



## Quality along the Dairy Chain for a Safe and Sustainable MILK PRIMA S2 – 2018

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| Deliverable number: | D3.3 of WP3                               |
| Deliverable title:  | Phytochemical profile of selected samples |

|                        |                   |
|------------------------|-------------------|
| Due date of milestone: | M9 – January 2020 |
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### Document information

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#### 5. Revision history

| Version | Date | Modified by | Comments |
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| 1       |      |             |          |
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#### 6. Dissemination level

|    |  |     |
|----|--|-----|
| PU | Public   | no  |
| CO | Confidential, only for members of the consortium | yes |

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## Executive Summary

|                                 |   |
|---------------------------------|---|
| <b>Background</b>               | Essential Oils (EOs) are secondary volatile metabolites biosynthesized by aromatic medicinal plants, characterized by strong odor and having a wide range of biological activities. Based on their antimicrobial potential against a wide range of bacterial strains, these phytoproducts could be used in the treatment or prevention of cow's udder mastitis. Thereby, we started the present study in vitro to assess the antimicrobial potential of 10 Tunisian EOs against two bacterial strains the most implicated in cow's udder mastitis in Tunisia, <i>Escherichia coli</i> and <i>Staphylococcus</i> : thyme ( <i>Thymus capitatus</i> ), bay laurel ( <i>Laurus nobilis</i> ), coriander ( <i>Coriandrum sativum</i> ), rosemary ( <i>Rosmarinus officinalis</i> ), wormwood ( <i>Artemisia herba alba</i> ), sage ( <i>Salvia officinalis</i> ), oregano ( <i>Origanum majorana</i> ), geranium ( <i>Pelargonium graveolens</i> ), prickly juniper ( <i>Juniperus oxycedrus</i> ), Nigella Sativa.   |
| <b>Objectives</b>               | The main objective of the deliverable was to determine chemical composition of the studied EOs.   |
| <b>Methods</b>                  | <p>Chemical composition of studied EOs was determined by gas chromatography (Agilent 7890 A series II) coupled to mass spectrometry (Agilent 5975 C mass spectrometer with electron impact ionization (70eV)). An apolar HP-5MS capillary column (30m x 0.25mm coated with 5% phenyl methylsilicone, and 95% dimethyl polysiloxane, 0.25 <math>\mu</math>m film thickness) was used. The oven temperature was programmed to rise from 50 °C to 240 °C at a rate of 5 °C/min. The transfer line temperature was 250 °C. The carrier gas was helium with a flow rate of 1.2 mL/min; the split ratio was 60:1. Scan time and mass range were 1 s and 40–300 m/z respectively.</p> <p>Essentials oils volatile compounds were identified by comparing their retention indexes (RI) related to (C9–C18) n-alkanes with those of authentic compounds (Analytical reagents, LabScan, Ltd, Dublin, Ireland) available in literature and in our laboratory and by matching their mass spectra fragmentation patterns with corresponding data stored in the mass spectra library of the GC–MS data system (NIST) and other published mass spectra (Adams, 2001). Relative percentage amounts of the identified compounds were obtained from the electronic integration of the FID peak area.</p> <p>All analyses were repeated three times and results represent mean values <math>\pm</math> standard deviation. Percentages of compounds were automatically calculated by using a HP-Chemstation software based on their respective areas.</p>  |
| <b>Results and implications</b> | <p>Gas chromatography coupled to mass spectrometry analyses of the three active EOs against studied bacterial strains showed 15 identified compounds for both thyme and coriander oils and 22 ones for laurel oil (Tables 1, 2 and 3; Figures 1, 2 and 3). Also, results revealed that Thyme EO is of carvacrol chemotype (70.62%) with appreciable amounts of <math>\gamma</math>-terpinene (7.58%), p-cymene (7.06%) and caryophyllene (4.54%). On the other hand, bay laurel EO is of 1,8-cineole/camphene chimiotype (43.85/13.81%) with 5.48% of <math>\alpha</math>-terpineol. As for the coriander EO it is of linalool chemotype (64.10%) with appreciable amount of <math>\alpha</math>-pinene (7.26%).</p> <p>The grouping of the identified volatile compounds in chemical classes showed that the three active EOs are characterized by the predominance of oxygenated monoterpenes. This chemical class represents 73.4%, 63.24% and 78.24% of thyme, laurel and coriander EOs respectively (Figure 4).</p> <p>Based on these results, it seems that the antibacterial activity of these selected EOs may be related to their richness in oxygenated monoterpenes and that the strong activity of thyme EO on both strains could be probably due to its high level of carvacrol. Based on these results, the three selected EOs and mostly the EO of thyme could be a promising alternatives to antibiotics used for the treatment of mastitis, nevertheless, further investigations, as planned in WP3, should be completed in order to confirm their innocuity.</p> <p>Finally, the chemical composition of the rest of EOs which did not show remarkable antibacterial effects is resumed below in tables 4, 5, 6, 7, 8 and 9 and their chromatographic profiles are given in figures 5, 6, 7, 8, 9 and 10.</p> |

Nigella sativa seeds were very poor in EO with a very low yield that will not be sufficient to do all the required experiences that's why we decided to extract fixed oil instead and which did not show good antibacterial activity.

