concentration of chrysosplenin was followed during various industrial processes applied to the leaves of *A. annua* [33]. α -Amyrin and β -amyrin were isolated from the non-polar fraction of *A. apiaceae* [34]. Other flavones were identified in *A. ludoviciana*: anthemidin [35] and several other guaianolides in the ssp. *mexicana* [36]. Jaceosidin was observed as an important compound in *A. ludoviciana* [7, 36] and jaceidin a trace compound in *Artemisia annua* [37].

Finally, γ -sitosterol was identified in genotypes of *A. annua* collected in the Sichuan province [38]. Again, as it was stated above, the identification made by a single method constitutes a short cut and is either questionable or at least must be confirmed.

Since higher molecular compounds could be irreversibly trapped on the DB-5 column as it is the case on the polar column, it is not possible to get a good idea of the quantitative importance of these guaianolides and flavones present in the microwave extract. The calculus is easier in the cases of the supercritical CO₂ extracts (Table 2). In samples C and D, 0.30 \pm 0.02 mg of the sum achillin + its isomer (leucomisin) are extracted from 3.53 and 1.26 g. The measured yields are 0.01 and 0.02% of fresh material, respectively. These figures must be compared to the 1.22 g of achillin extracted from 498 g of dried and milled plant material reported in *A. ludoviciana* [13]. Thus, these extracts are relatively rich in compounds with known pharmacological properties.

3.2 Some Evidence for New Compounds

For the three spectra having a base peak at an m/z value of 124 and parent peaks located at 376, 404, and 432, respectively, we propose the presence of three resorcinol derivatives: 5-*n*-nonadecyl-, 5-*n*-heneicosyl- and 5-*n*-tricosyl-1,3-benzenediols (Fig. 2). Their mass spectra are very similar to that of the known 5-pentyl-, 5-heptyl- [19], 5-*n*-pentadecyl- [39], and 5-*n*-heneicosyl-1,3-benzenediol (1,3-reorcinols) [19, 40]. Moreover, the known RI(DB-5MS) value for 5-pentylresorcinol was reported to be in the 1755-1770 range. Adding 16 methylene groups on the lateral alkyl chain would add 1600 units to this RI value. Thus, the expected RI(DB-5) value for 5-*n*-heneicosyl-1,3-benzenediol would be in the 3355-3370 range, in relatively good agreement of ~3400 observed in this work. Of course, other benzenediol isomers are available. However, the 4-*n*-alkyl-1,3-benzenediols have a base peak located at a m/z value of 123 rather than 124 [41].

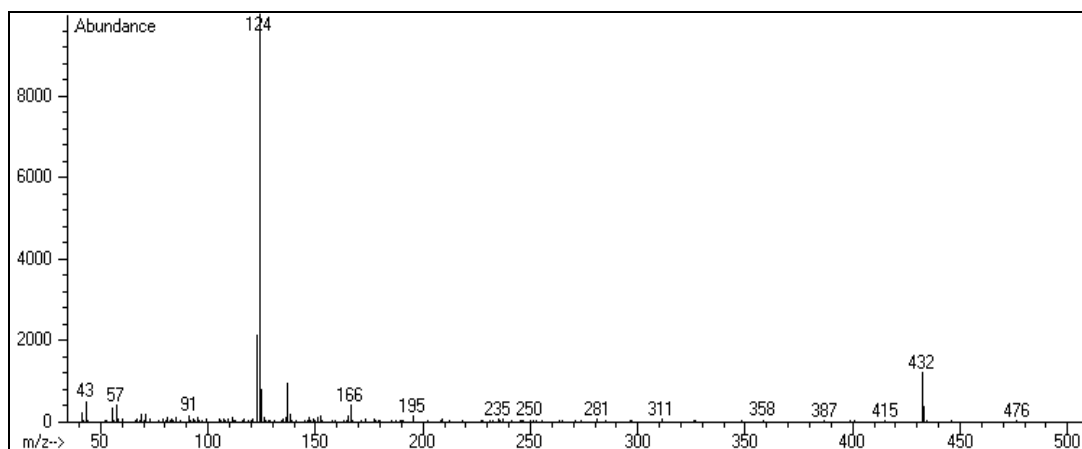


Fig 2: GC/MS of peak N° 103 of Table 2 and Fig. 1. This MS could be that of 5-*n*-tricosyl-1,3-benzenediol: see text. Peaks N° 94 and 101 have similar mass spectra except for their parent peaks at $m/z = 376$ and 404 amu.

