

## Full Length Research Paper

# Chemical analysis and antimicrobial effects of essential oil from *Limoniastrum guyonianum* growing in Tunisia

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**Medicinal *Limoniastrum guyonianum* (Plumbaginaceae) growing in Tunisia was studied for the first time for volatiles from leaves, flowers, seeds and roots. The chemical analysis of the essential oil obtained from different organs was performed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectroscopy (GC-MS). In roots and leaves oils, monoterpenes were found in almost equal level (22.56 and 26.21%, respectively). (3Z) hex-3-enylmethanoate was detected as the chemotype in roots, while furfural (14.63%), methyl-2,4-dimethylbenzoate (14.70%) and 3-phenylprop-2-enylpentanoate (15.05%) were the most abundant constituents from seeds, leaves and flowers, respectively. Furthermore, antibacterial effects of the indicated oils were evaluated against five sensitive bacteria: *Escherichia coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus epidermidis* NCIMB 8853, *Staphylococcus aureus* ATCC 29213 and *Micrococcus luteus* NCIMB 8166 using both disc diffusion and dilution methods.**

**Key words:** *Limoniastrum guyonianum* Bois, plumbaginaceae, essential oil, gas chromatography-flame ionization detector, gas chromatography-mass spectroscopy, antibacterial effects.

## INTRODUCTION

Essential oils are used for medicinal drugs, controlling harmful insects due to their antimicrobial and antifungal activities. Production of bioactive chemical compounds that escape into the environment occurs widely in natural plant communities. In continuation to our contribution to the valorization of natural medicinal plants. Braham et al. (2008), Hammami et al. (2009), the present work is dealing with the chemical and biological studies of *Limoniastrum guyonianum* Bois growing in Tunisia. It is a plant covered with concretions limestone's, of 20 to 40 cm of height, rower, in raised twigs, in linear, semi-cylindrical sheets (leaves), of 30 to 50 mm, (Floc'h, 1983). In south of Tunisia, the herb tea from leaves, branches and galls of *L. guyonianum* Bois has been used as a domestic folk medicine for the remedy of dysentery.

The importance of roots decoction is considerable owing to its applications as depurative, galls extracts are used for tanning leathers (Fintelmann and Weiss, 2004). During this study, hydrodistillation using cleverger type apparatus has been applied to different organs from *L. guyonianum* Bois collected in Monastir region (Tunisia) and chemical analysis has been performed for the first time using gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectroscopy (GC-MS) experiments. Antimicrobial effects of four samples have been evaluated against five bacteria species. Flowers volatile fraction was the most active against *Staphylococcus epidermidis* NCIMB 8853.

## MATERIALS AND METHODS

### Plant materials

*L. guyonianum* (Plumbaginaceae) was collected from Falaise of

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Monastir, Tunisia in March 2005. It was identified by Dr. F. Harzallah-Skhiri (Institute Supérieur de Biothechnologie de Monastir) and voucher specimens were deposited in Natural Substances and organic Synthesis Laboratory, Faculty of Sciences of Monastir.

### Volatile fractions preparation

Different organs from *L. guyonianum* (fresh flowers 246 g, leaves 351 g, stems 880 g and roots 432 g) were submitted successively to hydrodistillation in a Clevenger-type apparatus during five hours. Volatile fractions were collected and stored in a refrigerator at 4°C before analysis.

### GC-FID

Analysis has been investigated using a gas chromatograph HP 5890-series II equipped with: Flame ionisation detector (FID), HP-5 (5% phenyl 195% dimethylsiloxane) 30 m x 0.25 mm, 0.52 µm film thickness fused silica capillary column. The carrier gas was nitrogen (1.2 ml<sup>-1</sup>.min). The oven temperature programming was 1 min isothermal at 50°C, then 50 to 280°C at a rate of 5°C/min and held isothermal for 1 min. The injection port temperature was 250°C, detector 280°C. The volume injected is 0.1 µl of a 1% solution (diluted in hexane). Percentages of the constituents were calculated by electronic integration of FID peak areas without the use of response factor correction.

