Variation of concentrations of thymol and carvacrol in the essential oil of thymus satureoides during aging

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The aim of this work is to do a stability study of two chemo-typical active ingredients (thymol and carvacrol) of the essential oil of thyme “Thymus satureoides” collected from Azilal Region, Morocco. Specimen identification was performed for the raw obtained plant materials and the essential oil extraction carried out through initial and final chemical characterization of the essential oil - at t = 0 - and - at t = 6 months. Results show that a significant decrease (13%) in the initial thymol concentration was recorded during the assumed duration of the stability study (6 months). Carvacrol recorded an even larger drop and was approaching 25%, during the same study period.

Key words: Stability, concentration, essential oil, thymus satureoides, thymol, carvacrol.

INTRODUCTION

Thyme has more than 200 varieties with an endemism of about 46.6% in Morocco (Fennane et al., 2007). In Morocco, the genus Thymus (Lamiaceae) is represented by 21 species of which 12 are endemic (Benabid, 2000). The essential oil of thyme has various pharmacological properties, including antibacterial (Boscovic et al., 2017; Chraibi et al., 2016), antifungal (Ben et al., 2017; Jamali et al., 2012), and anti-oxidant properties (Tohidi et al., 2017; Kasrati et al., 2015; Jamali et al., 2012).

The practical study focuses on the analysis of the degree of conservation of the active principle (s), especially the two chemo-typical active ingredients of the plant (thymol and carvacrol) as well as the study and analysis of the variations of their rates and concentrations during the duration of their conservation. This relates to the stability and therefore the pharmacological, toxicological and ecological properties of the “essential oil”.

MATERIALS AND METHODS

Thyme satureoides - flowering aerial parts were collected at the zone level: Bin Elouidane, Azilal / Beni Mellal Province, Morocco, with the help of local collectors. The specimen was authenticated before and after harvesting according to the vascular plants of Morocco - Practical Flora of Morocco (Fennane et al., 2007) where a representative sample of the plant was deposited at the Scientific Institute of Rabat - Department of Herbal Botany and Ecology, 15
Initial chemical characterization of the essential oil EO / TS at t = 0

A first analysis (at t = 0) of the chemical composition of EO / TS was carried out at the National Center for Scientific and Technical Research "CNRST" / Division of Technical Support Units for Research Scientific "UATRS" - Rabat - Morocco, under the following conditions:

**GPC / MS Analysis Conditions** = UATRS Standard Conditions (Essential Oil)

**Apparatus:** Gas chromatograph (TRACE GC ULTRA) coupled to a mass spectrometer (Polaris Q MS with ion trap).

**Type of analysis performed:** Qualitative and quantitative.

**Type of ionization:** Electronic impact (70 eV)

**Solvent type:** n-Hexane or ethyl acetate

**Column type:** VB-5 (Methylpolysiloxane 5% phenyl), 30 m * 0.25 mm * 0.25 µm.

**Injection conditions:** Table 1

**Separation conditions:** Table 2

### Data processing

The data obtained after the analysis are processed in a computer, in the form of digital data. This allows the development of a spectrogram which is a graphical presentation of the spectral map (peaks) of the various components of the analyzed product. Special software, integrated into the GC-MS system, generally handles the acquisition and processing of digital data from the detector, while at the same time it can be interpreted by automatic comparative study with reference scientific data contained in laboratory-specific computerized libraries.

**Final chemical characterization (in post - aging) of the essential oil extract E/O / T.S at t = 6 months**

The essential oil sub-sample having undergone the above-mentioned conditions of accelerated aging was subjected to a final analysis of its chemical composition following the same procedure (analysis and treatment of results) mentioned above.**Comparing the individual areas of each substance separately (thymol and carvacrol), at t = 0 and t = 6 months and calculating the percentage difference**

Whereas all the parameters of the analysis (GC / MS) of all the samples at t = 0 and at t = 6 months are fixed and identical, particularly, those linked to the technique of analysis, equipment used, manipulation of the analyst, analytical environment (temperature, humidity, pressure ...), and the automatic interpretation of the software. We applied a very simple approach of calculation, by direct comparison of the individual areas corresponding to each substance separately, successively obtained at t = 0 and at t = 6 months. The calculation was done as follows:

**For carvacrol**

\[ A_{C0} \text{Area at } t = 0 \]

\[ A_{C6} \text{Carvacrol area at } t = 6 \text{ months} \]

Whereas \( A_{C0} \) corresponds to 100% of the substance.

