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# INFLUENCE OF SEASONAL VARIATION ON CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF THE ESSENTIAL OIL OF FIELD WORMWOOD *ARTEMISIA CAMPESTRIS*

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## Abstrat

**Description of the subject:** It is known that the quantitative chemical composition of plants and consequently their biological activities vary throughout the vegetative cycle.

**Objectifs:** Evaluate the season effect on the yield, chemical composition and antioxidant capacity of *A.campestris* EO.

**Methods:** EO isolated by hydrodistillation were analyzed by GC/SM. The antioxidant capacity of the EO was determined by DPPH free radical scavenging ability.

**Results:** 39 compounds have been identified. HE of the summer and winter season dominated by α-Pinene. EO of the spring season is a new chemotype detected in Algeria (τ-Muurolene chimotype).

**Conclusion:** *Artemisia campestris* essential oils has great potential as an antioxidant medicine.

**Keywords *:*** *A.campestris*; Seasonal variation; Essential oil; Chemical composition; Antioxidant activity.

# INFLUENCE DE LA VARIATION SAISONNIÈRE SUR LA COMPOSITION CHIMIQUE ET L'ACTIVITÉ ANTIOXYDANTE DE L'HUILE ESSENTIELLE DE L'ARMOISE CHAMPETRE *ARTEMISIA CAMPESTRIS*

## Resumé

**Description du sujet :** On sait que la composition chimique quantitative des plantes et par conséquent leurs activités biologiques varient tout au long du cycle végétatif.

**Objectifs :** Évaluer l'effet de la saison sur le rendement, la composition chimique et la capacité antioxydante de l'huile essentielle d'*Artemisia campestris*.

**Méthodes :** Les huiles essentielles isolées par hydrodistillation ont été analysées par CG/SM. La capacité antioxydante de l'huile essentielle a été déterminée par la capacité de balayage des radicaux libres DPPH.

**Résultats :** 39 composés ont été identifiés. HE de la saison l'été et de l'hiver a dominance α-Pinène. Huile essentielle de la saison de printemps est un nouveau chimotype détecté en Algérie (chimotype τ-Muurolene).

**Conclusion :** Les huiles essentielles d'*Artemisia campestris* présentent un grand potentiel en tant que médicament antioxydant.

**Mots-clés:** *A.campestris*; Variation saisonnière; Huile essentielle; Composition chimique; Activité antioxydante.

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## INTRODUCTION

The genus Artemisia belongs to one of the largest and most widely distributed genera of the family Asteraceae (Compositae). It is a diverse and economically important genus and it has more than 500 species. Most plants within this genus have a great importance as medication, foodstuff, ornamentals or soil stabilizers [1, 2, 3, 4]. *Artemisia* species are widely used in traditional medicine all over the world with different and wellknown therapeutic applications (stomach ache, diarrhoea, parasitism, intestinal and bronchial infections, angina, wounds, pimples, colds and coughs) [4, 5]. They exhibit anti-inflammatory, antitumor, antioxidant, antispasmodic, antimicrobial, insecticidal, antimalarial, antifungal and antioxidant activities [4, 2]. In the flora of Algeria According to [6], it is represented with 11 spontaneous *Artemisia* species *Artemisia campestris* commonly known as “dgouft” grown wild the arid and semiarid regions of Algeria.The leaves of this plant were used as hypoglycemic, cholagogue, choleretic, digestive, depurative, antilithiasic, and for the treatment of obesity and to decrease cholesterol. It was used as a decoction as antivenin, anti-inflammatory, anti-rrheumatic and antimicrobial [7, 10].The phytochemical screening of this species revealed the presence of tannins, polyphenols, flavonoids, saponosides, essential oil and minerals [11].

