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CHEMICAL COMPOSITION AND DIFFERENTIATION OF ESSENTIAL OILS OF MOROCCO'S DIFFERENT VARIETIES OF THYME

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ABSTRACT*:* *This study aims at analysing the chemical composition of the essential oils (E.O.) of four varieties of thyme (Thymus broussonetii, Thymus satureioides, Thymus zygis and Thymus vulgaris) and their comparison; through the principal components analysis (PCA) method; with new species of thyme (T. riatarum, lepobotrys T and T. munbyanus), previously studied by M. El Idrissi & all., 2012. As a matter of fact, this comparison is the identification or differentiation between species. Chromatographic analysis of thyme (E.O).has emerged different chemotypes. Thus, thymol, is both the chemotype T. zygis -Meknes 44.17% and T. vulgaris –Errachidia 35.87%. The chemotype T. brossenetii- Khémisset is carvone 46.70%, while that of Thymus satureoides from Agadir is the borneol 36.56%. (PCA) and hierarchical clustering (AHC) method shows a similarity of chémotye for T. vulgaris, T. leptobotrys and T. zygis, although the majority of compounds differ quantitatively. Same for the T. munbyanus, T. satureioides& T. riatarum which are chemotype "borneol"*

KEYWORDS*:* Essential Oils; Differentiation; Chemotype; ACP; GC / MS; thyme.

NTRODUCTION

Mediterranean regions are "hotspots" of biodiversity (Myers et al.2000).They have a remarkable diversity of vascular plants estimated at more than 48 000 species, of which 57% are endemic to these regions (Cowling et al.1996). Amongthe diversity of Mediterranean plants, there are many aromatic species, most of which belongs to the Lamiaceae family (about 260 kinds, for a number of species estimated between 6500 and 7000 (Spichiger, 2004).Thymus which means "flavor", rich in essential oils, is a genus with more than300 species and best known of the Lamiaceae family. This genus is native to southern Europe, although today it is grown all over the world. It is widespread in the Mediterranean basin. In Morocco, the thymus type is represented by21 species of which 12are endemic (Benabid, 2000).Essential oils of these species take a great part in countless pharmaceutical, cosmetic and aromatic compositions, hence the economic and scientific interest. Various species of this genus have been reported to have several powers, antibacterial (Imelouane 2009), (Rota 2008), (Martino 2009) and (Loziene 2007), antifungal (Pinto 2006), (Goncalves 2010), (Giordani 2008), (Karaman 2001), and antioxidant power (Sarikurkcu 2010), (Bounatirou 2007).

The chemical composition of essential oils of thyme species and especially their flavonoid capacity plays an important role in the taxonomy and the distinction between the different species (Boros 2010), (Horwath 2008).

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Intra-specific variability of the essential oils of the genus Thymus is well documented; (Stahl-Biskup 2002). Thymol and carvacrol are the main compounds in most thyme essential oils. Given this diversity, we found interesting, studying and seeking possible similarities among eight varieties chemically analyzed. The studied samples are*broussonetii Thymus, Thymus satureioides, Thymus zygis, Thymus vulgaris, T.riatarum, T.lepobotrys* and *T.munbyanus.*

MATERIALS AND METHODS

Plant material

•Sample Collection

The studied samples are from in formal settlements Collected in different areas ofMorocco.cf (Table 1).

Table 1 : Location of the collected species.

Species	Place ofcollection
T. vulgaris	Er-Rachidia
T. zygis	Meknès
T. broussonetii	Khémisset
T. satureoides	Agadir

METHODS

•Drying of plant material and calculation of humidity

These four thyme varieties were dried in open air, at room temperature. Only the aerial part was used then pulverized using a power mill. The moisture content was determined by drying; 5g of the plant material; in oven at 105° C until a constant weight.

•Extraction of the essential oil (E.O.)

The collected samples are dried for 7days in open air and in shade. The extraction of E.O. was performed by hydro distillation in a Clevenger-type apparatus (Clevenger, 1928), nominal capacity of 2L. After 3 hours of reflux. This oil was dried over Na2SO4. The essential oil obtained was stored at 4°C in the dark. The yield of essential oils expressed related to the dry matter (in ml / 100 g dry matter).

