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# Physico-chemical characterization and antimicrobial activity of an essential oil from the flowering umbels of wild *Daucus carota* L. subsp. *carota* Algeria

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**Abstract**

Aromatic and medicinal plants are an important source of molecules with antimicrobial effects, particularly in their volatile extracts. Species of the genus *Daucus* are considered among the richest plants in secondary metabolites, such as essential oils, which have an inhibitory effect on the development of bacterial colonies and harmful strains of fungi. Aiming at enhancing use of native plants in Algeria, we investigated essential oil from the flowering umbels of *Daucus carota* subsp. *carota* harvested in the region of Aith Laaziz (Algeria). Hydro-distillates were tested for their antimicrobial activities on bacteria and yeasts pathogenic to humans. The extraction produced a high yield of 3.65% essential oil. Analysis of the essential oil by GC (FID) and GC/MS allowed the identification of 47 compounds, four of which were predominant, represented by 53.4% of sesquiterpenes, carotol (10.38%), germacene D (9%). These secondary metabolites were shown to have moderate antimicrobial activity against *B. subtilis* and *S. aureus*, and yeast strains (*C. albicans* and *S. cerevisiae)*. However, it should be noted that the MIC values are quite low (0.125-0.5%) but MBC and MFC values are average (0.5-1%).The essential oil is not very active against Gram$^-$ bacteria *E.coli*, *P. aeruginosa* and *P. fluoresces* where the MIC values are low (0.5 to 1%)

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# 1. Introduction

The use of medicinal plants for the treatment of disease is very old and has developed during the history of mankind. It has now been shown that about 20% of plant species possess therapeutic or cosmetic powers and contain secondary metabolites with different biological properties; these bioactive molecules are used in various fields such as medicine, pharmacy, cosmetology, phyto-pharmacy and agri-food [1,2]. According to [3], the varied ecological and edaphic conditions in Algeria support a diverse flora but many species are little studied or unstudied. This study was undertaken to investigate the importance of secondary metabolites in Algerian plants to assess their antifungal and antibacterial properties. Searching for new sources of active compounds by extracting volatile components from these plants is a potentially important for human health. Plant secondary metabolites are the subject of much *in vitro* and *in vivo* research, in particular, the development of new natural products such as phenolic compounds and essential oils offer considerable promise. The emergence of side effects of synthetic-molecule medicines and the increase of resistance of pathogenic microorganisms to conventional antibiotics (following inappropriate usage) pose a serious public health problem. Much research is currently being directed towards exploring the potential of active biological extracts of aromatics from medicinal plants, and especially of essential oils. [4] and [5] note that research and the development of such bio-active molecules should be accelerated [6] show that the use of these natural substances is increasingly effective in controlling pathogenic microbes. These extracts have no long-term resistance characteristics in microorganisms. The complexity of the chemical constituents of essential oils make it extremely difficult for microbial strains to develop resistance. Research on the antimicrobial activity of essential oils extracted from plants in the genus *Daucus* has demonstrated powerful antimicrobial effects when these volatile substances are used against several pathogenic microbial agents. These effects are closely related to the broad-spectrum chemical profile of these volatile compounds [7-20]. In view of this, and given the diversity of the chemical composition of the essential oil extracted from the *Daucus carota*, as well as its wide geographical distribution and high availability in Algeria, this aromatic plant (*Apiaceae*) was selected for further research. This study reports on the essential oil extracted from the wild carrot *Daucus carota* subsp. *carota*. Though common in Algeria, this plant is of Asian origin, and is very rich in secondary metabolites. Determination of the essential oil yield and its physicochemical characterization by GC (FID) and GC/MS analysis are carried out on this plant in order to identify the chemical composition of this oil. The evaluation of *in vitro* antimicrobial activity was also carried out by measurement of inhibition diameters in cultures and by study of the minimum inhibitory concentration (MIC) and minimum bactericidal and fungicidal concentrations **(**MBC, MFC) of the essential oil on a variety of bacteria and yeasts pathogenic to humans.

# 2. Material and methods

### *2.1. Plant material*

In order to extract the essential oil of *Daucus carota* subsp. *carota*, gatherings of the flowering tops were made at the time of the flowering in May 2014 in the region of Aith Laaziz (Bouira). This region is characterized by perennial dry scrubland. Botanical identification of this species was carried out at the Department of Botany, National School of Agronomy of El-Harrach (Algiers).

### *2.2. Microbial material*

The essential oil was tested *in vitro* on both $Gram^+$ and $Gram^-$ bacterial strains, and on yeasts pathogenic to humans. These strains came from the Institute Pasteur of Algiers. The characteristics of the strains tested are given in table 1.

