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Discrimination of chemical compounds of the oil of Menthasuaveolens (L.) of Eastern Morocco by the GPC-MS and chemometric methods

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- ✓ MenthaSuaveolens(L.);
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- ✓ Principal component analysis(PCA)
- ✓ Descendinghierarchical classification (DHC).

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Abstract

The study of chemical variability of essential oils from Menthasuaveolens (L.), obtained by hydrodystillation, showedimportant quantitative and qualitative differences . Characterization and valuation were conducted by the determination of the chemical composition of the essential oils, using the gaz phase chromatographic (GPC) coupled with mass spectrometry (GPC/MS).Analysis of the essential oils of the plant Menthasuaveolens(L.) was harvested in nine communities in East Morocco. (Berkane, Trifa, Ahfir, Laayoun, Benioukil, Ain sfa, Jerada, GafaïtandTafoughalt) during the month of may 2015, has led us to identify 61 components. These results show the likely existence of majority chemical compounds, such as: thePulegone, the Piperitone, Piperitone ox, the ox Z-Piperitone, 1, 2-Eepoxymenty, Germacrene D, trans-Caryophyl and the Nepetalactene. To study the chemical variability of the essential oil of the plant from the Menthasuaveolens (L.), it is essential to perform processing chemometric, to the matrix formed by the percentages of the compositions of essential oils. For this, the two most tools frequently used are: principal component analysis. (PCA) and the descending hierarchical classification (DHC) [1]. The application of these tools has allowed us to have synthetic vision of the similarities and differences within a set of samples. So these two techniques allowed us to determine possible links between the essential oils composition and the geographical origins of the plant.

1. Introduction

The world production and consumption of essential oils are increasing very fast due to the fact that theycan be easily obtained from plants by several ecofriendly extraction methods. Moreover, they are efficiently used in a wide variety of applications such as cosmetics, medicinals, pharmaceuticals, perfumes, foods, antibacterialand antifongic industries. Mints (Lamiaceae) are herbaceous, perennial, very fragrant, plants that are particularly found in wetlands. There are many species of mint which some grow spontaneously in East Morocco. Suaveolens (L.) Mentha or peppermint to round leaves, belongs to the family of the Labiatae. It is an aromatic plant widely used in traditional medicine, a decoction of the leaves is very appreciated in the treatment of stomach pain and diarrhea, because it proceeds from the antibacterial and antifungal properties. It also acts as agent delaying reproduction of the malaria vector. Anopheles stephensis [2, 3]. It has cardiovascular effects. (Activity hypotensive, vasodilator, bradycardia), activity on the sympathetic nerve centers. (Relaxing, stimulating, anti depression) [4], it works against colds and respiratory infections as a poultice or inhaled. The leaves are recommended in case of fever [5].

The geographical origin (altitude, climate, …) of the plant is an essential parameterthat determine the quality and the efficiency of the essential oil. In this study, essential oils of Menthasuaveolens(L.), using gas phase chromatography (GPC) technique were characterized. However, GPC technique cannot directly give informationson the effect of the geographical origin of the plant's essential oil composition.In order to study

such effect, the idea of combining the results obtained using GPC with Chemometrics and more precisely, analysis multivariate, such as: analysis in principal components and Classification hierarchical descendant, to investigate the traceability of Mentha Suaveolens (L.) and its essential oil composition was developed.
The objective of this study is,first to determine the chemical composition of the essential oils of Menthasuaveolens (L.), growing in the wild in nine regions of the Eastern Morocco, using CPG technique. Secondly, to determine the characteristics of the oil and therelationship between its chemical composition and the region of the harvest. Principal components analysis (PCA) [6] was used to analyzechromatographic data and build maps factorials, bringing together groups of variables.

2. Material and methods

2.1.*plant material and extraction of the volatile constituents.*
Menthasuaveolens (L.) including the vernacular name is "Timarssat" in Arabic, is used in several domains such as dishes, confectionery, cosmetics and Perfumery. Its identification was very easy because of the characteristic shape of its leaves, round, thickand wrinkled. The typical stem of the Labiatae is square. The whole plant is covered with dense and whitish hairs that make it soft; like all mints, ithas a strong characteristic smell. The small flowers are collected in the spikes closing branches. The plant height varies from 25 to 80 cm, the flower is 5 mm long (figure 1).

Figure 1: Photo of Menthasuaveolens (L.) of the eastern Morocco.

Aerial of Menthasuaveolens parts were harvested during the month of may 2015 innine communities in Easten Morocco: Berkane, Trifa, Ahfir, Laayoun, Beni oil, Ain sfa, Jerada, Gafait, and Tafoughalt with the altitude respectively 200, 220, 232, 625, 450, 620,1045, 1045 and 1500 m. Specimens have been deposited in the Faculty of science ofthe University Mohamed first Oujda (Morocco).
A mixture of about Xg of Mentha suaveolens with Y ml of deionized water were subjected to hydrodistillation in a Clevenger-type apparatus (Figure 2) until no more essential oil was obtained.

