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Chemical Composition of Essential Oils from Leaves-stems, Flowers and Roots of *Inula graveolens* from Tunisia

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Abstract: This study lies within the scope of the valorisation of medicinal and aromatic plants of the Tunisian flora in the aim to find novel bioactive natural products. The essential oil constituents from the aerial part without flowers (stems and leaves), flowers and roots of *Inula graveolens* (L.) Desf., syn. *Dittrichia graveolens* (Desf.) Greuter, gathered in the area of Monastir (Tunisia) have been studied by GC-FID and GC-MS. Remarkable differences were found between the constituent percentages of the different studied organs. The most important compounds from the aerial part without flowers were τ -cadinol (9.2%), borneol (21.4%), bornyl acetate (33.4%). In the flowers oil, the main components found were Camphene (5.5%), τ -cadinol (11.3%), borneol (19.3%), bornyl acetate (39.6%). The major constituents in the oil roots were found to be carvone (5.0%), bornyl acetate (5.3%), p-mentha-1(7), 2-dien-8-ol (5.3%), β -selinene (11.5%) and a non identified compound (n°85, 22.2%). A comparison of these data with previous results reported in the literature showed surprising differences.

Key words: *Inula graveolens*, different organs, essential oil, hydrodistillation, GC-FID, GC-MS

INTRODUCTION

The genus *Inula* L. belongs to the Asteraceae family. Pottier-Alapetite^[1], considered for this genus four species occurring in Tunisia: *Inula viscosa* (L.) Ait, *I. graveolens* (L.) Desf., *I. crithmoides* L. and *I. montana* L. Var. *calycina* (Presl) Batt.

Inula graveolens, syn. *Dittrichia graveolens* (Desf.) Greuter, is one of the most conspicuous plants of late summer flowering in large profusion from September to December. It grows along road sides, on waste grounds, in humid soil and near subsalt lands. It is distributed in the Eastern part of the country and some scattered places in the north east, but never can it be found in the south. *Inula graveolens* is an annual plant that grows about 80 cm high. The stems are covered with small, soft hair, the leaves are oval and pointed, the flowers are small, yellow and have the smell of camphor. The roots are fleshy. The essential oil is known as the most effective oil for loosening mucous and deep congestion. It is also useful for acute and chronic respiratory conditions such as coughs, colds, sinusitis (sinus infections) laryngitis and

bronchitis. It supports lymphatic circulation as well as the immune system, it also reduces acneic skin inflammation^[2]. Le Floc'h^[3] and Bouket^[4] have not reported about its possible use in popular medicine in Tunisia. Several *Inula* species have been used in various treatments; for calculus, for eye, as diuretic, poison and sudorific^[5].

The essential oil composition of some species of this genus has been previously reported^[6-8]. Previous studies on *I. graveolens* revealed that studies on the essential oil composition of the aerial parts are very scarce. Most have been carried out on dried material. There are no studies on the composition of the essential oil of the roots. Nevertheless, different aerial part extracts have been chemically investigated and several sesquiterpene lactones^[9], benzoic acid derivatives and other compounds^[10, 11], were reported. Only one work has been done on *I. graveolens* from Tunisia and 17 constituents were identified in the aerial part essential oil showing, in mixture, antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*^[12].

The oil of aerial parts of *I. graveolens* growing in Corsica^[13], showed the occurrence of 13 main components

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from which bornyl acetate and borneol (65.8-75.6%) were always the two major compounds. Camphene, limonene, α -terpineol, 1, 5 and 1(7), 2-*p*-menthadienols were present at appreciable contents. (*E*)-caryophyllene and (*E*)-caryophyllene epoxide, caryophylladienol as well as τ -cadinol were the most important sesquiterpenes. The same authors showed that proportion of the compounds bornyl acetate, borneol and τ -cadinol changes throughout the life of the plant. Nevertheless, some differences were reported between oil compounds proportion of several plants collected in a restricted area. Marongiu *et al.*^[14] reported that the hydrodistilled essential oil of *I. graveolens* aerial parts was mainly made up of selin-11-en-4- α -ol (14.1%), 1,10-di-*epi*-cubenol (10.3%), cedr-8(15)-en-9- α -ol (10.3%), caryophyllene oxide (6.2%) and nerolidol acetate (5.9%). This study reports the composition of the essential oils obtained by hydrodistillation from the whole stems and leaves, flowers and roots of *I. graveolens*. It also discusses the differences between the three compositions.

