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Yield and chemical composition of oil isolated from Algerian *Hypericum perforatum* L. as influenced by the plant habitat, harvesting date and plant organ

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The changes in volatile oil chemical composition of *Hypericum perforatum* L. from Algeria were investigated according to harvesting locality and period, as well as plant organ. Aerial parts were collected from three different regions (Algiers, Medea and Blida), from 2009 to 2014. All oils were extracted by hydrodistillation and analyzed by GC and GC/MS. The main compounds were α-pinene and 2-methyl-octane. The flower and fruit parts gave the best yield (0.30%- 0.39%) and the highest concentrations of monoterpenes (32.7% to 35.9% of isolated oil) and non-oxygenated compounds (89.6% - 90.2%), while leave oils contained the highest concentrations of sesquiterpenes (52.9% to 59.5%) and oxygenated compounds (40.5% to 48.4%). From the aerial parts, the best oil yields were obtained for Blida (2014) and Medea (2011) samples. Volatile oil isolated from aerial partsof the Blida-2014 sample was examined for its antimicrobial activity.

Keywords: *Hypericum perforatum* L., volatile oil; chemical composition; harvesting year; plant organ; plant habitat

INTRODUCTION

The genus *Hypericum* (Hypericaceae) is widely distributed through temperate regions and depicted by approximately 450 species [1]. *Hypericum perforatum* L., commonly known as St. John's wort, is used in traditional medicine since ancient times [2]. Nowadays, most of the investigations on *Hypericum perforatum* L. focused on the anti-depressant properties of the isolated substances [3], as well as on their anti-inflammatory, antimicrobial and anti-proliferative activities [4]. Research also concerned the bioactive compound classes including naphthodianthrone derivatives (hypericin and pseudohypericin), acylated phloroglucinol derivatives (hyperforin and adhyperforin), and flavonoids such as quercetin, quercitrin, hyperoside, rutin, kaempferol, biapigenin, and amentoflavone [5]. Numerous scientific studies investigated the chemical composition [6] or the changes in chemical composition of volatile oils of *Hypericum perforatum* L. extracted by hydrodistillation [7]. The influence of geographical distribution on the chemical composition of oils was also scrutinized [8, 9]. Beside this, other aspects [10-12] were examined as the phenological cycle [13] and the type of plant organ [14-16]. However, little research is reported on the effect of the seasonal variations on the chemical composition of the essential oil except Smelcerovic *et al.* [17] who investigated the changes in chemical composition of *Hypericum perforatum* L. oil extracted by steam distillation during three nonconsecutive years, namely 1998, 2001 and 2003. Moreover, *Hypericum perforatum* L. from the Mediterranean area as Turkey [18], Tunisia [19], France [20] or Greece [21] was investigated, but to the best of our knowledge, no study was reported on Algerian species apart from our recent study [22]. The present contribution concerns the influence of (a) three harvesting localities in the north of Algeria (Algiers, Blida and Medea); (b) the harvesting year between 2009 and 2014 (year per year); (c) the plant organ (whole aerial part, leaves, flowers-fruits) on the chemical composition of volatile oils extracted by hydrodistillation. This study also characterizes the antimicrobial activity. The originality of the work is the simultaneous study of the influence of harvesting period, harvesting region and the plant organ subjected to hydrodistillation on the composition of volatile oil.

EXPERIMENTAL

Plant material and chemicals

To investigate the effect of plant habitat,

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Table 1. Basic characterization of sites where *Hypericum perforatum* L. (aerial parts) was collected. All harvesting areas have a warm and temperate climate.

Locality	Blida	Algiers	Medea
Altitude(m)	260	200	486
Latitude	36°28'12" N	36°44'11" N	36°24'46" N
Longitude	2°49'39" E	3°02'31" E	2°45'14" E
Mean annual temperature (°C)	17.9	17.7	14.4

Hypericum perforatum L. was collected from three different places in north Algeria (Algiers, Blida and Medea) (Table 1). For Blida locality, samples were typically harvested every year between May 25th and June 4th (flowering period) for six consecutive years (2009-2014). The plant samples were identified by the Head of the Herbarium of the National Institute of Agronomy (INA, El-Harrach-Algiers-Algeria) and dried in a shaded room at a temperature not exceeding 25 °C. Herbal scissors were used during the harvesting process and to separate leaves and flowers-fruits.