Figure 2: Assembly of extraction of essential oils by the hydro-distillation method.

2.2 *GPC – SM*
The extracted essential oil was analyzed thanks to GPC-MS technique using Perkin-Elmer turbo (quadrupole) spectrometer, coupled to a Perkin-Elmer Auto-System, equipped with a molten silica capillary. (Rtx - 1 and RTX-wax). Carrier gas: helium (1 ml / min), the ion source temperature: 150 ° C, the programmed oven temperature was increased from 60 to 230 ° C at a heating rate of 2 ° C/min, then maintained iso-thermal at 230 ° C during 35 min, the temperature of the injector: 280 ° C, the ionization energy: 70 eV, the mass of the ionization electron spectra were acquiredon the 35-350 mass range Da , mode split injection: 1 / 80, the injection volume: 0.22 µl of pure oil.

2.3*Chemometric method*

The term chemometric was introduced in 1972 by Swedish Svante would and the American Bruce.R. Kowalski [7]. According to the company international chemometric founded in 1974, the definition of chemometric is the discipline of chemistry which uses mathematical and statistical methods to design plan or select experiments and optimal procedures of measures. It provides maximum information by analyzing chemical data [8]. Nowadays the chemometric is becoming more and more important because it allows to create predictive models and experiences plans to replace some techniques in the industry by other less expensive, faster and non-polluting. For example,chemistry solutions dosages with burette are increasingly replaced by analyses at base of the spectra. These are used to predict the presence of contaminants and the quality of the products. The Chemometric has three main objectives [9]. The first is the description of the data in synthetic form, it is the case of the basic statistics (averages, variances,..), or descriptive methods such as principal components analysis [10], the second objective is the continuous values by regression prediction: Partial Least Squares [11], or the prediction of classes by discriminant analysis[12], the last is the planning of experiments[13]. Among the multi-varied analysis used, principal component analysis and descending hierarchical classification approaches were chosen in this work.

2.3.1 *Principal component analysis. (PCA)*

The principal components analysis is a technique of multi - varied analysis, it is based on the transformation of a group of quantitative variables original in a new space of variables formed from linear variables starting combinations, reducing thus the space. A range of 1000 wavelengths can be represented by a point in the new space.These combinations are called principal components which must be interpreted independentlyfromthe others, and they contain a portion of the variance that cannot be expressed in no other main component. (Lopez and al.) [14] and (Pla and al.) [15]. Geometrically, PCA technique is considered as a method of rotating the data so that the observer is best placed to understand the relationships between samples. Dataare represented in a new system of axis formed by the principal components. The first axis ischosen in the direction of the greater dispersion of samples: of greater variability, called principal component (PC1). The second axis is the second main component (PC2) which is orthogonal to the first. The process is repeated until the last new component (PCi) [16,17].

2.3.2 *Descending hierarchical classification (DHC)*

To classify means togatheritems (individuals or variables) that are similar, or separate those who differ according to certain criteria. That is to say to create homogeneous classes the most distant one from the other. There are a large number of classification techniques, whose main objective is to allocate a group of individuals in a number of sub groups. Each group must be differentiated from others. The technique is based on the distance between the individuals. The lower the distance is, the more properties and characteristics of individuals are similars. The classification is done on this basis regardless of the method used to obtain the sections within the main groups and then smaller subdivisions of these sections, and so forth. The descending hierarchical classification, known as "divided", is from a single class comprising all individuals, to divide it into two. This is repeated successively until all classes contain a single individual (singletons), or until a desired number of classes. The basic process of the hierarchical methods was given by Johnson, 1967 [18]. These methods seek to form a hierarchy of partitions from a given population within each other. A hierarchy can be seen as a set of nested graphically partitions. It is often represented by a tree structure represented by a hierarchical tree also known as dendrogram.

2.4. *Softwares*

2.4.1 Unscrambler

The software used is the Unscrambler which is a complete software of multi-varied statistics. It is developed by the company Norwegian CAMO, it establishes the standard of multi-varied data analysis software. It is the preferred tool of researchers, engineers and analysts of data around the world. Thanks to the powerand speed of the tool to make quick, easy and accurate matrices of large data and complex analyses. The Unscrambler software is well suited for the analysis of data in spectroscopy, experimental plans, quality control, optimization of processes, Predictions, etc. In this work, Unscrambler V9.2 software "(CAMO process AS, Oslo, Norway) was used to achieve a PCA and to get a card factorial linking the relationship between the region and the oil extracted from the harvested of the Oriental Morocco."

2.4.2 Statistical Package for the Social Sciences. (SPSS)

The SPSS is a software analysis and data processing that allow to develop several types of analysesbased on calculationsappropriate diagrams. SPSS 13.0versionwas used for thesegmentation of the regions where the harvest of our plant was made.