• Analysis of the chemical composition by gas chromatography coupled to GC / MS mass spectrometry.

The analysis of the essential oil was carried out by gas chromatography (GC Ultra Trace) coupled to a mass spectrometer (MS Polaris Q ion trap). Fragmentation is made by 70 eV electron impact. The column temperature (VB-5 (5% phenyl methyl polysiloxane), 30 m × 0.25 mm × 0.25 nm) is programmed from 40 to 300 ° C at 4 ° C / min. The carrier gas is helium at 1,4mL / min. The injection mode is split. The device is connected to a computer system running a mass spectra library NIST 98.

ISSN 2055-0073(Print) ISSN 2055-0081(Online)

•Analysis of the similarity between species by principal component analysis (PCA) method and hierarchical clustering (AHC)

ACP

The principal component analysis (PCA) is a method for simplifying data, the study of relationships between all variables to determine the similarities and differences between individuals. Thus, the analysis of the relationship between the chemical composition of E.O. and taxonomy of eight species of thyme was performed by PCR, only the discriminating variables were taken in to account.(I. Jolliffe, 2002).

AHC

The hierarchical classification CAH method is a complementary method of the CPA. It is about a dichotomous tree (Dendrogram) showing the successive aggregations groups at given levels (distance or similarity) up into a single class Fabien (Knight et al, 2012).

RESULTS AND DISCUSSION

Thyme humidity

Analysis of samples revealed a high humidity between 67.40 and 79.50%.

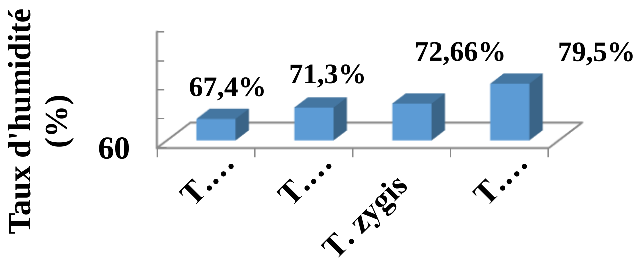

Figure 1: Humidity level of thyme (*Thymus vulgaris, Thymus broussonetii, Thymus satureioides* and *Thymus zygis)*

Average yield of essential oil of Thymus

The average yield (three trials for each sample) in essential oil for the various species is 1%, 1.3%, 1.5%, 1.75% respectively for T. *broussonetii, T. zygis, T. satureioides* and *T. vulgaris.*

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Composition of chemical species analysed