**Table 1:** Characteristics of the tested microbial strains

| Microorganisms | Morphology | Tested strains |
|---|---|---|
| Bacteria Gram$^-$ | Bacilli | *Escherichia coli* (A.T.C.C 4157) |
| | | *Pseudomonas aeruginosa* (A.T.C.C 9027) |
| | | *Pseudomonas fluoresces* (A.T.C.C 25923) |
| Bacteria Gram$^+$ | Bacilli | *Bacillus subtilis* (A.T.C.C 9372) |
| | Cocci | *Staphylococcus aureus* (A.T.C.C 6538) |
| Yeasts (fungi) | | *Candida albicans* (A.T.C.C 24433) |
| | | *Saccharomyces cerevisiae* (A.T.C.C 2601) |

### 2.3. *Essential oil isolation*

In order to extract the essential oil, the Cleavenger device modified according to [21] was used for the hydro-distillation method. Thus the essential oil, devoid of solvent, was separated into two phases, an aqueous phase and an oily phase. With the aid of a separating funnel, the low density oil rises to the surface and the hydrosol for decantation. The extract is stored in shaded bottles, sealed at 4 °C in the refrigerator before using to evaluate physico-chemical characteristics and antimicrobial activity.

#### *2.3.1. Calculation of the yield of essential oil ($Y_{EO}$ in %)*

The yield is the ratio between the mass of the extracted essential oil and the mass of the dry plant material. It is calculated according to [21], using the following equation:

$$\mathbf{Y_{EO}}\ (\%) = \left(\frac{\mathbf{Meo}}{\mathbf{Mdpm}}\right) \times 100$$

$\mathbf{M_{dpm}}$: Mass of the dry plant material (g).

$\mathbf{M_{eo}}$: Mass of extracted essential oil (g).

#### *2.3.2. Moisture rate Calculation (MC %)*

The dry and wet mass of plant material ($M_{dpm}$, $M_{fpm}$ in g) terms represent the water content in the plant material. This is considered important for characterizing the quality of the essential oil extract. It is obtained using the Dean and Strack apparatus to obtain the mass of the actual dry plant material used. It is calculated according to the [21] through the following equation:

$$\mathbf{M_{dpm} = M_{fpm} \times (1\text{-}MC)}$$

$\mathbf{M_{dpm}}$: Mass of the dry plant material (g).

$\mathbf{M_{fpm}}$: Mass of the fresh plant material (g).

### *2.4. Physico-chemical analysis of the essential oil of Daucus carota subsp. carota*

#### *2.4.1. Organoleptic and physico-chemical properties of the essential oil*

The determination of the organoleptic and physico-chemical properties of the essential oil was carried out in accordance with [22] and by comparing the results obtained with those already published on the essential oils extracted from the different plant parts of the same genus and of the same species. Essential oil parameters considered in this study are color, physical appearance, relative density (RD) at 20 °C, rotatory power (RP) at 20 °C., refractive index (RI) at 20 °C, acid index (AI), ester index (EI) and saponification index (SI).

### *2.4.2. Gas chromatography (GC)*

The chemical compounds were performed using a Hewlett Packard 6890 gas chromatography instrument. (Agilent Technologies) with a single injector and two flame ionization detector (FID), witn different stationary phases. Column: HP-5MS dimensions 30 m × 0.25 mm internal diameter, film thickness 0.25 µm. Oven temperature program: 60 °C for 8 min, ramp of 2 °C/min up to 250 °C and then held isothermal for 10 min, injector and detector temperature: 250 °C. Helium was the carrier gas at 0.5 ml/min and 0.2 µl injected by splitting ratio was 50:1

### *2.4.3. Gas chromatography-Mass Spectrometry (GC/MS)*

Chemical analysis of the extracted essential oil was carried out in the Gas Chromatograph: HP (Agilent Technologies) 6890 more, with silica column, interfaced with mass spectrometer (Agilent technologies) 5973C VL MSD, under the following operating conditions: Type column: HP-5MS, dimensions: 30 m × 0.25 mm internal diameter, film thickness 0.25 µm. Interface temperature: 280 °C, MS source temperature: 230 °C, MS quadrupole temperature: 150 °C, ionization energy 70 eV, scan range: to 34 at 450 *m/z*. identification of the various chemical compounds of the essential oil of *Daucus carota* subsp. *carota* relies on the retention indices (I.R) and their mass spectra. They are calculated using a linear interpretation with respect to the retention time of a series of n-alkanes from $C_7$ to $C_{26}$ [23,24].