Numerous studies in the litterature have reported on chemical composition of oils isolated from *Artemisia campestris* of different origins [12, 18], but Little has been published in connection with the effect of the harvest time in the composition of A. campestris oils. Essential oils and their components are gaining increasing interest in the food and pharmaceutical industries as natural antioxidants, because of their relatively safe status and their wide acceptance by consumers [19]. As commonly phenomena for many plant species, chemical composition and yield of the essential oil bearing plants have been mostly correlated to the environmental factors such as temperature, humidity, radiation, harvest time, effect of soil properties and wind as well as the maturation of the plant and its physiological and biochemical pathway [20, 23].

In order to increase technological and economical effectiveness in the uses of EOs their production should be optimized by a proper selection of pecies/varieties/cultivars and agrotechnological practices, for instance by selecting the most beneficial harvesting time. It is not clear in which of season Artemisia plants exhibit the highest concentration of the active compounds that are directly related with biological activities. For these reasons, the main goal of the present study was to determine the seasonl effect considering only summer (late vegetative stage), winter (full-flowering stage), spring (early vegetative stage) on the chemical composition and antioxidant properties of *A.campestris*.This paper reports the first study of the seasonal variation of the oil of A. campestris growing wild in Djelfa, Algeria.

## MATERIALS AND METHODS

### *1. Plant material*

The aerial parts of *A.campestris* that utilized in this study were collected during late vegetative stage (July, 2013), full-flowering stage (December 2013) and early vegetative stage (May, 2014) from its wild habitat in Ain Bel region in south-west of Djelfa -algerie. The identification of the species was realized by Professor Dahia Mustapha, botanist at the University Ziane Achour, Faculty of Sciences of Djelfa, Algeria.Samples were air-dried during 15 days in the laboratory at room temperature till the weight stay stable.

### *2. Essential oil extraction*

The aerial parts of individual plants were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The EO obtained was separated from water and dried over anhydrous sodium sulphate and kept in amber vials at 4 ◦C until chromatographic analysis. Yield percentage was calculated as weight (g) of EO per 100 g of plant dry matter.

### *3. Gas Chromatography-Mass Spectrometry (GC-MS)*

GC/MS is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample [24]. Then gas phase chromatographic analyses were carried out with the aid of a Trace GC Ultra apparatus equipped with one injector in Split Play, a TRB-5 MS column (30 m x0.25 mm, film thickness 0.25 μm). The operating conditions are as follows: carrier gas: helium; solvent: ethyl acetate; injection volume: 50 μL; flow rate: 1 mL/min; The operating condition of GC oven temperature was maintained as: initial temperature 40 °C for 1 min, programmed rate 5 °C/min up to final temperature 220 °C with isotherm for 1 min; injector temperature 280° C.The coupling with the mass spectrometer ITQ 900 was done with a temperature of 220 °C interface.The operating conditions are as follows: type electron impact ionization (70 eV); injector temperature was 200 °C. Mass spectra were recorded over 50–500 a.m.u. range.The database used was NIST M Search. The identification of the constituents was assigned on the basis of comparison of their retention indices and mass spectra with those given in the literature [25, 26].

### *4. DPPH• radical scavenging activity*

In this part of our work, the in vitro antioxidant activities of the leaves essential oils extracts stored at 4◦C have been evaluated based on the DPPH test. Antioxidant scavenging activity was studied using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) as described by Adedapo et al. [27] with some modifications: 2 mL of various dilutions of the plant extracts were mixed with an equal volume of 0.135 mM methanolic DPPH solution. After an incubation period of 30 min in the dark at room temperature, the absorbance at 517 nm and the wavelength of maximum absorbance of DPPH, were recorded as A (sample). A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as A(blank). Ascorbic acid is used as reference. Measurements were performed in triplicate. The free radical-scavenging activity of each solution was then calculated as percent inhibition according to the following equation: % inhibition = 100 (A (blank)– A(sample))/A(blank).

