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Physicochemical, chromatographic and spectral analyzes of essential oils of Hoggar Myrtle of Tamanrasset in southern Algeria

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Abstract

The *Myrtus nivellei* Batt. and Trab. is an endemic shrub of the central Sahara, known for its respiratory anti-infective and hypoglycemic properties. This work consists of a physicochemical, chromatographic and spectral analysis of essential oils extracted from leaves harvested in the region of Tamanrasset in southern Algeria.

The extractive yield is 1.6 ± 0.05 (ml/100g of dry plant), the refractive index is 1.4700 ± 0.0005 and the relative density is 0.9052 (g/ml). GC-MS made it possible to inventory thirty-six (36) compounds: 78.55% of monoterpenes, essentially oxygenated, including 1,8-Cineole which represents the majority compound at 33.95%; which could partly explain the anti-infectious properties of Hoggar Myrtle essential oil.

Keywords: *Myrtus nivellei*; Essential oils; Physical indices; GC-MS; 1.8 cineole

1. Introduction

The Myrtle of Tamanrasset or of the central Sahara or *Myrtus nivellei* Batt. and Trab. (In Tamashek: Tafeltest and in Tifinagh script: + ⊙ + I I] [+) was discovered at the beginning of the 20th century by Lieutenant Nivelle, *Myrtus nivellei* was described by Battandier and Trabut in 1911 (Figure.1) [1]. It is an endemic species of the central Sahara and distributed in southern Algeria within the massifs of Hoggar, Tassili n'Immidir (or Mouydir), Tassili n'Ajjer and Tefedest It can reach Tibesti and Chad (Figure 2) [2]. Morphologically very close to *Myrtus communis, Myrtus nivellei* is a shrub generally 50 to 120 cm high, sometimes it reaches 2 m high [2], it is characterized by linear and thick leaves with a single vein, 2 to 3 cm long by 3 mm wide, very fragrant when crushed [3], and by a longer fruiting peduncle. The white flowers and the blackish fruits when ripe are very similar to those of *Myrtus communis*, with however some microscopic characteristics which can be explored,

The epicuticular waxes are in greater quantity, and the chloroplasts apparently of smaller size [4]. Two subspecies have been described: *Myrtus nivellei subsp. Nivellei* Batt. &Trab. distributed in Algeria and *Myrtus nivellei subsp. tibesticus* (Quézel) exclusively in Chad [5].

The leaves harvested throughout the year are prepared in decoction and taken orally. They are used as a condiment [2,7], and also serve as a remedy for respiratory ailments, like common myrtle; especially for its anti-inflammatory properties [8] and in a poultice as an analgesic [9]. Added to barley meal, they are used against diarrhea and gonorrhea [8]. According to the healers interviewed, *Myrtus nivellei* could be used to fight against hypertension and as a hypoglycaemic in the same way as *Myrtus communis* [10]. It is also a good antifungal [8]. Myrtle leaves, macerated in melted butter, are used in the preparation of a brilliantine used in hairdressing [11], and to blacken the hair in the form of a decoction [12].

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Figure 1 Myrtus nivellei Batt. and Trab. (Shrub, leaves & fruits) [6]

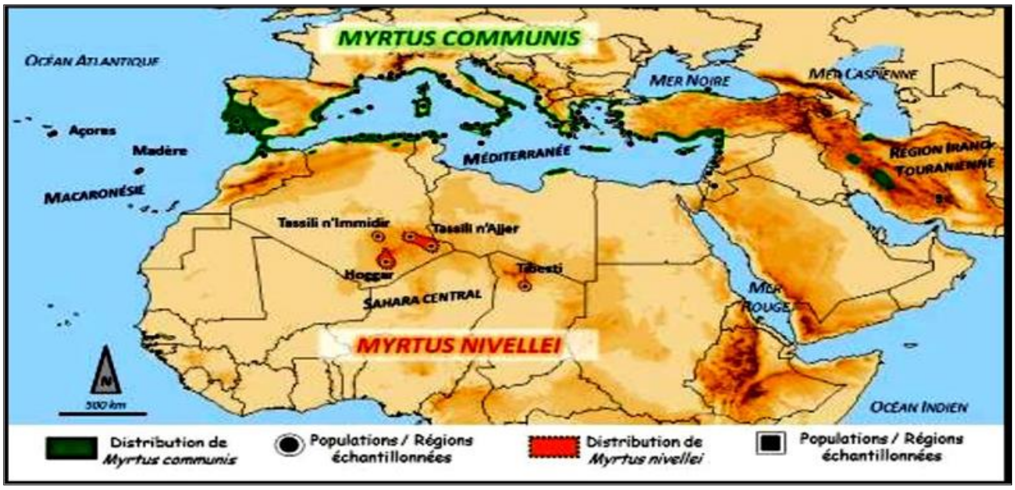

Figure 2 Geographical distribution of *Myrtus nivellei* Batt. and Trab [4]