## *2.5. Antimicrobial activity of the essential oil of Daucus carota subsp. carota*

The antimicrobial power of the essential oil was tested according to qualitative and quantitative analyses as follow:

### *2.5.1. Antimicrobial capacity according to quantitative and qualitative tests*

The antibiogram technique uses diffusion on agar medium [25]; the principle is based on the resistance of the tested strains against the extracts used [26]. The inhibitory activity of the antimicrobial agents is indicated by the appearance of a clear zone around a cellulose disk impregnated with extracted essential oil. The sterile disks are 9 mm diameter cellulose (reference disk: MN 640w filter paper, MACHEREY-NAGEL GmbH and co, KG Germany), injected with 20 µl of essential oil. Each moistened disc is then placed in a Petri dish on previously prepared agar, Muller Hinton for the bacteria and Sabouraud for the yeasts. The petri dishes are closed to allow the essential oil to diffuse at room temperature for 30 minutes. Afterwards, they are incubated in the oven at 37 °C for 18 to 24 h for bacteria and at 25 °C. for 48 h for yeasts [27]. According to [28], activity is assessed by measuring the diameter of the inhibition zone (halo) around each disc using a caliper. [29] note that the smaller the diameter of the halo, the more resistant the bacterium is. The estimation scale used for antimicrobial activity is given by [30]. Kanamycin, cephalexin, carbenicillin, bacitracin and amoxicillin were used as antibiotic references against both $Gram^+$ and $Gram^-$ bacterial strains while Fluconazol was used as positive controls for antifungal activity (10-30 µg/ml).

### *2.5.2. Qualitative analyses (MIC, MBC and MFC)*

The MIC is defined as the lowest concentration of essential oil needed to induce 90% of a reduction in microbial growth. Frequently, the MIC is not completely bactericidal (MBC), or fungicidal (MFC), since some of the incubation effect is capable of developing after disappearance of the inhibitory compound [31,32]. Dilutions of the essential oil were prepared in DMSO mixed with the culture media [33]. Ranges of binary concentrations were obtained ranging from 2 to 0.03% [34].

### *2.6. Data Analysis*

All experiments were performed in triplicate; the results are presented as means ± SD. The means were compared statistically using the SPSS 21 test (with $P \leq 0.05$).

## 3. Results and discussion

### *3.1. The essential oil yield*

After 3 hours extraction by hydro-distillation of *Daucus carota* subsp. *Carota* umbels the essential oil yield was 3.65%, with a moisture content of 64%. The main differences in these results by different workers are thought to be related to geographical origin, climatic and edaphic factors, i.e. temperature, moisture, and the nature of the soil. The parts of the plant used for testing, as well as the different stages of growth, the period of harvesting, the conservation of the plant material and the extraction method are all factors that could influence the oil yield (table 2). The results published by [37] confirm these variations between different species, between organs of the same species and between places of harvest of the same species, more research on these factors is clearly needed.

**Table 2:** The essential oil yield

| Species | Part of the plant | Extraction mode | Authors | Sites & Country | Yield (%) |
|---|---|---|---|---|---|
| *D. carota* subsp. *carota* | Flowering umbels | Hydro-distillation | This work-2017 | Aith-Laziz (Algeria) | 3.65 |
| *Daucus rebaudii* | Aerial parts | Hydro-distillation | Djarri et *al*., 2006 [7] | El Kala (Algeria) | 0.4 |
| *D. carota L., cultivar ''Chanteney''* | Seeds | Hydro-distillation<br>Supercritical $CO_2$ | Glišić et *al*., 2007 [8] | Northern Serbia | 0.69<br>1.17 |
| *D. carota sativa* | Seeds | Hydro-distillation | Imamu et *al*, 2007 [9] | Uzbekistan | 2.2 |
| *D. carota* subsp.*carota* | Flowering umbels<br>Ripe umbels with mature seeds | Hydro-distillation | Maxia et *al*., 2009 [11] | Portugal | 0.7<br>1 |
| *Daucus carota* subsp. *hispanicus* | Aerial parts<br>Roots | Hydro-distillation | Bendiabdellah et *al*, 2013 [16] | Tlemcen (Algeria) | 0.4-2.2<br>0.13-1.6 |
| *D. carota gummifer*<br>*D. carota carota* | Aerial parts (stems and leaves) | Hydro-distillation | Meliani et *al*., 2013 [17] | Tlemcen (Algeria) | 1.52<br>1.64 |
| *D. carota* | Seeds | Hydro-distillation | Rokbeni et *al*., 2013 [18] | Northern Tunisia | 0.5-2.6 |
| *D. carota sativus* | Seeds initiation stage<br>Green seed stage<br>Light brown seed Full bloom stage<br>Full brown seed | Hydro-distillation | Verma et *al*., 2014 [19] | Foothilis (India) | 1.8<br>1.5<br>1.3<br>1.1 |
| *D. gracilis* | Aerial parts | Hydro-distillation | El Kolli et *al*., 2015 [20] | Skikda (Algeria) | 0.56 |
| *D. carota* | Umbels | Hydro-distillation | Behidj et *al*., 2015 [35] | Bouira (Algeria) | 4.65 |
| *D. carota* subsp. *carota* | Ripe umbels with seeds | Hydro-distillation | Alves-Silva et *al*., 2016 [36] | Portugal | 0.9 |

### *3.2. Physico-chemical and organoleptic characterization*

The results of the organoleptic and physicochemical characteristics of the essential oil extracted from flowering umbels of the wild carrot used in this study are given in table 3.

