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Chemical Composition of the Essential oil of *Brocchia cinerea* Grown in South Eastern of Algeria

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The aerial parts of *Brocchia cinerea* collected from the region of Ouargla, which is located in the southeastern of Algeria, were investigated to obtain the essential oil of this plant by hydrodistillation method. The first sample HE1 was obtained by decantation of the distillate while the second HE2 was extracted from the distillate by *n*-hexane. The samples were analyzed by GC-FID on two capillary columns: a non-polar (HP1) and a polar one (Innowax). The HE1 extract was also analyzed by GC-MS on a DB5 column. The two samples presented nearly the same composition, the main components identified in both were: thujone (47.72 %), camphor (10.54 %), santolinatriene (8.00 %), eucalyptol (6.37 %) and in second order lyratyl acetate (4.17 %), terpinen-4-ol (2.77 %).

Key Words: Asteraceae, *Brocchia cinerea*, Essential oil, GC-MS.

INTRODUCTION

Brocchia cinerea (Fig. 1) belongs to the *Asteraceae*[1], it grows abundantly after the rain season in the southeastern of Algerian Sahara (Ouargla city) and surroundings[2,3], its local name is "chihia". This plant is traditionally used as a remedy to treat various ailments such as spasm, colic, cough and in the same way in the treatment of other broncho-pulmonar affections.

Fig. 1. *Brocchia cinerea*

In previous studies, it has been demonstrated that the plant showed interesting biological activities: febrifugal, antibacterial, antifungal, analgesic and antiinflammatory[4-7]. Nomad people also add it to tea to perfume it and filter goats butter through it in order to better preserve it.

To the best of our knowledge, no previous investigations were performed about the volatile fraction chemical composition of this plant except a short communication which reported a study of the essential oil of *Brocchia cinerea* growing in Egypt[8].

The objectives of the present paper are the determination of the essential oil chemical composition of this plant. Further more a comparison of the chemical composition of the two samples, HE1 and HE2 which are respectively the pure essential oil and the one obtained with *n*-hexane.

EXPERIMENTAL

Extraction of the essential oils: The plant material of *Brocchia cinerea* was collected in March 2005, during the flowering stage, at 20 km north from Ouargla city. Plant identification was carried out by Dr K. Maiza, medical botanist in medical botanic laboratory, faculty of medicine, Algiers University, Algeria.

The aerial parts of *Brocchia cinerea* were air-dried at room temperature in a dark place. The essential oils were obtained by hydrodistillation using a modified Clevenger-type apparatus for 2 h. The sample HE1 was collected directly by decantation of the distillate and stored while HE2 was obtained by liquid-liquid extraction of the distillate with *n*-hexane. After evaporation of *n*-hexane, both samples HE1 and HE2 were stored at 4 ºC.

Analysis of HE1 and HE2: In a first time, HE1, HE2 were analyzed using gas chromarography with flame ionization detection (GC-FID) on two types of capillary column: HP1 and innowax. Then, gas chromarography coupled at mass spectrometry (GC-MS) on DB5 capillary column, was used to analyze the samples.

GC-FID analysis: The GC-FID analyses were carried out using an Agilent HP5975 Series C gas chromatograph equipped first with HP1 a fused silica capillary column coated with a non-polar phase (50 m*200 µm id, film thickness 0.33 µm). An Innowax column coated with a polar stationary phase (50 m × 200 µm id, film thickness 0.5 µm) was then used to separate both samples.

With both columns, helium was the carrier gas with a flow rate of 1.3 cm^3 min^{-1} while the oven temperature was programmed as follows: 80 ºC during 8 min, then increased up to 200 ºC at a 2 ºC/min rate and finally to 285 ºC at 10 ºC/min. The injector and detector temperatures were 250 ºC and 300 ºC, respectively.

