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Chemical study of the aromatic fraction of floral water from *Origanum compactum* Benth

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Abstract
A new method of extraction of aromatic water was applied to the oregano with compact flowers (*Origanum compactum* Benth.), it acts of the extraction in solid phase or S.P.E. This method was compared with the traditional method which relates to the extraction liquid-liquid. This study, enabled us to note that method S.P.E remains profitable, as well, with regard to time necessary to the extraction that at the cost of the operation.
In addition, a qualitative and quantitative analysis of various essential oils of the aromatic water of the oregano with compact flowers (*Origanum compactum* Benth.) was realized by using gas chromatography, coupled to a detector with ionization of flame (C.P.G-F.I.D) then with the mass spectrometry (C.P.G-S.M) and this, in order to identify the principal chemical components characterizing the essential oil of the aromatic water of oregano with compact flowers.

Keywords: Oregano with compact flowers, floral water, essential oil, S.P.E.

Introduction
Oregano with compact flowers is endemic to Morocco and southern Spain (Jahandiez and Maire, 1932). It is a species that grows in the forests, scrub and rocky pastures of the lowlands and low mountains. Biogeographically *Origanum compactum* is found in Morocco (grows in Rif, Tangier, Morocco central-northern, north western Morocco, west of southern Morocco, the Haouz, the Haut atlas and south of the Iberian Peninsula (Jahandiez et Maire, 1932). Flowering occurs in May-July (Benabid, 2000) [5].
O. *compactum* belongs to the compactum section where successive verticillastres are reconciled fake ears contracted terminal, short and globular (spicastre). It is a perennial, chamephyte generally pubescent, hairy stems, covered with long hair (3 mm). The stem leaves are oval-ovoid, large, up to 35 mm, clearly stalked (2-8 mm). Hairy leaves, more on the lower side than on the upper side, hair margins with long hairs (≥ 1mm).. The inflorescences are in dense spikes and short, very purple, large flowers opposite (5-12 mm), sessile. The chalice is usually hairless, 3-4 mm long, 5 subequal triangular teeth (0.5-1 mm). The exerte corolla covered with very fine hairs (≤ à 0,1 mm). The floral bracts are lanceolate oval -ovoid not membranous, stiff, leathery, sessile, truncate base, 6-8 mm long, glabrous on 2 sides, with inconspicuous glands, higher margin (top edge) ciliotée (eyelashes 0.2-0.5 mm). The floral bracts overlap each other from the base to the top of the ear and hide chalices (Atbib, 1985).
In Morocco, oregano is considered a panacea. It has centuries of traditional use in therapy; its local name ‘‘zaa tar’’ probably meaning ‘‘wind chaser’’ in Berber. Due to its high quality, this oregano was exported in the past to countries as far as China. Currently, this plant is commonly sold in markets and herbal shops in Morocco (Jeannot, 2003) [11].
Oregano has been used a long time by Moroccan population for medicinal properties and food preparation purposes. It is mainly used as a culinary condiment and largely employed in popular medicine for the treatment of ailments such as dysentery, colitis, bronco-pulmonary affections, gastric acidity, and gastro-intestinal diseases (Bouyahya *et al*. 2016) [7]. Against colds, O.R.L diseases and bronchitis, it is also administered in the form of fumigations. It is also used against diseases of the mouth (mouth ulcers and gingivitis) and stimulating the appetite (Bellakhdar, 1997) [4].
Many scientific studies have been conducted to examine and justify the practices associated with oregano. In effect, these studies have demonstrated that essential oils and extracts from this species have many biological activities as antimicrobial, antioxidant, cytotoxic, anti-tumoral, anti-corrosion, etc. (Bouyahya *et al*. 2016) [7].

There have been numerous studies conducted on the chemical composition of O. compactum essential oil (Belkamel at al. 2013, Bellakhdar, 1997; Benjilali *et al*., 1986; Charai and Mosaddak, 1996; Herouart, 1987; Rougelin, 1993; Van Den Broucke and Lemli, 1980) [4, 3, 6, 8, 10, 14, 17].

The essential oil of oregano has antibacterial, antiviral and antifungal powers. This oil has an activity on certain mould that can produce mycotoxins whose presence in food constitutes a health risk for the consumer. Oregano oil proved to be able to stop growth at a concentration of 0.1% in the medium (Tantaoui-Elaraki, 1997) [16].