3. Results and Discussion

3.1. Properties of oils

The compounds identified in the essential oils of Menthasuaveolens (L.) from the nine regions of interest are listed in Table 1.

Table 1: Chemical composition of Menthasuaveolens(L.) of Eastern Morocco.

compounds	H1: Ahfir	H2: Trifa	H3: Laayoun	H4: Tafoughalt	H5: Beni Oukil	H6: Jarada	H7: Ain sfae	H8: Berkane	H9: Tgafayat
α-Pinene	0	0	0.2	0.9	0	0.4	0.2	0	0.3
Camphene	0	0	0.1	0.4	0	0.1	0.2	0	0.1
1-Octen-3-ol	0.3	1.1	1.1	1.3	1.1	0.8	0.5	0.65	0.7
Sabinene	0	0	0.2	0.5	0	0.5	0.1	0	0.4
β-Pinene	0	0	0.3	1	0	0.6	0.2	0	0.6
Myrcene	0	0	0.4	0.8	0	0.7	0.1	0	0.8
a-Terpinene	0	0	0.3	0.4	0	0.1	0	0	0.1
p-Cymene	0	0.2	0.5	0.2	0.2	0.2	0	0.1	0.1
Limonene	0	0	0.5	0.8	0	4	0.4	0	2
1.8-Cineole	0.1	0.1	0	0	0.3	1.8	0	0.1	2
Z-b-Ocimene	0	0.1	0.3	0.4	0.1	0.2	0	0	1.8
g-Terpinene	0	0.1	0.8	0.8	0.2	0.3	0	0	0.5
E-hydrate Sabinene	1.5	0.7	1.6	1.5	1.4	4.9	1.9	1.2	5
p-Cymenene	0	0	0	0.3	0	0	0	0	0
Terpinolene	0	0	0.2	0.3	0	0.1	0	0	0.1
Linalol	0.4	0.3	0.8	0.3	0.5	0	0	0.3	0.1
1-Octen-3-yl-acetate	0.2	1.2	0.7	1.2	0.3	1.1	0.2	0.7	0.9
cis-p-Menth-2-en-1-ol	0	0.3	0.3	0.2	0.2	0.1	0.1	0.2	0.1
trans-p-Menth-2-en-1ol	0	0.2	0.2	0.1	0.1	0	0	0.1	0
Borneol	1.5	1	1.6	1.5	2.2	0.5	2.8	1.2	0.4
Nonanol	0	0	0	0.4	0	0	0	0	0
p-Cymen-8-ol	3.2	2.3	2.6	6.5	0.4	0.1	0.3	2.7	0.1
Terpinen-4-ol	0.8	5	3.4	2.4	3	1	1.1	2.5	1
α-Terpineol	0.4	0.7	0.6	0.7	0.4	0.4	0.4	0.5	0.5
8.9-Dehydrothymol	0.9	0.6	0.5	1	0	0	0	0.7	0
Pulegone	0	0	0	0	14.3	0.5	0.2	0	0.4
Piperitone	0	0	1	0.3	0	0.6	11.7	0	0.5
E-Piperitone oxyde	0.5	1.2	0.1	0	0.2	0.1	0.9	1	0.1
Z-Piperitone oxyde	2.9	13.3	0.2	0	4	0.3	0.1	10	0.2
Bornylacetate	0.3	0.5	0.5	0.5	0.2	0.2	0.1	0.2	0.3
Thymol	0.4	1.3	0	0	0	0	0.3	0.9	0.1
Diosphenol	0.8	1.1	0	0	0	0	0	1	0
Piperitenone	3.7	0.7	0.7	1	1.2	4.1	0.3	2	4
Piperitenone oxyde	24.4	11.7	59.3	33.1	52.3	64.4	61	17	65
Z-Jasmone	0.6	0.9	0.5	0.3	0.5	0.6	0.4	0.7	0.5
a-Copaene	0.6	0.9	0.2	0.2	0.3	0	0	0.8	0