GC-MS analysis: An Agilent HP6890 GC-MS system was used equipped with a mass-selective detector using electron impact ionization. The separations were performed on a DB5 capillary column (30 m × 0.25 mm id, 0.25 µm film thickness), which was directly interfaced into the ion source of the MS system. The oven temperature program used for analysis was as follows: the initial temperature was maintained at 45°C for 8 min and then increased up to 250 ºC at 2 ºC/min rate. 250 ºC was maintained for 14 min. Helium was the carrier gas at a 0.5 cm^3 min^{-1} flow rate and the split ratio was (1:80). Both injector and detector temperatures were 250 ºC.

The electron impact ionization energy was 70 eV and the mass range scanned was 29-550 (*m/z*). The retention indices (Ir) for all the compounds were determined according to Van Den Dool method using retention times of *n*-alkanes (C5-C31), that had been injected after the essential oils under the same chromatographic conditions.

The components were first identified by comparison of their retention indices with those reported in published works[9-27] then confirmed by comparison of their mass spectra with those of standards in Wiley, NIST and aromas CNRS libraries.

RESULTS AND DISCUSSION

The hydrodistillation of *Brocchia cinerea* aerial parts, gave a yellow like brown essential oil (HE1) and a pale yellow essential oil (HE2), with a yield of : HE1 (0.75 % v/w), HE2 (0.85 % v/w) this last higher yield could be explained by the presence of *n*-hexane trace.

Data obtained from qualitative and semi-quantitative determinations of the two investigated oil samples are shown in Tables 1 and 2. GC-FID chromatograms are very similar for both samples, as it can be seen in Table-1, except for α-terpinene and *trans*-β-farnesene which are present in HE1 but

TABLE-1
CHEMICAL COMPOSITION OF HE1 AND HE2 ANALYZED BY GC-FID

Identified Compounds	Ir (Innowax)	Ir ref (pol)	Reference	Ir HP1	Ir ref (apol)	Reference	%HE1/HP1	%HE2/HP1
Ethanol	-	-	-	-	-	-	0.03	0.05
Acetique acid	-	-	-	-	-	-	0.04	0.03
Hexanal	-	-	-	-	-	-	0.02	0.02
cis-Hex-3-en-1-ol	-	-	-	831	859	15	0.08	0.08
cis-Salvene	892	892	15	842	856	15	0.06	0.03
Santolinatriene	1030	1011	19	903	908	9	**8.00**	**5.8**
Tricyclene	1016	1003	20	909	927	15	0.07	0.04
α-Thujene	1030	1021	16	924	930	15	0.63	0.18
Benzaldehyde	1743	1495	16	929	936	21	0.04	0.02
β-Pinene	1030	1024	13	932	932	9	1.21	0.66
Camphene	1075	1066	13	945	947	9	1.67	1
Isopropyl methyl benzene	1134	1279	22	946	1026	23	0.16	0.15
Sabinene	1127	1120	13	966	973	23	0.38	2.36
β-Pinene	1116	1110	13	972	976	9	0.54	0.39
Myrcene	1162	1159	13	981	986	9	0.12	0.26
α-Phellandrene	1185	1164	13	997	1005	9	0.09	0.06
Isoamyl isobutyrate	-	-	-	1002	1000	22	0.02	0.01
α-Terpinene	1190	1179	13	1011	1015	9	0.14	-
p-Cymene	1274	1269	13	1013	1026	23	1.20	0.54
Limonene	1206	1201	16	1023	1025	12	0.27	0.25
1,8-Cineole	1220	1209	13	1023	1030	9	6.37	**5.9**
γ-Terpinene	1250	1243	13	1049	1053	12	0.71	0.12
trans-Thujanol 4	1464	1459	13	1054	1068	21	0.77	0.23
Terpinolene	1280	1281	13	1080	1088	9	0.15	0.04
cis-Thujanol 4	-	-	-	1086	1078	24	0.62	0.22
α-Thujone	1431	1422	13	1088	1102	15	0.63	0.54
β-Thujone	1457	1442	13	1104	1114	15	**47.72**	**55.4**
cis-p-Menth-2-en-1-ol	-	-	-	1110	1122	22	0.10	0.07
Camphor	1528	1514	13	1125	1126	12	**10.54**	**11.28**
Inconnu sur HP1	1767	-	-	1140	-	-	1.86	1.53
Borneol	1695	1698	13	1150	1166	9	0.48	0.25
Lavandulol	1667	1677	16	1150	1181	15	0.18	0.18
Terpinen-4-ol	1602	1596	13	1164	1177	11	**2.77**	**2.18**
α-Terpineol	1692	1694	13	1173	1189	11,15	0.74	0.7
Cuminaldehyde	-	-	-	1212	1224	16	0.07	0.02