**Table 3:** Organoleptic and physico-chemical characteristics of essential oil

| Organoleptic and physico-chemical properties | | This work-2017 Aith-Laziz (Bouira) Flowering umbels | Sour El Ghozlane (Bouira) Flowering umbels [35] | Algiers Aerial parts [38] | Pologne Flowering umbels [23] | Seeds [22] |
|---|---|---|---|---|---|---|
| Organoleptic properties | Aspect | Clear liquid | Clear liquid | / | / | Liquide limpide |
| | Color | Yellow clear | Yellow gold | / | / | Yellow clear to shaded |
| | Odor | Characteristic, pungent and pleasant | Characteristic, pleasant and spicy | / | / | Characteristic |
| Physical properties at 20°C | Relative density | 0.937 | 0.926 | 0.930 | 0.9701 | 0.900-0.945 |
| | Refractive index | 1.487 | 1.429 | 1.464 | 1.4715 | 1.480-1.493 |
| | optical rotation | -35.65° | +0.24° | +0.33° | -27.0° | -30 to -4° |
| Chemical properties | Ester index | 33 | 35 | / | / | 15-55 |
| | Saponification index | 50 | 45 | / | / | / |
| | Acid index | 17 | 10 | / | / | Min 5 |

According to international standards, the European Pharmacopoeia, ISO and Afnor, the determination of physico-chemical parameters is a necessary step in assessing the quality of essential oils. But they remain insufficient to characterize an essential oil without the determination of the chemical constituents. The latter evaluate the quality of the chemical functions, namely acids, esters, and alcohols. Values of the physical properties measured in the essential oil of *Daucus carota* subsp. *carota* are cited by [23] are similar to those of the present study. However, the refractive index value determined in the present study is greater than 1.487 compared to that reported by [35] [38], which were respectively 1.464 and 1.429. But they approximate to the value of [22]. Which varies from 1.48 to 1,493. The values of relative density, acid index, ester index, saponification index, color, odor and appearance are similar to those reported in the studies cited above. It is noteworthy that the rotatory power value for the extracted oil is very low (-35.65) (Table 3)but is close to that quoted by [22] and [23]. Thus, it is very different from the results obtained by [35, 38]. According to [39], an essential oil which is characterized by a high ester index cannot be preserved for a long time. Thus, density is a criterion of purity which is closely related to the chemical composition.

### *3.3. GC/MS analysis*

According to table 4, GC/MS analysis identified 47 compounds, ie 98.33%, of which the dominance of sesquiterpenes was 53.4%.

**Table 4:** Chemical composition and essential oil retention index of *Daucus carota* subsp. *carota*

| Number of pick | Compounds | Retention index | Total % |
|---|---|---|---|
| 1 | α-Thujene | 924 | 0.49 |
| 2 | α-Pinene | 935 | 6.03 |
| 3 | Camphene | 946 | 0.21 |
| 4 | Sabinene | 969 | 4.33 |
| 5 | β-Pinene | 977 | 0.9 |
| 6 | β-Myrcene | 988 | 3.24 |
| 7 | δ.3-Carene | 1008 | 0.29 |
| 8 | P-Cymene | 1016 | 0.38 |
| 9 | Limonene | 1024 | 13.49 |
| 10 | β-Ocimene | 1035 | 0.24 |
| 11 | γ-Terpinene | 1046 | 0.42 |
| 12 | α-Terpinolene | 1077 | 0.25 |
| 13 | Linalool | 1085 | 0.94 |
| 14 | Terpinene-4-ol | 1169 | 0.44 |
| 15 | α-Terpineol | 1177 | 0.13 |
| 16 | Geraniol | 1240 | 0.28 |
| 17 | α-Bornyl Acetate | 1280 | 0.61 |
| 18 | α-Longipinene | 1350 | 0.4 |
| 19 | α-Copaene | 1371 | 0.3 |
| 20 | Geranyl Acetate | 1390 | 12.26 |
| 21 | α-Cedrene | 1404 | 5.27 |
| 22 | Z-α-Bergamotene | 1407 | 0.18 |
| 23 | β-Caryophyllene | 1419 | 2.94 |
| 24 | Z-β-Farnesene | 1434 | 0.45 |
| 25 | E-α-Bergamotene | 1445 | 0.64 |
| 26 | β-Humulene | 1450 | 1.44 |
| 27 | E-β-Farnesene | 1456 | 0.6 |
| 28 | β-Cadinene | 1472 | 1.12 |
| 29 | Germacrene D | 1475 | 9 |
| 30 | β-Selinene | 1485 | 0.22 |
| 31 | Bicyclogermacrene | 1488 | 0.59 |
| 32 | α-Selinene | 1490 | 0.43 |
| 33 | Z-α-Bisabolene | 1492 | 5.43 |
| 34 | β-Himachalene | 1495 | 0.72 |
| 35 | β-Bisabolene | 1500 | 0.15 |
| 36 | E-α-Farnesene | 1507 | 0.72 |
| 37 | Germacrene A | 1511 | 2.41 |
| 38 | γ-Bisabolene | 1518 | 2.37 |
| 39 | E-α- Bisabolene | 1520 | 0.68 |
| 40 | β-Sesquiphellandrene | 1523 | 1.41 |
| 41 | α-Cadinene | 1525 | 0.95 |
| 42 | Caryophyllene Oxide | 1559 | 0.42 |
| 43 | β-Asarone | 1561 | 3.08 |
| 44 | Aromadendrene epoxide | 1565 | 0.22 |
| 45 | Carotol | 1594 | 10.38 |
| 46 | α-Cadinol | 1630 | 0.49 |
| 47 | β-Bisabolol | 1660 | 0.39 |