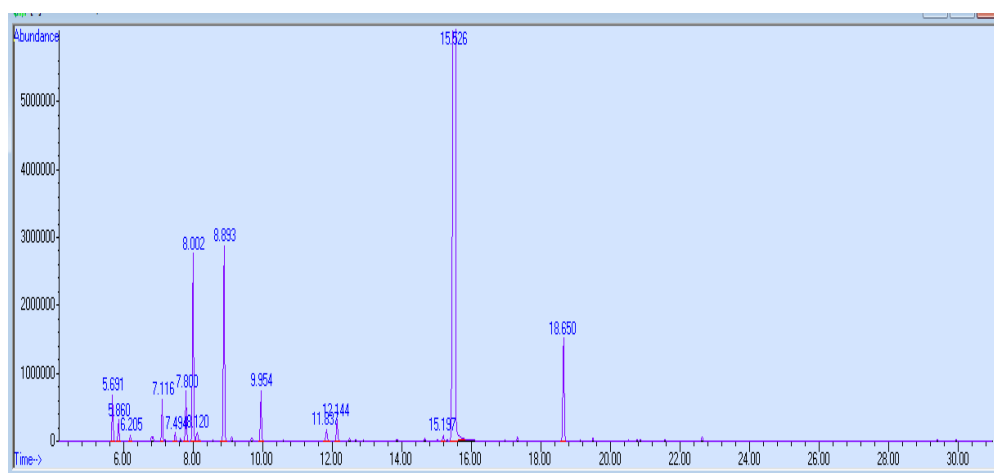
## Detailed content

Gas chromatography coupled to mass spectrometry analyses of the three active EOs against studied bacterial strains showed 15 identified compounds for both thyme and coriander oils and 22 ones for laurel oil (Tables 1, 2 and 3; Figures 1, 2 and 3)). Also, results revealed that Thyme EO is of carvacrol chemotype (70.62%) with appreciable amounts of  $\gamma$ -terpinene (7.58%), *p*-cymene (7.06%) and caryophyllene (4.54%). On the other hand, bay laurel EO is of 1,8-cineole/camphene chimotype (43.85/13.81%) with 5.48% of  $\alpha$ -terpineol. As for the coriander EO it is of linalool chemotype (64.10%) with appreciable amount of  $\alpha$ -pinene (7.26%).

**Table 1. Chemical composition of thyme (*Thymus capitatus*) essential oil**

| N° | Compound              | % of total volatiles |
|----|-----------------------|----------------------|
| 1  | $\alpha$ -thujene     | 1.49 $\pm$ 0.06      |
| 2  | $\alpha$ -pinene      | 0.71 $\pm$ 0.01      |
| 3  | Camphene              | 0.21 $\pm$ 0.00      |
| 4  | $\beta$ -myrcene      | 1.42 $\pm$ 0.05      |
| 5  | l-Phellandrene        | 0.30 $\pm$ 0.00      |
| 6  | $\alpha$ -terpinene   | 1.84 $\pm$ 0.07      |
| 7  | <i>p</i> -cymene      | 7.06 $\pm$ 0.15      |
| 8  | $\beta$ -phellandrene | 0.55 $\pm$ 0.01      |
| 9  | $\gamma$ -terpinene   | 7.58 $\pm$ 0.16      |
| 10 | Linalool              | 1.95 $\pm$ 0.07      |
| 11 | Borneol               | 0.57 $\pm$ 0.01      |
| 12 | Cyclohexen-1-ol       | 0.91 $\pm$ 0.02      |
| 13 | Thymol                | 0.24 $\pm$ 0.00      |
| 14 | Carvacrol             | 70.62 $\pm$ 1.07     |
| 15 | Caryophyllene         | 4.55 $\pm$ 0.08      |

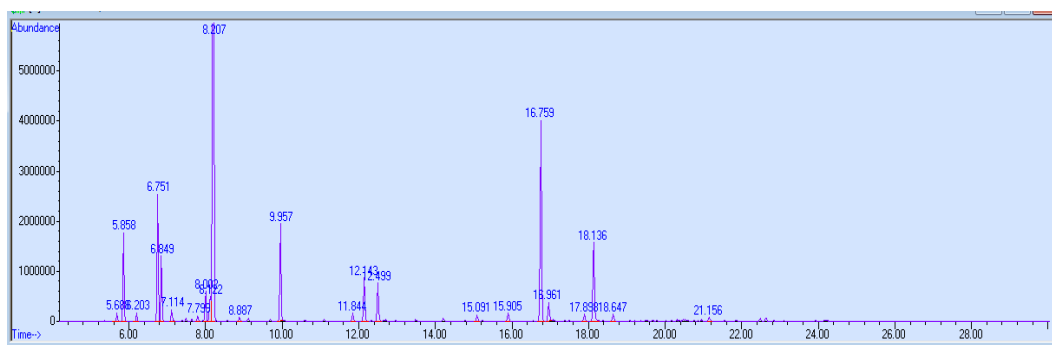
**Figure 1. Chromatogram of thyme essential oil**