However, O. compactum essential oil must be used carefully and over the short term only because of its irritant properties (direct application on skin must be avoided) and its potential hepatotoxicity at high and repeated levels (Franchomme and Penoel, 1990) [9]. Consequently, the use of *O. compactum* hydrolat, which should contain active substances in lower concentrations, is of great. Although the chemical composition of the essential oil from *O. compactum* is well studied, no research has so far been conducted concerning the hydrolat (Jeannot, 2003) [11].

In this work, we looked at the essential oil part that makes up the aromatic water of oregano with compact flowers (*Origanum compactum*). For this, we have studied and compared two methods of extraction:

- Liquid-liquid extraction (official method)
- Solid Phase Extraction (S.P.E) used for the first time to isolate the essential oil from the floral water.

In addition, a qualitative and quantitative analysis of the various essential oils of the aromatic waters obtained was carried out using gas chromatography, coupled with a flame ionization detector (CPG-FID) and then with mass spectrometry (GC-MS), in order to identify the main chemical components characterizing the essential oil of the aromatic water of Oregano with compact flowers.

Material and methods

1. The floral waters

Floral waters, also called Hydrolats or Hydrosols, are slightly aromatic products that contain very small amounts of essential oil (about 1/1000). The hydrolat is obtained by prolonged impregnation of the essential oil in pure water (Roulier, 1993) [15] and this, by recovery, after condensation, of the distillation water (2 to 4 litres of water for one kg of plants fresh).

Otherwise, aromatic hydrolat is a natural by-product of distillation whose therapeutic properties are complementary to those of the essential oil (Mailhebiau, 1994) [13].

To study and analyze this aromatic fraction, it was necessary to extract it from the aromatic water. To do this, two extraction methods were applied, which were compared in order to select the one that allows a valid yield of the aromatic fraction and which ensures an economic cost. For each method used, 10 different extractions were carried out and the respective averages of the concentrations of the different molecules identified in the aromatic fractions were taken into account.

2. Liquid-liquid extraction (E.L.L)

This is the most commonly used method. In addition, it is recommended by the official text of January 4, 1978 established by the French Ministry of Agriculture (Anonymous, 1978) [1]. Its principle is based on the distribution of a solute between two immiscible solvents and its affinity or solubility in each of them (Mahuzier, 1999) [12]. The choice of solvents depends on the desired extraction result.

In the case of the floral waters analyzed, the extraction of the aromatic fraction present in the floral water (or aqueous phase) is carried out by an organic phase. In the laboratory at two organic solvents, a polar solvent, ether and an apolar solvent pentane were used.

In general, a single extraction does not fully extract the essential oil present in the aromatic hydrolat. It is then necessary to carry out several extractions of the floral water until obtaining a satisfactory yield (Mahuzier, 1999) [12].

Extraction protocol

Reagents: NACL, Pentane or diethyl Ether

Material: 250 ml Erlenmeyer flask, separating funnel, pleated filters n ° 70.

Operating mode

150 ml of unfiltered floral water + 37.5 g of sodium chloride (to saturate the water and release the aromatic compounds) were introduced into the Erlenmeyer flask and then dissolved by stirring. The solution with 37.5 ml of solvent (pentane or ether) was then used with a magnetic stirrer for 10 min and then decanted for 1 hour, in a separating funnel.

The aqueous phase is then withdrawn into the Erlenmeyer flask. A second extraction with 37.5 ml of solvent and then a third with 15 ml of solvent was carried out under the same conditions as above.

The three organic fractions (Pentanic or Ethereal) were collected in the clean and dry separating funnel and then washed with a few ml of distilled water. The wash water is removed and the final organic phase filtered on filter paper (rinsing beforehand with a little solvent). The filter was rinsed again with the same solvent. The organic phases were evaporated and left about 3 ml while avoiding total evaporation.

The extractions thus carried out are combined and then evaporated in order to finally have a sufficient concentration of product to be analyzed by Gas Chromatography (C.P.G). Although this is an easy-to-use method, multiple extractions takes about 4 hours and large quantities of solvents.

3. Solid phase extraction (S.P.E)

In order to overcome the various problems of the liquid-liquid extraction method which are related to the cost and the time required, another method of extracting the aromatic fraction of the floral water has been sought. This is the method of S.P.E. or Solid Phase Extraction. This method has been applied for the first time to our knowledge, as part of the extraction of oil from aromatic water.

Its principle is based on the adsorption phenomenon (Mahuzier, 1999) [12]. The latter corresponds to the more or less energetic fixation of a solute on a solid surface. This phenomenon involves complex forces between the solutes and the adsorbent. However, for this adsorption to be used for separative purposes, it is necessary that this binding is reversible.