2. Material and methods

2.1. Localisation, Period and Identification of the plant :

The leaves of *Myrtus nivellei* were harvested in full bloom between the months of September and October on twigs at Oued Iguioui in the Tagmart region (45 km north of Tamanrasset : Latitude North : 23°13'20″ and Longitude East : 05°28' 36″) at 1600 meters altitude, the twigs also bore mature and immature flowers and fruits. The identification of the plant was carried out according to the new flora of Algeria and the southern desert regions (Quezel P. and Santa S. (1977), and the flora of the Sahara (Ozenda P., 1983), by Pr. Boukhalfa D. and Mr. Belghoul M. (biologist, ONPCA), it was confirmed by Mr. Abdelkrim H., professor of botany (National School of Agronomy of El Harrach).

2.2. Extraction of essential oils

The vegetable matter, weighed beforehand (50gr), is placed in a glass column, which surmounts a balloon filled with water. The two-liter flask is filled to 2/3 with distilled water, a glass column surmounts it, and the whole is brought to 60°C at atmospheric pressure. The steam flow is kept constant throughout the duration of the extraction. The mass of oil recovered is determined by weighing, the yield is expressed in relation to the mass of dry matter [**13**].

2.3. **Physical indices**

2.3.1. Refractive index at 20°C

Is determined by methods that comply with AFNOR standards, 2011 [**14**]. The Refractometer allows direct reading of refractive indices between 1.3000 and 1.7000, with an accuracy of ± 0.0002[**8**].

2.3.2. Relative density

It is the ratio of the mass of a certain volume of an essential oil at 20 °C , to the mass of an equal volume of distilled water at 20°C. This quantity is dimensionless and its symbol is d_{20}^{20}. The apparatus is represented by standard laboratory equipment and, in particular, the glass pycnometer, with a minimum nominal capacity of 5 ml, the thermostatic bath, maintained at a temperature of 20°C ± 0.2°C, the precision, graduated from 10°C to 30°C, with divisions at 0.2°C or 0.1°C and the analytical balance, accurate to within 0.001 g [8].

2.4. **Gas chromatograph coupled with a mass spectrometer (CPG/MS)**

The gas chromatograph used is a Hewlett-Packard (HP) Palo Alto CA, USA (Agilent technologies) 6800 plus. The sample is introduced with a microsyringe (split-splitless set at 250°C, injection mode: Split 50:1, Volume injected: 0.2 µl the injector has a dual function: it brings the sample to the vapor state). Hewlett Packard-5MS type column, apolar (length 30 m, internal diameter: 0.25 mm, film thickness 0.25 µm). Stationary phase: 5% phenyl 95% dimethylpolysiloxane. Detector: mass spectrometer (MS) from HP (Agilent technologies), type MSD 5973, triple quadripolar mass filter (QQQ). Data reading and processing are carried out by a computer system managing a NIST 05 [14] mass spectra bank and the Adams RP guide [15].

3. Results and discussion

3.1. **Extractive yield and physical indices**

The average yield of essential oil, calculated according to the mass of the dry plant material to be treated, the refractive indices and the relative densities for all the species studied are represented in Table 1 below:

Table 1 Summary table of the physical indices of the essential oils of *Myrtus nivellei*

	Average yield(ml /100 gr)	**IRnx20**	**Relative density (g/ml)**
Myrtus nivellei	1.6 ±0.05	1.4710 ±0.0005	0.9052

- The essential oil yield of this species (1.6 ± 0.05%) is higher than that of the literature, which is 0.5 to 0.9%. However, it is low compared to the yield of the same species harvested in the wilaya of Djanet, (2.91%) [10]. The discrepancy is probably due to the difference in geographical area, the drying conditions of the plant and the picking period. Myrtle leaves from Morocco give a low yield of essential oil and vary from 0.3 to 0.4% [12]. Myrtle leaves from southern and southwestern Iran give an essential oil yield close to that of our work (from 0.7 to 1.5% [13].
- The refractive index of the essential oil of this species is clearly higher than that of water, it is identical to that of cineole myrtle (Myrtus communis L. cineoliferum) which is 1.461 to 1.489, it varies essentially with the content of monoterpenes (78.55%) and oxygenated derivatives (55.751%)[Zhiri, 2005].
- Relative density: 0.9052 at 20°C; it is close to that of common myrtle EO (density 0.890 to 0.915) and complies with AFNOR standards (2011), it is also identical to that of cineole myrtle (0.878 – 0.895) [Satrani B et al, 2006].