### GC/MS

Analysis of the volatile compounds was run on a Hewlett-Pakard GC/MS system (GC: 5890 series II, MSD 5972). The fused-silica HP-Innowax capillary column (30 m x 0.25 mm with a film thickness of 0.25 µm) was directly coupled to the MS. Helium was the carrier gas having a flow rate of 1.2 ml<sup>-1</sup>.min. Oven temperature was programmed as follows (50°C for 1 min, 50 to 280°C at 5°C/min) and subsequently, held isothermal for 20 min. Injector port is 0.1 µl of a 1% solution (diluted in hexane). Mass spectrometer: HP5972 recording at 70 eV, the scan time is 1.5 s, mass range is 40 to 300 amu. Software adopted to handle mass spectra and chromatograms was a Chemstation.

### Identification of volatile compounds

Different constituents from the indicated oils have been investigated by comparison of their mass spectra with those of computer library (Wiley 275 library). Further confirmation was done from retention index data generated from a series of alkanes retention indices (relative to C<sub>9</sub>-C<sub>28</sub>) (Adams, 1995).

### Biological tests

#### Antibacterial assay, disc diffusion method

Antibacterial activity of the volatile fractions was screened against five pathogenic bacteria (Table 2). The inhibitory effect on bacterial growth was determined using agar-disc diffusion assay (Boussaada et al., 2008; Bagamboula and Uyttendaele, 2004). The bacterial cultures were first grown on Muller Hinton agar (MH) plates at 37°C for 18 to 24 h prior to seeding onto the nutrient agar. One or several colonies of the respective bacteria were transferred into API suspension medium (bioMerieux) and adjusted to 0.5 McFarland turbidity standards with a Densimat (bioMerieux) (Saïdana et al.,

2008). The inoculate of respective bacteria were streaked into MH agar plates using a sterile swab and were then dried at 37°C during 15 min. A sterile filter disc having 6 mm of diameter was placed on the MH agar surface and 7 µl of the essential oil was dropped onto each Whatman paper disc, (Bel et al., 2008). The treated Petri dishes were incubated at 37°C for 18 to 24 h. Antibacterial activity was evaluated by measuring the clear zone surrounding the Whatman paper.

#### Antibacterial assay, dilution method

The minimal inhibitory concentrations (MIC) of volatile fractions were determined using Muller Hinton broth (MHB) dilution method (Fernandes et al., 2007). All tests were performed in MHB supplemented with DMSO 5%, (Lavallée et al., 2010). Bacterial strains were cultured overnight in MHB at 37°C. Tubes of MHB containing various concentrations of oils were inoculated with 10 µl bacterial inoculums and adjusted to 10<sup>6</sup> to 10<sup>7</sup> CFU/ml. They were incubated under shaking conditions (120 rpm) at 37°C for 24 h, (Saïdana et al., 2008; Boussaada et al., 2008). Control tubes without tested samples were prepared. The essays were performed in triplicate. MIC is defined as the lowest concentration preventing visible growth (Hammer et al., 1999; Delaquis et al., 2002). To determine minimal bactericidal concentrations values (MBC), (Fernandes et al., 2007), a lapful 10 µl of tested samples were streaked on the surface of TSA agar medium discs in order to visualize no bacterial growth. The discs were incubated at 37°C during 18 to 24 h (Canillac and Mourey, 2002). MBC is defined as the concentration to which 99.9% or more, the initial inoculums are killed (Fernandes et al., 2007; Canillac and Mourey, 2002).

## RESULTS AND DISCUSSION

Volatiles from roots, seeds, leaves and flowers of *L. guyonianum* Bois were obtained after hydrodistillation in a Clevenger-type apparatus with 1.4.10<sup>-2</sup>, 1.10<sup>-2</sup>, 2.5.10<sup>-2</sup> and 2.8.10<sup>-2</sup>% yields, respectively. Chemical analysis of the volatiles from different organs, was performed by GC-FID and GC-MS. Composition of the oils can be seen in Table 1. Thirty five constituents were identified in roots essential oil representing 87.28% of the total volatiles, 25.67% from which are terpenoids. The chemical classes represented in roots oil were as follows: 2.43% amines, 2.78% alcohols, 13.33% ketones, 29.84% esters, 1.41% ethers, 21.71% aldehydes, 4.73% sulphur derivatives, 3.11% fatty acids, 2.3% anhydrides and 5.64% of phenolic derivatives. (3Z) hex-3-enylmethanoate has been identified as the chemotype of the oil. On the other hand, thirty one components were identified in seeds essential oil, this fraction was characterized by a high content of non terpenic compounds 78.34% containing mainly esters and aldehydes (31.52 and 18.64%, respectively).