Nepetalactone	7.1	2.9	3.2	9.9	1	0.8	0.7	0	0.7
b-Bourbonene	0	0	0.9	0.7	0	0	0	0	0
b-Elemenene	0.6	0.7	0.3	0.5	0.2	0.2	0.2	0.6	0.1
1.2-Epoxymenthyl acetate	5.5	13.4	0	0	0.7	0	0	10	0
a-Gurjunene	0.6	0.4	0	0	0.2	0	0	0.5	0
trans-Caryophyllene	9.9	5.3	3.7	7.8	3.1	2	2.8	6	1.8
Cadina-3.5-diene	0.5	0	0	0	0	0	0.4	0	0.1
E-b-Farnesene	1.4	1.6	0.2	0.2	0.6	0.3	0.2	1.5	0.2
a-Humulene	1.2	0.7	0.5	0.9	0.4	0.3	0.3	1	0.4
cis-Muurola-4(15).5diene	1.6	1.6	0.3	1	0.4	0.4	0.7	1.6	0.4
Phenethyl 2-methylbutyrate	0.1	0.2	0	0	0	0	0	0.1	0
g-Muurolene	0.2	0.4	0	0	0	0	0	0.3	0
Germacrene D	9.3	4.7	3.5	5	2.1	2.9	3.9	7	3
Amorpha-4.7(11)-diene	0.6	0.3	0.1	0.57	0	0	0	0.5	0
a-Muurolene	0.1	0.3	0	0	0	0	0	0.2	0
g-Cadinene	0.3	0.6	0.2	0.2	0.2	0.1	0.1	0.5	0.1
Calamenene	0.6	1.4	0.2	0.4	0.3	0.2	0.3	1	0.2
d-Cadinene	0.9	1.6	0.3	0.4	0.3	0.1	0.2	2.2	0.1
a-Cadinene	0.3	0.4	0	0.81	0	0	0.1	0.3	0
Caryophyllene oxyde	0.8	0.9	0.3	0.4	0.4	0	0.1	0.8	0
1-10-diepiCubenol	0.7	1.1	0.2	0.4	0.3	0.2	0.4	0.9	0.1
tau Cadinol	0.8	1.3	0.3	0.4	0.3	0.3	0.4	1	0.3
Hinesol	0.3	1.1	0.6	0.8	0.1	0	0	0.7	0
a-Cadinol	1.1	2.3	0.7	0.9	0.5	0	0	1.7	0
E-Phytol	0.5	0	0	0.3	0	0	0	0.2	0

Sixty-one compounds have been identified (Table 1); the essential oils of Mentha suaveolens are mainly composed of : Pulegone. Piperitone, Z-Piperitone ox Piperitone. 1. 2-Eepoxymenty. Germacrene D. trans-Caryophyl and Nepetalactene with different percentages. This difference in the content of essential oils componentsis due to abiotic factors such as: the specificclimate in the regions of origin of the samples, the geography iethe altitude and the nature of the soil [19].

In a previous work, the authors demonstrated that the chemical composition of the essential oils of Mentha suaveolens (L.) can also vary depending on the extraction technique used [20].

To better analyse the results concerningthe chemical composition of the extracted oils of the plant Menthasuaveolens(L.)shown in table 1 a multi-varied analysis were made. The results of the chemical composition of the oils of the plant were submitted first to a principal components analysis PCA and then to a descending hierarchical classification DHC.

3-2Majority variables.

Using PCA technique, one can see from the figure 3 ofgraphic Scores (Origin T2) that no atypical sampleswere detected. Indeed, anomalies may be dueto acquisition problems during GPC analysis, handling of the samples, etc ….). This has to be check first in order to ensure that the sample is homogeneous with theother samples 'outliers' and will not be a source of errors when processing chemometric.

From figure 3 we note that all samples are inside the ellipse of the graph of the Scores (Origin T2). So no samples are considered as atypical and all the samples were kept for the PCA analysis.

Figure 4 shows the result of PCA which gives the graph of the Scores according to vector PC1 and PC2. These two principal components gathered 73% of overall variability. Clearly, the analysis shows that the samples can be separated into three groups.

The first group (I) includes five localities (BeniOukil. Jerada. Gafait. Ain Sfa and Laayoun). It has been defined by high levels of Pulegone and the Piperitone (ox) as shown in figure 3. The second group (II) includes two localities (Trifa and Berkane). In comparison with the Group (I). It is characterized by a higher content of Z-Piperitone (ox) and 1. 2-Eepoxymenty. The third group (III) includes two localities (Ahfir and Tafoughalt). It is characterized by percentages more important of Germacrene D. Trans-Caryophyl and Nepetalactene (figure 5).