3.2. Gas chromatography coupled with mass spectrometry (CPG-MS):

The GC-MS made it possible to determine the chemical composition and the chemotype of the essential oil of *Myrtus nivellei*, by comparing their mass spectrum with those of the data in the bibliography.

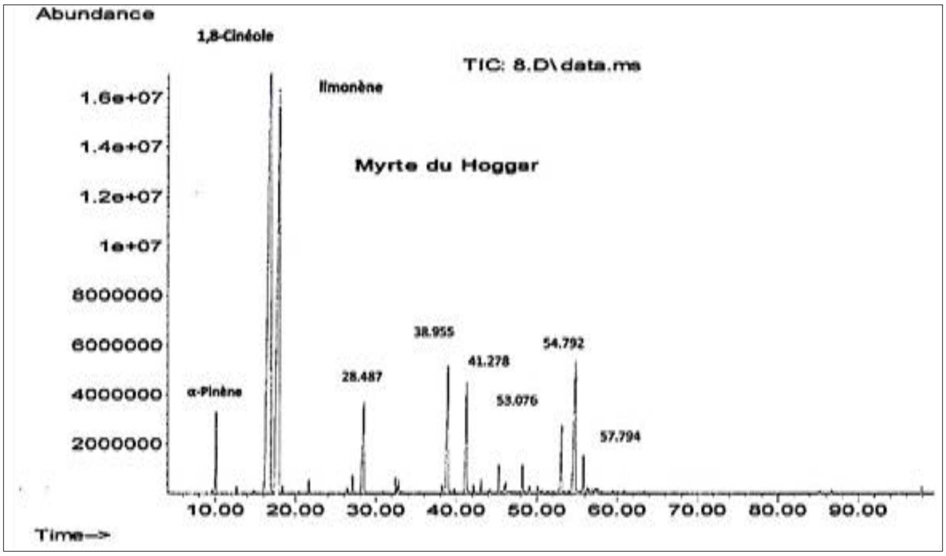

Figure 3 Chromatographic profile of the essential oil extract of *Myrtus nivellei*

Table 2 Chemical composition of the essential oil of *Myrtus nivellei*

N°	Compounds identified	TR	IR	%
01	α- thuyène	9.64	926	0.11
02	α-pinène	10.07	933	2.29
03	β-pinène	12.61	975	0.22
04	α-phellandrène	14.06	999	0.09
05	γ-3-carène	14.85	1010	0.11
06	1,8-cinéole	16.86	1038	33.95
07	dl-limonène	17.04	1040	19.76
08	γ.-terpinène	18.36	1059	0.21
09	α-terpinolène	20.39	1087	0.10
10	Linalool	21.67	1105	0.41
11	p-mentha-1,3-dien-8-ol	26.46	1171	0.29
12	terpinène-4-ol	27.06	1180	0.67
13	α-terpineol	28.48	1199	5.78
14	linalyl acetate	32.44	1256	0.43
15	Geraniol	32.66	1259	0.18
16	méthyl citronellate	32.83	1262	0.42
17	exo-2-Hydroxycinéole acetate	38.19	1341	0.34
18	α-terpinenyl acetate	38.95	1352	7.03
19	néryl acetate	39.76	1367	0.17
20	géranyl acetate	41.27	1389	5.85
21	(E)-β.-caryophyllène	43.08	1416	0.47
22	α.-humulène	45.26	1451	0.93

23	4,4-Dimethyl-2-phenylthio-2-pentene	45.97	1463	0.28
24	2(1H)-naphthalenone, octahydro-8a-(1-propenyl)-,cis-	46.14	1466	0.36
25	(+)-aromadendrène	47.75	1478	0.09
26	N-ethyl-1,3-dithioisoindoline	48.25	1500	1.19
27	selina-3,7(11)-diène	50.54	1542	0.10
28	germacrène B	51.41	1554	0.12
29	dérivé à base de 5-(Morpholino) pent-2-en-4-ynal	53.07	1582	3.08
30	NI	53.99	1597	0.11
31	Lasiocarpenonol	54.79	1612	9.79
32	ethanone, 1-[4-(1-hydroxy-1-methyl ethyl)phenyl]-	55.79	1623	1.32
33	γ-eudesmol	56.24	1638	0.32
34	β-eudesmol	57.30	1657	0.14
35	2,6-diisopropylnaphthalène	60.86	1722	0.14
36	benzoate de benzyl	63.34	1769	0.15
Total (%)				97.00