Furfural was the most abundant constituent in seeds volatile fraction. Esters were found in almost comparative levels in leaves and flowers essential oils (40.74 and 41.68%, respectively). Terpenes represented 36.4% of the constituents of leaves oil and 21.04% of those from flowers. Methyl-2,4-dimethylbenzoate (14.70%) and 3-phenylprop-2-enylpentanoate (15.05%) were the major

**Table 2.** Antimicrobial effects of *Limoniastrum guyonianum* oils at a concentration of 20 mg.mL<sup>-1</sup>.

| Bacteria/ samples of oils                    | Roots | Seeds | Leaves | Flowers |
|--|-------|-------|--------|---------|
| <i>Escherichia coli</i> ATCC 35218           | -     | -     | -      | -       |
| <i>Pseudomonas aeruginosa</i> ATCC 27853     | -     | -     | -      | -       |
| <i>Micrococcus luteus</i> NCIMB 8166         | 6     | 6     | 6      | 6       |
| <i>Staphylococcus epidermidis</i> NCIMB 8853 | -     | -     | -      | -       |
| <i>Staphylococcus aureus</i> ATCC 29213      | -     | 7     | 7      | 8       |

**Table 1.** Chemical composition (%) of *L. guyonianum* roots, stems, leaves and flowers oils.

| No | Compounds   | RI polar | Roots (%) | Stems (%) | Leaves (%) | Flowers (%) | Identification |
|----|---|----------|-----------|-----------|------------|-------------|----------------|
| 1  | 2-methylprp-2-enal  | 973      | 8.54      | -         | -          | -           | MS.RI          |
| 2  | Methyl-2-methylbutanoate                                  | 1007     | 1.08      | 2.93      | -          | -           | MS.RI          |
| 3  | Hexan-3-one   | 1049     | 1.68      | 1.26      | -          | -           | MS.RI          |
| 4  | ethylbenzen   | 1252     | -         | -         | -          | 2.11        | MS.RI          |
| 5  | (3Z) hex-3-enylmethanoate                                 | 1254     | 9.18      | -         | -          | -           | MS.RI          |
| 6  | 2-methylbutyl-2-methylbutanoate                           | 1285     | 3.15      | -         | -          | -           | MS.RI          |
| 7  | 2-methylbutyl-3-methylbutanoate                           | 1296     | 2.56      | -         | -          | -           | MS.RI          |
| 8  | 4,8-dimethylnona-1,3,7-trien                              | 1297     | -         | 3.08      | -          | -           | MS.RI          |
| 9  | (3E)-Hex-3-enylethanoate                                  | 1311     | -         | -         | -          | 1.30        | MS.RI          |
| 10 | 2,3-dimethyl-1,4-diazin                                   | 1318     | 2.43      | -         | -          | -           | MS.RI          |
| 11 | Nonan-3-one   | 1354     | -         | 2.01      | -          | -           | MS.RI          |
| 12 | 2,6-dimethylhept-5-enal                                   | 1356     | 3.79      | -         | 1.82       | -           | MS.RI          |
| 13 | butylhexanoate  | 1408     | 2.07      | -         | -          | 1.82        | MS.RI          |
| 14 | Hexyl-2-methylbutanoate                                   | 1424     | 1.71      | -         | -          | -           | MS.RI          |
| 15 | ethyloctanoate  | 1425     | -         | 1.18      | -          | -           | MS.RI          |
| 16 | Prop-2-enyldisulfide                                      | 1435     | -         | 3.4       | -          | -           | MS.RI          |
| 17 | 1-(methylthio)-ethylprop-2-enyldisulfide                  | 1437     | 1.73      | -         | -          | -           | MS.RI          |
| 18 | furfural  | 1462     | -         | 14.63     | 1.15       | -           | MS.RI          |
| 19 | (2E) hex-2-enylbutanoate                                  | 1465     | 1.18      | -         | -          | -           | MS.RI          |
| 20 | 2-isopropyl-5-methylcyclohexanone                         | 1477     | -         | 3.04      | -          | -           | MS.RI          |
| 21 | di-prop-2-enyldisulfide                                   | 1489     | -         | 1.04      | -          | -           | MS.RI          |
| 22 | 1-methyl-3-propyltrisulfide                               | 1494     | 1.47      | 2.00      | -          | -           | MS.RI          |
| 23 | 5-methyl-2-(1-methyl-1-enyl)cyclohexylethanoate           | 1585     | -         | -         | 3.43       | -           | MS.RI          |
| 24 | 3-isopropyl-2,6-dimethylphenol                            | 1586     | -         | -         | -          | 8.17        | MS.RI          |
| 25 | 5-methyl-2-(1-methyleth-1-enyl) cyclohexanone             | 1588     | 1.76      | -         | -          | -           | MS.RI          |
| 26 | 2-methyl-5-(1-methyleth-1-enyl) cyclohexanone             | 1614     | -         | -         | -          | 2.00        | MS.RI          |
| 27 | 5-isopropylbicyclo [3.1.0]hexan-2-one                     | 1627     | 1.57      | -         | -          | -           | MS.RI          |
| 28 | benzylformate   | 1674     | -         | 1.31      | -          | -           | MS.RI          |
| 29 | 1,7,7-trimethylbicyclo[2.2.1]heptan-2-ylpropanoate        | 1675     | 1.34      | -         | -          | -           | MS.RI          |
| 30 | (3Z) Non-3-en-1-ol  | 1687     | 1.36      | -         | -          | -           | MS.RI          |
| 31 | benzylethanoate   | 1714     | -         | 1.1       | 3.65       | -           | MS.RI          |
| 32 | 3,4-dimethylfuran-2,5-dione                               | 1730     | 2.3       | 5.17      | -          | -           | MS.RI          |
| 33 | (2E, 4Z) deca-2,4-dienal                                  | 1744     | 8.31      | -         | -          | -           | MS.RI          |
| 34 | 2-(1-hydroxy-1-methylethyl)-5-methylcyclohexanone         | 1769     | 1.00      | -         | -          | -           | MS.RI          |
| 35 | Benzyl-2-methylpropanoate                                 | 1771     | -         | 6.2       | -          | 1.37        | MS.RI          |
| 36 | 3,7-dimethyloct-6-enylbutanoate                           | 1786     | 1.00      | 2.12      | -          | -           | MS.RI          |
| 37 | (2E)-3,7-dimethylocta-2,6-dienyl-2-methylpropanoate       | 1788     | -         | -         | 2.59       | -           | MS.RI          |
| 38 | hexyloctanoate  | 1802     | -         | -         | -          | 1.01        | MS.RI          |
| 39 | 2-methyl-5-(1-methyleth-1-enyl) cyclohex-2-enol (carveol) | 1804     | 1.42      | 1.55      | -          | -           | MS.RI          |

