

## EXTRACTION AND STUDY OF ESSENTIAL OIL OF *Salvia officinalis* FROM TUNIS (TUNISIA)

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### ABSTRACT

The composition of the essential oils of the aerial parts of *Salvia officinalis*, obtained by steam distillation, collected from an area in the northern suburb of Tunis, was analyzed by GC-MS and GC-FID. The essential oils of the organic phase and those extracted from the aqueous phase by steam distillation have a different chemical composition. More than 95% of the total of components was detected. The main components are:  $\alpha$ -thujone (21,13%-33,52%),  $\beta$ -thujone (7,11%-6,24%), eucalyptol (1,19%-12,06%), camphor (20.69%-54,29%) and the caryophyllene (0,61%-6,75%). The chemotype of the *Salvia officinalis* studied is the  $\alpha$ -thujone.

**Keys words:** Chemotype, essential oil, medicinal plants, *Salvia officinalis*,  $\alpha$ -thujone

### INTRODUCTION

The Mediterranean climatic conditions of Tunisia hasten the wild aromatic plant development as well (rosemary, thyme, sage...) that cultivated (marjoram, mint...). Unfortunately, among more than 2500 species that count the Tunisian flora, until now, only a small number has been studied<sup>1-8</sup> and recognized as having some known therapeutic properties since the middle age and even before.

The valorization of these natural resources can have some considerable effect on the economy of our country. Indeed, different plants contain molecules to added strong values. Among these molecules, one finds compounds having an olfactory activity. They represent what one calls the essential oils that are used often in the pharmaceutical and cosmetic industries.

For the goal of exploiting plant pushing in Tunisia and well-known for their medicinal virtues, different studies have been achieved in our laboratory<sup>1,4,5</sup>. The *Salvia officinalis* belongs to the

family labiatae<sup>1</sup>. It is an aromatic plant, used fresh, dried or as an essential oil. The essential oil of *salvia officinalis* is largely used in traditional medicine<sup>2</sup>. The essential oil of *salvia officinalis* has an industrial interest<sup>2</sup> and especially the Dalmatian sage (*salvia officinalis*) which is considered to possess the finest and the most characteristic aroma<sup>4</sup>. More recent studies on the biological activity of sages showed that the essential oil and some of its constituents own antimicrobial and antioxidant properties<sup>5,6</sup>. It has tonic stimulant properties and it is used in perfumery, in cosmetics<sup>7</sup> and for liquors. The chemical composition of *salvia officinalis* oil has been the subject of considerable study<sup>8,9</sup>.

The commercial *salvia officinalis* essential oil has also been studied<sup>8-10</sup>. It contains:  $\alpha$ -pinene (1,5-4,0%), camphene (3,2-6,7%),  $\beta$ -pinene (0,5-1,4%), myrcene (0,6-1,1%), limonene (1,3-2,5%), eucalyptol (8,0-12,0%), p-cymene (0,2-1,1%),  $\alpha$ -thujone (19,9-37,2%),  $\beta$ -thujone (3,5-14,2%), camphor (12,3-27,7%), bronyl acetate (0,9-3,5%),  $\beta$ -caryophyllene (2,8-6,0%),  $\alpha$ -humulene (0,3-6,9%) and borneol (1,9-5,3%).

Different works showed that the outputs of the essential oil extraction obtained from *salvia officinalis* and their chemical compositions are closely bound to many parameters as the technique of extraction<sup>11,12</sup>, the geographical origin of the plant<sup>13</sup>, and the part of the plant treated<sup>13,14</sup>.

In this survey, we are interested in studying the proprieties, the yield and the composition of the *salvia officinalis* oil obtained by steam distillation process. This study concerns the essential oils extracted from the organic phase and those extracted from the aqueous phase.