- Gas Chromatography-Mass Spectrometry (GC-MS) allowed the detection of thirty-six (36) compound were counted in the EO of Myrtus nivellei. from the central Sahara representing a total of 97.00%. The essential oil of Myrtus nivellei is relatively rich in monoterpenes (78.55%) of which 55.75% are oxygenated monoterpenes, 1.8 cineole is the majority compound with 33.95%, α-terpinenyl acetate (7.03%), geranyl acetate (5.85%) and α-terpineol (5.78%). Hydrogenated monoterpenes represent 22.80% of which dl-limonene is the main compound (19.76%) and α.-pinene (2.29%) …). Finally, sesquiterpenes form 18.45% with 16.08% of oxygenated sesquiterpenes (lasiocarpenonol (9.79%)) … and 2.37% of hydrogenated form, including ((E)-β.-caryophyllene (0.47%) and α-humulene (0.93%).
- GC/MS showed some similarity in the essential oil composition of our sample and that analyzed by Bouzabata et al (2013). It showed a high content of 1.8 cineole (33.95%), this compound perfectly characterizes the communis species, in various countries (Table 3), but at variable levels, with the exception of Myrtus communis from Morocco, which has a level almost similar to that of our sample (23 to 36%).
- The period, the harvesting and drying conditions could be the cause of this variability, The limonene content of the essential oil of Myrtus nivellei is clearly higher than those of Myrtus communis from Morocco and Tunisia; the harvest period and the analysis conditions could also be the cause of this difference. On the other hand, Myrtus nivellei is less rich in α-pinene (2.28-4.5%) against 20 to 28% for Myrtus communis from Morocco (table 3).

Most likely Compounds 29 and 30, Table 2; are those, respectively, 5-(morpholino) pent-2-en-4-ynal1-hydroxy-1-(3-methylbutoxy)-2-acetoxy-3,5,5-trimethyl-3-cyclopentene. (MPHTC 29 (KI: 1582) and 1-hydroxy-1-(3-methyl-2-butenoxy)-2-acetoxy-3,5,5-trimethyl-3-cyclopentene (HTBATC) (KI:1594), identified as Bouzabata et al (2013).

1,8 cineole, α-terpineol and geranyl acetate are the most constant compounds, they are found in the two species of the Genus Myrtus, whatever the geographical area.

Table 3 Comparison between the chemical compositions of the essential oil of *Myrtus nivellei* with the *Myrtus communis* of the Mediterranean region

Compound	***Myrtus communis* (French pharmacopy, 2013)**			***Myrtus nivellei (Algeria)***			
				Hoggar		**Djanet**	
	Balkans	**Morocco**	**Tunisia**	**Bouzabata and al. 2013**	**Boukhalfa and al 2017 [16]**	**Touaibi and al;2014**	**Bouzabata and al.2013**
α-pinene	10-25	10-28	47-57	5.2	2.28	-	3.1
Limonene	8-15	8-15	6 -11	19.9	19.76	-	25
1,8 cineole	18-29	23- 43	15-25	39	33.95	12.06	37.5
Linalool	7-19	1.5- 5	2 - 4	0.8	0.41	-	1.7
α-terpineol	1- 4	2-5	1 - 3	5.0	5.77	13.01	5.0
Myrtenol	≥0.3	≥ 0.3	≤0.5	-	-	-	-
myrtenyl acetate	13-21	13- 25	≤ 0.5	-	-	-	-
geranyl acetate	1- 4.0	1.5 -3	1.5- 3	3.6	5.81	-	5.1
methyl-eugenol	≤ 1.5	≤ 1.5	≤ 1	-	-	-	-
acetate linalyl	-	-	-	1.6	0.43	-	4.2
α-terpinyl acetate	-	-	-	4	7.03	-	3.8
1,6 octadien-3-ol,3,7-dimethyl	-	-	-	-	-	2.42	-
δ-elemene	-	-	-	-	-	15.69	-
Citral	-	-	-	-	-	11.66	-
1,2-benzenediol ,3,5-	-	-	-	-	-	5.36	-
Azulene	-	-	-	-	-	6.18	-
α-patchoulene	-	-	-	-	-	2.87	-
MPHTC	-	-	-	6.7	3.08	-	-
HTBATC	-	-	-	1.5	0.11	-	-