MATERIALS AND METHODS

Plant material: *Imula graveolens* was identified according to the flora of Tunisia^[1]. A specimen was collected, dried and a voucher specimen was deposited at the herbarium (Asteraceae, n°23) of the Ecole Supérieure d'Horticulture et d'Élevage de Chott-Meriem, Université du Centre, Sousse, Tunisia. Plants were gathered at the flowering stage in November 2004 in the area of Monastir (latitude 35°46'0" N, longitude 10°59' 0" E, coastal region, east of Tunisia, with a sub-humid climate). The fresh plant was separated in three parts: stems and leaves (SL), flowers (F) and roots (R). Each part was divided in little peaces and weighted before extraction of essential oils.

Extraction of essential oils: Hydrodistillation of the fresh material was performed in a Clevenger-type apparatus for 4 h up to the point at which the oil contained in the matrix was exhausted. The oil obtained was collected and dried over anhydrous sodium sulphate and stored in sealed glass vials in a refrigerator at 4-5°C prior to analysis. Yield based on fresh weight of the sample was calculated.

Analyses of the essential oil

Gas Chromatograph: HP 5890-series II equipped with: Flame ionization detectors (FID), HP-5 (BP-1) (5% phenyl+95% dimethylpolysiloxane) 30 m x 0.25 mm ID, 0.25 μ m film thickness fused capillary column and HP Innowax (BP-20) (polyethylenglycol) 30 m x 0.25 mm ID, 0.25 μ m film thickness fused capillary column. The carrier

gas was nitrogen (1.2 mL min⁻¹). The oven temperature program was 1 min isothermal at 50°C, then 50-280°C at rate of 5°C/min and held isothermal for 1 min. The injection port temperature was 250°C, detector 280°C. Volume injected: 0.1 μ L of 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: The analyses of the volatile constituents were run on a Hewlett-Packard GC-MS system (GC: 5890 series II; MSD 5972). The fused-silica HP-5 MS capillary column (30 mx0.25 mm ID, film thickness of 0.25 μ m) was directly coupled to the MS. The carrier gas was helium, with a flow rate of 1.2 mL min⁻¹. Oven temperature was programmed (50°C for 1 min, then 50-280°C at 5°C/min) and subsequently, held isothermal for 20 min. Injector port: 250°C, detector: 280°C, split ratio 1:50. Volume injected: 0.1 μ L of 1% solution (diluted in hexane).

Mass spectrometer: HP5972 recording at 70 eV; scan time 1.5 sec; mass Range 40-300 amu. Software adopted to handle mass spectra and chromatograms was a ChemStation.

Identification of the compounds: The components of the oils were identified by comparison of their mass spectra with those of a computer library (Wiley 275 library). Further confirmation was done by referring to Kováts Index data generated from a series of alkanes (C₉-C₂₆)^[15,16].

RESULTS AND DISCUSSION

I. graveolens do not yield the same amount of oil from the different parts. The total yield of essential oil obtained from (SL), (F) and (R) was 0.07, 0.17 and 0.06 (w/%), respectively. The three oils were light yellow, liquid at room temperature, their odours are agreeable. The composition of the volatile oils extracted by hydrodistillation from the different parts of the plant is reported in Table 1 together with the Kováts' Indices (KI) calculated for each compound, the percentage composition and the identification methods. The constituents are arranged according to their elution on the polar BP-20 capillary column. A total of 88 constituents were found from which 5 are Not Identified (NI).