Elution (or desorption) consists in extracting, from the solid phase, the substance adsorbed with a solvent which, in this case, is called the eluent. There is a break in the bonds between the adsorbent and the initially fixed molecules which are replaced on the adsorption sites by that of the eluent.

The relations between the solute (essential oil), the adsorbent and the eluent imply that there are a number of common properties between them.

First, it must have electrostatic attractions between the organic ions and the adsorbent substance to allow a very strong attachment between them.
Then it is also necessary that the organic molecules are endowed with polarity, that is to say that they carry oxygenated groups, nitrogenous or halogenated. This polarity is even greater than the number of functional groups is greater. The electrostatic attractions between the polar molecules are less strong but of the same type as the attractions due to the ions.
In the case of aromatic hydrocarbons, their electron cloud is formed in the vicinity of polar substances and they behave as more polar substances than their aliphatic counterparts. This character increasing the number of cycles allows the polycyclic molecules to adsorb very easily.
Another property is the structural configuration. This influences the adsorption. Thus, the adsorption facility increases with the flatness of the molecules and some adsorbents more easily fix linear hydrocarbons than branched hydrocarbons.

Extraction protocol
Reagents: methanol, distilled water, ethyl ether.
Material: Bond Elut C8 cartridge, adapter for S.P.E cartridge, 50 ml syringe.

Operating mode
- Packaging: by passing 5 ml of methanol through the cartridge
- Washing: rinse the cartridge with 5 ml of distilled water
- Introduction of 150 ml of floral water to treat
- Elution with 3 ml of ether.

Throughout the handling, we kept a constant and moderate flow. After each step, the cartridge is dried by a passage of air through it (for the elimination of any residues).

Results and discussion
1. Qualitative and quantitative study of the fraction of essential oil contained in *Origanum compactum* floral water
The analyses carried out on the products obtained, using liquid-liquid and S.P.E extractions, enabled us to identify 8 main and different molecules in the two types of extracts. The 8 compounds, were identified using the techniques of the C.P.G.-F.I.D and C.P.G.SM, they are in the order of their elution as follows:
Cineole-1-8, 1-octen-3-ol, linalool, terpinen-4-ol, alpha terpineol, borneol, thymol, carvacrol (Table 1):

Table 1: Chemical composition of the essential oil contained in aromatic water of oregano with compact flowers

			extraction S.P.E			liquid-liquid Extraction		
Composants	**N° des Peaks**	**Ik**	**m(X)**	**standard deviation**	**C.V(X)**	**m(X)**	**standard deviation**	**C.V(X)**
Cineole-1-8	1	1229	0,2	0,05	22,6	0,36	0,18	49,63
1-octen-3-ol	2	1457	0,31	0,02	7,5	0,28	0,01	5,27
Linalol	3	1555	1,17	0,03	2,48	1,15	0,02	2,06
Terpinen-4-ol	4	1624	0,51	0,03	5,96	0,51	0,03	6,24
alpha-terpineol	5	1716	0,61	0,01	1,73	0,61	0,02	3,49
Borneol	6	1723	0,34	0,01	3,77	0,34	0,02	4,72
Thymol	7	2190	19,55	0,09	0,44	19,27	0,24	1,22
Carvacrol	8	2223	76,75	0,21	0,27	75,44	1,05	1,39

I.k : Kovatz index
m (X): average over 10 samples C.V (X): correlation coefficients

Table 1 shows that the major components are, as in the case of pure essential oil, the two phenols which are carvacrol and thymol. These two compounds show equivalent concentrations in both types of extracts; Carvacrol has an average concentration of 76.75% in the extract obtained by SPE extraction, compared with 75.5% in the liquid-liquid extract, whereas the thymol shows a relatively similar concentration in both types of extract, is about 19.5%.
Unlike the pure essential oil, we note the absence of para-cymene and gamma terpinen. Moreover, the presence of linalool in this aromatic part of the hydrolat, its rate is equivalent to that recorded in the pure essential oil. Its concentrations are 1.17% in the extract S.P.E and 1.15% in the liquid-liquid extract against 1.55% in the pure essential oil.
It should be noted that only compounds with concentrations greater than or equal to 0.07% have been taken into consideration in the table of values. This choice was made in order to avoid integrating the artifacts responsible for background noise during the chromatographic analysis.