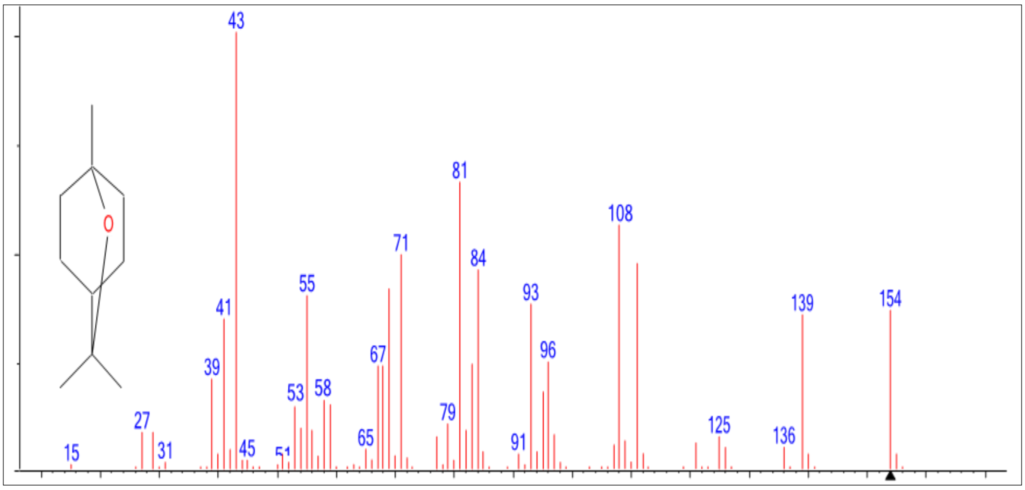

Figure 4 Mass spectrum of 1,8-cineole of the essential oil of Myrtus nivellei

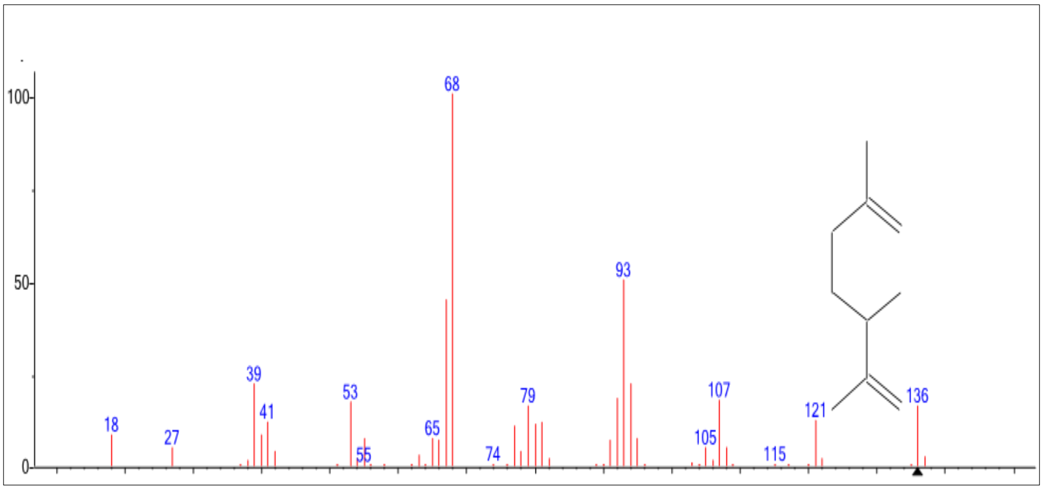

Figure 5 Limonene mass spectrum of Myrtus nivellei essential oil

Sometimes it is very difficult to separate limonene and 1,8-cineole, they are co-eluted. We can clearly see in the mass spectrum of the sample fragments that come from limonene (68 and 138) and others that are specific to 1,8-cineole, however, the latter is in the majority in this sample.

4. Conclusion

The Ahaggar, a mountain range in the central Sahara, is subject to a double climatic influence, that of a temperate Mediterranean climate and that of a tropical Saharo-Arabic climate,

Biogeographically, this work focuses on a species of Mediterranean origin, which has become endemic in this region, the *Myrtus nivellei* (*Myrtaceae*). The particular environment of the plant under study has greatly influenced its chemical composition.

The study of secondary metabolites showed that for the essential oil, the yield is quite high (1.6 ± 0.05%), which confirms the peculiarity of the morphological appearance of desert species.

The chemotype (chemotype) of the essential oil of this species is confirmed, since the 1-8, cineole is predominant in this sample.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

The authors and all co-authors declare that they have no conflicts of interest in connection with this document, and the material described is not in the process of being published nor is it intended for publication elsewhere.

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