Hydrodistillation apparatus and procedure

Conventional hydrodistillation apparatus (Clevenger-type) according to the *European Pharmacopeia* [23] was employed. A quantity of 300 g of *Hypericum perforatum* for 1.5 to 2 L of distilled water was used during 240 min hydrodistillation from the first drop of distillate until the raw material has been completely exhausted. The essential oil was collected, dried over anhydrous sodium sulfate, and stored in a dark place at 4°C for further analysis. Each extraction was performed at least three times, and a standard deviation was calculated. The extraction yield was calculated according to eq. 1.

$$\text{Extraction yield (\%)} = \left(\frac{\text{mass of collected oil}}{\text{mass of dry material}}\right) \times 100 \quad \text{(eq.1)}$$

GC and GC-MS identification

GC analysis of volatile compounds was carried out using Hewlett Packard HP6890 series II system coupled to an ionization flame detector (FID). The compounds were separated on a HP-5 capillary column (5 % phenylmethylpolysiloxane, 30 m × 0.25 mm i.d., 0.25 μm film thickness). The gas chromatographic parameters were set up as follows: initial temperature 60°C for 5 min, rate 2°C min^{-1}, final temperature 250°C, held for 10 min; injector and transfer line temperatures were set at 250°C. Nitrogen was used as the carrier gas at a flow rate of 1 mL/min; injection volume, 1 μL; split ratio, 1:20. The percent compositions were determined from electronic integration measurements using flame ionization detection (FID, 250°C). GC–MS analysis was carried out using Varian 3900 chromatograph coupled to a Saturn 2100T mass spectrometer. Samples were analyzed on a fused-silica capillary column. The non-polar column was HP-5MS (30 m × 0.25 mm × 0.25 μm film thickness). The spectra were obtained using the following conditions: carrier gas helium at a flow rate of 1 mL/min; split mode 1:20; 1 μL injection volume; injection temperature 250°C. The oven temperature program was: 60°C for 5 min, rate 2°C/min to 250°C and held for 10 min. The ionization mode used was electronic impact at 70 eV. The constituents were identified by comparison of their GC linear retention indices (RI), determined with reference to a homologous series of C_5–C_{32} n-alkanes. The identification was confirmed by computer matching against commercial libraries (Wiley and NIST) and by comparison with mass spectra from literature data [24].

Antimicrobial activity

The antimicrobial activity of the essential oil obtained by hydrodistillation of the plant sample collected at Blida in 2014 was examined by the paper disk diffusion method [25] and by determination of the minimal inhibitory concentrations (MICs) according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing [26]. The antibacterial activity of *Hypericum perforatum* L. essential oil was tested against four bacterial strains including Gram-positive bacteria: *Micrococcus luteus* ATCC 9314 (Ml), *Bacillus subtilis* ATCC 6633 (Bs), *Staphylococcus aureus* CIP 7625 (Sa) and the Gram-negative bacterium: *Klebsiella pneumoniae* (CIP 8291 (Kp1). The antifungal activity of the essential oil was investigated using two filamentous fungi: *Fusarium oxysporum albedinis* CURZA (Foa) and *Aspergillus carbonarius* M333 (Ac), and two yeast strains: *Candida albicans* (Ca) and *Candida glabrata* (Cg). All tests were performed in triplicate. The microorganisms were regenerated twice before use in the manipulations by culturing bacterial strains on Mueller-Hinton, and fungal strains on Sabouraud agar. The paper disk diffusion assay was

performed according to the protocol described by Lass-Flörl *et al.* [27] The minimal inhibitory concentrations (MICs) were determined by the conventional agar dilution method described in [27].

Statistical considerations

The data were analyzed using Statgraphics software (Centurion version). One-way analysis of variance with $p \leq 0.05$ was performed to identify significant differences among the different samples. All results presented are the mean of triplicate.

RESULTS AND DISCUSSION

Variability in oil chemical composition of Hypericum perforatum L.