Sesquiterpenes are represented by carotol (10.38%) and germacene D (9%) as the major compounds, and Z-α-bisabonene (5.43%) and α-cedrene (5.27%) were secondary constituents. While β-asarone (3.08), α-caryophyllene (2.94%), germacene A (2.41%) and γ-bisabolene (2.37) are poorly represented. Among the major identified monoterpenes, one limonene (13.49%) and geranyl acetate (12.26%) are notable. α-pinene (6.03%), sabinene (4.33%) and β-myrcene, (3.24%) are moderately present. These monoterpenes contribute 44.93% on all the chemical constituents of this essential oil (figure 1).

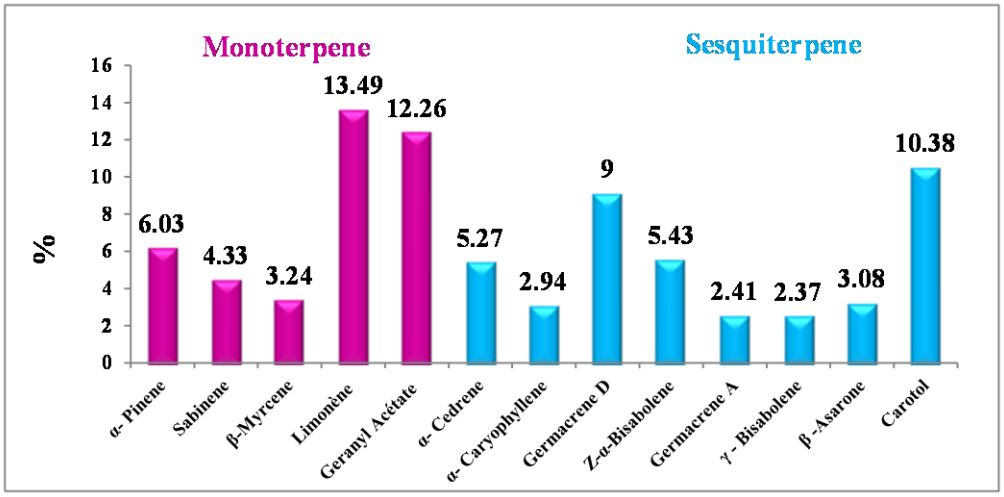


**Figure 1.** Majority chemical compounds essential oil of *Daucus carota* subsp. *carota*

Other study carried out on the essential oil of *Daucus carota* showed a great diversity in its chemical composition according to the species, the habitat and the different parts of the plant studied. According to [10, 7, 12, 17, 19, 23, 36], the essential oil obtained by hydro-distillation of the aerial parts of different spices of *Daucus* in several regions of the world contains a significant amount of monoterpenes, composed essentially of α-pinene, sabinene, limonene and geranyl acetate. [11] report that the essential oil extracted by hydro-distillation from the mature umbels with seeds of Portugal consists predominantly of 88% monoterpenes, the main components of which are geranyl acetate (65%) and α-pinene (13%). The essential oils of flowering umbels contain α-pinene and geranyl acetate, which are also the main compounds, respectively 37,9% and 15% out of a total of 73,7% of monterpenes. Analysis of the essential oil of the same species from Italy shows that, under the same operating conditions, a composition of 78.5% sesquiterpenes was obtained for flowering umbels, namely 17.6% β-bisabolene, 25.1% carotol and 21.6% of 11-α-(H)-himacal-4-en-1-β-ol. Whereas for umbels with seeds, 73.6% sesquiterpenes were found, ie 51% β-bisabolene and 10% (E)-methyl isoeugenol. Supercritical extracts contain low amounts of monoterpenes and higher amount of sesquiterpense hydrocarbons. [16] showed that yields of essential oils of *Daucus carota* subsp. *hispanicus* Gouan from different regions of Tlemcen (Algeria) present phenylpropanoids in abundance with a high rate of 83.8% myristicin in flowers, a rate of 80.2% in leaves, a rate of 66.9% In the stems and 73.2% in the aerial parts. In contrast, in the roots the apiol is represented by 80.3% and myristicin by 16.6%. According to [18], seed essential oils taken from ten wild populations of *Daucus carota* distributed over northern Tunisia consist of a total of 36 constituents with a predominance of sesquiterpene hydrocarbons in most samples (22.63 to 89.93%), with 39.33% of β-bisabolene, sabinene (8.53%), the geranyl acetate (7.12%), and elemicine (6.26%). [35] report that tne chemical analyzes of the essential oil of the flowering tops of *Daucus carota* collected in Sour El Ghozlane (Algeria) highlighted the monoterpenes as a major component with two dominant components. They noted α-pinene (22.3%) and limonene