Identified Compounds	Ir (Innowax)	Ir ref (pol)	Reference	Ir HP1	Ir ref (apol)	Reference	%HE1/HP1	%HE2/HP1
cis-3-Hexenyl isovalerate	-	-	-	1216	1243	25	0.30	0.34
Lyratyl acetate	1642	-	-	1254	-	-	**4.17**	**2.96**
Bornyl acetate	1585	1575	13	1270	1289	15	0.78	0.46
Lavandulyle acetate	1620	1617	26	1270	1272	27	0.09	0.09
Carvacrol	-	-	-	1276	1299	15	0.08	0.02
Neryl acetate	-	-	-	1342	1362	15,16	0.04	0.04
Geranyl acetate	-	-	-	1357	1382	16	0.07	0.05
cis-Jasmine	-	-	-	1366	1393	15	0.19	0.43
trans-β-farnesene	-	-	-	1447	1457	15	0.12	-
Spathulenol	-	-	-	1565	1574	10	0.05	0.06
Oxide de caryophyllene	-	-	-	1576	1577	10	0.03	0.03
cis-Methyl jasmonate	-	-	-	1608	1626	16	0.03	0.04
Inconnu sur Innowax	1505	-	-	-	-	-	1.94	1.66
Total							96.37	96.77

Ir (Innowax): retention indice on Innowax column. Ir ref (pol) : reference retention indice on polar column; Ir HP1 : retention indice on HP1 column. Ir ref (apol) : reference retention indice on apolar column; %HE1/HP1 : percentages of HE1 sample constituents. % HE2/HP1: percentages of HE2 sample constituents

not in HE2. The main compounds were β-thujone (47.72 %), camphor (10.54 %), santolinatriene (8.00 %) eucalyptol (6.37 %), lyratyl acetate (4.17 %) and terpinen-4-ol (2.77 %).

It can be noticed that the relative percentages of most constituents are higher in HE1 than in HE2, while the two main components are more concentrated in HE2. Their relative percentages in HE2 and HE1 are, respectively: 55.4 %-47.72 % for β-thujone and 11.28 %-10.54 % for camphor.

More than 50 % of the chemical composition; including the main compound are also identified by Fournier *et al.*[8] but with difference in percentages. That probably returns to the different collection origins of this plant.

As it can be seen on the histogram (Fig. 2), more than 90 % of *Brocchia cinerea* essential oil is constituted approximately by 30 % of monoterpenic hydrocarbons, 42 % of oxygenated monoterpenes, 4 % of oxygenated sesquiterpenes and 24 % of other substances.

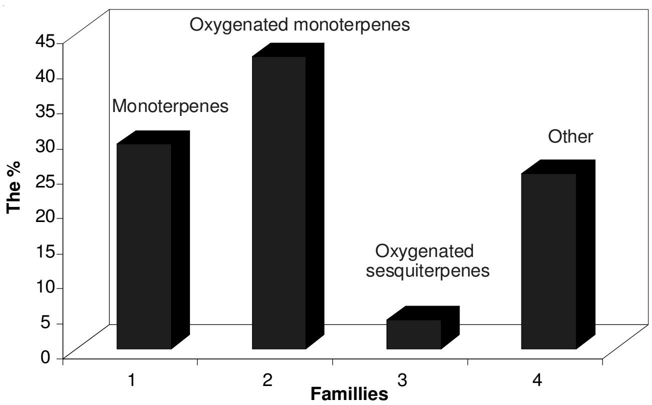

Fig. 2. Histogram of the present chemical families

Regarding the chemical composition of this plant, its interesting biological activities can be explained by the high content of oxygenated monoterpenoids, in particular eucalyptol and camphor, which are well-known for their antiseptic properties and their efficiency in the treatment of respiratory diseases[17]. On the other hand, the relatively high concentration of thujone should be also highlighted, this compound being known as a toxic substance[18].