2. Comparative study between pure essential oil and essential oil derived from aromatic water
When the composition of the aromatic portion of the hydrolat is compared to that of the pure essential oil obtained directly after distillation by steam distillation, it is noted that all the components of the aromatic water are also part of the water essential oil, after GC-MS analysis.
The detailed analysis of the essential oil of the compact flower oregano was conducted by our research team in a recent study (Belkamel *et al*. 2013) [3].
It is therefore the passage of a few fractions of essential oil in the hydrolat during the distillation. According to Franchomme and Penoel (1990) [9], the water of entrainment retains in solution some odorous principles, which are hydrophilic (table 2):

Table 2: Qualitative comparison between the pure essential oil and the essential oil of the Moroccan oregano floral water (Origanum compactum)

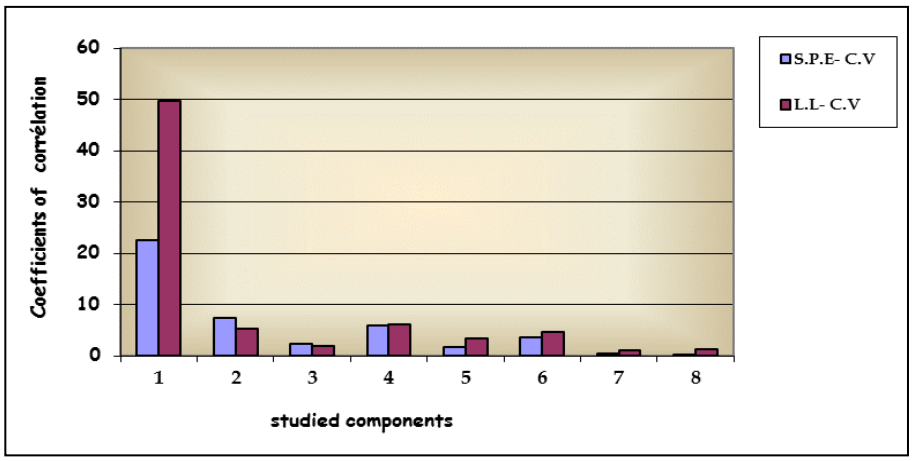
Composition	H.E	E.F	Composition	H.E	E.F
alpha - thujene	*		Terpinolene	*	
alpha - pinene	*		1-octen-3-ol	*	*
camphene	*		Camphor	*	
beta - pinene	*		Linalol	*	*
delta-3-carene	*		terpinene-4-ol	*	*
myrcene	*		beta – caryophyllene	*	
alpha - phellandrene	*		alpha – terpineol	*	*
alpha terpinene	*		Borneol	*	*
limonene	*		delta – cadinene	*	
Cineole - 1,8	*	*	Caryophyllene oxyde	*	
gamma - terpinene	*		Thymol	*	*
para - cymene	*		Carvacrol	*	*

E.F: floral water H.E: essential oil *: presence of the compound

It must be said that the essential oil of floral water (hydrolat) consists mainly of phenols and a small percentage of alcohols, that is to say compounds with polar groups. As with floral water, the pure essential oil is mostly composed of phenols.
Jeannot (2003) [11] studied the hydrolat of *Origanum compactum* hydrolat and shows that it contains as major compounds thymol and carvacrol which are the main active substances of essential oil. It is also free from mono and sesquiterpene hydrocarbons. The concentration of the aromatic fraction is relatively high with approximately 500 mg/l.

3. Study of the reproducibility of the method by S.P.E

To demonstrate the reproducibility of this new method (SPE) compared to the official one (liquid-liquid), we carried out 10 extractions, using the two methods, then we took into account, the standard deviations, the coefficients of the variations and averages taken for each of the components compared to the 10 samples taken for the statistical significance (table 1).
In the same table also, the different molecules identified as well as their corresponding Kovatz indices. The objective is to be able to select the best method that would be interesting to apply, both qualitatively and quantitatively and which could be adopted for the various extractions necessary for quality control of aromatic waters intended for marketing and to the various industrial products.
In the light of the results obtained by looking at the correlation coefficients for each molecule in the two extracts, it is found that 6 out of 8 compounds (numbers: 1, 4, 5, 6, and 8), have very satisfactory coefficients in the case these coefficients have lower values in the SPE method compared to the liquid-liquid method.
For the remaining 2 components (numbers 2 and 3), we note indeed that the correlation coefficients are relatively lower in the liquid-liquid extract compared to those found in the SPE extract. However, the differences remain very not significant (7.50 and 2.48 in the extract SPE against, respectively 5.27 and 2.06 in the liquid-liquid extract).
Graph 1 illustrates the good reproducibility of the S.P.E method, after comparing the correlation coefficients corresponding to the 8 molecules identified in the two types of extracts studied (Table 1)