Influence of the plant habitat. Samples of *Hypericum perforatum* L. aerial parts from three different regions, Blida, Algiers and Medea (Table 2), collected during 2011 season and subjected to hydrodistillation, were investigated. From Table 2, it is clear that there is a significant difference in the obtained yields and percentages of chemical classes, namely total oxygenated, total non-oxygenated monoterpenes and sesquiterpenes according to the plant habitat. The oil yields varied from 0.049 % for the Blida sample to 0.136 % for the Medea sample. These values are in agreement with those of Pirbalouti *et al*. [28] for *Hypericum perforatum* L. oil extracted by hydodistillation from Iranian species and with those of Cossuta *et al.* [29] for Hungarian species isolated by supercritical fluid extraction. The more important compounds were: 2-methyl-octane (13.8 %), α-pinene (15.9 %), α-amorphene (14.6 %) for the Blida sample and α-pinene (11.04 %), α-amorphene (16.6 %) and n-tetradecanol (10.7 %) for the Algiers sample. For the Medea sample the major compounds were 2-methyl-octane (12.1 %), α-pinene (22.03 %) and β-selinene (10.9%). α-Pinene appears to be one of the most abounded compounds in the oils originating from the three investigated regions while α-amorphene is the major compound in the volatile oil isolated from Blida and Algiers samples.

On the other hand, the concentration of 2-methyl-octane is higher in the Blida and Medea samples. It should be noted that there seems to be a relation between the altitude of the harvesting region and the proportion of α-pinene in the extracted oil. The content of α-pinene was proportional to the altitude of the sample collection site. The higher the altitude, the higher amounts of α-pinene were detected (Table 1). An inverse trend was observed for n-tetradecanol and α-amorphene for which the proportions in the extracted oil decreased with altitude. Table 2 also indicates that volatile oil extracted from Algiers sample is rich in sesquiterpenes (62 %) and oxygenated compounds (31 %) and that those from both Blida and Medea are rich in sesquiterpenes (44.4 % and 48.9 %, respectively). Hajdari *et al.* [12] showed that the altitude of the harvesting locality has an effect on oil composition. Marrelli *et al*. [30] tested the phototoxicity of Italian (south Apennine) *Hypericum perforatum* L. extracts harvested in several altitudes.

Table 2. Percentage of classes of compounds identified in the volatile oils from *Hypericum perforatum* L. (aerial parts) originated from the three investigated regions.

Classes of chemical compounds/ regions	Blida	Algiers	Medea
Total monoterpenes	21.2	12.6	25.7
Hydrogenated monoterpenes	20.4	12.3	25.5
Oxygenated monoterpenes	0.9	0.3	0.2
Total sesquiterpenes	44.4	61.7	49.0
Hydrogenated sesquiterpenes	34.0	45.8	39.9
Oxygenated sesquiterpenes	10.4	15.8	9.1
Total non-terpenes	34.2	24.2	25.1
Oxygenated non-terpenes	11.9	15.0	6.7
Non oxygenated non-terpenes	22.4	9.3	18.4
Total oxygenated compounds	23.1	31.1	16.0
Total non-oxygenated compounds	76.7	67.4	83.8
Total identified%	99.8	98.5	99.7
Yield %	0.049	0.055	0.136

The values in the table represent the relative percentages (relative content %) against the total percentage of identified compounds. All results presented are the mean of triplicate. The standard deviations were systematically less than 1%.

The results showed that the best antiradical and antioxidant activities are obtained for the plants collected at an altitude of 370 m, which is close to the height (486 m) of the collection site of our Medea samples.

Xenophontos *et al.* [31] pointed out a great difference in total hypericins content according to altitude of habitat. A clear trend showing an increase of the total hypericins content with increasing altitude was observed.

Influence of harvesting year between 2009 and 2014. Volatile oil composition of *Hypericum perforatum* L. aerial parts from the locality of Blida, according to six consecutive years (2009-2014) are presented in Table 3 as classes of compounds. The obtained yields, ranging between 0.050 % and 0.131 %, are in agreement with those obtained by Helmja *et al.* [11] for Estonian species (from 0.068 % to 0.188 %). Year per year, the major compounds are displayed in Table 4. It should be stated that two compounds are systematically present with high proportions: 2-methyl-octane and α-pinene apart from 2012, where 2-methyl-octane is present in lower proportions (Figure 1). Non-terpene compounds were also identified. For example, in 2011 and 2012, the oil contained between 22.4 % and 15.9 % of alkanes and between 9.5 % and 12.3 % of alcohols, between 34.2 % and 29 % of non-terpenes, for the two years, respectively.