**Table 2. Chemical composition of bay laurel (*Laurus nobilis*) essential oil**

| N° | Compound                   | % of total volatiles |
|----|----------------------------|----------------------|
| 1  | $\alpha$ -phellandrene     | 0.46 $\pm$ 0.03      |
| 2  | $\alpha$ -pinene           | 4.72 $\pm$ 0.20      |
| 3  | Camphene                   | 0.48 $\pm$ 0.02      |
| 4  | Sabinene                   | 7.03 $\pm$ 0.21      |
| 5  | $\beta$ -pinene            | 3.63 $\pm$ 0.15      |
| 6  | $\beta$ -myrcene           | 0.70 $\pm$ 0.02      |
| 7  | $\alpha$ -terpinene        | 0.32 $\pm$ 0.02      |
| 8  | p-cymene                   | 1.81 $\pm$ 0.07      |
| 9  | D-limonene                 | 1.76 $\pm$ 0.06      |
| 10 | 1,8-cineole                | 43.85 $\pm$ 0.82     |
| 11 | $\gamma$ -terpinene        | 0.24 $\pm$ 0.01      |
| 12 | $\beta$ -linalool          | 5.94 $\pm$ 0.24      |
| 13 | 2,5-dimethyl-2,4-hexadiene | 0.54 $\pm$ 0.02      |
| 14 | $\alpha$ -terpineol        | 5.48 $\pm$ 0.19      |
| 15 | Endobornyl acetate         | 0.44 $\pm$ 0.03      |
| 16 | $\delta$ -terpinyl acetate | 0.54 $\pm$ 0.03      |
| 17 | Camphene                   | 13.82 $\pm$ 0.32     |
| 18 | Eugenol                    | 1.37 $\pm$ 0.05      |
| 19 | $\beta$ -Elemene           | 0.50 $\pm$ 0.02      |
| 20 | Methyl eugenol             | 5.63 $\pm$ 0.1       |
| 21 | Caryophyllene              | 0.46 $\pm$ 0.03      |
| 22 | $\delta$ -cadinene         | 0.29 $\pm$ 0.01      |

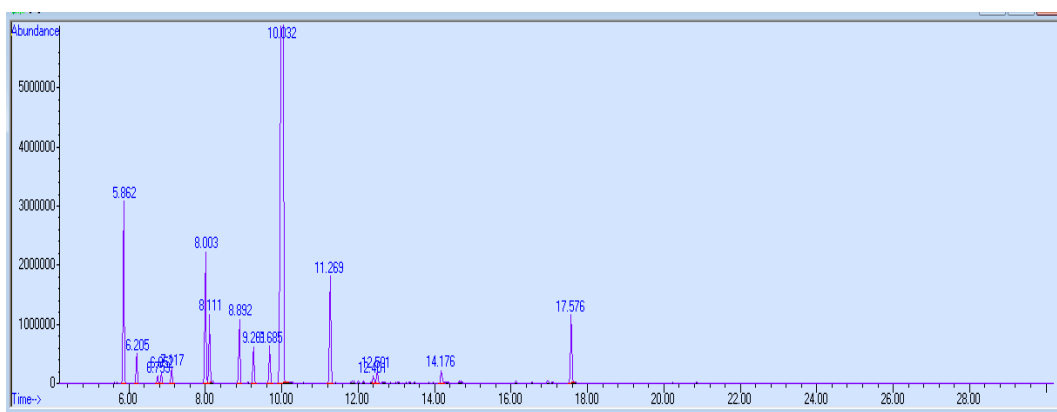
**Figure 2. Chromatogram of Bay laurel essential oil**