## EXPERIMENTAL

### Plant material

The *Salvia officinalis* or sage of garden is a plant to leaves green silvery, oblong texture, covered of a white down, and to purple-blue flowers (Fig. - 1). It is a plant of the labiates family belonging to mentholated plants. The sage is reputed to be poisonous to high dose; it is an example for the case of the *Salvia Officinalis* of thyone chemotype, which provokes an influx of blood in the abdominal organs, can be harmful to the nervous system. One assigns him, however numerous medicinal virtues: antiseptic, antispasmodic, calming, cephalic, digestive, etc. It is also used in cosmetic, in perfumes<sup>14,15</sup> and in feeding as condiment. The Latin appellation demonstrates well the importance of the sage in the traditional Pharmacopeias. Indeed, *salvia* in Latin means to heal and *salver* to save and its name in Arabic mean the language of camel or *nâama* (ostrich).

All along this work we used the medicinal sage (*Salvia Officinalis*), a variety pushing in the north suburb of Tunis, gathered in the garden of the Preparatory Institute of Scientific Studies and Techniques (IPEST). The leaves have been picked in November (year 2000). After harvesting, Once the plant material (*salvia officinalis* leave's) was picked (mass  $m$ ), it was placed in the shade for 7 days until the stabilisation of its weight. These conditions were sufficient to eliminate any trace of water and we obtained dried plant (mass  $m'$ ). The rate of hydration was calculated as follows:

$$RH = [(m - m') / m] * 100$$

The minimal hydration rate (RH=4.8%).

### Extraction

According to literature, the chemical composition of the essential oil and their yield depend on the isolation method<sup>16,17</sup>. The essential oils have been extracted by classic steam distillation for five hours. Using one kilogram of dried, *salvia officinalis*, leaves. The plant matter, placed in a spherical flask (volume= 10 litres), and 5 litres of distilled water was placed in another flask.

Steam distillation was chosen because, this technique is the most frequently applied method to isolate essential oils, yielding pure oils free of resins, tars and other unwanted compounds. Two phases were obtained, an organic phase and an aqueous phase (aromatic water). The first one, totally composed of essential oil (EOop), has been treated with sodium sulphate ( $\text{Na}_2\text{SO}_4$ ) to eliminate all water traces. The second (aromatic water) contained a worth considering quantity of essential oil, either under soluble form or in the form of fine droplets that are dispersed<sup>11</sup>. The aromatic waters are processed with petrol ether to extract the essential oil of the aqueous phase (EOap).

### Outputs of extraction are defined as follows:

#### REOop =

mass of the EOop / mass of the green plant material (fresh)

#### REOap =

mass of the EOap / mass of the green plant material (fresh)

#### Rtot =

REOop + REOap

The oil samples were stored in a refrigerator at 5°C until analysed. All experiments were made in triplicate.

### Analysis

The composition of the essential oils was investigated by GC and GC-MS. The quantification of essential oil components was carried out by GC analysis using a HP 5890 gas chromatograph apparatus equipped with a DB-5 fused silica capillary column (25m x 0,25 mm i.d., 0,25 µm film thicknesses). The oven temperature was held for 1mn at 50°C, and then programmed from 50°C to 280°C at 9°C/min. The operating temperature for the injection was 240°C. The carrier gas was helium



**Fig. - 1 : *Salvia officinalis***

at a flowing rate of 1,2 ml/mn. The samples were injected in Split mode and the volume injected was 0,2  $\mu$ l (1% solutions diluted in hexane). The relative amounts of the individual components are based on the peak area obtained, with FID response factor correction.

The identification of the essential oil components was performed by GC-MS analysis, using a HP 5890 gas chromatograph coupled to a HP 5972 mass spectrometer under the same conditions as in GC analysis but using a 30 m DB-5 column. Mass spectra were recorded at 70 eV. The oil components were identified by comparison of their mass spectra with those of a computer library of the HP Chemstation database: HP Wiley 275.L, or with authentic compounds and confirmed by comparison of their retention either with those of authentic compounds or with data published in literature<sup>18</sup>.