Table 3 shows that the six oils are rich in sesquiterpenes (from 25.3 to 52.2%), especially hydrogenated sesquiterpenes (from 17.5 to 42.9%), as well as in non-oxygenated compounds (from 76.3 to 89.2%) while they are poor in oxygenated monoterpenes (from 0.4% to 3.2%). These ranges are in agreement with those of Hosni *et al.* [9] who studied the extraction of Tunisian *Hypericum perforatum* L. oil harvested in 2006. The obtained oil was composed from 38.3% of hydrogenated sesquiterpenes and 20.5% of hydrogenated monoterpenes. As in our study, the authors reported low proportions in oxygenated monoterpenes (2%) and oxygenated sesquiterpenes (3.6%). More recently, Hajdari *et al.* [12] pointed out low proportions in oxygenated monoterpenes (from 1.3 to 2.4%) in *Hypericum perforatum* L. essential oil originating from Kosovo.

Variability in the chemical composition according to the plant organ. To investigate the influence of plant part subjected to extraction on oil composition a study was conducted on two parts of the plant: leaves and flowers-fruits in 2011 for the localities of Medea (M) and Blida (B). As it was expected, the extraction of volatile oils from flowers-fruit gave a higher oil yield ranging from 0.3% to 0.4% with high proportions of *α*-pinene (24.6% - 28.9%) and 2-methyl-octane (13.6% - 15.3%). Akhbari *et al.* [14] also reported a high proportion in *α*-pinene (11.34 %) for the oil extracted from fruits of Iranian *Hypericum perforatum* L. species together with *α*-amorphene (15.9%).

Table 3. Percentage of classes of compounds identified in volatile oils of *Hypericum perforatum* L. (whole aerial parts – region of Blida) obtained by hydrodistillation between 2009 and 2014

Classes of chemical compounds / years	2009	2010	2011	2012	2013	2014
Total monoterpenes	27.1	38.7	21.2	18.6	24.4	32.8
Hydrogenated monoterpenes	26.7	35.5	20.4	17.4	21.7	31.2
Oxygenated monoterpenes	0.4	3.2	0.9	1.2	2.7	1.6
Total sesquiterpenes	27.8	25.3	44.4	52.2	37.6	36.8
Hydrogenated sesquiterpenes	20.1	17.5	34.0	42.9	31.6	32.2
Oxygenated sesquiterpenes	7.7	7.7	10.4	9.3	6.0	4.6
Total non-terpenes	44.2	34.1	34.2	29.0	36.3	29.4
Oxygenated non-terpenes	3.6	1.2	11.9	13.0	8.2	3.7
Non oxygenated non-terpenes	40.6	32.9	22.4	16.0	28.1	25.7
Total oxygenated compounds	11.6	12.1	23.1	23.5	16.9	9.8
Total non-oxygenated compounds	87.6	85.9	76.7	76.3	81.4	89.2
Total identified %	99.2	98.1	99.8	99.8	98.2	99.0
Yield %	0.050	0.048	0.049	0.082	0.064	0.131

The values in the table represent the relative percentages (relative content %) against the total percentage of identified compounds. All results presented are the mean of triplicate. The standard deviations were systematically less than 1%.

Table 4: Major identified compounds in volatile oils extracted from *Hypericum perforatum* L. between 2009 and 2014.