**Table 3. Chemical composition of coriander (*Coriandrum sativum*) essential oil**

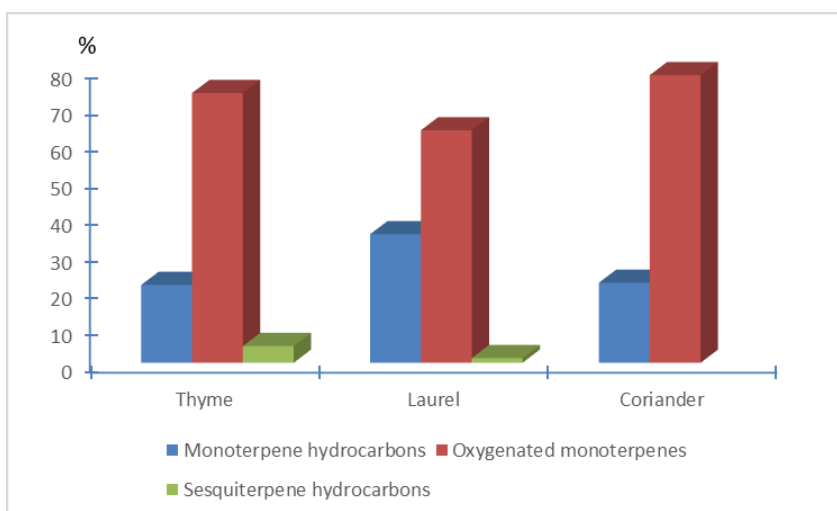
| N° | Compound            | % of total volatiles |
|----|---------------------|----------------------|
| 1  | $\alpha$ -pinene    | 7.26 $\pm$ 0.22      |
| 2  | Camphene            | 1.23 $\pm$ 0.07      |
| 3  | Sabinene            | 0.29 $\pm$ 0.01      |
| 4  | $\beta$ -pinene     | 0.48 $\pm$ 0.03      |
| 5  | $\beta$ -myrcene    | 0.63 $\pm$ 0.05      |
| 6  | o-cymene            | 5.91 $\pm$ 0.15      |
| 7  | D-Limonene          | 3.02 $\pm$ 0.09      |
| 8  | $\gamma$ -terpinene | 2.94 $\pm$ 0.09      |
| 9  | Linalool oxide      | 3.53 $\pm$ 0.12      |
| 10 | Linalool            | 64.10 $\pm$ 0.68     |
| 11 | Camphor             | 5.50 $\pm$ 0.12      |
| 12 | Terpenediol         | 0.37 $\pm$ 0.03      |
| 13 | $\alpha$ -terpineol | 0.62 $\pm$ 0.05      |
| 14 | Geraniol            | 0.78 $\pm$ 0.06      |
| 15 | Geranyl acetate     | 3.35 $\pm$ 0.10      |

**Figure 3. Chromatogram of coriander essential oil**



The grouping of the identified volatile compounds in chemical classes showed that the three active EOs are characterized by the predominance of oxygenated monoterpenes. This chemical class represents 73.4%, 63.24% and 78.24% of thyme, laurel and coriander EOs respectively (Figure 4).

**Figure 4. Main chemical classes of Thyme, Laurel and Coriander essential oils (%)**



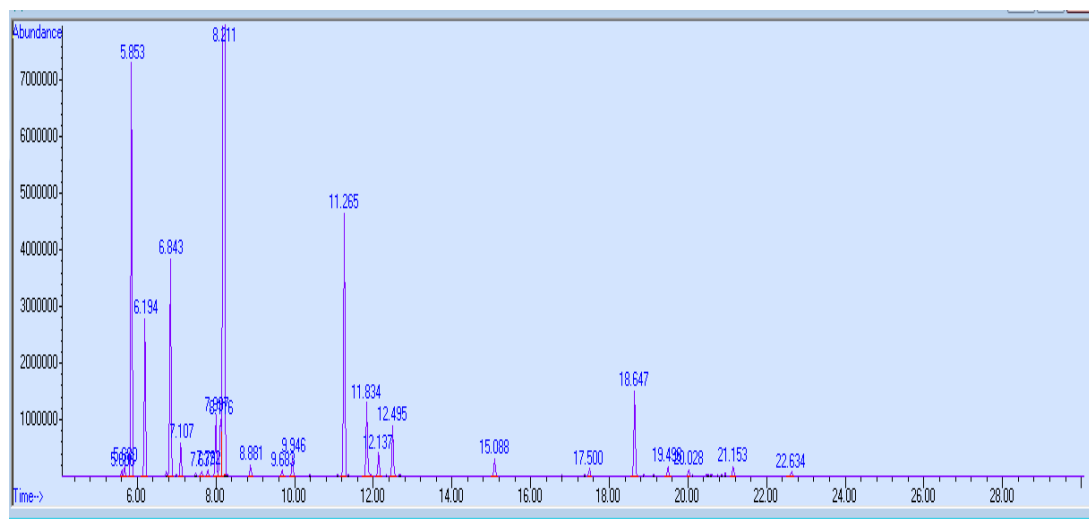
Based on these results, it seems that the antibacterial activity of these selected EOs may be related to their richness in oxygenated monoterpenes and that the strong activity of thyme EO on both strains could be probably due to its high level of carvacrol. Based on these results, the three selected EOs and mostly the EO of thyme could be a promising alternatives to antibiotics used for the treatment of mastitis, nevertheless, further investigations, as planned in WP3, should be completed in order to confirm their innocuity.