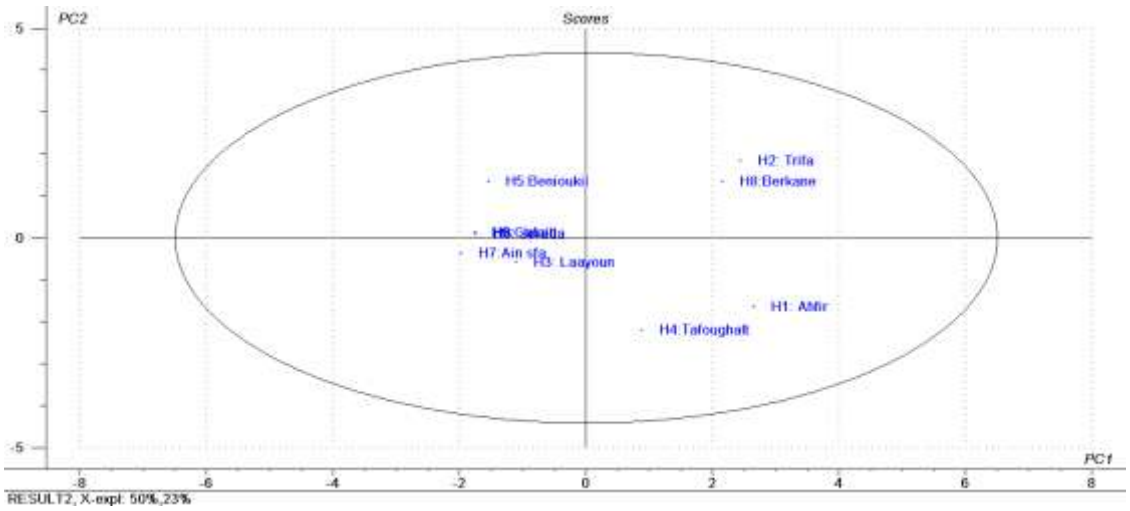

Figure 3: Graph of Scores according to Origin T2.

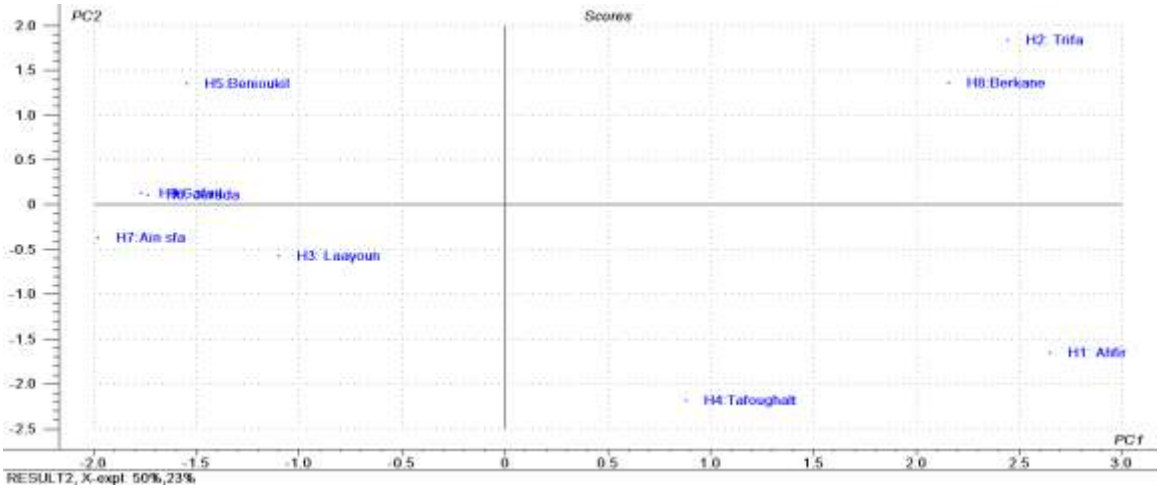

Figure 4: Graph of the Scores according to PC1 and PC2 vectors.

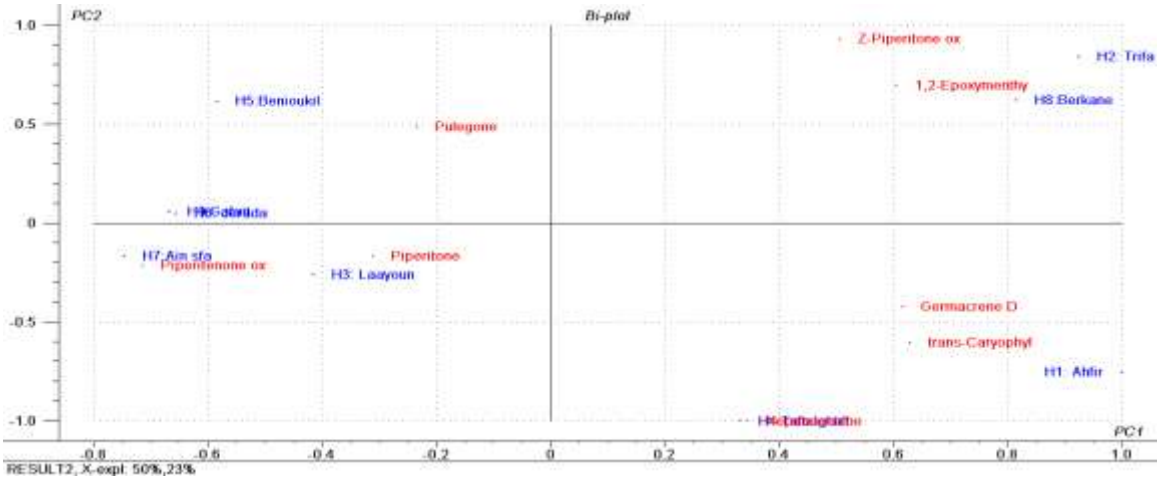

Figure 5: Graph of the biplot according to PC1 and PC2 vectors.