Oils obtained from (SL) and (F) were characterized by the predominance of oxygenated monoterpenes in comparison with the rest of the constituents (Fig. 1). On the contrary, oil obtained from roots (R) was riche in oxygenated sesquiterpenes and sesquiterpene hydrocarbons (Fig. 1). Monoterpene hydrocarbons and

Table 1: Percentage composition of the essential oils from different parts of *I. graveolens*

| N° | Compound | KI (BP-1) | KI (BP-20) | Percentage | | | Identification |
|----|----------------------------|-----------|------------|------------|------|------|----------------|
| | | | | SL | F | R | |
| 1 | Tricyclene | 926 | 1015 | 0.1 | 0.2 | 0.1 | MS, KI |
| 2 | α -Thujene | 931 | 1020 | 0.1 | 0.1 | tr | MS, KI |
| 3 | α -Pinene | 939 | 1026 | tr | tr | tr | MS, KI |
| 4 | Camphene | 944 | 1064 | 1.2 | 5.5 | 0.2 | MS, KI |
| 5 | Cis-dihydroocimene | 982 | 1088 | - | 0.1 | - | MS, KI |
| 6 | β -Pinene | 976 | 1107 | 0.2 | 1.0 | 0.1 | MS, KI |
| 7 | δ -3-Carene | 1010 | 1148 | tr | tr | tr | MS, KI |
| 8 | Myrcene | 991 | 1158 | tr | tr | tr | MS, KI |
| 9 | α -Phellandrene | 1002 | 1160 | 0.1 | 0.1 | 0.1 | MS, KI |
| 10 | α -Terpinene | 1015 | 1176 | 0.1 | 0.1 | 0.2 | MS, KI |
| 11 | Dehydrocineole | 978 | 1187 | 0.2 | 0.3 | 0.1 | MS, KI |
| 12 | Limonene | 1016 | 1196 | tr | tr | tr | MS, KI |
| 13 | β -Phellandrene | 1020 | 1206 | tr | tr | 0.1 | MS, KI |
| 14 | γ -Terpinene | 1057 | 1239 | 0.1 | tr | tr | MS, KI |
| 15 | (Z)- β -Ocimene | 1035 | 1244 | tr | tr | tr | MS, KI |
| 16 | p-Cymene | 1011 | 1265 | 0.1 | tr | tr | MS, KI |
| 17 | Terpinolene | 1078 | 1276 | tr | tr | 0.1 | MS, KI |
| 18 | Tridecane | 1300 | 1300 | tr | 0.1 | 0.1 | MS, KI |
| 19 | Tetradecane | 1400 | 1400 | tr | tr | tr | MS, KI |
| 20 | p-Cymenene | 1071 | 1430 | 0.1 | 0.1 | 0.1 | MS, KI |
| 21 | α -Copaene | 1378 | 1484 | tr | tr | 0.5 | MS, KI |
| 22 | Artemisia alcohol | 1066 | 1495 | 0.1 | 0.1 | 0.1 | MS, KI |
| 23 | Clovene | 1376 | 1496 | 0.1 | 0.1 | tr | MS, KI |
| 24 | Camphor | 1120 | 1508 | 0.1 | 0.1 | 0.1 | MS, KI |
| 25 | β -Bourbobene | 1380 | 1513 | 0.1 | 0.1 | tr | MS, KI |
| 26 | Linalool | 1081 | 1535 | 0.1 | tr | 0.3 | MS, KI |
| 27 | β -Caryophyllene | 1420 | 1567 | 0.3 | 0.4 | 2.4 | MS, KI |
| 28 | Fenchol | 1098 | 1571 | 0.1 | 0.1 | 0.5 | MS, KI |
| 29 | Bornyl acetate | 1272 | 1578 | 33.4 | 39.6 | 5.3 | MS, KI |