Finally, the chemical composition of the rest of EOs which did not show remarkable antibacterial effects is resumed below in tables 4, 5, 6, 7, 8 and 9 and their chromatographic profiles are given in figures 5, 6, 7, 8, 9 and 10.

**Table 4. Chemical composition of rosemary (*Rosmarinus officinalis*) essential oil**

| N° | Compound       | % of total volatiles |
|----|----------------|----------------------|
| 1  | Tricyclene     | 0.19 ± 0.01          |
| 2  | α-thujene      | 0.31 ± 0.02          |
| 3  | α-pinene       | 12.77 ± 0.56         |
| 4  | Camphene       | 5.00 ± 0.20          |
| 5  | β-pinene       | 7.17 ± 0.09          |
| 6  | β-myrcene      | 1.03 ± 0.03          |
| 7  | δ-3-carene     | 0.18 ± 0.01          |
| 8  | α-terpinene    | 0.22 ± 0.01          |
| 9  | p-cymene       | 2.20 ± 0.06          |
| 10 | D-limonene     | 2.21 ± 0.03          |
| 11 | 1,8-cineole    | 46.37 ± 0.79         |
| 12 | γ-terpinene    | 0.37 ± 0.03          |
| 13 | α-terpinolene  | 0.22 ± 0.01          |
| 14 | Linallool      | 0.69 ± 0.06          |
| 15 | Camphor        | 9.97 ± 0.14          |
| 16 | Borneol        | 2.94 ± 0.06          |
| 17 | Terpineol      | 0.86 ± 0.04          |
| 18 | α-Terpineol    | 1.91 ± 0.05          |
| 19 | Bornyl acetate | 0.67 ± 0.03          |
| 20 | α-copaene      | 0.27 ± 0.01          |
| 21 | Caryophyllene  | 3.23 ± 0.09          |

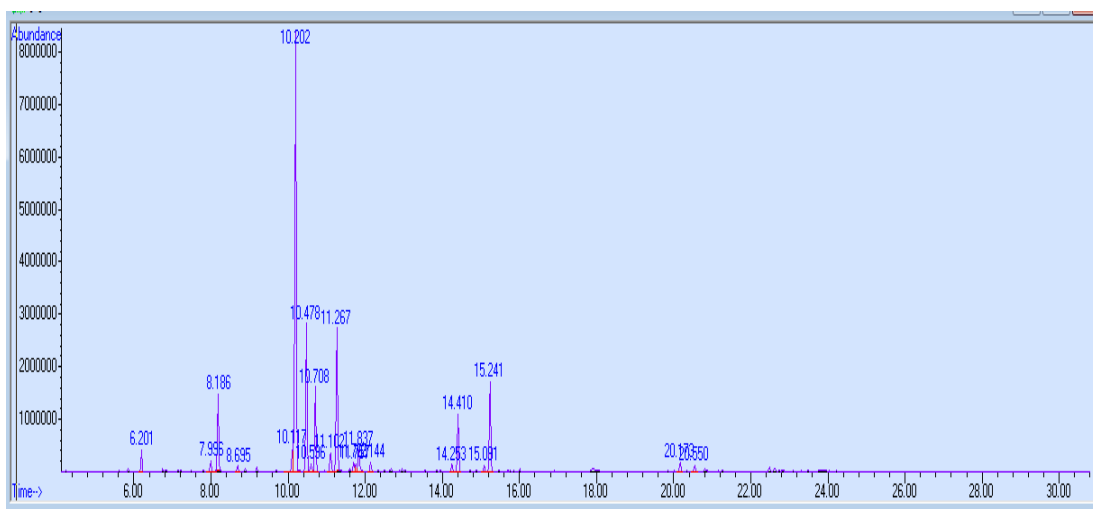
**Figure 5. Chromatogram of rosemary essential oil**



**Table 5. Chemical composition of wormwood (*Artemisia herba alba*) essential oil**

| N° | Compound                                | % of total volatiles |
|----|---|----------------------|
| 1  | Camphene                                | 1.38 ± 0.06          |
| 2  | <i>p</i> -Cymene                        | 0.89 ± 0.03          |
| 3  | 1,8-Cineole                             | 5.69 ± 0.11          |
| 4  | Filifolone                              | 1.97 ± 0.07          |
| 5  | $\beta$ -thujone                        | 38.27 ± 0.53         |
| 6  | $\alpha$ -Thujone                       | 11.58 ± 0.17         |
| 7  | 1-terpineol                             | 0.67 ± 0.02          |
| 8  | Chrysanthenone                          | 6.76 ± 0.10          |
| 9  | <i>Trans</i> -Verbenol                  | 1.77 ± 0.05          |
| 10 | Camphor                                 | 11.88 ± 0.20         |
| 11 | <i>Trans</i> -1,2-Dihydroperillaldehyde | 0.73 ± 0.08          |
| 12 | Pinocarvone                             | 0.71 ± 0.04          |
| 13 | endo-Borneol                            | 2.07 ± 0.06          |
| 14 | 4-Terpineol                             | 0.75 ± 0.03          |
| 15 | Piperitone                              | 0.60 ± 0.01          |
| 16 | Bornyl acetate                          | 5.08 ± 0.09          |
| 17 | Sabinyl acetate                         | 7.24 ± 0.10          |
| 18 | Germacrene-D                            | 0.90 ± 0.03          |
| 19 | Bicyclogermacrene                       | 0.64 ± 0.01          |