(15.8%). The sesquiterpenes are represented mainly by carotol to 21.7%. Generally, the main components in the essential oil of aerial part in full flowering of Algerian *Daucus carota* subsp. *carota*, were α-pinene (21.3 %) and asarone (18.4 %), while the essential oils isolated from flowers and both leaves and stems were dominated by asarone (9.8-9.4 %), α-pinene (10.9-10.6 %) and β-bisabolene (7.6-9.3 %) respectively. Before flowering and after flowering, the major constituents were the linalool (11.2 %) and asarone (20.8 %) respectively [38]. [40], worked on the essential oils of flowering umbels of *Daucus carota* subsp. *carota* harvested in Tunisia in two different bioclimatic areas. Sejnane is located in the humid bioclimatic zone and Tunis is part of the sub-humid bioclimatic zone. The analyzed essential oils show a great chemical variability in their compositions according to the geographical origin as well as the extraction process. The same authors mention that the essential oil of Sejnane obtained by hydro-distillation is characterized by the dominance of sesquiterpenes 65,3% with 14,5% of α-pinene, 12,7% of 11-α-(H)-himacal-4-en-1-β-ol a, b and 7.4% α-selinene. While the essential oil extracted by supercritical $CO_2$ contains 73.1% sesquiterpenes with relatively different levels of major compounds, ie 17.4% of 11-α-(H)-Himacal-4-en-1-β-Ol a, b, 12% α-pinene and 8.6% α-selinene. However, the essential oil extracted by hydro-distillation belonging to the Tunis region has a high content of 55.5% sesquiterpenes, ie 48% carotol and 31.5% phenylpropanoids. While the essential oil obtained by supercritical $CO_2$ has remarkable amounts of 60.5% sesquiterpenes, 55.5% of carotol and 35.5% of phenylpropanoids are noted. Thus, it is found that the contents of the majority compounds of the oils obtained from these two extraction methods are connected, since they do not show a great difference in the chemical composition. In the light of the results obtained, there are some important variations in the chemical composition of the essential oil of *Daucus carota*. Thus, the components of the essential oil of the flowering heads show marked variability according to the harvesting region, the extraction mode and also the part of the plant used. As a result, it can be suggested that this species is characterized by considerable chemical polymorphism. The geographical origin, temperature, relative humidity, edaphic nature of the soil, plant organ used, stage of growth affected by the harvest, period of harvesting, conservation of the plant and method of extraction are parameters which play a determining role in the rate of components and in the variation of the chemical composition of the essential oil of each studied plant species [2, 37, 41].

### *3.4. Antimicrobial Activity*

The results of the antimicrobial effect of the essential oil of *Daucus carota* subsp. *carota* and standard antibiotics on the growth of tested microbial strains are shown in table 5 and table 6.

**Table 5:** Qualitative and quantitative evaluation of the antimicrobial activity of the essential oil against the tested microbial strains

| Strains | Inhibition diameter (mm) | MIC (%) | CMB CMF | Action |
|---|---|---|---|---|
| *Escherichia coli* | 11 | 0.5 | > 2 | ND |
| *Pseudomonas aeruginosa* | 10.33 | 1 | > 2 | ND |
| *Pseudomonas fluoresces* | 10.33 | 1 | > 2 | ND |
| *Bacillus subtilis* | 16.5 | 0.125 | 1 | 8 |
| *Staphylococcus aureus* | 16.33 | 0.125 | 0.5 | 4 |
| *Candida albicans* | 16 | 0.25 | 1 | 4 |
| *Saccharomyces cerevisiae* | 16 | 0.125 | 0.5 | 8 |