To verify the dispersion of groups obtained in figure4, descending hierarchical classification was used. The latter confirmed the existence of three groups (figure 6) as clearly evidencein (figure 4). Indeed. the first group includes five localities (BeniOukil. Jerada. Gafait. Ain Sfa and the Laayoun of the eastern). The second group two localities (Trifa and Berkane) and the last group is made up of two communities (Ahfir and Tafoughalt).

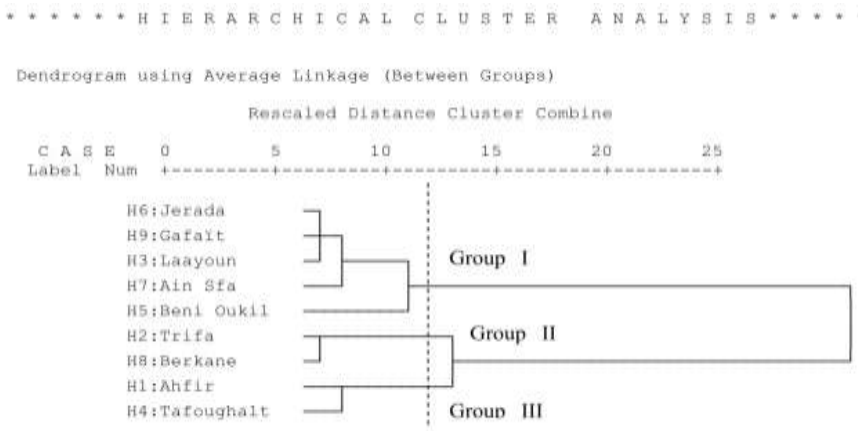

Figure 6: Dendrogramrepresenting the distribution of MenthaSuaveolensessential oils (L.) From Easten Morocco following the majority variables.

3-3 Minority variables

The treatment of the minority variables underwent the same process as that of the majority variables. The calculation to check if there are abnormal samples (atypical) was carried out. Figure 7 shows that all samples are localized inside the ellipse. So no samples will be removed in the chemometric calculations.

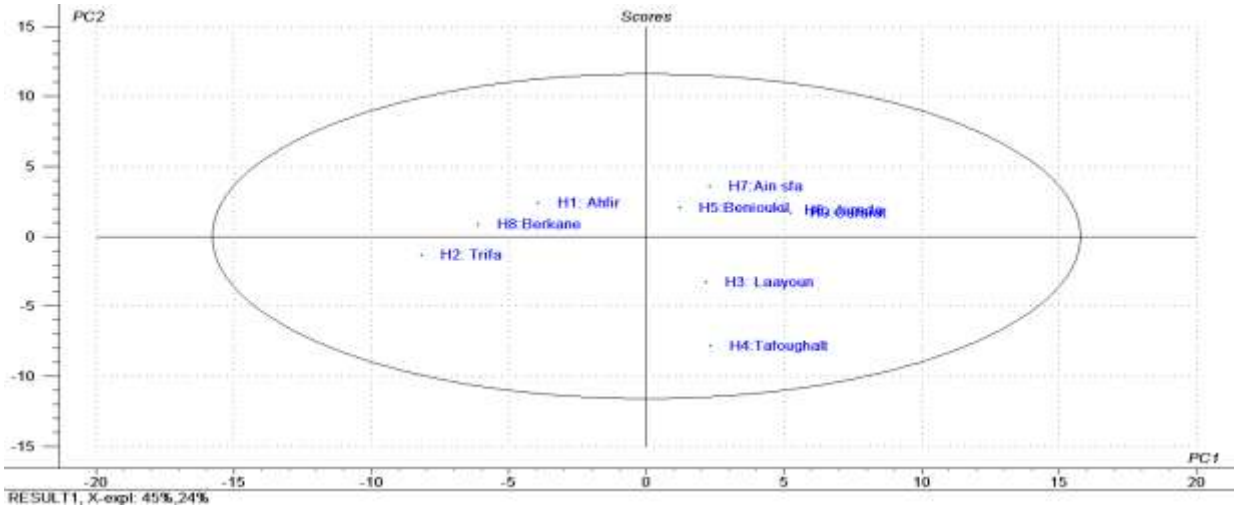

Figure 7: Graph of Scores according to Origin T2.

Figure 8 shows the graph of the Scores according to the vectors PC1 and PC2. thesetwo principal components gathered 69% of overall variability. Three separate groups are clearly detected. The first group (I) includes three localities (Trifa. Berkane and Ahfir). The second group (II) includes four localities (BeniOukil. Jerada. Gafait and Ain Sfa) and the third group (III) includes two localities (Laayoun and Tafoughalt).