## RESULTS AND DISCUSSION

### Physic-chemical characteristics and chemical compositions of essential oils

Table -1 shows yield and physic-chemical characteristics of essentials oils extracted by steam distillation from *Salvia officinalis* collected in "La Marsa" Physical-chemicals proprieties. The quantity of oil carried out the aqueous phase is very important, it represent 44% of the total oils weight.

In this study, the total yield was (0,944 % (w/w)). Compared to another studies, the yield obtained is lower. In fact Mastelic [19], using the hydrodistillation process, obtained an yield of (1,42%) and an yield of 1,39% using the extraction with pentane steam and an yield of (1,40%) using the ether steam. In another study, Chalchat and col.<sup>20</sup> proved that the yield of extraction of the essential oils for four hours from the *Salvia*

**Table -1: Physico-chemical characteristics of *S. officinalis* essentials oils**

Essential oils	EOop	EOap	ONIPPAM <sup>(1)</sup>
Yields (%) (w/w)	0,531	0,413	-
% of oil	56,25	43,75	-
Density at 20°C (d <sup>20</sup> )	0,921	0,918	0,910 ; 0,930
pH at 20°C	3	3,5	-
Refractive index at 20°C	1,4765	1,4631	1,4580 ; 1,4740
[ $\alpha_D$ ] $\lambda$ 20°C (rotator index)	+26,3	+18,8	+2° ; +30°
AI (Acid index)	1,34	0,56	-
Ester Index	7,87	3,64	-

(<sup>1</sup>) ONIPPAM : Office Nationale Interprofessionnelle des Plantes à Parfums Aromatiques et Médicinales, France.

**Table -2 : Chemical composition of *S. officinalis* oils (EOop and EOap)**

Components	RI*	HEa %	HEb %
$\alpha$ -pinene	933	Tr	Tr
Bicyclo[3,1,1]hept-2-ene3,6,6-trimethyl	946	Tr	—
Camphene	954	2,18	0,78
Cyclohexene,4-methylene-1-(methylethyl)	960	0,66	—
Sabinene	962	0,51	—
$\beta$ -pinene	966	0,33	0,12
Cyclohexene,1-methyl-4-(1-methylethylene)	1011	Tr	—
Eucalyptol (1-8,Cineol)	1030	12,06	1,19
$\alpha$ -phellandrene	1033	0,51	—
$\alpha$ -thujone	1090	33,52	21,13
$\beta$ -thujone	1104	7,11	6,24
Camphor	1129	20,69	54,29
Borneol	1163	0,70	Tr
Isoborneol	1165	—	6,63
Linalyle acetate	1242	Tr	Tr
Isobornyle acetate	1287	Tr	Tr
$\beta$ -caryophyllene	1420	6,75	0,61
$\alpha$ -caryophyllene	1414	2,83	0,88
$\alpha$ -humulene	1459	1,11	Tr
Aromadendrene	1455	Tr	1,36
Ledene	1622	0,25	0,25
Epimanool	2031	1,08	0,78
<b>% Total</b>		90,30	94,25
<b>% oxygen components</b>		75,11	90,26
<b><math>\alpha</math>-thujone/<math>\beta</math>-thujone</b>		4,71	3,38
<b><math>\alpha</math>-thujone/camphre</b>		1,62	0,38

RI\*: Retention index on a DB5 column

Tr : Trace  $\leq$  0,1

Table - 3: Comparison of chemical composition of *Salvia officinalis* essential oils