Two constituents were detected by GC-FID but could not be identified they were eluted on the Innowax column at the retention indices 1505 and 1767, while the second one had a retention index of 1140 on the HP1 capillary column.

Because of its high sensitivity, GC-MS was very useful in the qualitative analysis of our samples. More than 50 peaks were detected, among them 32 compounds were identified and reported on Table-2, 21 of them being already detected by GC-FID analysis.

The results show that the main compounds are α-thujone, camphor and eucalyptol. Other characteristic terpenic substances were identified in this work such as *p*-menth-1-en-8-ol, sabinene, α-thujenal, *trans*-piperitol and β-pinene.

β-Thujone was not detected by GC-MS, but α-thujone was present with a relatively high percentage, as it can be seen on the chromatogram shown in Fig. 3.

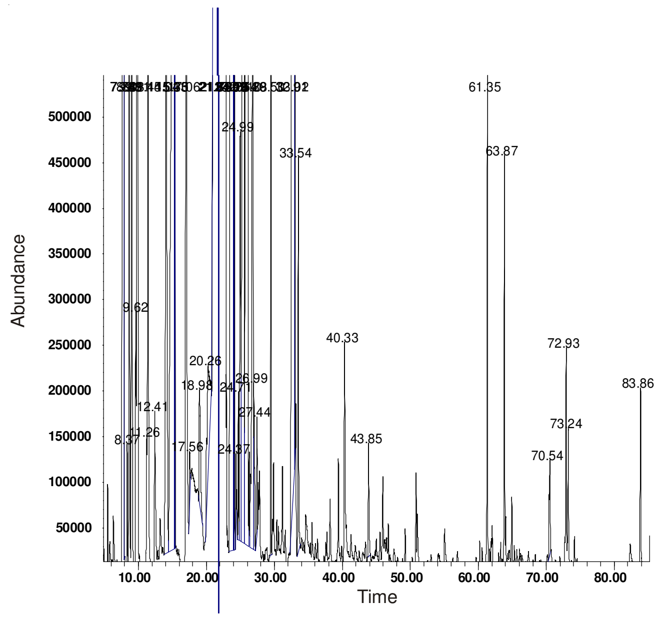

Fig. 3. Chromatogram obtained by GC-MS

It is important to notice that 21 other constituents were also detected by this analysis. Among these non-identified components, some of them are present in relatively high concentration as it can be seen on the chromatogram (Fig. 3).