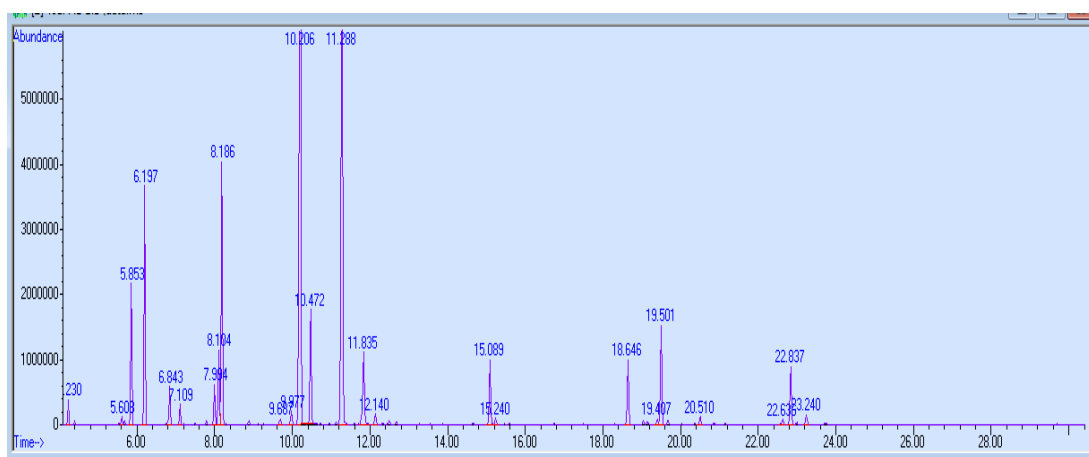
**Figure 6. Chromatogram of wormwood essential oil**



**Table 6. Chemical composition of sage (*Salvia officinalis*) essential oil**

| N° | Compound            | % of total volatiles |
|----|---------------------|----------------------|
| 1  | <i>Cis</i> -salvene | 0.60 ± 0.01          |
| 2  | Tricyclene          | 0.23 ± 0.01          |
| 3  | α-pinene            | 4.08 ± 0.10          |
| 4  | Camphene            | 7.22 ± 0.11          |
| 5  | β-pinene            | 1.40 ± 0.05          |
| 6  | β-myrcene           | 0.60 ± 0.01          |
| 7  | <i>p</i> -cymene    | 1.30 ± 0.04          |
| 8  | Limonene            | 2.58 ± 0.05          |
| 9  | 1,8-cineole         | 8.84 ± 0.11          |
| 10 | α-terpinolene       | 0.26 ± 0.01          |
| 11 | Linalool            | 0.50 ± 0.02          |
| 12 | β-Thujone           | 27.36 ± 0.50         |
| 13 | α-Thujone           | 3.90 ± 0.09          |
| 14 | Camphor             | 25.25 ± 0.47         |
| 15 | Endo-borneol        | 3.14 ± 0.07          |
| 16 | Terpineol           | 0.40 ± 0.02          |
| 17 | Bornyl acetate      | 2.39 ± 0.05          |
| 18 | Saninyl acetate     | 0.26 ± 0.01          |
| 19 | Caryophyllene       | 2.47 ± 0.05          |
| 20 | Acethylbnzene       | 0.23 ± 0.01          |
| 21 | β-selinene          | 3.85 ± 0.09          |
| 22 | Ledene              | 0.29 ± 0.01          |
| 23 | Caryophyllene oxide | 0.24 ± 0.01          |
| 24 | Viridiflorol        | 2.29 ± 0.05          |