Table 2: Chemical composition of the essential oil of Thymus

Constituents	Formula	(%)			
		T. v **Err.**	***T. b*** **Khm.**	***T. s*** **Ag.**	***T. z*** **Mek.**
α-Pinene	$C_{10}H_{16}$	0,01	-	0,66	0,02
Camphene	$C_{10}H_{16}$	0,48	0,20	14,04	0,70
Sabinene	$C_{10}H_{16}$	0,52	0,62	-	0,78
β-Pinene	$C_{10}H_{16}$	1,64	-	0,54	1,17
2-Carina	$C_{10}H_{16}$	-	0,05	-	-
α-Phellandrene	$C_{10}H_{16}$	0,69	0,81	0,19	1,21
3-Carina	$C_{10}H_{16}$	0,86	5,18	8,11	0,84
α-Terpinene	$C_{10}H_{16}$	1,88	-	-	1,79
p-Cymene	$C_{10}H_{14}$	14,19	3,90	2,45	15,45
β-Phellandrene	$C_{10}H_{16}$	0,16	0,17	1,63	0,19
γ-Terpinene	$C_{10}H_{16}$	12,86	5,67	0,41	11,30
hydrate trans-Sabinene	$C_{10}H_{18}O$	1,37	0,75	-	1,59
Terpinolene	$C_{10}H_{16}$	-	1,18	-	-
α-Campholenal	$C_{10}H_{16}O$	-	-	0,69	-
Borneol	$C_{10}H_{18}O$	1,52	0,29	36,56	1,69
Terpinen-4-ol	$C_{10}H_{18}O$	0,10	0,21	1,58	0,13
Myrtenol	$C_{10}H_{16}O$	-	0,06	-	-
Isobornyl formats	$C_{11}H_{18}O_2$	0,03	-	1,04	-
Isothymolmethylether	$C_{11}H_{16}O$	-	-	0,20	0,13
Bornylacetate	$C_{12}H_{20}O_2$	-	-	2,98	-
Thymol	$C_{10}H_{14}O$	35,87	23,90	-	44,17
Carvacrol	$C_{10}H_{14}O$	18,62	-	-	13,48
α-Terpinylacetate	$C_{12}H_{20}O_2$	-	-	14,47	-
Caryophyllene	$C_{15}H_{24}$	4,69	3,77	2,89	3,44
Guaia-3,9-diene	$C_{15}H_{24}$	-	0,13	0,07	-
Aromandendrene	$C_{15}H_{24}$	-	-	0,09	-
α-Guaiene	$C_{15}H_{24}$	0,10	-	0,11	0,07
β-Cadinene	$C_{15}H_{24}$	-	0,13	-	-
γ-Gurjunene	$C_{15}H_{24}$	1,10	1,53	-	-
α-Isomethyl ionone	$C_{14}H_{22}O$	-	0,11	-	-
Germacrene-D	$C_{15}H_{24}$	0,14	0,90	-	0,06
γ-Methylionone	$C_{14}H_{22}O$	-	-	0,04	-
10s, 11s-Himachala-3(12),4-diene	$C_{15}H_{24}$	-	0,18	-	-
β-Guaiene	$C_{15}H_{24}$	0,15	0,21	0,17	0,06
Valencene	$C_{15}H_{24}$	0,19	0,71	-	-
γ-Cadinene	$C_{15}H_{24}$	0,17	0,38	0,32	0,07
carvone	$C_{10}H_{14}O$	-	46,70	-	-

ISSN 2055-0073(Print) ISSN 2055-0081(Online)

δ-Cadinene	$C_{15}H_{24}$	0,34	0,90	0,22	0,16
α-Muurolene	$C_{15}H_{24}$	-	-	0,04	-
Selina-3,7(11)-diene	$C_{15}H_{24}$	-	-	-	0,12
Spathulenol	$C_{15}H_{24}O$	1,86	0,87	-	-
Caryophellene oxide	$C_{15}H_{24}O$	-	-	2,69	1,06
Cedreneoxide	$C_{15}H_{24}O$	-	-	5,90	-
(-)-Spathulenol	$C_{15}H_{24}O$	0,21	-	-	-
Aromadendrene oxide	$C_{15}H_{24}O$	-	-	0,25	-
δ-Cadinol	$C_{15}H_{26}O$	0,04	-	0,16	-
γ-costol	$C_{15}H_{24}O$	0,05	-	1,12	-
Germacrone	$C_{15}H_{22}O$	-	0,03	-	-
Total		**99.84%**	**99.54%**	**99.62%**	**99.68%**
number of components		**28**	**28**	**29**	**24**

The results of the chromatographic analysis of essential oils of thyme are shown in the table below. Thus, the chromatographic analysis of the essential oil of *T. vulgaris* identified 28 constituents corresponding to about 99.84% of the chemical composition of the essential oil extracted. The major constituents are 35.87% thymol, carvacrol 18.62%, p-cymene 14.19% and 12.86% γ-terpinene. *Thymus broussonetii* contains 28 components corresponding to 99.54% of the chemical composition of the essential oil. This composition is characterized by the predominance of carvone 46.70% Thymol (23.90%), γ-terpinene (5.67%), 3-Carene (5.18%) and p-cymene (3.90%).

29 constituents equivalent to 99.62% are identified by GC E.O. *Thymus satureioides* of Agadir. This composition is characterized by the predominance of Borneol (36,56), α-Terpinylacetate (14.47%), Camphene (14.04%) and 3-carene (8.11%).