Graph 1: Reproducibility and Comparison of Correlation Coefficients in both extraction methods: S.P.E and Liquid-Liquid

This comparative study shows us, thanks to the coefficients obtained, a very satisfactory reproducibility for this new technique called S.P.E used in the extraction of essential oils contained in aromatic waters.
It can be concluded that the S.P.E method remains more interesting than the liquid-liquid method (official), in fact, the S.P.E is more economical and faster, it has the following advantages: It is easy to use; it is four times faster than the liquid-liquid method; it is much more profitable than the first from an economic point of view, since it does not require the use of many solvents. The amount of solvent used is 3 ml of ether by extraction, against 95 ml of solvents for the liquid-liquid method.
Finally, it can be said that, thanks to its reproducibility, both qualitatively and quantitatively, the S.P.E could be adopted while substituting it for the method officially recognized to date (liquid-liquid extraction).
In the practice of this method (S.P.E), we worked on two samples of oregano hydrolat with compact flowers.

The extracts analyzed (aromatic fractions), shows us on the qualitative pan the existence of the 8 molecules already identified previously in the essential oil of Moroccan oregano. Quantitatively, there are significant differences between these two samples from two different producers. Concerning the main molecules (carvacrol and thymol) in the two samples, there are differences in important concentrations.

In batch I, the concentration of thymol is 19.5%, while its percentage is much higher in batch II, it is 40.6%, a difference of more than 100% in favor of batch II (Table 3).

Regarding the concentration of carvacrol, it is found that it is much higher in batch I than in batch II, it is about 76.7% against 55% respectively. However, it is noted that the total of the two phenols remains practically the same in both batch. The sum of the thymol and carvacrol levels is 96% in batch I and 95.5% in batch II.

The content of thymol is high, when that of carvacrol is low and vice versa, when the thymol decreases, carvacrol increases. This may be a biosynthetic correlation between the thymol biosynthetic pathway and that of carvacrol.

We can conclude, saying that the two batches of floral water from Morocco could correspond to two different periods of cuts. Also, we note that the aromatic waters of Moroccan oregano are very rich in phenols (more than 95%).

As regards alcohols, their contents hardly exceed 1.2%. In this respect, it is noted that there are very slight fluctuations in the levels between the two types of floral waters. For batch I, the total amount of alcohols identified is 3.14%, while for batch II it is 2.39%. This variation is mainly due to the 1-octen-3-ol and alpha-terpineol contents, which are twice as high in batch I as in batch II. In addition, the linalool content is less than 1% in batch II, whereas it is greater than 1% in batch I (Table 3).

Table 3: Comparison of chemical compositions of H.E of two batch floral water of Moroccan oregano *(Origanum compactum)*

identified compounds	N° of peaks	batch I	batch II
		Average (%)	Average (%)
cineole-1,8	1	0,2	0,17
1-octen-3-ol	2	0,31	0,16
linalol	3	1,17	0,93
terpinene-4-ol	4	0,51	0,56
alpha terpineol	5	0,61	0,35
borneol	6	0,34	0,22
thymol	7	19,55	40,59
carvacrol	8	76,75	55,15

Conclusion

The aromatic waters (hydrolats) resulting from the distillation of aromatic plants have various uses. The quality control of these waters requires above all isolating the aromatic part, consisting of the essential oil that saturates these hydrolats. Through the study carried out, it was possible to apply a new method adapted to the extraction of this odorous part, it is the application of the extraction in solid phase or SPE This method has been compared elsewhere to the classical and official method, which concerns liquid-liquid extraction.

This study has shown that the S.P.E method remains profitable, both in terms of the time required for extraction and the cost of the operation.

Finally, in addition to the traditional use of O. *compactum* hydrolat as medicine to treat gastrointestinal tract disorders, it could have applications for skin and mouth problems. This is because the hydrolat contains the same active substances as the essential oil but in a lower concentration. A further point, aside from the interest in aromatherapy, is that this hydrolat has good keeping qualities. Indeed, unlike some other hydrolats, O. *compactum* can be stored under the right conditions for at least two years without microbial contamination due to its high concentration of phenols (Jeannot, 2003) [11].

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