## RESULTS

### *1. Essential oil yields*

With the growing interest in the use of essential oils in both food and pharmaceutical industries, the yield of their extraction is an important parameter for their industrial exploitation. The essential oil (EO) yield of a given species is influenced by intrinsic parameters (such as growth stages) and extrinsic ones (suchas pedoclimatic conditions and extraction methods). As shown in Table 1, variable essential oil concentration as calculated on the basis of dry plant weight (v/w) was observed for *A.campestris* depending on the different seasons. According to our results, the observed yield of essential oils increases significantly from summer to spring. Indeed, the lowest yield of essential oil is recorded for the samples collected in spring (0.37%) while the highest level is observed from samples collected in summer with a mean value of 0.41%.

### *2. Chemical composition of essential oils*

Essential oil composition of *A. campestris*, along with the retention indices and the quantitative data are listed in Table1. A total of 39 compounds, covering more than 96% of the total integrated GC peak area were identified (Fig. 2). Comparison of the analytical data revealed notable quantitative and qualitative differences between oil samples. Thirty-five components accounting for 96.95% of the total composition were identified in the summer (Late vegetative stage). The major constituents of this oil were α-Pinene (39,71 %), a-Eudesmol (10,94 %), o-Cymene (10,2 %), Santolina triene (7,36 %), (-)-Spathulenol (6,98 %), cis-Lanceol (3,69 %), τ-Terpinen (2,63 %).

In the samples collected in winter (Full flowering stage), 34 compounds were identified amounting 99.12% of total components with α-Pinene (29,55 %), τ-Muurolene (9,54 %),santolina triene (9,26 %), τ-Gurjunene (8,67 %), o-Cymene (8,38 %), τ-Terpinen (6,3 %), (-)-Spathulenol (5,74 %), (-)-Isoledene (4,1 %), 3-Carene (3,93 %), a-Eudesmol (3,51 %), cis-Lanceol (2,65 %) were identified. Finally, In the spring (Early vegetative stage), 32 compounds amounting 99.48 % of total components were identified which included τ-Muurolene (37,12 %), α-Pinene (23,95 %), 3-Carene (8,8 %), Santolina triene (8,55 %), τ-Gurjunene (3,23 %), a-Farnesene (2,61 %), a-Eudesmol (2,45 %),β-Cadinene (2,26 %).

Table 1: Percentage composition of essential oils obtained from aerial parts of *A. campestris* at different season.