GC of the essential oil of *Thymus zygis* spring, 24 constituents represent about 99.68% of the chemical composition. The latter is characterized by the predominance of thymol (44.17%), p-cymene (15.45%), carvacrol (13.48%) and γ-terpinene (11.30%).

Table 3: Major Constituent of essential oil of thymus

Constituent	***Tz* Mek.**	***Tv* Erra.**	***Ts* Aga.**	***Tb* Khem.**
Camphene	-	**-**	**14,04**	**-**
3-Carene	-	**-**	**8,11**	**5,18**
p-Cymene	**15,45**	**14,19**	**-**	**3,90**
γ-Terpinene	**11,30**	**12,86**	**-**	**5,67**
Borneol	**-**	**-**	**36,56**	**-**
Thymol	**44,17**	**35,87**	**-**	**23,90**

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Carvacrol	**13,48**	**18,62**	**-**	**-**
α-Terpinylacetate	**-**	**-**	**14,47**	**-**
Caryophyllene	**3,44**	**4,69**	**-**	**3,77**
carvone	**-**	**-**		**46,70**
Cedreneoxide	**-**	**-**	**5,90**	**-**

Comparison of chemical species analyzed with those of the literature

Indeed, previous work by El Idrissi (El Idrissi & all, 2012) on the chemical composition of the essential oil of four thymus species; namely *T..munbyanus* (Itzer / Midelt), *T. munbyanus* (Chqua / Taza), *T. leptobotrys* (Azilal), T.riatarum (Taza) shows that camphor is the major component of the species *T. munbyanus* two areas; Itzer (24.87%) and Chqua (27.76%).The essential oil of *T. leptobotrys* shows a group of compounds whose majority are: carvacrol (31.98%), thymol (18.96%) and α-pinene (12.17%), p-cymene (5.89%).The major constituents of the essential oil of *T.riatarum* are: borneol (31 .13%), camphene (16.4%), p-cymene (9.18%).The comparison between these chemical species reported in the literature by El Idrissi, and those analyzed by our care, was made by the two methods aroused namely ACP & CAH.

Analysis of the similarity between species through ACP and CAH

• Principal Component Analysis

To achieve this analysis we chose the first two factorial axes. Species dispersion of thyme in the plane formed by the two axes can explain 68.10% of the variability, including 47.15% on the first axis and 20.95% on the second axis. Three groups of species are formed in the two axis systems.