Table 1. Contd.

| No | Compounds   | RI polar | Roots (%) | Stems (%) | Leaves (%) | Flowers (%) | Identification |
|----|---|----------|-----------|-----------|------------|-------------|----------------|
| 40 | 1,2-dimethoxy-4-methylbenzene                           | 1805     | -         | -         | 5.40       | -           | MS.RI          |
| 41 | 2-methyl-5-(1-methyleth-1-enyl)cyclohex-2-enylethanoate | 1814     | -         | -         | -          | 2.12        | MS.RI          |
| 42 | Ethyl-2-hydroxybenzoate                                 | 1816     | 2.36      | 2.95      | -          | -           | MS.RI          |
| 43 | Methyl-2,4-dimethylbenzoate                             | 1821     | -         | -         | 14.70      | -           | MS.RI          |
| 44 | 2-hydroxy-3-methylcyclopent-2-enone                     | 1835     | -         | 2.94      | -          | -           | MS.RI          |
| 45 | 2-acetyl-5-methylol                                     | 1845     | -         | 1.54      | -          | -           | MS.RI          |
| 46 | Dihydro- $\beta$ -ionone                                | 1852     | 4.81      | -         | -          | -           | MS.RI          |
| 47 | 2-phenylethyl-2-methylpropanoate                        | 1855     | -         | 5.03      | 4.43       | 1.04        | MS.RI          |
| 48 | Prop-2-enylpropylsulfide                                | 1866     | 1.53      | -         | -          | -           | MS.RI          |
| 49 | 3-isopropyliden-4a,5-dimethyldecahydronaphtalene        | 1867     | -         | -         | -          | 3.15        | MS.RI          |
| 50 | 1-(7,7-dimethylbicyclo [3.1.1] heptanyl)methanol        | 1871     | -         | 1.02      | 8.12       | -           | MS.RI          |
| 51 | (2Z)-3-phenylprop-2-enal                                | 1889     | -         | 1.17      | -          | -           | MS.RI          |
| 52 | 2-(4'-methylcyclohex-3'-enyl)propan-1-ol                | 1905     | -         | 1.02      | 1.26       | 1.16        | MS.RI          |
| 53 | 4,7-dimethyl-1-(1'-methylethyl)-1,2-dihydronaphtalene   | 1917     | -         | -         | 1.05       | -           | MS.RI          |
| 54 | 3,7-dimethylocta-2,6-dienyl-3-methylbutanoate           | 1918     | 2.04      | -         | -          | -           | MS.RI          |
| 55 | 2-ethylhexanoic acid                                    | 1953     | 1.07      | -         | -          | -           | MS.RI          |
| 56 | (2E) 3,7-dimethylocta-2,6-dienylpentanoate              | 1965     | -         | -         | 5.11       | 2.66        | MS.RI          |
| 57 | 3-hydroxy-2-methyl-4H-pyran-4-one                       | 1966     | -         | 1.11      | -          | -           | MS.RI          |
| 58 | pentadecanal  | 2002     | 1.07      | -         | -          | -           | MS.RI          |
| 59 | 1-(1,5-dimethylhex-4-enyl)-4-methylcyclohex-3-enol      | 2014     | -         | -         | 2.74       | -           | MS.RI          |
| 60 | (2E)-3-phenylprop-2-enal                                | 2019     | -         | -         | -          | 1.69        | MS.RI          |
| 61 | 2-phenylethylpentanoate                                 | 2037     | -         | -         | 1.37       | -           | MS.RI          |