| 30 | (E)-Caryophyllene | 1419 | 1588 | 0.3 | 0.3 | 0.4 | MS, KI |
| 31 | Terpinen-4-ol | 1161 | 1590 | 1.7 | 0.9 | 1.1 | MS, KI |
| 32 | Camphene hydrate | 1133 | 1596 | 0.1 | tr | tr | MS, KI |
| 33 | Hexadecane | 1600 | 1600 | 0.2 | 0.2 | tr | MS, KI |
| 34 | Allo-aromadendrene | 1459 | 1633 | 0.5 | 0.4 | tr | MS, KI |
| 35 | (E)-2-Decanal | 1236 | 1634 | 0.1 | tr | 0.7 | MS, KI |
| 36 | α -Humulene | 1457 | 1658 | 0.1 | 0.1 | 0.3 | MS, KI |
| 37 | γ -Muuroolene | 1471 | 1677 | 0.2 | 0.1 | 0.4 | MS, KI |
| 38 | α -Terpineol | 1171 | 1685 | 0.1 | 0.1 | tr | MS, KI |
| 39 | Borneol | 1149 | 1690 | 21.4 | 19.3 | 1.5 | MS, KI |
| 40 | Heptadecane | 1700 | 1700 | 0.1 | 0.1 | 0.4 | MS, KI |
| 41 | Zonarene | 1519 | 1702 | 0.4 | 0.3 | 0.3 | MS, KI |
| 42 | p-Mentha-1,5dien-8-ol | 1145 | 1708 | 0.6 | 0.4 | 2.6 | MS, KI |
| 43 | α -Muuroolene | 1492 | 1712 | 1.4 | 1.2 | 0.6 | MS, KI |
| 44 | Dihydrocarveol | 1188 | 1713 | 0.1 | 0.1 | tr | MS, KI |
| 45 | α -Bisabolene | 1498 | 1715 | 0.1 | 0.1 | 0.2 | MS, KI |
| 46 | β -Selinene | 1483 | 1718 | 0.1 | 0.3 | 11.5 | MS, KI |
| 47 | Germacrene-D | 1478 | 1721 | 0.5 | 0.2 | 4.3 | MS, KI |
| 48 | Carvone | 1215 | 1727 | 0.3 | 0.1 | 5.0 | MS, KI |
| 49 | Piperitone | 1227 | 1732 | 0.8 | 0.5 | 1.2 | MS, KI |
| 50 | δ -Cadinene | 1514 | 1747 | 2.1 | 1.3 | 0.9 | MS, KI |
| 51 | α -Amorphen | 1527 | 1752 | 0.1 | 0.1 | 2.7 | MS, KI |
| 52 | γ -Cadinene | 1507 | 1758 | 0.1 | 1.5 | 0.2 | MS, KI |
| 53 | 4-Methylacetophenone | 1154 | 1770 | 0.1 | 0.1 | 0.2 | MS, KI |
| 54 | p-Mentha-1(7), 2-dien-8-ol | 1168 | 1773 | 0.3 | tr | 5.3 | MS, KI |
| 55 | Cadina-1,4-diene | 1527 | 1776 | 0.1 | 0.1 | tr | MS, KI |
| 56 | α -Cadinene | 1530 | 1783 | 0.1 | 0.1 | tr | MS, KI |
| 57 | Cis-Calamenene | 1517 | 1827 | 0.5 | 0.3 | 0.6 | MS, KI |
| 58 | Trans-Calamenene | 1517 | 1830 | 0.1 | 0.1 | 1.3 | MS, KI |
| 59 | Geranyl acetone | 1426 | 1848 | 0.1 | tr | 0.2 | MS, KI |
| 60 | p-Cymen-8-ol | 1158 | 1852 | 0.3 | 0.2 | 0.1 | MS, KI |
| 61 | Nonadecane | 1900 | 1900 | tr | tr | 0.1 | MS, KI |
| 62 | α -Calacorene | 1539 | 1922 | 0.1 | 0.2 | 0.1 | MS, KI |
| 63 | β -Ionone | 1462 | 1933 | 0.3 | 0.4 | tr | MS, KI |
| 64 | β -Calacorene | 1548 | 1955 | 0.4 | 0.2 | tr | MS, KI |
| 65 | Caryophyllene oxide | 1572 | 1979 | 0.4 | 0.8 | 0.1 | MS, KI |