Calculate the percentage (X) of \( A_{C6} \) t6 compared to \( A_{C0} \) t0, through the equation (rule of three):

\[ X = A_{C6} / A_{C0} \times 100\% \]

Calculate afterwards the difference "E_{C}" (in percentage) between \( A_{C0} \) t0 and \( A_{C6} \) t6:

\[ E_{C} = 100\% - X \]

**For the thymol**

\[ A_{T0} \text{Thymol area at } t = 0 \]

\[ A_{T6} \text{Thymol area at } t = 6 \text{ months} \]

Whereas \( A_{T0} \) corresponds to 100% of the substance.

Calculate the percentage (Y) of \( A_{T6} \) t6 compared to \( A_{T0} \) t0, through the equation (rule of three):

\[ Y = A_{T6} / A_{T0} \times 100\% \]

Calculate, afterwards, the difference "E_{T}" (in percentage) between \( A_{T0} \) t0 and \( A_{T6} \) t6:

\[ E_{T} = 100\% - Y \]
Table 2. Representation of separation conditions.

<table>
<thead>
<tr>
<th>Ramp (°C /min)</th>
<th>Final Temperature (°C)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>40</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>180</td>
<td>0.00</td>
</tr>
<tr>
<td>20</td>
<td>300</td>
<td>2.00</td>
</tr>
</tbody>
</table>

Figure 1. Initial spectrogram (graphic presentation of the spectral map (peaks)) of the different components of *T. satureoides* essential oil at *t* = 0. Retention times: RT = 16.57 and RT = 18.55 correspond successively to "Carvacrol" and "Thymol". Source: Elkacimi I. (2016).

RESULTS

Extraction step

Approximately 2.5 ml (2.22 g) of *T. satureoides* essential oil (EO / TS) was extracted from almost 100 g of the raw material for two days (at the rate of 1.5 ml for the first day and 1 ml for the second day) in the laboratory of the Faculty and in accordance with the above academic procedure.

Initial analysis step: initial chemical characterization (at *t* = 0) of the essential oil E.O / T.S.

The result of initial qualitative analysis (at *t* = 0) of the studied essential oil using gas chromatography coupled with mass spectrometry (GC / MS) is summarized in the

Final analysis step: Final chemical characterization (in post-aging) of the essential oil extract E.O / T.S

The result of the final qualitative analysis (at *t* = 6 months after aging under: *t* = 40 °C and HR = 75%) of the essential oil using gas chromatography coupled with mass spectrometry (GC / MS) is summarized in Figure 2.

Calculation of individual percentages and deviations

The quantitative results of the two main components (carvacrol and thymol) are listed in Table 3. These results are in accordance with the following basic principles of calculation:
Figure 2. Spectrogram (graphic presentation of the spectral map (peaks)) of the various components of the saturated thyme essential oil at \( t = 6 \) months after aging under: \( t = 40^\circ C \) and HR = 75%. Retention times: RT = 16.58 and RT = 18.55 correspond successively to "Carvacrol" and "Thymol".


Table 3. Representation of the results of the quantitative analysis (at \( t = 6 \) months after aging under: \( t = 40^\circ C \) and HR = 75%) of carvacrol and thymol compared to the initial composition (100%) of the same substances in essential oil of \( T. \) satureoides (EO / TS).

<table>
<thead>
<tr>
<th>Retention time (RT)</th>
<th>Substance</th>
<th>Individual percentage of the substance at ( t = 6 ) months relative to its initial concentration at ( t = 0 )</th>
<th>Difference (in percentage) between the concentration of the same substance between ( t = 0 ) and ( t = 6 ) months</th>
</tr>
</thead>
<tbody>
<tr>
<td>( T = 0 ) / ( t = 6 ) months</td>
<td>Carvacrol</td>
<td>76.51%</td>
<td>23.49%</td>
</tr>
<tr>
<td>16.57 / 16.58</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( T = 0 ) et ( t = 6 ) months</td>
<td>Thymol</td>
<td>86.94%</td>
<td>13.06%</td>
</tr>
<tr>
<td>18.55</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


