

Quality Attributes of the Thyme (*Thymus numidicus* Poiret.) Essential Oil

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Abstract: One of the main factors limiting the increase, trade and market penetration by essential oils has been the lack of quality standards. Thyme is among the most important essential oil of Algeria that are used by the perfume, food and pharmaceutical industry. This study was undertaken to determine the physicochemical and organoleptic properties of the essential oil of Thyme (*Thymus numidicus* Poiret.) growing spontaneously in Algeria. The evaluation of the yield of essential oil extracted by hydrodistillation is: 1,1%. The qualitative and quantitative analysis by (GC / MS) of the essential oil allowed to identify 65 compounds which represent: 95.70%, the main ones being: carvacrol (27.388%), thymol (22.476%), *p*-cymene (9.411%), delta.3-Carene (8.424%), β -Pinène (5.971%), α -terpinolène (5.808%), Limonène (1.917%), α -pinène (1.743%), β -Cubebène (1.651%), α -Copaene (1.385%), α -terpinène (1.353%), α -thujen (1.166%), totaling approximately: 88,693%. The density is: 0.900 ± 0.005 . The measurement of the calculated refractive index and reduced to 20°C is of low refraction to light: 1.4830 ± 0.003 . The boiling and evaporation index are: (233-255°C).

Keywords: Thyme, *Thymus numidicus* Poiret., Essential Oil, Chemical Composition, Density, Refractive Index, Boiling and Evaporation Index

1. Introduction

Essential oils possess many biological activities. They have organoleptic properties: (appearance, color, odor, flavor) and physicochemical properties: (chromatographic profile, density, refractive index and melt index).

These indices are criteria of purity which indicates the presence of foreign bodies the case of the density, and which informs us on the purity and the group of the essential oil the case of the index of refraction [1].

The methods used to determine the physicochemical indices are those indicated by the French Standards Association (AFNOR) Code of Standards. The purpose of the present work was to determinate the organoleptic and physicochemical properties of Algerian Thyme essential oil. And relate them with their chemical composition, for further application in food and pharmaceutical industries as natural valuable products.

2. Materials and Methods

2.1. Selection of Plant Material

The samples come from the region of Azzaba located at Skikda city (North-east of Algeria), the harvest was carried out in June 2013. The identification of Thyme (*Thymus numidicus*) was made by: Dr. Djeddi Samah, department of biology university of Annaba, according to the following flora: (Dobignard and Chatelain, 2010-2013) [2], (Quézel and Santa, 1963) [3]. The choice for this species is justified by the fact that it is endemic and rich in essential oils and phenolic compounds including flavonoids known for their various biological activities.

2.2. Extraction of the Essential Oil

The simple hydrodistillation consists in directly immersing the plant material to be treated (intact or

possibly ground) in the water which is then brought to a boil. The heterogeneous vapors are condensed on a cold surface and the essential oil separates by difference in density (Bruneton, 1995) [4].

Essential oils were obtained in a Clevenger type device [5]. Distillation was carried out by boiling, for an hour and a half, 200 g of fresh plant material with one liter of water in a two-liter flask surmounted by a 60 cm long column connected to a condenser. The yield of essential oil is expressed by the volume of oil (in milliliter) obtained for the mass 100 g of dry vegetable matter. The essential oil was stored at 4°C in the dark in the presence of anhydrous sodium sulfate.

2.3. Measurement of Relative Density at 20°C

The relative density of the essential oil is defined as the ratio of the mass of a certain volume of oil at 20°C. and the equal mass of volume of distilled water at 20°C. This size is dimensionless and its symbol is d_{20}^{20} . The density is measured using a pycnometer of volume: 5 ml at the temperature of 20°C (Standard NF T 75 - 111) [6].

2.4. Measurement of the Refractive Index

The measurements were carried out using a Prisma-CETI convex refractometer. When the determination is carried out at a temperature other than 20°C., the correction is carried out at 20°C. using the formula (Standard NF T 75 - 112) [7]:

$$I_{20} = I_t + 0,00045 (T - 20^\circ\text{C})$$

I_{20} = Index at 20°C.

I_t = Temperature or ambient temperature.

T = Ambient temperature.

2.5. Melting Point Determination

The principle is based on the heating of a capillary tube containing a test portion of the essential oil in a heating and the notation of the melting temperature (AOCS, 1997. In Soares *et al.*, 2009) [8].

2.6. Chromatographic Analysis

The GC-MS analysis was performed using a Hewlett Packard 5973-6800 system operating in EI mode (70 eV) equipped with a split/splitless injector (250°C), a split ratio 1/50, using a fused silica HP-5 MS capillary column (30 m × 0.25 mm (i.d.), film thickness: 0.25 µm. The temperature program for the HP-5 MS column was from 60°C to 280°C at a rate of 2°C/min. Helium was used as a carrier gas at a flow rate of 0.5 ml/min. Injection volume of the sample was 0.2 µl. The identification of the components was conducted in an IS system managing a library of spectrum wiley 7n.l. The GC-MS analysis was performed at the Scientific and Technological Scientific Research Center on Physico Chemical Analysis (CRAPC), Bab ezzouar (Algiers, Algeria).