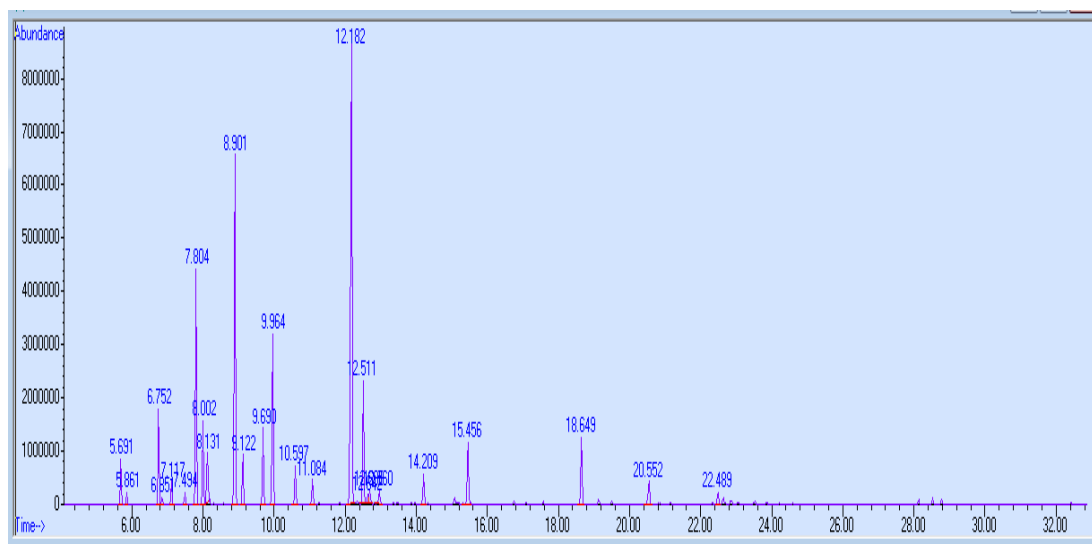
**Figure 7. Chromatogram of sage essential oil**



**Table 7. Chemical composition of oregano (*Origanum majorana*) essential oil**

| N° | Compound                     | % of total volatiles |
|----|------------------------------|----------------------|
| 1  | $\alpha$ -thujene            | 1.64 $\pm$ 0.05      |
| 2  | $\alpha$ -pinene             | 0.47 $\pm$ 0.01      |
| 3  | Sabinene                     | 3.73 $\pm$ 0.08      |
| 4  | $\beta$ -pinene              | 0.32 $\pm$ 0.01      |
| 5  | $\beta$ -myrcene             | 0.88 $\pm$ 0.03      |
| 6  | Phellandrene                 | 0.49 $\pm$ 0.02      |
| 7  | $\alpha$ -terpinene          | 9.83 $\pm$ 0.14      |
| 8  | <i>p</i> -cymene             | 3.50 $\pm$ 0.07      |
| 9  | Sabinene                     | 2.82 $\pm$ 0.05      |
| 10 | $\gamma$ -terpinene          | 15.57 $\pm$ 0.18     |
| 11 | <i>p</i> -menth-8-en-ol      | 2.18 $\pm$ 0.04      |
| 12 | $\alpha$ -terpinolene        | 3.40 $\pm$ 0.05      |
| 13 | <i>Cis</i> -sabinene hydrate | 7.97 $\pm$ 0.11      |
| 14 | Cyclohexen-1-ol              | 1.77 $\pm$ 0.05      |
| 15 | Terpinen-1-ol                | 1.12 $\pm$ 0.04      |
| 16 | Terpineol                    | 26.89 $\pm$ 0.40     |
| 17 | $\alpha$ -terpineol          | 5.95 $\pm$ 0.09      |
| 18 | Estragol                     | 0.66 $\pm$ 0.02      |
| 19 | Piperitol                    | 0.70 $\pm$ 0.03      |
| 20 | Linalyl acetate              | 1.44 $\pm$ 0.05      |
| 21 | $\gamma$ -terpinene          | 2.99 $\pm$ 0.05      |
| 22 | Caryophyllene                | 3.29 $\pm$ 0.06      |
| 23 | Bicyclgermacrene             | 1.37 $\pm$ 0.05      |

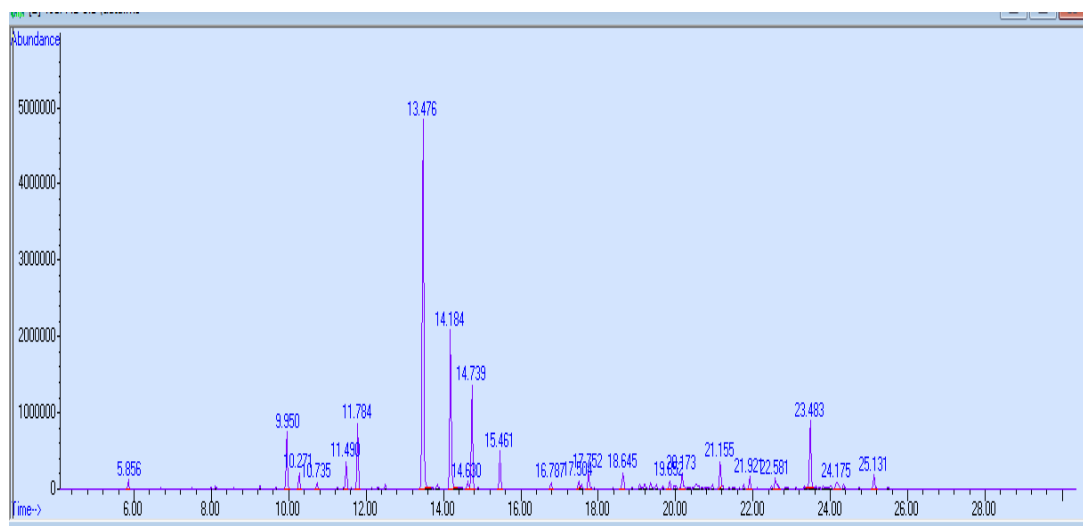
**Figure 8. Chromatogram of oregano essential oil**