| 62 | Pentyl-2-hydroxybenzoate                                | 2075     | -         | -         | -          | 4.53        | MS.RI          |
| 63 | Hexadecane-2-one  | 2091     | 2.51      | 1.1       | -          | -           | MS.RI          |
| 64 | 1,2,4-trimethoxybenzene                                 | 2099     | -         | -         | -          | 1.65        | MS.RI          |
| 65 | (3Z) hex-3-enylbenzoate                                 | 2106     | -         | -         | -          | 4.98        | MS.RI          |
| 66 | 1-(4-methoxyphenyl)ethanone                             | 2113     | -         | -         | -          | 1.35        | MS.RI          |
| 67 | Oct-7-enoic acid  | 2150     | -         | -         | -          | 2.36        | MS.RI          |
| 68 | (2E) hex-2-enylbenzoate                                 | 2168     | 1.06      | 2.65      | 1.90       | 2.91        | MS.RI          |
| 69 | 4-isopropylphenol                                       | 2177     | -         | -         | -          | 1.39        | MS.RI          |
| 70 | 2-methoxy-4-(prop-1-enyl)phenol                         | 2184     | 2.30      | -         | -          | -           | MS.RI          |
| 71 | Decahydro-6-isopropyl-4,8a-dimethylnapht-1-ol           | 2216     | -         | -         | -          | 1.10        | MS.RI          |
| 71 | (3Z) hex-3-enyl-2-hydroxybenzoate                       | 2232     | -         | 1.59      | -          | -           | MS.RI          |
| 73 | 2-methoxy-4-(prop-2-enyl)phenylethanoate                | 2260     | -         | -         | -          | 1.94        | MS.RI          |
| 74 | (2E) 3-phenylprop-2-en-1-ol                             | 2264     | -         | -         | 9.62       | -           | MS.RI          |
| 75 | 2-formyl-1,1,5-trimethylcyclohexa-2,4-dien-6-ol         | 2293     | -         | -         | 2.13       | 1.82        | MS.RI          |
| 76 | (Z) 3,7-dimethylocta-2,6-dienoic acid                   | 2298     | 2.04      | -         | -          | -           | MS.RI          |
| 77 | (2E) 3,7-dimethylocta-2,6-dienoic acid                  | 2312     | -         | 1.72      | -          | -           | MS.RI          |
| 78 | 2-(4-methoxyphenyl) ethanol                             | 2342     | -         | -         | 1.33       | -           | MS.RI          |
| 79 | 3-phenylprop-2-enylpentanoate                           | 2347     | -         | -         | -          | 15.05       | MS.RI          |
| 80 | 2,3-(1',2'-dimethoxy-3'-allyl)benzyliden-1,4-dioxolane  | 2369     | -         | -         | -          | 1.17        | MS.RI          |
| 81 | Benzoic acid  | 2424     | -         | 1.44      | -          | 1.01        | MS.RI          |
| 82 | benzophenone  | 2466     | -         | -         | -          | 7.10        | MS.RI          |
| 83 | (9Z) Ethyloctadec-9-enoate                              | 2472     | -         | -         | -          | 1.89        | MS.RI          |
| 84 | 4-(prop-2-enyl)-2-dimethoxyphenol                       | 2493     | -         | -         | -          | 1.43        | MS.RI          |
| 85 | 5-hydroxymethylfurfural                                 | 2515     | -         | 2.84      | -          | -           | MS.RI          |
| 86 | (9Z,12Z,15Z) ethyloctadeca-9,12,15-trienoate            | 2614     | -         | -         | -          | 1.97        | MS.RI          |