By the same token, two unidentified compounds with parent and base peaks located at $m/z = 418$ and 138 , respectively, and RI(DB-5) values of 3340 and >3440 (compounds 96 and 101 in Table 2), have similar spectra to that of 3-*n*-heptadecyl-5-methoxyphenol (Fig. 3) [40]. These compounds have a CH₂ group added to the 5-*n*-heneicosanylresorcinol. There are at least three different places to add this methylene group:

1. On the side chain. In that case, the base peak of the molecule should be located at $m/z = 124$.
2. On the benzene moiety, one hydroxyl group is replaced by a methoxy group, giving rise to the formation of 3-heneicosanyl-5-methoxyphenol or
3. One hydrogen atom of the benzene ring is replaced by a methyl group leading to the formation of 5-heneicosanyl-*x*-methyl-1,3-benzenediol with $x = 2$ or 4 .

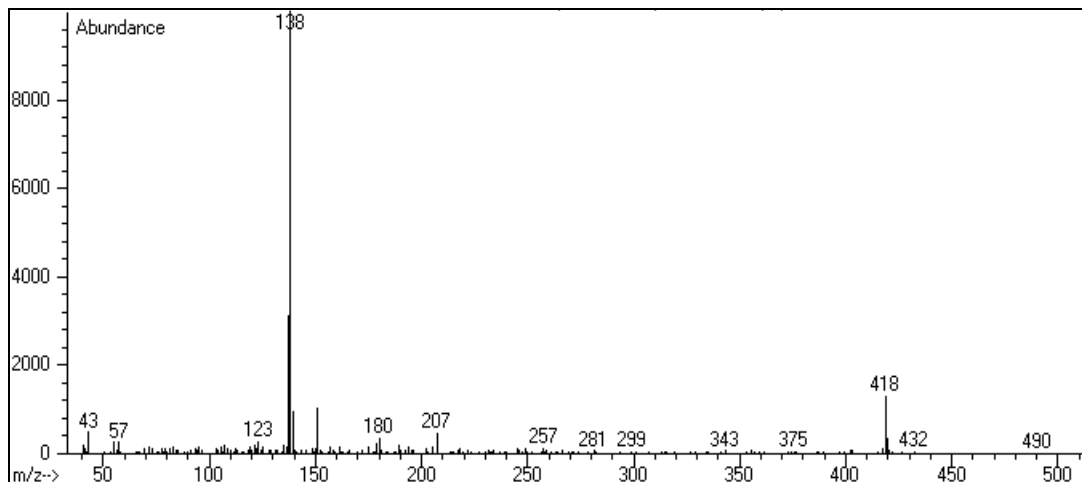


Fig 3: GC/MS of peak N° 102 of Table 2 and Fig. 1. This could be the MS of 3-*n*-heneicosanyl-*x*-methyl-1,3-benzenediol: see text. MS of peak 97 is very similar, except that peak $m/z = 138$ is about 40% higher.

The two above-mentioned peaks could correspond to the two last cases. The second case was retained for 3-*n*-heptadecyl-5-methoxyphenol [40]. This does not tell us if it corresponds to the peak with a RI value of 3340 or that with a RI value >3440. Using the additive properties of the retention index system, substituting an H atom by a methyl group, for example going from benzene to toluene, RI value increases by *ca.* 100 units. Alternatively, substituting a hydroxyl group by a methoxy group, for example going from phenol to methoxybenzene, there is a decrease of the RI value by *ca.* ±60 units. Applying these observations, the first peak with a RI value of 3340, could correspond to the second case described above. The other peak with a RI value > 3440 could correspond to the third case, that means to the 5-*n*-heneicosanyl-*x*-methyl-1,3-benzenediol molecule. This discussion does not preclude the presence of other benzenediols such as the 4-*n*-alkyl-2-methoxyphenol, for example 4-*n*-nonadecyl-2-methoxyphenol [41].

4. Conclusion

The composition of the essential oil, the volatile components, is quite classical. Microwave as well as CO₂ supercritical extracts show a rich variety of non-volatile components. From literature data, all the major identified compounds observed with retention indices higher 2000 over the non-polar column is not surprising. The most interesting fact resides in the variety and the relative concentration of these compounds.

5. Conflict of Interest.

The authors declare no conflict of interest.

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