| **Compounds** | tr (min) | Percentage composition (%) | | |
|---|---|---|---|---|
| | | Summer | Winter | Spring |
| Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (±)- | 7,72 | 0,02 | 0,01 | 0,01 |
| Camphene | 8,47 | 0,07 | 0,15 | 0,04 |
| α-Pinene | 9,51 | 39,71 | 29,55 | 23,95 |
| α-Phellandrene | 10,11 | 0,42 | 0,23 | 0,11 |
| Terpinolen | 10,47 | 0,67 | 0,44 | 0,23 |
| o-Cymene | 10,77 | 10,2 | 8,38 | 1,51 |
| Santolina triene | 10,92 | 7,36 | 9,26 | 8,55 |
| 3-Carene | 11,42 | 1,33 | 3,93 | 8,8 |
| τ-Terpinen | 11,75 | 2,63 | 6,3 | 4.00 |
| Cyclohexene, 4-methyl-3-(1-methylethylidene)- | 12,58 | 0,50 | 0,49 | 0,6 |
| 1,7,7-Trimethylbicyclo[2.2.1]hept-5-en-2-ol | 13,64 | 0,14 | 0,05 | 0,01 |
| (-)-cis-Sabinol | 14,04 | 0,43 | 0,14 | 0,01 |
| Bicyclo[3.1.0]hexan-2-ol, 2-methyl-5-(1-methylethyl)-, (1à,2à,5à)- | 15,17 | 0,98 | 0,49 | 0,09 |
| p-Mentha-2,4(8)-diene | 15,54 | 0,3 | 0,09 | 0,03 |
| Myrtenal ± | 15,67 | 0,71 | 0,25 | 0,02 |
| Myrtenol | 15,71 | 0,33 | 0,03 | 0.00 |
| 1,4-Pentadiene, 3-methyl- | 16,82 | 0,15 | 0,08 | 0,06 |
| 3-Cyclohexene-1-carboxaldehyde, 1,3,4-trimethyl- | 18,04 | 0,14 | 0,05 | 0,01 |
| 2-Isopropylidene-3-methylhexa-3,5-dienal | 18,87 | 0,15 | 0,07 | 0,02 |
| 1,5-Decadiyne | 19,76 | 0,15 | 0,07 | 0,03 |
| τ-Muurolene | 21,74 | 1,62 | 9,54 | 37,12 |
| (+)-Camphene | 22.00 | 1,22 | 0,21 | 0,4 |
| α-Cubebene | 23,85 | 0,17 | 0,84 | 0,24 |
| α-Gurjunene | 24,87 | 0,13 | 0,47 | 0,31 |
| (-)-Isoledene | 26,10 | 0,73 | 4,1 | 0.00 |
| (+)-Cuparene | 26,18 | 1,22 | 0.00 | 0.00 |
| τ-Himachalene | 26,27 | 0,25 | 0,35 | 0.00 |
| τ-Gurjunene | 26,87 | 0.00 | 0.00 | 3,23 |
| α-Muurolene | 26,93 | 0,12 | 8,67 | 0,29 |
| à-Farnesene | 27,42 | 0.00 | 0.00 | 2,61 |
| β-Cadinene | 28,03 | 0.00 | 0.00 | 2,26 |
| Davana ether | 28,32 | 0,26 | 1,03 | 0.00 |
| Tricyclo[5.2.2.0(1,6)]undecan-3-ol, 2-methylene-6,8,8-trimethyl- | 29,36 | 0,78 | 0,37 | 0.00 |
| Elemene | 29,93 | 0,31 | 0,28 | 0.00 |
| (-)-Spathulenol | 30,55 | 6,98 | 5,74 | 1,23 |
| cis-Lanceol | 32,08 | 3,69 | 2,65 | 0,6 |
| a-Eudesmol | 34,02 | 10,94 | 3,51 | 2,45 |
| 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol | 35,58 | 2,14 | 1,3 | 0,41 |
| 5-Methoxy-2,2,6-trimethyl-1-(3-methyl-buta-1,3-dienyl)-7-oxabicyclo[4.1.0]heptanes | 37,82 | 0.00 | 0.00 | 0,25 |
| Total oil (%) | | **96,95** | **99,12** | **99,48** |
| Yield (%, v/w) | | **0,41** | **0,39** | **0,37** |