Components	<i>S. officinalis</i> from Tunisia % area HE <sub>a</sub>	HE <sub>b</sub>	Valeurs d'ONIPPAM	Commercial <i>S. officinalis</i> <sup>24,29,30</sup>	Serbia <sup>27</sup>	Portugal <sup>28</sup>	Montenegro <sup>27</sup>	Bulgaria <sup>26</sup>	Italy <sup>25</sup>
$\alpha$ -Pinene	Tr	Tr	1 - 6,5	1,5 - 4,0	3,02	4,22	4,58	5,10	1,21
Camphene	2,18	0,78	1,5 - 7	3,2 - 6,7	5,28	2,52	3,47	3,60	0,85
$\beta$ -Pinene	0,33	0,12	-	0,5 - 1,4	0,52	2,22	1,34	2,20	7,22
Myrcene	-	-	-	0,6 - 1,1	0,75	0,92	0,36	0,90	0,75
Limonene	-	-	0,5 - 3	1,3 - 2,5	-	1,59	-	Tr	0,67
1,8-cineol	12,06	1,29	5,5 - 13	8,0 - 12,0	6,35	6,47	12	12,50	7,73
P-cymene	-	-	-	0,2 - 1,1	1,89	0,11	0,58	0,70	0,51
$\alpha$ -Thujone	33,52	21,13	18 - 43	19,9 - 37,2	19,90	25,50	8,47	29,40	39,32
$\beta$ -Thujone	7,11	6,24	3 - 8,5	3,5 - 14,2	3,79	3,89	1,33	17,40	3,07
Camphor	20,69	54,29	4,50 - 24,50	12,30 - 27,70	24,80	19,51	7,62	11,70	2,12
Bronyl Acetate	Tr	Tr	0 - 1	0,9 - 3,5	4,91	1,15	2,23	-	0,28
Terpinen-4-ol	-	-	-	0,2 - 4,4	0,46	0,23	0,33	0,30	0,39
$\beta$ -caryophyllene	-	-	-	2,8 - 6,0	Tr	3,17	Tr	1,10	9,05
$\alpha$ -caryophyllene	2,83	0,88	-	-	Tr	-	Tr	-	-
$\alpha$ -humulene	1,12	Tr	0 - 12	0,3 - 6,9	3,97	7,46	5,94	2,9	12,42
Borneol	0,7	Tr	-	1,9 - 5,3	5,40	0,06	8,50	1,60	0,67
Epimanool	1,08	0,78	-	-	-	-	-	-	-

officinalis, using a Clevenger method is function of the plant origin: France (2,05%), Hongrie (2,50%), Portugal (2,90%), Portugal (2,30%). In present investigation, the yield obtained may be because the method of extraction, la period of the collection of the plant materials. For example, the sage used in this study was collected in November and those used by Mastelic was collected in July.

The GC-MS analysis allowed the identification of 22 components representing more than 90% of the total oils. The components of the volatile oils and their relative percentages are listed in Table - 2, in the order of their retention indices. A comparative study of the oil composition of (*Salvia officinalis*) EO<sub>op</sub> and EO<sub>ap</sub> extracted by steam distillation showed wide differences in the relative percentages of the same components. In fact, the main constituents of the oil extracted from the organic phase, are  $\alpha$ -thujone (33,52%), camphor (20,69%), eucalyptol (12,06%),  $\beta$ -thujone (7,11%) and  $\beta$ -caryophyllene (6,75%), and from the aqueous phase are camphor (54,29%),  $\alpha$ -thujone (21,13%),  $\beta$ -thujone (6,24%) and isoborneol (6,63%).

#### It was also observed that

- $\alpha$ -pinene, and  $\beta$ -pinene are present in a weak rate in the two fractions of essential oils obtained, however sabinene,  $\alpha$ -phallandrene and cyclohexane-4-methylene-1 (1-methylethyl) are present only in the organic phase.
- The components having a high molar mass as aromandendrene,  $\alpha$ -humulene, and epimanool are present with a same amount (2,4%) on the organic and aqueous essential oil obtained by steam distillation.
- The camphor has a very good affinity with water since it is always present in the Eoap with a big percentage. In addition, one recovers it in the Eoap (54,29%) with a rate greater than the one of Eoop (20,69%).