For carvacrol

\( A_C \) \( t_0 \): Carvacrol area (at \( t = 0 \)) = 67097757
\( A_C \) \( t_6 \): Carvacrol area (at \( t = 6 \) months) = 51339704
Whereas \( A_C \) \( t_0 \) corresponds to 100% of the substance.
Calculation of the percentage (X) of \( A_C \) \( t_6 \) with respect to \( A_C \) \( t_0 \), through the equation (rule of three):
\[
X = \frac{A_C \ t_6}{A_C \ t_0} \times 100\% = \frac{51339704}{67097757} \times 100\% = 76.51\%
\]

Calculation, afterwards, of the difference \( "E_C" \) (in percentage) between \( A_C \) \( t_0 \) and \( A_C \) \( t_6 \):
\[
E_C = 100\% - X = 100 - 76.51\% = 23.49\%
\]

For the thymol

\( A_T \) \( t_0 \): Thymol area (at \( t = 0 \)) = 302397036
\( A_T \) \( t_6 \): Thymol area (at \( t = 6 \) months) = 262920106
Whereas \( A_T \) \( t_0 \) corresponds to 100% of the substance.
Calculation of the percentage (Y) of AT t6 with respect to AT t0, through the equation (rule of three):

\[ Y = \frac{AT\ t6}{AT\ t0} \times 100\% = 262920106/302397036 \times 100\% = 86.94\% \]

Calculation, afterwards, of the difference "ET" (in percentage) between AT t0 and AT t6:

\[ E_T = 100\% - Y = 100 - 8.94\% = 13.06\% \]

**DISCUSSION**

In the first place and according to spectrograms (Figures 1 and 2) resulting from the GC / MS analysis of the essential oil (EO / TS), the specific identity of this variety of thyme can be confirmed. Because the concentrations of both thymol and carvacrol are more compared to those of other components including that of "borneol", which is not the case of the variety of the plant generally known by its chemotype rich in borneol, it is therefore deduced that it is a second (non-ordinary) variety of saturated thyme as reported by Benjilali et al. (1987). This hypothesis is verified by other research carried out on essential oils of T. satureioides (EO / TS) from different regions of Morocco. This is the case of EO / TS, from Ifrane Region, whose major component is "P-cymene" (Elouali et al., 2013) or the case of EO / TS from Taroudant Region that is rich in "borneol" (Ramzi et al., 2017). For the EO / TS essential oil extraction, it has a total yield of about 2.22%, with more than 1.1% obtained by Elouali et al. (2013).

On the other hand, by making a comparative study of the quantitative results relating to the levels of the two components (thymol and carvacrol) obtained following the stability study of the essential oil (between t = 0 and t = 6 months under accelerated aging), we found a significant decrease of more than 13% in the initial thymol concentration over the assumed duration (6 months), from 100% at t = 0 to 86.94% at t = 6 months. Carvacrol decreased even more, approaching 25% over the same study period, from 100% to t = 0 to 76.51%. t = 6 months.

It can be deduced that, in general terms, this decrease, no matter how large, in the concentration of the two substances during the study period (6 months) may be essentially due to their degradation under the sudden climatic and environmental factors (mainly redox reactions) effects on one hand and, on the other hand, to the use of the essential oil as it is (traditional preservation) without being subject to any industrial preservation (under vacuum or under nitrogen). It should also be noted that the degradation is greater in the case of carvacrol with more than 23% compared to that of thymol (13%). This led us to suggest that carvacrol may be more sensitive to degradation factors than thymol with a difference of more than 10%.

**Conclusion**

From the foregoing, it can be concluded that the essential oil of T. satureioides (HE / TS) is subject to rapid deterioration in the case of artisanal conservation, in time and under the effect of climate degradation factors. This encourages us to recommend complementary studies in the same direction, so as to include, among others:

- Stability study of vacuum conditioned EO / TS.
- The stability study of the EO / TS conditioned under nitrogen.
- The stability study of the EO / TS added conservator (s).

This will serve to form a general and more precise idea of the stability status of the thyme essential oil (EO / TS) for each type of conditioning and preservation and especially, for comparative study using traditional packaging and preservation, which is the approach adopted in this study. The ultimate goal sought here is to find, with tangible evidence to support, the best way to keep the essential oil of T. satureioides. This could be valid for other essential oils.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**REFERENCES**