3. Results and Discussion

3.1. Rendement

The results obtained indicate that the extraction yield of the essential oil by hydrodistillation is 1.1%. The results are agreed with those of Hadeif, 2004 [9]: The essential oil yields of *Thymus numidicus* obtained from the dry matter of the aerial part of the plant (Annaba region in Algeria) is 1.10%, rich in thymol and carvacrol.

In contrast, this performance was revealed different than that given by the same species from different regions in Algeria; (Tizi Ouzou [10] and Annaba [11]) whose performance can reach 1.58% to 1.92%.

This difference can be attributed to several factors such as climate and geographical conditions and the period of harvesting and drying conditions. It has been shown that drying affects the performance of essential oil: a plant dried in non-optimal condition may lose all of its essential oil.

3.2. Organoleptic Properties

The following table (Table 1) shows the characteristics of the essential oil obtained (color, odor, flavor, solubility) in the species studied.

Table 1. Organoleptic characteristics of *Thymus* essential oil.

Plant	Color	Odour	Flavor	Solubility
Thyme	Clear, clear liquid	Colorless to reddish brown	Pleasant smell thymol	pungent
	Liposoluble, soluble in 2 volumes of alcohol 80%			

3.3. Physico-Chemical Properties

3.3.1. Chemical Composition

Qualitative and quantitative analysis (GC / MS) of the essential oil identified 65 compounds representing a total of 95.70% (Table 2 and Figure 1). The essence of *Thymus numidicus* consists mainly of: carvacrol (27.388%), thymol (22.476%), *p*-cymene (9.411%), delta.3-Carene (8.424%), β -Pinene (5.971%), α -terpinolene (5.808%), Limonene (1.917%), α -pinene (1.743%), β -Cubebene (1.651%), α -Copaene (1.385%), α -terpinene (1.353%), α -thujen (1.166%), totaling approximately 88.693%.

Table 2. Chemical composition of *Thymus* essential oil (Principal constituents).

N°	Compound	Retention Time	%
01	α -Thujen	9.862	1.166
02	α -Pinene	10.266	2.914
03	β -Pinene	14.081	5.971
04	α -Terpinene	15.518	1.353
05	<i>p</i> -Cymene	16.385	9.411
06	α -Terpinolene	16.551	1.917
07	Thymol	36.676	22.476
08	Carvacrol	37.879	27.388
09	β -Cubebene	47.048	1.651
10	α -Copaene	49.576	1.385

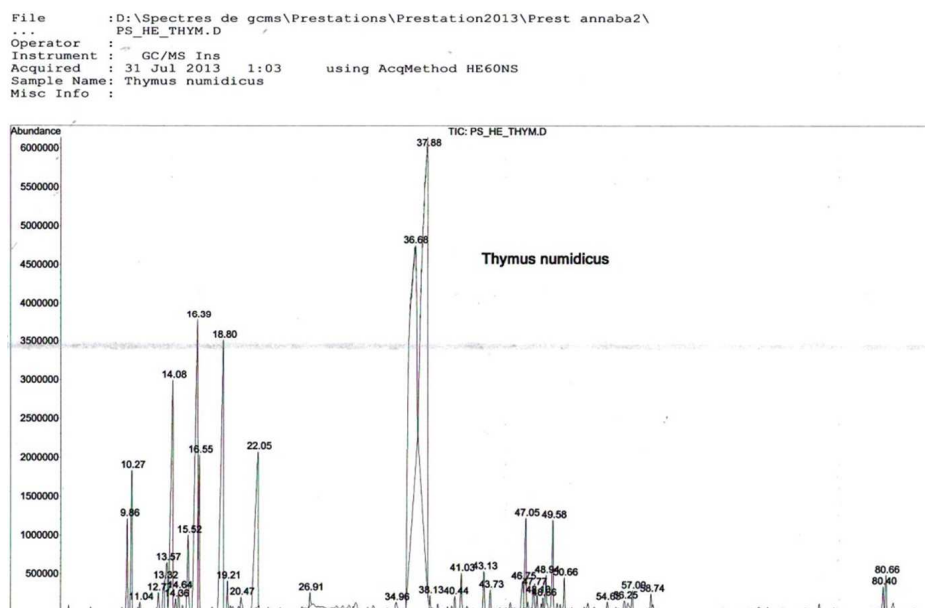


Figure 1. Chromatographic profile of the essential oil of the Thyme analyzed by GC / MS.

The chemical composition of the essential oil of *Thymus numidicus* grown in the region of Azzaba, Skikda city (Algeria) shows some differences by some Work Table 3.

Table 3. Chemical composition of the essential oil of *Thymus numidicus* growing in different regions in Algeria.