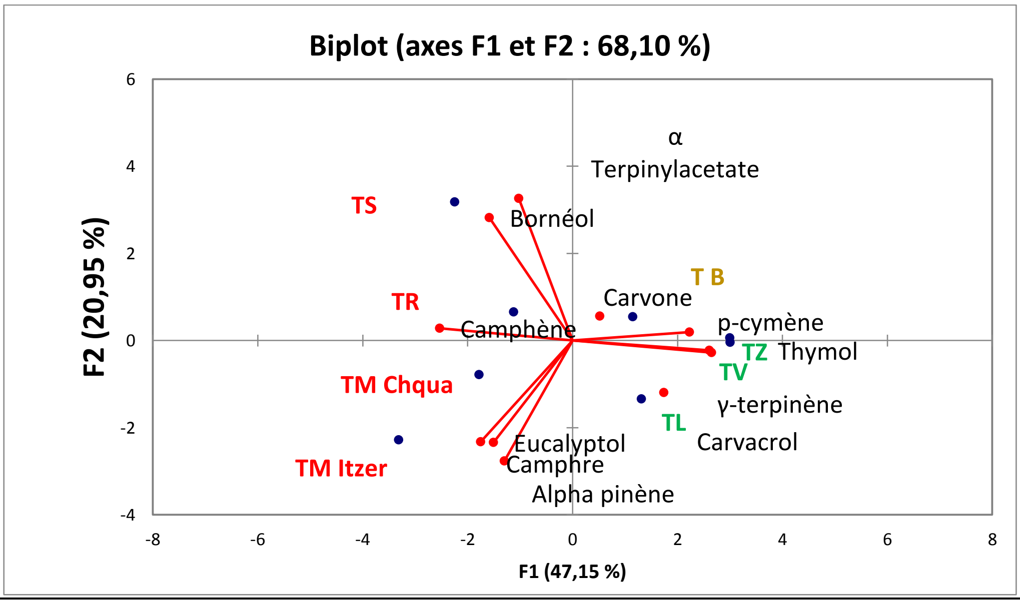

Figure 2: Principal components analysis for the main compounds of Essential Oil of thyme

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• Group 1 formed by the 4 species of thymus *T. munbyanus* (Itzer), *T. munbyanus* (Chqua), *T. riatarum* and *T. satureioides*. This group is borneol chemotype. It is distinguished from other groups by the unique presence of camphene.

• Group 2 formed by three species; *T. leptobotrys*, *T.zygis* and *T.vulgaris*. This group is characterized by Thymol chemotype, and significant levels of carvacrol, p-cymene and gamma terpinene.

• *Thymus brossounetti* form an isolated group (group 3) other groups with unique and major presence of carvone. However, there are some similarities with group 2 thanks to the high rate of thymol and considerable amounts of p-cymene and gamma terpinene.

•Analysis CAH

Figure 3 includes all the samples using Ward technique with a measure of the Euclidean distance. The Joint horizontal line indicates a truncation level defining a partition in three groups of all studied, namely {T.*munbyanus* -Izer, T.*munbyanus*-chqua, T*riatarum*r, T.*satureioides*}, {T.*broussonetii*}, {T.*leptobotrys*, T.Zygis, T.Vulgaris}.

This technique confirms the results obtained by the CPA.

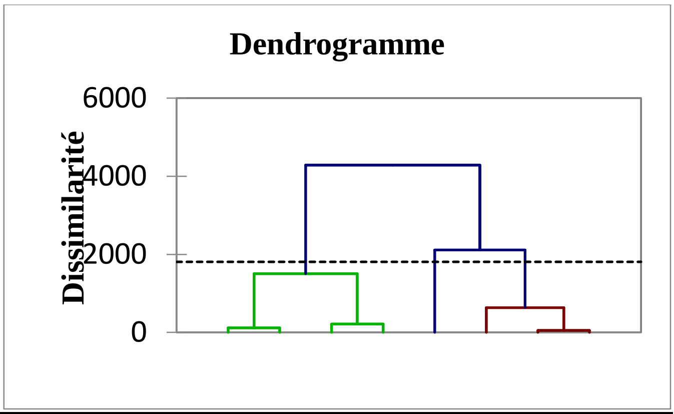

Figure 3: Dendrogram obtained from the analysis of the composition of the E.O. of 8 species of thyme.

CONCLUSION

In conclusion, in relation to the ACP, chemical profiles *T.munbyanus* (itzer, chqua); *T.riatarum*r and *T.satureioides,* are quite similar in terms of content and Borneol and camphene. *T. broussonetti* presents a profile rich and unique in carvone compared to other species thym. *T. leptobotrys, T. Zygis* and *T. Vulgaris* have a very similar profile richer in thymol and carvacrol than other species.

It should be noted that the similarity between T. and T. vulgaris Zygis is absolute and it is verified by both methods ACP & CAH: At Figure 2 (superposition of points representing TZ & TV) and -Figure 3 (the trait that is almost coincident with the x axis).

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AHC method affirms CPA data and allows distinction between three groups: group 1; represented by *T. riatarum*r; is formed by *Tmunbyanus*, *T.riatarum*r and *T. satureioides*, group 2; represented by *T. Vulgaris*; is formed by *T. leptobotrys*, *T. Zygis* and *T.Vulgaris*. For group 3, it is distinct and trained by *T. broussonetti*.

These results open interesting perspectives for taxonomy and distinguish between different species of plants. We plan to continue this study to provide a comparison of both the chemical and morphological profile of the species.

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