Year	Major compounds
2009	2-Methyl-octane (30.67%),α-Pinene (23.01%),β-Caryophyllene (7.20%)
2010	2-Methyl-octane (22.32%),α-Pinene (23.83%),Myrcene (6.81%)
2011	2-Methyl-octane (13.79%),α-Pinene (15.89%), α-Amorphene (14.59%)
2012	α- Pinene (13.65%), α-Selinene (16.98%), n-Tetradecanol(11.9%)
2013	2-Methyl-octane (21.67%),α-Pinene (15.74%), γ-Himachalene (12.053%)
2014	2-Methyl-octane (20.21%),α-Pinene (20.77%),Germacrene-D (10.768%)

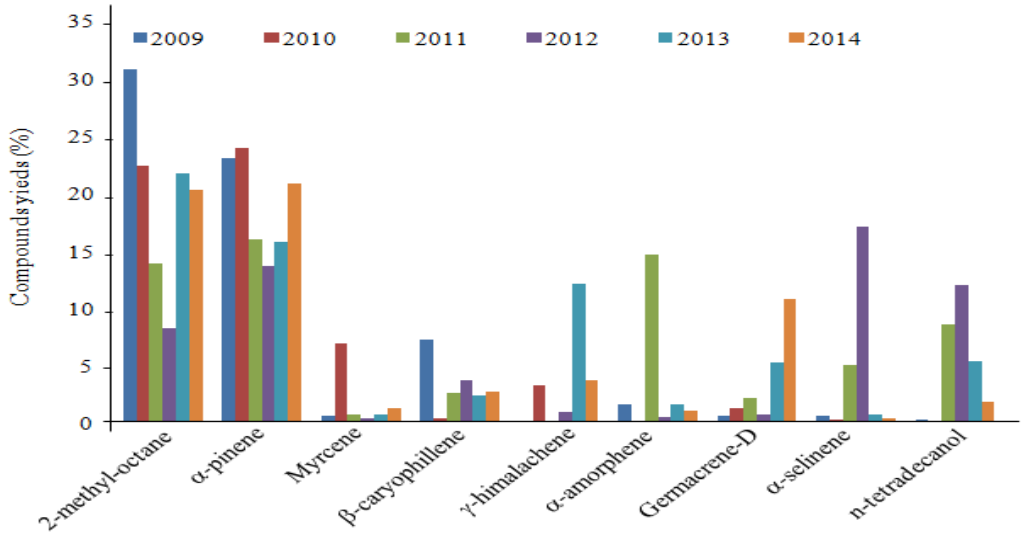

Figure1. Main compounds identified in volatile oils from *Hypericum perforatum* L. (aerial parts) between 2009 and 2014.

Table 5. Percentage of classes of compounds identified in volatile oils from *Hypericum perforatum* L. Leaves-B and flowers-fruit-B are plants that originated from Blida and leaves-M and flowers-fruit-M that originated from Medea

Classes of chemical compounds /Organs	Leaves-B	Flowers & Fruits-B	Leaves-M	Flowers & Fruits-M
Total monoterpenes	7.6	32.7	3.6	36.0
Hydrogenated monoterpenes	6.9	32.2	3.3	35.3
Oxygenated monoterpenes	0.7	0.5	0.3	0.6
Total sesquiterpenes	53.0	41.6	59.5	37.0
Hydrogenated sesquiterpenes	36.4	36.1	39.8	31.2
Oxygenated sesquiterpenes	16.5	5.5	19.8	5.8
Total non-terpenes	34.9	24.1	32.5	25.7
Oxygenated non-terpenes	23.3	2.7	28.4	2.1
Non oxygenated non-terpenes	11.7	21.4	4.2	23.6
Total oxygenated compounds	40.5	8.7	48.4	8.6
Total non-oxygenated compounds	55.0	89.6	42.2	90.2
Total identified %	95.5	98.4	95.6	98.7
Yield %	0.062	0.39	0.067	0.301

The values in the table represent the relative percentages (relative content %) against the total percentage of identified compounds. All results presented are the mean of triplicate. The standard deviations were systematically less than 1%.

Oxygenated monoterpenes exhibited the lowest percentages whatever the plan organ considered (from 0.23% to 0.87%). This is in accordance with the results presented by Radusiene *et al*. [16] in which the oxygenated monoterpenes in oils isolated from flowers varied from 0.1% to 0.9 % and almost the same percentages (from traces to 0.9%) in oils from leaves. However, for sesquiterpene hydrocarbons, our results showed equivalent percentages in the two plant parts (from 31.2 % to 39.8%) while according to Radusiene *et al.* [16] they were higher in flowers than in leaves.