TABLE-2
CHEMICAL COMPOSITION OF HE1 ANALYZED BY GC-MS

Identified compounds	tr (min)	Ir (exp)	MS (exp)	MS (refe)
Hexanal	4.24	800	44(100), 56(79), 41(60), 43(55), 29(35), 27(33), 72(15)	44(100), 56(82), 41(66), 43(53), 29(38), 27(33), 72(19)
cis-Salvene	5.5	837	41(100), 67(98), 81(96), 68(80), 69(60), 55(50), 39(45), 109(40)	41(100), 67(72), 81(64), 55(38), 109(22), 95(10), 124(1)
Isoamyl acetate	6.33	864	43(100), 70(45), 55(42), 41(20), 42(18), 32(12), 61(10)	43(100), 55(43), 73(38), 41(37), 42(33), 61(27)
Santolinatrienne	7.88	907	93(100), 121(80), 79(70), 91(50), 77(47), 67(40), 105(32), 41(25)	93(100), 41(72), 79(70), 121(55), 77(51), 67(49), 53(34)
Tricyclene	8.36	916	93(100), 79(32), 91(30), 79(32), 92(30), 77(30), 41(29), 121(25), 136(20), 39(20)	93(100), 91(31), 92(27), 79(25), 41(24), 39(22), 77(22), 121(20).
α-Thujene	8.68	921	93(100), 91(60), 77(47), 92(43), 79(15), 136(15), 41(10)	93(100), 77(23), 91(21), 92(20), 41(15) 39(14), 79(10), 121(5)
α-Pinene	9.08	928	93(100), 91(35), 92(32), 77(29), 79(25), 121(19), 41(15), 39(10)	93(100), 92(35), 91(32), 77(29), 79(23), 41(20), 39(19), 121(14)
Camphene	9.91	942	93(100), 121(75), 79(40), 91(37), 67(35), 107(32), 77(30), 41(20)39(18), 136(15)	93(100), 121(58), 79(40), 91(37), 39(34), 41(32), 67(29), 77(28)
4-Methylene-1-(1-methyleth-eyl)bicyclo[3,1,0] Hexane.	11.26	965	93(100), 77(50), 91(50), 41(40), 57(39), 79(40), 39(30), 136(15)	93(100), 41(61), 69(35), 39(32), 91(31), 77(28), 79(27), 27(21)
β-Pinene	11.45	968	93(100), 69(40), 41(39), 79(37), 77(36), 91(36), 121(18), 136(15)	93(100), 41(61), 69(35), 39(32), 91(31), 77(28), 79(27), 27(21)
α-Phellandrene	13.19	998	93(100), 91(60), 77(55), 92(40),136(30), 41(25), 32(22), 39(20), 57(18), 79(18), 85(15)	93(100), 91(33), 77(31), 91(25), 136(16), 41(14), 39(11), 27(10)
Eucalyptol	15.30	1027	43(100), 81(98), 108(90), 154(85), 111(80), 139(75), 71(70), 84(68), 93(65), 55(50)	43(100), 93(55), 81(55), 71(47), 69(40), 84(38), 68(38), 108(36)
γ-Terpinene	17.06	1053	93(100), 91(50), 77(35), 136(35), 121(30), 43(20), 39(10)	93(100), 91(33), 77(32), 136(29), 121(27), 39(23), 43(23), 27(23)
α-Thujone	21.48	1115	110(100), 81(95), 95(80), 67(60), 69(57), 68(55), 109(55), 41(48)	110 (100), 81(88), 95(70), 67(68), 68(58), 41(58), 69(53), 109(43)
8-Methylene-3-oxatri-cyclo[5,2,0,0(2,4)]nonane	22.88	1136	79(100), 91(95), 96(75), 77(73), 39(55), 109(55), 81(50), 41(50), 69(53), 93(45)	79(100), 92(83), 39(69), 91(62), 77(57), 41(50), 27(47), 93(41)
Camphor	23.92	1150	95(100), 81(82), 108(45), 69(40), 152(42), 55(35), 41(38)	95(100), 41(73), 81(74), 108(39), 69(39), 55(38), 27(35), 39(34)
Borneol	24.99	1166	95(100), 41(20), 110(22), 55(12), 67(11), 139(10), 121(8), 93(9)	95(100), 41(18), 110(16), 93(12), 55(11), 67(10), 139(10), 121(9)
α-Thujenal	26.29	1186	79(100), 77(40), 107(35), 105(30), 108(18), 106(17), 91(17), 41(15), 43(14)	79(100), 107(51), 77(33), 105(29), 108(29), 106(28), 41(17), 43(16)
p-Cymen-8-ol	26.53	1189	43(100), 135(85), 91(27), 67(20), 39(15), 41(17), 79(18), 55(12), 82(9), 105(7)	43(100), 135(52), 91(21), 39(9),