constituents of leaves and flowers volatile fractions, respectively. Comparative study of the oils from *L.*

*guyonianum* organs was performed by GC-FID and GC-MS, differences in composition were detected, in the

**Table 3.** Antibacterial effects of *L. guyonianum* oils - dilution method.

|                                  | Roots oil                                   |   | Seeds oil                                   |   | Leaves oil                                  |   | Flowers oil                                 |   |
|----------------------------------|---|---|---|---|---|---|---|---|
|                                  | MIC<br>( $\mu\text{g}\cdot\text{ml}^{-1}$ ) | MBC<br>( $\mu\text{g}\cdot\text{ml}^{-1}$ ) | MIC<br>( $\mu\text{g}\cdot\text{ml}^{-1}$ ) | MBC<br>( $\mu\text{g}\cdot\text{ml}^{-1}$ ) | MIC<br>( $\mu\text{g}\cdot\text{ml}^{-1}$ ) | MBC<br>( $\mu\text{g}\cdot\text{ml}^{-1}$ ) | MIC<br>( $\mu\text{g}\cdot\text{ml}^{-1}$ ) | MBC<br>( $\mu\text{g}\cdot\text{ml}^{-1}$ ) |
| <i>E. coli</i> ATCC 35218        | 20  | -   | 20  | -   | 20  | -   | 20  | -   |
| <i>M. luteus</i> NCIMB 8166      | 20  | -   | 20  | -   | 20  | -   | 20  | -   |
| <i>S. epidermidis</i> NCIMB 8853 | 20  | -   | 20  | -   | 20  | -   | 20  | -   |
| <i>S. aureus</i> ATCC 29213      | 20  | -   | 20  | -   | 20  | -   | 20  | -   |

other hand, 2-(4-methylcyclohex-3-enyl) propanol has been identified as common monoterpenol constituent of roots, leaves and flowers (Table 3). In general, it was found that proportions of sesquiterpenes increased in the order stems (completely absent), roots (3%), flowers (7%) and leaves (10%), whereas the proportions of monoterpenes decreased from leaves to roots, flowers and stems (26, 23, 10 and 6%). Additionally it was noted that non terpenic derivatives were present in large amount in stems (79%), flowers (65%), roots (61%) and leaves (47%), respectively.

Biological assays showed that *L. guyonianum* prevented visible growth of all tested bacteria at a lower concentration ( $\text{MIC}=0.02 \text{ mg}\cdot\text{ml}^{-1}$ ). This may be attributed to the abundance of esters and aldehydes. In fact, aldehydes are known in literature for their significant antibacterial effects (Chang and Chan, 2001). However, in many cases, the whole volatile fraction is shown to be more active than its major constituents, this suggested that the contribution of the less abundant components should be considered as well as the synergistic effect between all components, (Gill et al., 2002). As an example, furfural carboxaldehyde is the chemotype of stems essential oil (14.63%), this compound is cited in literature to have antimicrobial effects, the same aldehyde is less abundant in leaves (1.15%) and quite absent in roots and flowers active volatile fractions. In addition, we noticed that the results of both disc diffusion and dilution tests for the antimicrobial effects are slightly different. The MIC appears to be lower than the concentration that inhibits bacterial growth in disc diffusion method. This may be explained by the fact that the size of the inhibition zone does not reflect the real antibacterial effectiveness of any compound. This depends and is affected by the solubility of the oil and the diffusion range in the agar. Comparing to other plants, *L. guyonianum* volatile fractions having an MIC equal to  $0.02 \text{ mg}\cdot\text{ml}^{-1}$  are more active than *Limonium echioïdes* and *Suaeda fruticosa* belonging to the same Plumbaginaceae family and having an MIC of  $0.5 \text{ mg}\cdot\text{ml}^{-1}$ , (Saïdana et al., 2008).

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