Author's	Region	Major Constituent
Hadef, (2004) [09]	Annaba (Algeria)	thymol (60.80%), <i>p</i> -cymene (10.32%), linlol (8%), γ -terpinene (7.60%) and carvacrol (5.07%).
Kouch <i>et al.</i> , (2014) [11]	Annaba (Algeria)	thymol (77.5%), <i>p</i> -cymene (10.1%) and γ -terpinene (6.37%).
Kabouche <i>et al.</i> , (2004) [12]	Constantine (Algeria)	thymol (68.2%), carvacrol (16.9%), linalool (11.5%) and <i>p</i> -Cymene (01.0%).
Zeghib <i>et al.</i> , (2013) [13]	Constantine (Algeria)	thymol (23.92%), linalool (17.20%), <i>o</i> -cymene (11.41%), γ -terpinene (10.84%), thymol methyl ether (6.73%) and carvacrol (6.02%).
Benayache <i>et al.</i> , (2014) [14]	Constantine (Algeria)	thymol (54.1%), <i>p</i> -cymene (15.3%), linalool (5.4%), carvacrol (3.8%), thymoquinone (3.7%), α -pinene (2%), thymol methylether (1.7%) and <i>b</i> -caryophyllene (1.8%).
Saidj <i>et al.</i> , (2008) [15]	Yakouren, Tizi Ouzou (Algeria)	thymol (51.0%), carvacrol (9.4%), linalool (3.3%), thymol-methyl-ether (3.2%) and iso-caryophyllene (2.7%).
Messara <i>et al.</i> , 2017 [10]	Tifrit, Tizi Ouzou, (Algeria)	thymol (40.40%) and carvacrol (13.37%).

This great diversity and variability in the chemical composition of thyme essential oil is well known. There are several factors responsible for this variability, the most important of which are the climate, the soil, the harvest period and the method of conservation and extraction. Genetic factors and the vegetative cycle also take part in this chemical diversity of the volatile oils of *Thymus numidicus*.

According to Pibiri, 2006 [16] in aromatherapy, the use of CPG is essential to differentiate in the same species chemical variations induced by different factors that have an influence on plant biosynthesis, such as sunshine, altitude, nature and the composition of the soil. They define the CHEMOTYPE or chemical race of the species concerned. Indeed, the observation of a massif of Thyme and the knowledge of its origin are not enough to characterize its essential oil.

3.3.2. Density

The density of an essential oil is a very important criterion for evaluating the quality of an essential oil in different areas of life (cosmetics, pharmacy, food industry, chemical, etc.). It

can easily give insight into the naturalness of the product as well as attempts at fraud and alteration.

Table 4. Density value of the essential oil (mean \pm standard deviation).

Plant	Density (Found)	Density (AFNOR NF T. 75-202)
Thyme	0,900 \pm 0,005	0,894 to 0,930

From the result (Table 4) of the obtained density, it can be said that the oil conforms to international standards. (According to the French Association of Standardization).

3.3.3. Refractive Index

The refractive indices were calculated and brought to 20°C. Using an Abbot refractometer and are shown in the table below (Table 5).

Table 5. Value of the refractive index at 20°C (mean \pm standard deviation) of the essential oil.

Plant	Refractive Index (Found)	Refractive Index (AFNOR)
Thyme	1,4830 \pm 0,003	1,4830 to 1,5100

The refractive index is the ratio between the celerity of light in the vacuum and the celerity of light in the medium under consideration. This report indicates the ability of the essential oil to reflect light. The refractive index of the sample corresponds to the AFNOR standards. It indicates its low refraction to light.

3.3.4. Melt Index

The melt index is usually found directly on the majority essential oil compounds after separation and not on the oil itself. Therefore, a separation of the major components of the oil is indispensable and indicated to determine this index.

Table 6. Value of the melting index of the essential oil.

Plant	Boiling Temperature (Found)	Evaporation T° (Found)	Melting Index (Literature)
Thyme	233	255	Thymol 233, carvacrol 237.2

4. Conclusion

Essential oils used for centuries often have a properties interesting, very little work has been done on the study of the organoleptic and physicochemical characteristics of aromatic fractions of Thyme (*Thymus numidicus* Poiret.) grown in Algeria. The safety of pharmaceutical and cosmetic products containing essential oils is largely linked to the quality of raw materials used and the formulation of the finished product. Because of its profile, the essential oil extracted by hydrodistillation of this plant possesses properties very appreciated in perfumery and will be very coveted in the sector of the food, pharmaceutical and cosmetic industry. And in order to determine whether the essential oil of Thyme complies with the standards of the European Pharmacopoeia, a series of tests has been carried out following as far as possible the conditions described according to the French Standards Association (AFNOR) Code of Standards and the values obtained for the different physicochemical indices comply with those cited in the literature.

Competing Interests

The authors declare there that they have no competing interests.

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