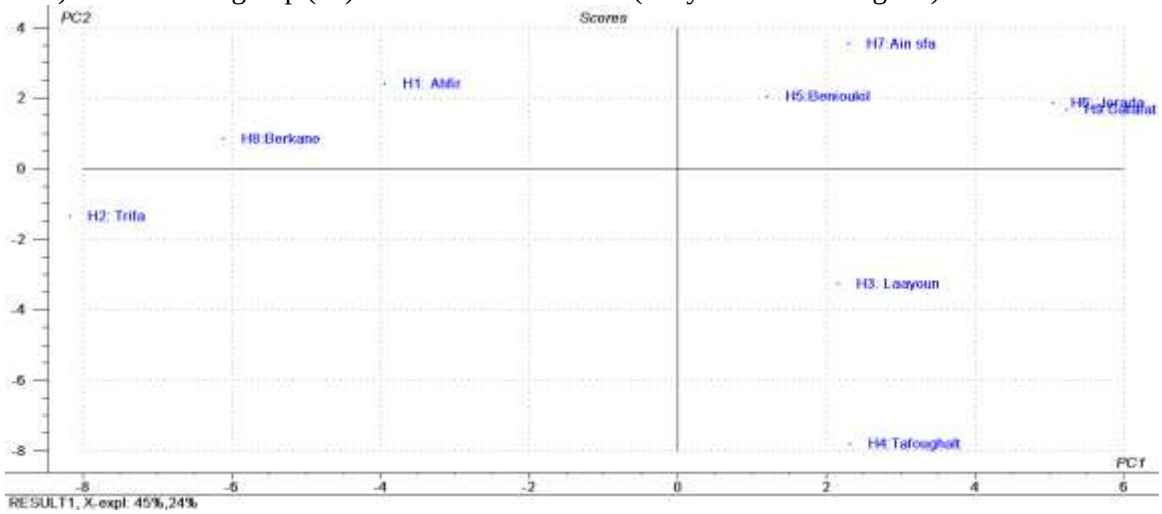

Figure 8: Graph of the Scores according to PC1 and PC2 vectors.

To verify the dispersion of groups shown in figure 8, the descending hierarchical classification (DHC) was carried out. The latter confirmed the existence of these last three groups (figure 9. The first group comprises three localities (Trifa. Berkane and Ahfir). The second group four localities (BeniOukil. Jerada. Gafait and Ain Sfa) and the last is formed of two localities (Laayoun and Tafoughalt).

Figure 10 shows the biplot of the distribution of minority according to PC1 and PC2 vectors. It clearly showsthat the variables that are in the Group (A) are highly correlated to the area of Trifa, entirely anti-correlated tothe oil composition from Group(D), located in the region of Jerada and Gafait. Similarly group (B) compounds are very abundant in the regionsof Berkane and Ahfir and those of Group (C) are present in the regions of Tafoughalt. Ain Sfa. Laayoun and BeniOukil. This distribution is strongly related to the fertility of the soilwhich increasesfrom theright to the left and also the altitude which increases from the left to the right (table 3).

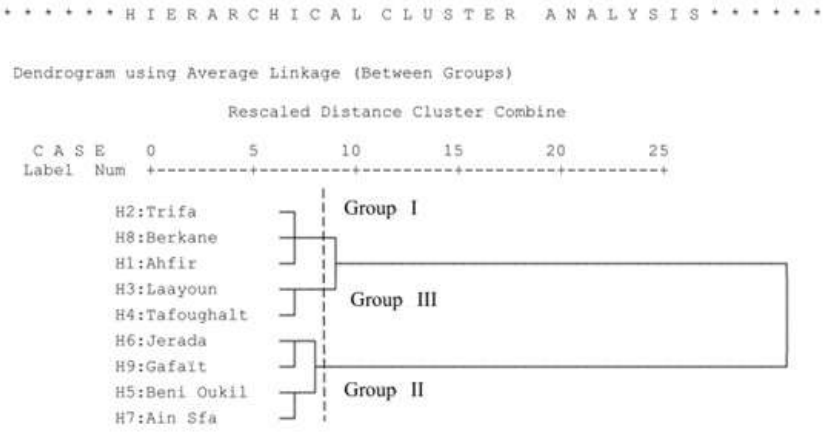

Figure 9: Dendrogram representing the distribution of MenthaSuaveolensessential oils (L.) From Easten Morocco following the minority variables.