**Table 6**: Qualitative evaluation of the antimicrobial activity of the antibiotic extracts against the tested microbial and fungal strains

| Strains | Antibiotics | Inhibition diameter (mm) |
|---|---|---|
| *Escherichia coli* | Kanamycin | 14.33 |
| | Cephalexin | 18.5 |
| | Carbenicillin | 23 |
| *Pseudomonas aeruginosa* | Carbenicillin | 14.33 |
| *Pseudomonas fluoresces* | Cephalexin | 18.5 |
| *Bacillus subtilis* | Bacitracin | 11.5 |
| | Amoxicillin | 21 |
| | Kanamycin | 23 |
| *Staphylococcus aureus* | Cephalexin | 20.33 |
| | Amoxicillin | 24.33 |
| *Candida albicans* | Fluconazol | 36 |
| *Saccharomyces cerevisiae* | Fluconazol | 35 |

Moderate inhibition ranging from 16 to 16.5 mm has been reported for $Gram^+$ bacteria, namely *Bacillus subtilis* and *Staphylococcus aureus*, as well as for both yeasts, *Candida albicans* and *Saccharomyces cerevisiae*.

It should be noted that the obtained MIC values are quite low, ranging between 1 and 0.125%, which indicates a broad spectrum of activity of the essential oil tested against all these $Gram^+$. A small zone of inhibition around the discs was observed with respect to *Escherichia coli*, *Pseudomonas aeroginosa* and *Pseudomonas fluoresces*. A diameter of inhibition zone ranging between 10.33 mm to 11 mm was noted. These $Gram^-$ bacteria possess a very high resistance potential against the antimicrobial action of the essential oil of *Daucus carota* subsp. *carota*. These microorganisms are considered to be slightly inhibitory. The values of MIC are average (0.5 to 1%). While CMB and CMF are between 0.5 and 1%. The $Gram^-$ bacteria have a very high potential for resistance against the antimicrobial action of essential oil *Daucus carota* subsp. *carota*, which is considered slightly inhibitory, with the CMB are superior to 2.

Reports CMB/CMI (=4) show that essential oil of *Daucus carota* subsp.*carota* is bactericidal against *Staphylococus aureus* and *Candida albicans*. The report of CMB/MIC and CMF/MIC equal to 8, and highlights the nature of bacteriostatic against *Bacilus subtilis* and *Saccharomyces cerevisiae*. This essential oil was more efficient than Bacitracin but less active than other synthetic antibiotics. [8] reported that the antimicrobial effects of essential oils of Serbian *Daucus carota* seeds obtained by hydro-distillation and supercritical $CO_2$ may be due to increased sensitivity of bacterial and fungal strains to carotol which represents 20% and 30.3%, respectively. This biological effect can be caused by the synergistic activity of the carotol with the other compounds of higher molecular weight present in the tested extracts. It turns out that $Gram^+$ bacteria; *Bacillus cereus*, *Bacillus subtilis* and *Rhodococcus equi* and the yeast *Candida albicans* are more sensitive than $Gram^-$ bacteria *Escherichia coli* et *Pseudomonas aeruginosa*. Fairly weak MICs are obtained with $Gram^+$ bacteria and the yeasts. Thus, they range from 80 to 640 µg/ml while $Gram^-$ bacteria have MICs> 1280 µg/ml. However, [10] demonstrated the effectiveness of essential oils of *Daucus carota* subsp. *halophilus* from Portugal, Cabo de S.Vicente, and Arrifana with respect to several species of *Candida* with MICs and CMBs of 1.25 to 2.5µl /ml. Concerning the evaluation of MICs and CMBs, [11] report that the essential oils of leaves and stems of *Daucus carota* extracted by hydro-distillation and supercritical $CO_2$ show varying degrees of inhibition against different types of fungi studied as *Candida albicans*, *Aspergillus aureus*, with MICs of 2.5 to 25 µl/ml, and