It was observed that the oxygenated component represents more than 80% in the essential oils of *salvia officinalis*, which explains the antioxidant activity of this essential oil<sup>5,6</sup>. The rate of the oxygenated components on the aqueous oil is higher than that on the organic oil.

This result can be anticipated since the oxygenated components have a good affinity with water. On the other hand the hydrocarbons are more present in the organic essential oil.

The highest quality of a sage essential oil is linked to the amount of  $\alpha$ - and  $\beta$ -thujone (>50%) and camphor (<20%)<sup>21,22</sup>. Then the quality of the organic phase oils is better to the one obtained from the aromatic water. The EOap obtained by steam distillation are similar to those find by Putievsky<sup>23</sup> and Lawrence<sup>24</sup>. Theirs results described a relatively low total thujone contents (16-37%) and a high camphor contents (24-41%).

To compare the results obtained to another study with *Salvia officinalis* collected in Italy<sup>25</sup>, Bulgaria<sup>26</sup>, Serbia<sup>27</sup>, Montenegro<sup>27</sup>, Portugal<sup>28</sup> and study of commercial *salvia officinalis* oils<sup>24,29,30</sup> (Table -3). In fact, the *Salvia* essential oil collected in Italy, contient essentially of  $\alpha$ -thujone (39,32%),  $\alpha$ -humulène (12,42%), 1,8-cineol (7,73%),  $\beta$ -pinene (7,22%),  $\beta$ -thujone (3,07%) and camphre (2,12%) . The essential oil of the *Salvia officinalis* from Portugal composed  $\alpha$ -thujone (25,50%), camphor (19,51%),  $\alpha$ -humulene (7,46%), 1,8-cineol (6,47%),  $\alpha$ -pinene (4,22%),  $\beta$ -thujone (3,89%) and camphene (2,57%). The main components of *Salvia officinalis* essential oils from Serbia are: camphor (24,80%),  $\alpha$ -thujone (19,90%), 1,8-cineol (6,35%), camphene (5,28%), bornyle acetate (4,91%),  $\beta$ -thujone (3,79%) and  $\alpha$ -humulene (3,97%), and the principal components in the essential oil of *salvia officinalis* from Montenegro are: 1,8-cineol (12%),  $\alpha$ -thujone (8,47%), camphor (7,62%),  $\alpha$ -humulene (5,94%),  $\alpha$ -pinene (4,58%), camphene (3,47%) bornyl acetate (2,23%). All the studies shows that the chemotype of *Salvia officinalis* from the mediterranen sites is the  $\alpha$ -thujone only those from Serbia, the main component is the camphor (24,80%) and from Montenegro, the main component is 1,2-cineol (12%).

Reports on the essential oil composition of this specie have been published by the authors cited, the variations reported in the sage essential oil composition depending on climate conditions<sup>31,32</sup>, organ, culture site<sup>33</sup> and season<sup>34</sup>. For example, the plant material treated in our study

was collected in November, that from Bulgaria was collected in June and those from Italy was collected in April. The chemical composition of essential oil, depend to the extraction process<sup>35</sup>. Essential oils are usually obtained from condiments, spices and herbs by the traditional vapour extraction method. But the steam distillation from acidic medium can cause important change in composition and transformation is influenced by the condition formed by other volatile compounds. However, high extraction temperatures may cause considerable loss of aroma quality. We have been extract the

essential oil of Tunisian *salvia officinalis* using the steam distillation method, M. Couladis and col. used the hydrodistillation to extract the essential oil of *salvia officinalis* collected in Montenegro and Serbia. The hydrodistillation with a Clevenger apparatus was the process used to obtain the *salvia officinalis* from Italy, Portugal and Bulgaria.

It was also found that chemical composition of Tunisian *Salvia officinalis* essential oil is conformed to the composition presented by the ONIPPAM<sup>1</sup>.

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