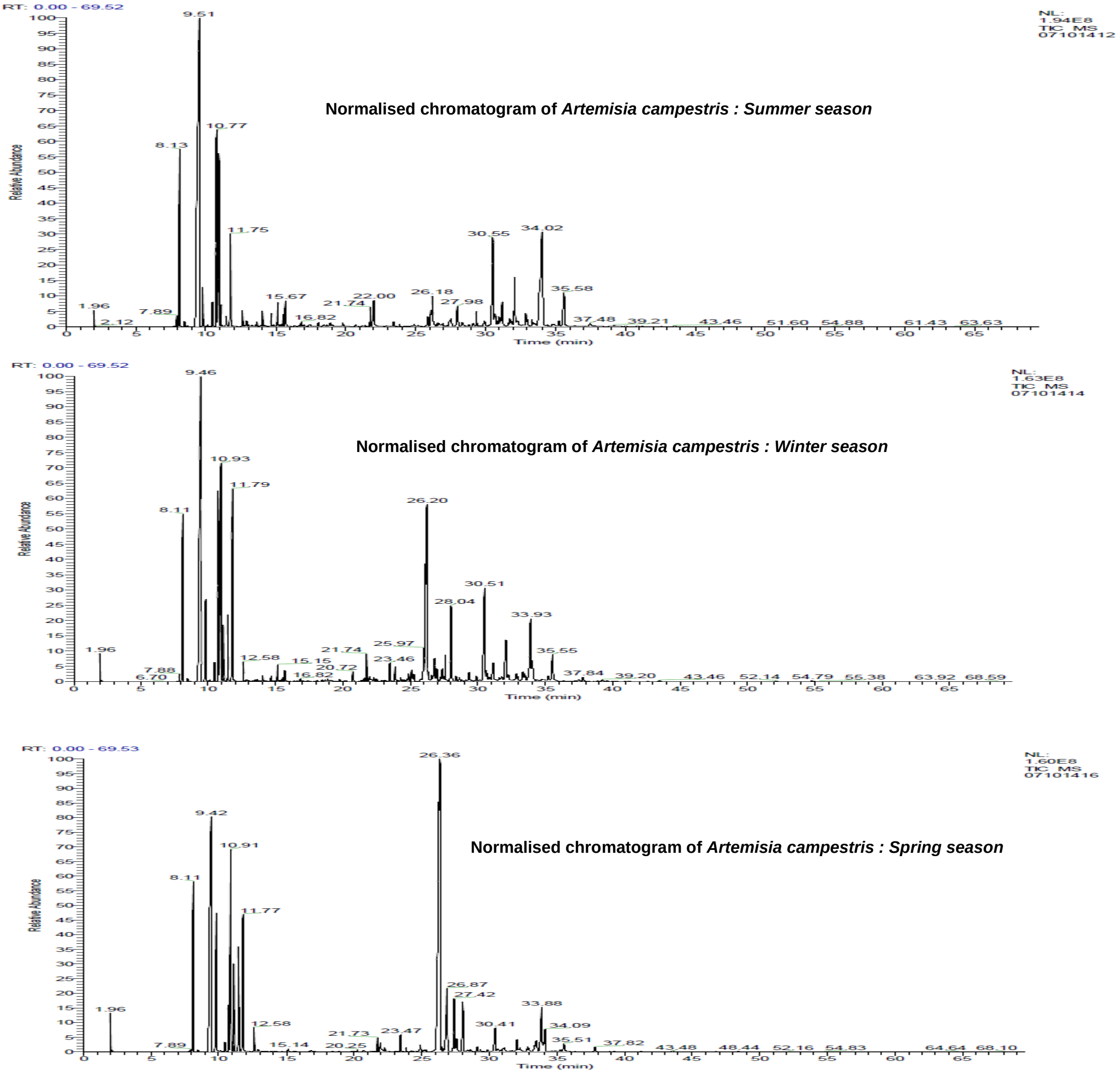

Figure 1 : Chromatographic profiles (GC) of *A. campestris* essential oil.

### 3. *Antioxidant activity*

Studies concerning the natural products to replace the synthetic additives and preservatives in the food industries are growing intensively. The antioxidant activity of Artemisia oils suggests their possible role in providing protection against oxidative diseases and their use as a natural antioxidant in food, flavor, and pharmaceutical industry [28]. In this work, the different essential oils extracted from *A. campestris* were screened for their antioxidant activity using DPPH method.

Our results highlight an important in vitro antioxidant activity of the essential oils of *A. campestris* which vary according to the season (Fig. 2). The essential oils extracted from aerial parts collected in summer were characterized by the best antioxidant activity (IC50= 21,33 ± 1,44 mg/l). The weakest antioxidant activity was observed for oils obtained in winter (Full -flowering stage) from flowering aerial parts with IC50 = 29,87 ± 1,42 mg/l. The oil obtained from leaves collected at the vegetative stage in spring showed intermediate antioxidant activity (IC50= 25,19 ± 2,00 mg/l).