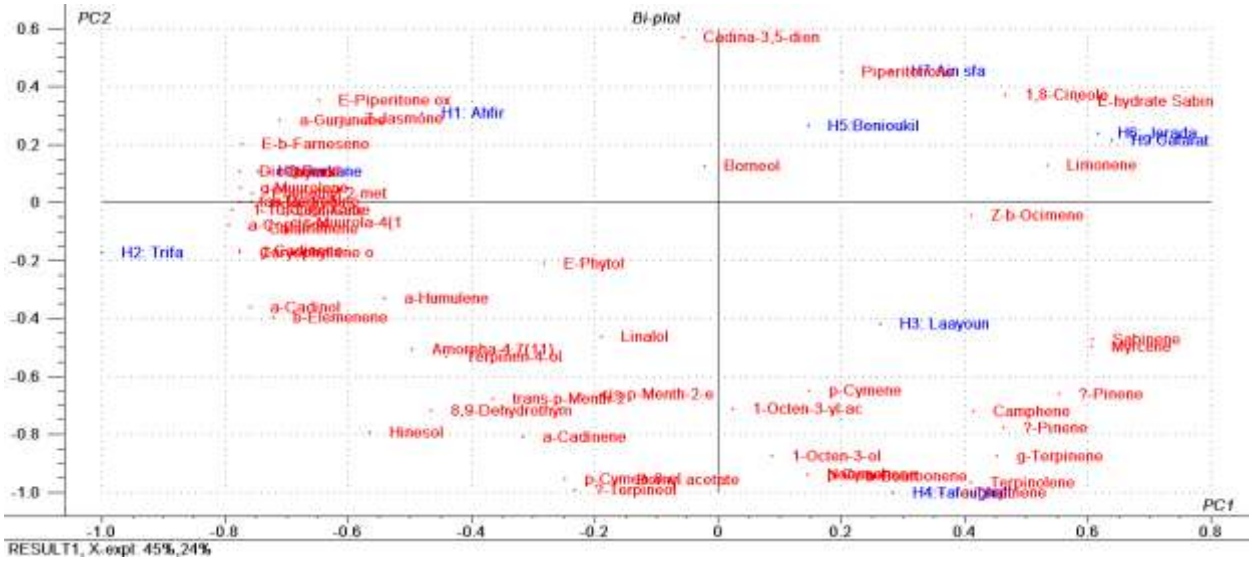

Figure 10: Graph of the biplot according to PC1 and PC2 vectors.

Table 2: Distribution of the minority in four groups (A. B. C. D).

Group A	E-Piperitoneoxyde. Thymol. Diosphenol. Z-Jasmone. a-Copaene. b-Elemenene. a-Gurjunene. E-b-Farnesene. a-Humulene. cis-Muurola-4(15).5-diene. Phenethyl 2-methylbutyrate. g-Muurolene. a-Muurolene. g-Cadinene. Calamenene. d-Cadinene. Caryophylleneoxyde. 1-10-diepi-Cubenol. tau Cadinol. Hinesol. a-Cadinol.
Group B	Linalol. cis-p-Menth-2-en-1-ol. trans-p-Menth-2-en-1-ol. p-Cymen-8-ol. p-Cymen-8-ol. Terpinen-4-ol. 8.9-Dehydrothymol. Bornyl acetate. Amorpha-4.7(11)-diene. a-Cadinene. E-Phytol.
Group C	Camphene. 1-Octen-3-ol. a-Terpinene. p-Cymene. Z-b-Ocimene. p-Cymenene. Terpinolene. Borneol. Nonanol. Piperitenone. b-Bourbonene. Cadina-3.5-diene.
Group D	α -Pinene. Sabinene. β -Pinene. Myrcene. Limonene. 1.8-Cineole. Z-b-Ocimene. g-Terpinene. E-hydrate Sabinene. Terpinolene.

Table (3): Altitude from different parts of the harvest.

Regions	Altitude (meter)
Berkane	**200**
Trifa	**220**
Ahfir	**232**
Laayoun	**625**
Beni OUKIL	**450**
Ain SFA	**620**
Jerada	**1045**
Gafaït	**1045**
Tafoughalt	**1500**

Conclusions

In May 2015,plantsof MenthaSuaveolens (L.),fromnine regions of the Eastern Morocco: (Berkane. Trifa. Ahfir. Laayoun. Benioukil. Ain sfa. Jerada. Gafait and Tafoughalt) atdifferent altitude, were collected. The essential oils extraction wasperformed by hydro-distillation method. Chemical analysis of essential oil from MenthaSuaveolens (L.) wasperformed by GPC/SM. Chemical analysis revealed the presence of 61 constituents dominated the Pulegone. the Piperitone. the Piperitone-ox. Z-Piperitone-ox. 1. 2-Eepoxymenty. Germacrene D. trans-Caryophyl and the Nepetalactene at different concentrations.

Principal Component Analysis (PCA) and the Descendant Hierarchical Classification (DHC) were exploited to obtain as mathematical models for classifying the different components of essential oils as well as their repartitionaccording to the harvest region. The results demonstrate that difference in the componentsdistribution of the samples is highly dependent on the altitude and the quality of the soil.

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