Table 1: (Continued)

| | | | | | | | |
|----|---|------|------|------|------|------|--------|
| 66 | Eicosane | 2000 | 2000 | tr | tr | tr | MS, KI |
| 67 | (E)-Nerolidol | 1544 | 2032 | 0.2 | 0.2 | tr | MS, KI |
| 68 | Heneicosane | 2100 | 2100 | 0.1 | tr | tr | MS, KI |
| 69 | 6-Eudesmen-4- α -ol | 1604 | 2167 | 0.1 | 0.1 | 1.1 | MS, KI |
| 70 | τ -Cadinol | 1628 | 2167 | 9.2 | 11.3 | 2.1 | MS, KI |
| 71 | δ -Muurolol | 1640 | 2177 | 0.7 | 0.4 | 0.3 | MS, KI |
| 72 | Thymol | 1266 | 2188 | 0.3 | 0.3 | 0.2 | MS, KI |
| 73 | Docosane | 2200 | 2200 | 0.3 | 0.2 | 0.1 | MS, KI |
| 74 | Carvacrol | 1273 | 2210 | 0.2 | 0.8 | 0.5 | MS, KI |
| 75 | α -Cadinol | 1640 | 2227 | 0.5 | 0.1 | 1.1 | MS, KI |
| 76 | Tricosane | 2300 | 2300 | 0.5 | 0.4 | 0.4 | MS, KI |
| 77 | α -Cyperone | 1729 | 2348 | 2.8 | 1.8 | 0.3 | MS, KI |
| 78 | Tetracosane | 2400 | 2400 | 1.5 | 0.4 | 0.1 | MS, KI |
| 79 | NI MW 220 (C ₁₅ H ₃₄ O) | 2215 | 2452 | 0.5 | 0.2 | 4.8 | MS, KI |
| 80 | Pentacosane | 2500 | 2500 | 1.1 | 0.9 | tr | MS, KI |
| 81 | NI MW220 (C ₁₅ H ₃₄ O) | 2248 | 2573 | 0.5 | 0.2 | 4.1 | MS, KI |
| 82 | Hexacosane | 2600 | 2600 | 0.8 | 0.3 | 0.4 | MS, KI |
| 83 | Heptacosane | 2700 | 2700 | 0.6 | 0.2 | tr | MS, KI |
| 84 | Octacosane | 2800 | 2800 | 0.4 | 0.1 | tr | MS, KI |
| 85 | NI MW 232 (C ₁₅ H ₃₀ O ₂) | 2358 | 2812 | 3.7 | 0.6 | 22.2 | MS, KI |
| 86 | NI MW 232 (C ₁₅ H ₃₀ O ₂) | 2440 | 2833 | 1.8 | 0.2 | 4.7 | MS, KI |
| 87 | 87NI MW 234 (C ₁₅ H ₃₂ O ₂) | 2580 | 2876 | 0.2 | tr | 0.1 | MS, KI |
| 88 | Nonacosane | 2900 | 2900 | 0.2 | 0.1 | 0.2 | MS, KI |
| | Monoterpene hydrocarbons | | | 2.3 | 7.2 | 1.0 | |
| | Oxygenated monoterpenes | | | 60.1 | 62.9 | 24.2 | |
| | Sesquiterpene hydrocarbons | | | 8.3 | 7.5 | 27.4 | |
| | Oxygenated sesquiterpenes | | | 20.6 | 15.9 | 41.8 | |
| | Hydrocarbons | | | 5.8 | 3.0 | 1.8 | |
| | others | | | 0.5 | 0.5 | 0.4 | |

The components and their percentages are listed in order of their elution on polar column (BP-20); tr : traces (<0.1%); (-) compound absent in the oil, SL: stems and leaves, F: Flowers; R: Roots.

Compound n° 79: EIMS *mz* (rel. intensity) 220 [M⁺] (12.0); 55 (23.8); 67 (24.3); 79 (32.8); 91 (26.4); 93 (53.2); 107 (30.4); 121 (100); 122 (22.8); 149 (27.6).

Compound n° 81: EIMS *mz* (rel. intensity) 220 [M⁺] (58.4); 55 (26.5); 67 (27.3); 77 (24.2); 79 (37.6); 91 (31.67); 93 (54.32); 107 (33.1); 121 (100); 122 (22.0); 149 (33.8).

Compound n° 85: EIMS *mz* (rel. intensity) 232 [M⁺] (15.6); 71 (45.5); 77 (30.5); 79 (16.1); 103 (18.1); 105 (36.9); 133 (31.7); 145 (28.4); 162 (100).