CMBs of 0.64 to 20 µl/ml. According to [12], the most important antibacterial activity of the essential oil of the *Daucus carota* umbels of Serbia is recorded in Gram$^+$ bacteria such as *Bacillus cereus* and *Pseudomonas aeruginosa* with a MIC of 5 ml/ml and a 10 ml/ml CMB. On the other hand, *Escherichia coli* has identical MIC and CMB values (10 ml/ml). These values are higher than those found in Gram$^-$ bacteria. [16], showed that the essential oils of the aerial parts and roots of *Daucus carota* subsp. *hispanicus* Gouan from different regions of Tlemcen (Algeria) tested on four different microorganisms give very remarkable effects. They are characterized by strong activity against *Candida albicans* with an average diameter of 26 to 30 mm and MIC values of 0.078 to 0.125 mg/ml. Concerning *Bacillus subtilis*, the diameter of the inhibition zone is between 14 and 16 mm with MIC values ranging from 1.2 to 1.5 mg/ml. This activity is low with regard to *Staphylococcus aureus* or a diameter of the inhibition zone of 8 to 10 mm and MIC values varying from 4.2 to 4.8 mg/ml. For *Escherichia coli*, a diameter of the inhibition zone of 6 mm was noted with MIC> 5 values. According to the same authors, the strong fungicidal activity of these essential oils is mainly due to two compounds; the phenylpropanoids ie the apiole with 93.6 to 97.1% and the myristicin ie 67.2 to 86.3% respectively in the roots and aerial parts. [17], demonstrated that the essential oil recovered from the aerial parts of *Daucus carota* subsp. *carota* of Algeria presents variable MICs according to the tested microbial strain. It should be noted that *Escherichia coli* and *Staphylococcus aureus* have identical MICs of 2.5 mg/ml. Thus, they are very sensitive to the tested extract. While *Pseudomonas aeruginosa* is moderately sensitive to this essential oil with a MIC of 5 mg/ml. *Bacillus cereus* is considered the most resistant pathogen to tested essential oil with a MIC> 6 mg/ml.
[23] cited MIC values of 3, 4, 5, 8 and > 8 µl /ml respectively in these different microorganisms, namely *Bacillus subtilis*, *Staphylococcus aureus*, *Candida albicans*, *Escherichia coli*, and *Pseudomonas aeroginosa* with respect to the essential oil of the florewing umbels of *Daucus carota* subsp. *carota* of Poland. The antimicrobial activity of the essential oil of *Daucus carota* subsp. *carota* of ripe umbels with seeds from Portugal was evaluated against several Gram$^+$, Gram$^-$ bacteria and yeasts. The MIC and the MLC were evaluated showing a significant activity towards *Bacilus subtilis* and *Staphylococus aureus* 0.32 and 0.64 µl/ml respectively, while *Escherichia coli* show the MIC and MLC<10 µl/ml. The oil inhibited more than 50% of *Candida albicans* at concentrations as low as 0.04 µl/ml (MIC/128) [36]. According to the work of [40], the CMI and MCC values of the essential oils of the flowering umbels of *Daucus carota* subsp. *carota* harvested in two different sites of Tunisia are of the order of 2.5% (v/v) compared to the two tested microorganisms, *Candida albicans* and *Aspergillus aureus*. These results are in agreement with those of Falleh *et al*., [43] who emphasized the effectiveness of essential oils against Gram$^+$ bacteria compared to Gram$^-$ bacteria. It is important to note that the absence of the outer membrane, Gram$^+$ bacteria develop a sensitivity to external environmental changes, such as temperature, pH, and the nature of the extract tested. Gram$^-$ bacteria possess intrinsic resistance to biocidal agents due to their outer double membrane. The latter is composed of lipopolysaccharides which form a barrier impermeable to hydrophobic compounds [44]. According to [45], the antimicrobial activities of the essential oil of the genus *Daucus* are probably due to carotol, which is the main component of this species. The carotol revealed a fungicidal activity against the phytotoxics of *Alternaria alternata* isolated from the surface of the seeds cultivar Perfekcja, a variety widely distributed in horticulture in Poland which inhibited the radial growth of fungi by 65% . The strongest antifungal activity was carrot seed oil is the source of sesquiterpenes carotol, daucol and β-caryophyllene. According to [5] and [42], the most efficient and broad spectrum chemical compounds are phenols, which have the reputation of being the main? antibacterial molecules of the plant world. They are rather rare and specific to certain essential oils such as thymol and carvacol, in particular alcohols, such as α-terpineol, terpinen-4-ol, and linalool, aldehydes and ketones.

## 4. Conclusion

The flowering umbels of *Daucus carota* subsp. *carota* from the region of Aith Laaziz (Bouira- Algeria) extracted by hydro-distillation contain a high percentage (3.64%) of essential oil. The physico-chemical analyzes of this oil showed an important degree of variability in chemical composition and in the nature of the compounds of identified. Composition is characterized by the dominance of sesquiterpenes (53.4%) mainly of carotol (10.38%), germacene D (9%), monoterpene (44.93%), limonene (13.49%), geranyl-acetate (12.26%) and α-pinene (6.03%). This distribution reflects the presence of a new chemotype. The moderate antimicrobial activity is directly related to the chemical profile, which is characterized by low levels of alcohols and ketones (monoterpenes), compounds that are essentially highly antimicrobial. Given the importance of the yield of the essential oil by this species and despite its moderate antimicrobial activity, with fairly low MIC values, this plant deserves to be more valued and exploited, in the pharmaceutical, medical, phytosanitary, and agri-food sectors.

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