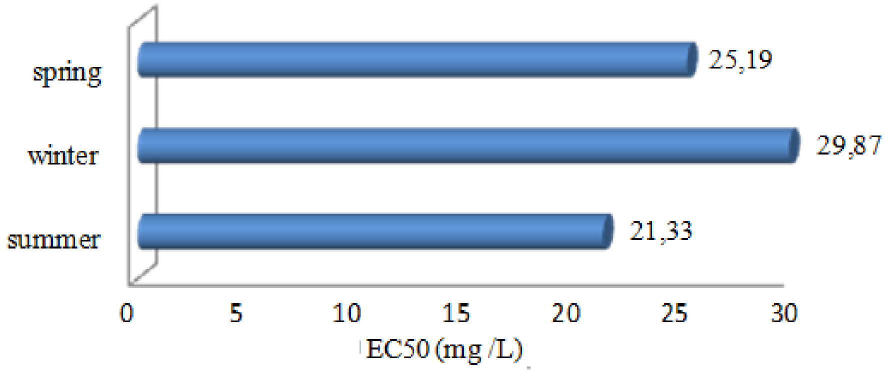

Figure 2 : Antioxidant activity of *A.campestris* of essential oils during three different seasons

## DISCUSSION

The crop yield and the quality of plant material are the most important characteristics in commercial cultivation of spices and aromatic herbs. It is very important to determine the optimal harvesting period, which may differ depending on climatic conditions and plant properties [29]. According to Abad et al. [30] the quality and yield of the essential oils from the Artemisia species is influenced by the harvesting season. Indeed the climatic conditions and water availablity in the soil have an effect on the vegetal secondary metabolism and, consequently, alter the composition of essential oils, through the seasons of the year [31]. Little has been published in connection with this matter, although there is some controversy concerning the EO yielded by different Artemisia species at different phenological stages. According to our results mature leaves (summer) produced more essential oil than young leaves (spring). Similar results were reported by Singh et al. [28] in *Artemisia scoparia*. Also, Ghanmi et al. indicated that the EO content in *Artemisia herba alba* was found to be higher in the month of June (summer). But according to Verdian-Rizi [32], the yields of EO at different growth stages of *Artemisia annua* were in the order of pre-flowering < post-flowering < full-flowering. Also, Mallavarapu et al. [33] indicated that the EO content was found to be higher at the full emergence of flower heads in Davana (*Artemisia pallens* Wall.). Concerning the genus Artemisia a low yield of 0.17% was recorded for the species *A. scoparia* [34] whereas this yield reach 0.5% for *A. mesatlantica* [35]. Higher levels were obtained for *A. herba alba* species in Tunisia (0.68–1.93%) [36] and *A. arborescens* L. in Lebanon (1.7%) [37]. According to our results, it seems that chemical composition of *A.campestris* EO varied significantly with physiological stage of the plant. In this way, *A.campestris* EOs could be divided into three groups according to the amounts of the major constituents:

- α-Pinene > a-Eudesmol > o-Cymene > Santolina triene > (-)-Spathulenol > cis-Lanceol >τ-Terpinen.
- α-Pinene > τ-Muurolene > santolina triene > τ-Gurjunene > o-Cymene > τ-Terpinen > (-)-Spathulenol > (-)-Isoledene > 3-Carene > a-Eudesmol > cis-Lanceol
- τ-Muurolene> α-Pinene > 3-Carene > Santolina triene > τ-Gurjunene > a-Farnesene > a-Eudesmol > β-Cadinene

α -Pinene was the most abundant component for samples collected in summer and winter (39.71 %, 29.55 % respectivly). Several authors reported the presence of α-pinene as a major constituent of *A. campestris* oils [38, 39, 40, 15, 18]. The sample collected in spring contained the highest amount τ-Muurolene (37.12 %).A comparison of chemical composition of the essential oil of *A. campestris* of spring to other studies shows that there are a new Algerian *A. campestris* chemotype (τ-Muurolene chemotype).