Compound n° 86: EIMS *mz* (rel. intensity) 232 [M⁺] (28.3); 71 (20.4); 77 (15.6); 91 (23.4); 105 (12.4); 115 (51.5); 133 (9.1); 145 (100); 146 (12.1); 162 (51.5).

Compound n° 87: EIMS *mz* (rel. intensity) 234 [M⁺] (10.4). 71 (36); 77 (5.6); 91 (6.9); 105 (4.7); 115 (7); 131 (9.3); 133 (5.3); 135 (6.9); 145 (64.2); 146 (100).

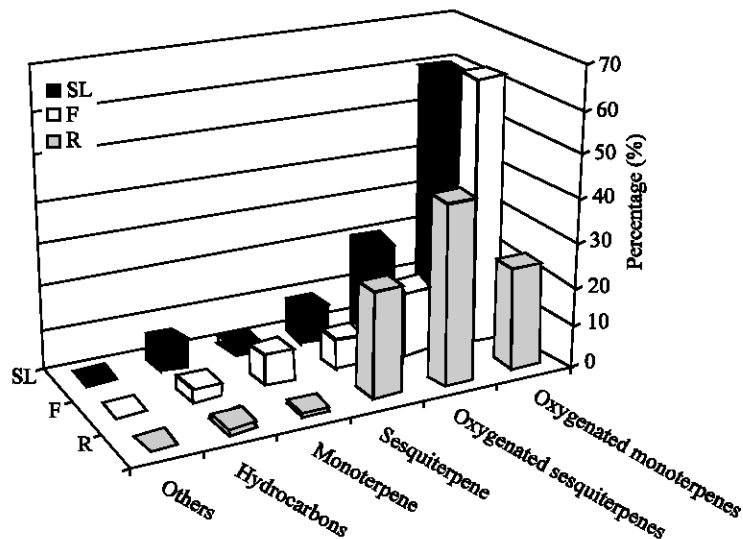


Fig. 1: Repartition of terpene compounds, hydrocarbons and others in the different organs of *Imula graveolens* (SL: Stems and leaves; F: flowers; R: roots)

hydrocarbons are not representative in all the studied organs (Fig. 1). Figure 1 also shows that the aerial parts (SL and F), are richer in oxygenated monoterpenes (60.1-62.9%), monoterpene hydrocarbons (2.3-7.2%) and hydrocarbons (5.8-3.0%) than roots. The latter contained more oxygenated sesquiterpenes (20.6-15.9%) and sesquiterpene hydrocarbons (8.3-7.5%). Oil from (SL) showed as main constituents τ -cadinol (n°70, 9.2%), borneol (n°39, 21.4%), bornyl acetate (n°29, 33.4%) (Fig. 2a). Hydrodistilled oil of flowers (F) contained mainly camphene (n°4, 5.5%), τ -cadinol (n°70, 11.3%), borneol (n°39, 19.3%), bornyl acetate (n°29, 39.6%) (Fig. 2b). The main components found in the oil obtained from roots were carvone (n°48, 5.0%), bornyl acetate (n°29, 5.3%), p-mentha-1(7), 2-dien-8-ol (n°54, 5.3%), β -selinene (n°46, 11.5%) and an unknown compound (n°85, 22.2%) (Fig. 2c).

We report differences between percentages of several constituents identified in (SL) and in (F) oils (Table 1). The latter was richer in camphene (n°4, 5.5%), bornyl acetate (n°29, 39.6%) and τ -cadinol (n°70, 11.3%) than (SL). Percentages in borneol (n°39, 21.4%), δ -cadinene (n°50, 2.1%) and compound 85 (n°85, 3.7%) were more important in the (SL) essential oil.