Antimicrobial activity

The antimicrobial activity of *Hypericum perforatum* L. (aerial parts collected from Blida in 2014) oil was tested against eight microorganisms using the paper disc diffusion method and by determining the minimal inhibitory concentrations (MIC). The results (Table 6) showed that it exhibited weak antibacterial (against Gram-positive bacteria) and antifungal (against filamentous fungi) activities. The Gram-negative bacteria and the yeast strains tested were resistant. The MIC values of the collected oils against all target microorganisms were higher than 100 µg/ml, confirming the weak activity of the extracted oil. The weak antimicrobial activity of the volatile oils could be related to the presence of hydrogenated monoterpenes (31.23%) including α-pinene (20.77%) and *β*-pinene (3.19%). Moreover, oxygenated monoterpenes and 2-methyl-octane (20.21%) as alkane, as well as germacrene-D (10.77%) as hydrogenated sesquiterpene may explain the slight antimicrobial properties.

Some studies reported that a good antimicrobial activity was often related to the presence of oxygenated terpenes, especially oxygenated monoterpenes [32, 33]. These compounds were detected in small percentages (0.23 – 0.87%) in this study. The weak antimicrobial activity of essential oils isolated from *Hypericum perforatum* L. was reported by Rančić *et al.* [34] against seven strains of bacteria and six strains of fungi. The diameters of inhibition zones obtained against the Gram-positive bacteria were weak; 6 mm (1 µl) and 10 mm (5 µl) for *Micrococcus luteus*, 5 mm (1 µl) and 11 mm (5 µl) for *Staphylococcus aureus*, 4 mm (1 µl) and 9 mm (5 µl) against *Staphylococcus epidermidis*. In the same work, weak activity against four Gram-negative bacteria (*Escherichia coli. Pseudomonas tolaasii. Salmonella enteritidis. S. typhimurium*) was also reported. Values of inhibition zone diameters ranging between 7 and 12 mm were obtained. The authors attributed the weak antimicrobial activity of the *Hypericum perforatum* L. volatile oil to its chemical composition, which is characterized by the dominance of nonane (63.8%).

Table 6: Antimicrobial activity of *Hypericum perforatum* L. essential oil by the paper disc diffusion method and minimal inhibitory concentrations.

Microorganisms	Diameters of the inhibitions zones (mm)		Minimal inhibitory concentrations (µg/ml)	
Gram-positive bacteria	Volatile oil	Gentamicin	Volatile oil	Gentamicin
Micrococcus luteus	11	28	> 100	1
Bacillus subtilis	10	27	> 100	2
Staphylococcus aureus	13	27	> 100	2
Gram-negative bacteria				
Klebsiellapneumoniae	0	25	> 100	2
Filamentous fungi	Volatile oil	Amphotericin B	Volatile oil	Amphotericin B
Aspergillus carbonarius	9	15	> 100	2
Fusarium oxysporum albedinis	11	14	> 100	1.5
Yeasts	Volatile oil	Itraconazole	Volatile oil	Itraconazole
Candida albicans	0	18	> 100	1
Candida glabrata	0	16	> 100	1.5

Values of inhibition zones include the diameter of disk (6 mm).. All results presented are the mean of triplicate. The standard deviations were systematically less than 1%.

CONCLUSIONS

The aim of this study was to assess the variability of chemical composition of *Hypericum perforatum* L. essential oil according to plant habitat, to the harvesting year between 2009 and 2014 and to plant organ subjected to extraction. 2-Methyl-octane and α-pinene were identified as the most abundant constituents. Considerable

qualitative and quantitative variations were observed in the chemical profile of essential oils. The harvesting localization reflected by the altitude of the plant habitat favorized the global yield of essential oil. The monitoring of the essential oil composition between 2009 and 2014 indicated that the above compounds were predominantly present but according to the harvesting year other important compounds may be present, as α-selinene, α-amorphene and γ-himachalene.

As found in our previous study on Algerian species originated from Blida [35], these compounds greatly contribute to the antioxidant activity of *Hypericum perforatum* L. essential oil making this plant an important source of natural antioxidants.

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