Also, the essential oil composition of A. campestris L. leaves, occurring in different regions of Tunisia, at different vegetation periods afforded variable volatile content, for which the main components were: ß-pinene (24- 49.8%), p-cymene (2.3-22.3%), α-pinene (4.1-12.5%), camphor (10.3%), spathulenol (1.2-10%), γ-muurolene (0.5-9.6%) and limonene (4.9-9.3%) [41, 42, 43, 12, 13, 39] .However, the seasonal chemical profile of essential oils hydrodistillated from flowers of A. campestris L. subsp. glutinosa originated from different regions of Italy revealed the existence of the components: β-pinene (6.9-57.2%), germacrene D (5.9-28.6%), bicyclogermacrene (3.9- 14.5%), myrcene (3.8- 11.2%) and α-pinene (5.3-9.2%) [44].From Iran, the oils extracted from leaves, stems and flowers were predominated by spathulenol (15.8-29.2%), α-pinene (23-29.2%), β-pinene (4.5-12.6%) and bicyclogermacrene (9.1-12%)[45] .Oils of aerial part of A. campestris L. subsp. campestris collected from numerous regions of Lithuania at the flowering stage were characterized by the major terpenics: caryophyllene oxide (3.7-38.8%), germacrene D (3.8-31.2%), γ-curcumene (4-14.8%), β-pinene (3.9-13.8%), α-pinene (4-11.4%) and humulene epoxide II (3.7-11.7%) [47,46]. The essential oil of A. campestris L. subsp. campestris from Poland investigated on different organs (inflorescences, flowers, stems, roots and flowers) and over five ontogenesis phases, was mainly dominated by (Z)-falcarinol (19-38.8%), germacrene D (9.7-28%) and γ-humulene (4-8.2%) [48, 49].

Compared to other studies, the composition of essential oil of A. campestris L. growing in different countries showed that this plant displays an intraspecific variation in the terpenic composition, which is dependent upon the phenological stage, plant part, geographic location, chemotype or subspecies. Consequently, A.campestris L. essential oil exhibited various chemotypes, mainly correlated to the place of growing of the plant and the subspecies studied.

For all seasons, the A.campestris oils obtained from aerial parts showed an important scavenging activity against DPPH radical whose the highest activity was obtained from samples collected in summer.There are many studies that demonstrate the antioxidant action of A. campestris extracts [50, 51].Our results are in agreement with what has been previously reported by Bakchiche and Gherib [52] whose IC50=39 μg/ml.According to Akrout et al. [13] the antioxidant activity of leaf essential oils estimated by the DPPH test system was too low (IC50 = 1874 μg/ml) comparatively to that of ascorbic acid (IC50=2.5μg/ml).The same observation was made with the antioxidant effect of essential oil from the aerial part,that has an IC50=94500 μg/ml, which is judged to be very feeble when compared to standards: ascorbic acid (IC50=240μg/ml), quercetin (IC50= 280μg/ml) and BHT (IC50=840μg/ml) [43].The highest antioxidant activities were recorded for semi Arid bioclimatic stage (Djelfa) where plants are facing heat and drought stress resulting in an antioxidant stress, and then they need to optimize their secondary metabolism to synthesize more antioxidant compounds [53].The variation of essential oil antioxidant capacity observed between the studied seasons is attributed to their chemical polymorphism.Indeed, it is difficult to attribute the antioxidant effect of a total essential oil to one or few active compounds; both minor and major compounds should make a significant contribution to the oil’s activity which is the interaction result of their chemical composition [54].Variations of environmental factors (rainfall, winter cold stress, summer precipitation, summer drought stress, evapotranspiration and humidity…) among the three studied seasons seem to have influenced the chemical polymorphism of the studied essential oils.This had clearly affected the antioxidant power of those oils.

## CONCLUSION

The appropriate harvest timing based on the scientific criteria is a key factor in determination of greatest essential oil potential and best antioxidant properties.A comparison of chemical composition of the essential oil of *A.campestris* at three season shows that there are significant differences in composition and major components.

Thus, the time of harvesting of this plant has major effects the yield and chemical composition of EO. This study has demonstrated that the essential oil of *A.campestris* exhibits seasonal changes owing to changes in weather conditions. Additionally, these changes in the qualitative and quantitative features resulted in modification of the antioxidant activity of the essential oil. For industrial use, if a particular composition, the results show is not possible recommended the best time for collecting of campestris herb because the chemical profile of essential oils were greatly affected by season.but for better antioxidant activity desired, the factor season must be considered. In the case of A. campestris essential oil obtained at summer could be the best choice for further use substitute for synthetic additives in food production process.

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