p-menth-1-en-8-ol	26.89	1194	59(100), 93(60), 121(49), 136(45), 81(33), 43(31), 67(20)	59(10), 93(60), 121(49), 136(45), 81(33), 43(31), 67(16), 92(17)
trans-Piperitol	27.82	1207	84(100), 83(40), 41(35), 139(32), 55(25), 91(20), 79(20), 67(15)	84(100), 41(27), 83(27), 136(26), 93(25), 43(22), 55(20), 77(18), 69(17), 67(8)
cis-3-Hexenylisovalerate	29.5	1232	82(100), 67(99), 57(98), 41(35), 85(32), 55(20), 54(9)	82(100), 67(77), 57(73), 85(55), 41(48), 55(27), 43(22), 29(22)
Lyratyl acetate	32.91	1280	119(100), 43(67), 91(50), 93(47), 79(45), 77(38), 105(35), 134(22)	119(100), 43(67), 134(47), 93(42), 91(41), 79(34), 105(26), 77(22)
Bornyl acetate	33.02	1281	95(100), 43(58), 93(48), 121(45), 136(40), 41(20), 80(18), 55(18)	95(100), 43(76), 93(45), 136(39), 121(34), 41(27), 80(17), 55(15)
Lavandulyl acetate	33.53	1289	69(100), 43(85), 93(75), 41(65), 68(45), 91(25), 121(23), 67(20), 136(10)	69(100), 43(74), 93(73), 41(50), 68(46), 121(24), 67(20), 136(12)
Carvacrol	34.04	1296	135(100), 150(35), 91(38), 43(48), 32(45), 79(28), 77(25), 107(19), 115(18), 136(10)	135(100), 150(31), 91(13), 136(10), 77(7), 107(7), 117(6), 115(5)
Neryl acetate	38.19	1359	69(100), 43(67), 41(65), 3(45), 68(40), 67(27), 121(25), 80(19)	69(100), 41(67), 43(45), 68(37), 93(35), 67(18), 80(13), 121(11)
Geranyl acetate	39.43	1379	69(100), 43(65), 41(62), 68(45), 93(24), 121(18), 138(14), 67(20)	69(100), 43(65), 41(63), 68(49), 93(24), 136(22), 121(14), 67(13)
cis-Jasmone	40.33	1392	79(100), 91(75), 110(75), 164(68), 55(65), 149(62), 41(61), 93(60), 77(60), 122(58), 135(58)	79(100), 91(98), 39(97), 77(64), 164(60), 91(58), 93(54), 53(50), 55(49), 110(45), 149(42), 135(40), 122(40)
Spathulenol	50.82	1566	43(100), 41(63), 91(62), 119(57), 205(50), 93(48), 105(48), 159(38)	43(100), 41(63), 205(61), 119(57), 91(50), 93(48), 159(43), 105(40)
Caryophyllène oxide	51.04	1570	43(100), 41(97), 79(82), 93(70), 91(54), 69(45), 55(42), 109(35), 121(25), 135(10)	43(100), 41(97), 79(82), 93(70), 91(54), 69(45), 55(42), 109(35), 121(25), 135(10)
Methyl jasmonate	55.05	1643	83(100), 41(58), 151(40), 67(40), 95(40), 55(43), 93(35), 109(38)	83(100), 41(53), 151(46), 67(33), 95(32), 55(29), 93(28), 109(28)

tr (min): retention time. Ir (exp) : experimental retention indice. MS(exp) : experimental mass spectra. MS(refe) : referential mass spectra.

Conclusion

This study about the chemical composition of the plant *Brocchia cinerea* species collected from the region of Ouargla, southeastern Algeria, was carried by extraction of its essential oil which has a rich and varied chemical composition; 30 % of monoterpenic hydrocarbons, 42 % of oxygenated monoterpenes, 4 % of oxygenated sesquiterpenes and 24 % of other substances. *n*-Hexane has proved to be a good solvent for this purpose. It allowed the identification of most constituents of *Brocchia cinerea* essential oil.

The high level of oxygenated monoterpenoids, such as eucalyptol and camphor, confirms the antimicrobial and bactericidal properties of this plant and the importance of further investigations to evaluate its potential applications.

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