**Table 8. Chemical composition of geranium (*Pelargonium graveolens*) essential oil**

| N° | Compound                  | % of total volatiles |
|----|---------------------------|----------------------|
| 1  | $\alpha$ -pinene          | 0.63 $\pm$ 0.03      |
| 2  | Linalool                  | 4.59 $\pm$ 0.08      |
| 3  | Rose Oxide                | 1.31 $\pm$ 0.05      |
| 4  | Non identified            | 0.55 $\pm$ 0.02      |
| 5  | Non identified            | 2.29 $\pm$ 0.06      |
| 6  | Menthone                  | 5.67 $\pm$ 0.092     |
| 7  | $\beta$ -citronellol      | 36.88 $\pm$ 0.45     |
| 8  | Geraniol                  | 15.02 $\pm$ 0.16     |
| 9  | $\alpha$ -citral          | 0.6 $\pm$ 0.02       |
| 10 | Citronellyl formate       | 8.74 $\pm$ 0.10      |
| 11 | Geraniol formate          | 3.22 $\pm$ 0.07      |
| 12 | $\alpha$ -cubebene        | 0.61 $\pm$ 0.02      |
| 13 | $\alpha$ -copaene         | 0.64 $\pm$ 0.03      |
| 14 | $\beta$ -bourbonene       | 1.64 $\pm$ 0.06      |
| 15 | Caryophyllene             | 1.65 $\pm$ 0.06      |
| 16 | Geranyl acetate           | 0.74 $\pm$ 0.03      |
| 17 | Germacrene-D              | 1.53 $\pm$ 0.06      |
| 18 | $\delta$ -cadinene        | 2.25 $\pm$ 0.06      |
| 19 | Butanoic acid             | 1.00 $\pm$ 0.05      |
| 20 | Phenyl Tiglate 2          | 1.40 $\pm$ 0.06      |
| 21 | 8-epi- $\gamma$ -eudesmol | 6.40 $\pm$ 0.09      |
| 22 | Indene                    | 1.18 $\pm$ 0.06      |
| 23 | Geranyl tiglate           | 1.39 $\pm$ 0.05      |

**Figure 9. Chromatogram of geranium essential oil**



**Table 9. Chemical composition of prickly juniper (*Juniperus oxycedrus*) essential oil**

| N° | Compound                      | % of total volatiles |
|----|-------------------------------|----------------------|
| 1  | $\alpha$ -pinene              | 60.49 $\pm$ 0.90     |
| 2  | $\beta$ -pinene               | 1.05 $\pm$ 0.04      |
| 3  | $\beta$ -myrcene              | 1.89 $\pm$ 0.06      |
| 4  | Phellandrene                  | 1.2 $\pm$ 0.04       |
| 5  | $\delta$ -3-carene            | 3.43 $\pm$ 0.07      |
| 6  | <i>p</i> -cymene              | 1.91 $\pm$ 0.07      |
| 7  | $\beta$ -phellandrene         | 9.08 $\pm$ 0.13      |
| 8  | $\alpha$ -terpinolene         | 0.73 $\pm$ 0.02      |
| 9  | $\alpha$ -campholenal         | 0.59 $\pm$ 0.01      |
| 10 | <i>Trans</i> -pinacarveol     | 0.56 $\pm$ 0.01      |
| 11 | <i>p</i> -menta-1,5-dien-8-ol | 0.62 $\pm$ 0.02      |
| 12 | $\alpha$ -terpineol           | 1.05 $\pm$ 0.04      |
| 13 | 4-carene                      | 4.45 $\pm$ 0.08      |
| 14 | Germacene-D                   | 2.69 $\pm$ 0.06      |
| 15 | $\alpha$ -bisabolene          | 0.51 $\pm$ 0.01      |
| 16 | Manoyl oxide                  | 2.96 $\pm$ 0.07      |
| 17 | Phenanthrene                  | 0.51 $\pm$ 0.01      |
| 18 | Lovopimaradiene               | 0.44 $\pm$ 0.01      |
| 19 | Octahydrophenanthrene         | 1.34 $\pm$ 0.04      |
| 20 | Abletadiene                   | 4.21 $\pm$ 0.08      |

**Figure 10. Chromatogram of prickly juniper essential oil**