Roots essential oil shows great differences in the percentages of its constituents compared to those from the aerial parts without flowers (SL). It is especially richer in piperitone (n°49, 1.2%), β -caryophyllene (n°27, 2.4%), p-mentha-1, 5-dien-8-ol (n°42, 2.6%), α -amorphen (n°51, 2.7%), germacrene-D (n°47, 4.3%), carvone (n°48, 5.0%), p-mentha-1(7), 2-dien-8-ol (n°54, 5.3%), β -selinene (n°46, 11.5%) and in the unknown compounds 79, 81, 85, 86, 87, but exhibited less percentages in β -pinene (n°6, 0.1%), camphene (n°4, 0.2%), α -cyperone (n°77, 0.3%), α -muurolene (n°43, 0.6%), δ -cadinene (n°50, 0.9%), borneol (n°39, 1.5%), τ -cadinol (n°70, 2.1%) and bornyl acetate (n°29, 5.3%).

The chemical oil composition of the investigated samples exhibited significant differences with that extracted from the air-dried aerial parts of *I. graveolens* from Southern Sardinia^[14]. It was mainly constituted of selin-11-en-4- α -ol (14.1%), 1,10-di-*epi*-cubanol (10.3%), cedr-8(15)-en-9- α -ol (10.3%), nerolidol acetate (5.9%) not identified in our samples and caryophyllene oxide (6.2%). Essential oil for the same species growing in Iran shows also differences in its main constituents and their percentages^[17]. Those main constituents were borneol (60.7%), β -caryophyllene (8.3%), bornyl acetate (6.8%),

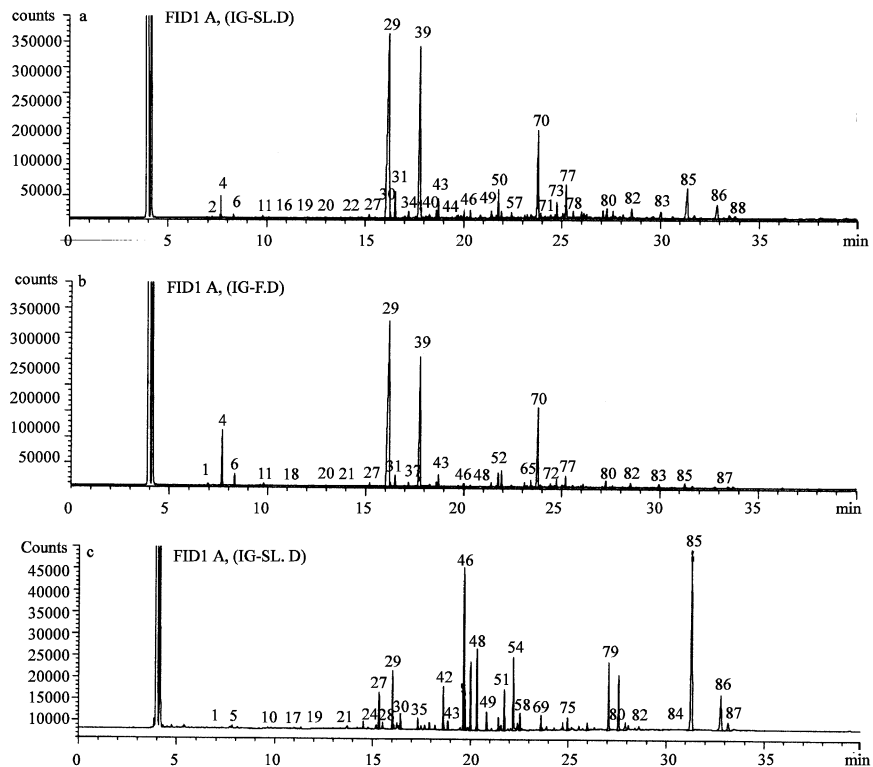


Fig. 2: Chromatograms *I. graveolens* (IG) essential oil of whole stems and leaves (SL) (a), flowers (F) (b) and roots (R) (c), run on a BP-20 capillary column

acetate (6.8%), τ -cadinol (5.2%) and caryophyllene oxide (4.3%). This comparative study shows clearly the effect of the plant origin on the composition of the corresponding essential oil. On the other hand, our results show more concordance with those given by Blanc *et al.*^[13] done on the same species gathered in Corsica and permit to confirm the occurrence of the most constituents identified in *I. graveolens* commercial oil.

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