Chemical composition and bioactivity of essential oils and Ethanolic extracts of *Ocimum basilicum* L. and *Thymus algeriensis* Boiss. & Reut. from the Algerian Saharan Atlas

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**Abstract**

**Background:** There is increasing interest in the pharmaceutical and food industries to substitute synthetic chemicals with naturally occurring compounds possessing bioactive properties. Plants are valuable sources of bioactive compounds. The present study investigates the chemical composition and antioxidant, antimicrobial, and anticancer activities of ethanolic extracts (EEs) and essential oils (EOs) from two species in the Lamiaceae family, *Ocimum basilicum* L. and *Thymus algeriensis* Boiss. & Reut., cultivated in the Algerian Saharan Atlas.

**Methods:** The total flavonoid contents of the plants’ ethanolic extracts were determined by the aluminium chloride method, while the total phenols were determined using the Folin-Ciocalteu method. Essential oils were obtained by hydrodistillation of the aerial parts of the plants and were analysed by GC-MS. The free radical-scavenging ability and antioxidant potential of the plants’ EEs and EOs were probed using the 2, 2-diphenyl-picrylhydrazyl radical-scavenging, ABTS radical-scavenging, ferric-reducing power and phosphomolybdenum assays. The antimicrobial activities were evaluated against several pathogens characteristic of gram-negative bacteria (three species), gram-positive bacteria (three species) and fungi (two species). The microdilution method was used to estimate the minimum inhibitory concentrations (MICs). The oils’ anticancer potential against several cancer types was also studied using the MTT assay and reported as the toxic doses that resulted in a 50% reduction in cancer cell growth (LD50).

**Results:** Phenolic compounds in the EEs from both plants were analysed by HPLC and demonstrated a rich flavonoid content. Chemical analysis of the essential oil from *Ocimum basilicum* revealed 26 unique compounds, with linalool (52.1%) and linalyl acetate (19.1%) as the major compounds. A total of 29 compounds were identified in the essential oil from *Thymus algeriensis*, with α-terpinyl acetate (47.4%), neryl acetate (9.6%), and α-pinene (6.8%) as the major compounds. The ethanolic extracts and essential oils from both plants exhibited moderate antioxidant activities and moderate to weak antimicrobial activities. Furthermore, anticancer activities against the examined human cancer cell lines were associated with only the EOs from both plants, with LD50 values ranging between 300 and 1000 μg/mL.

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Background
Among the numerous medicinal plants, some prevalent species are particularly popular in folk medicine due to their antioxidant properties and health benefits [1]. The Lamiaceae family is one of the most extensively studied plant families containing members with proven human health benefits. Antimicrobial [2–4], antioxidant [5, 6], anti-inflammatory, anti-depressive, and antiproliferative effects [7, 8] have been demonstrated for the active compounds found in many plants from this family. Rosemary, thyme, basil, and sage are famous members of the Lamiaceae family and are among the most commonly used plants in traditional Mediterranean remedies, mainly for the treatment of gastritis, infections, dermatitis, bronchitis, and inflammation [3, 4, 9, 10].

Ocimum basilicum, an aromatic herb that belongs to the Lamiaceae family and the Nepetoideae subfamily [9], has been used in folk medicine for the treatment of gastrointestinal and respiratory diseases [10]. This species was also reported as having beneficial effects on kidney malfunctions, warts and worm infestations [10]. Various chemical functionalities characteristic of anthocyanins, flavonoids, monoterpenes, phenolic acids and their esters, phenylpropanoid derivatives, phytosterols, tannins, and triterpenes have been identified in different O. basilicum extracts [11, 12]. Essential oils extracted from its leaves and flowers can be used as flavouring agents in food, medicine and cosmetics [10, 11].

Another renowned genus of the Lamiaceae family is the Thymus genus. It contains approximately 215 to 350 species that are remarkably predominant in the Mediterranean area [13, 14]. The aerial parts and the volatile components of Thymus species are traditionally used as herbal teas and condiments and for numerous medicinal purposes, e.g., as antispasmodic, antibacterial, antiviral, expectorant and antioxidant agents [15]. Compounds in several classes have been identified in Thymus species, such as carvacrol, thymol, geraniol, γ-terpineol, thujone and linalool [16].

The aim of this study was to investigate the chemical composition and biological activities of essential oils and ethanolic extracts from Ocimum basilicum and Thymus algeriensis cultivated in the Algerian Saharan Atlas (Laghouat region).

Materials and methods
Plant materials
Ocimum basilicum and Thymus algeriensis were harvested in their flowering stage between April and May 2016 in the area of the Algerian Saharan Atlas (Laghouat region). The species were characterized at the Department of Biology, Faculty of Science, University of Laghouat (Algeria), and the voucher specimens were banked at the Laboratory of Process Engineering, University of Laghouat, with the numbers LGP Ob/04/16 and LGP Ta/05/16, respectively. The plant materials were air-dried for 15 days and stored at room temperature (25 ± 2 °C) without exposure to direct sunlight.

Preparation of the ethanolic extracts
Dried plant aerial parts (leaves) were pulverized. Each 15 g of ground sample was placed into a separate Erlenmeyer flask. Then, 100 mL of ethanol (100%) was added, and the samples were incubated in a water bath at 55 °C for 6 h. Separation of the extraction mixture from the residue was achieved by filtration through Whatman No. 1 filter paper. Each plant residue was re-extracted in triplicate with ethanol. After filtration, the two portions were mixed. The residual solvent in the ethanolic extracts were removed under reduced pressure at 48–49 °C using a rotary evaporator (Rotavapor IKA VB 10, Germany). Water in the extracts was lyophilized using a freeze dryer (Thermo Savant Modulyo D, USA) for 8 h at −50 °C and 0.040 mbar. The yields of these fractions were 20.16% (Ocimum basilicum) and 15.78% (Thymus algeriensis).

Total phenolic content
The total phenolic content was determined with Folin-Ciocalteu reagent using the method described by Singleton et al. [17]. Briefly, to 200 μL of each sample (three replicates), 1 mL of 10% (v/v) Folin-Ciocalteu reagent and 800 μL of Na2CO3 (7.5%, w/v) were added and incubated at 25 °C for 30 min. The absorbance of each sample was measured at 760 nm using a spectrophotometer (OPTI-ZEN 2120 UV). An ethanol solution of gallic acid was tested in parallel to obtain a calibration curve. Values were expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g dry weight).
Total flavonoid content
The total flavonoid content was estimated using the aluminum chloride colorimetric method of Boulanouar et al. [18]. Briefly, 1 mL of diluted extract was mixed with 1 mL of a 2% AlCl₃ methanol solution. After incubation at room temperature for 15 min, the absorbance was measured at 415 nm using a spectrophotometer (OPTIZEN 2120 UV). The total flavonoid content was calculated from the calibration curve of rutin and expressed as milligrams of rutin equivalents per gram of dry weight (mg RE/g dry weight).

Analysis of phenolic contents by HPLC
The HPLC procedure described by Caponio et al. [19] was adopted for the analysis of phenolic contents. The conditions utilized were as follows: a C-18 column (CTO-10ASVp, 4.6 mm × 250 mm, 5 μM), an injection volume of 20 μL, a mobile phase composed of solvent A (3% formic acid in distilled water) and solvent B (100% acetonitrile), gradient elution from 15 to 100% B over a run time of 45 min at a flow rate of 1 mL/min, and a UV-Vis detector. For analysis, the samples were dissolved in ethanol, and 20 μL of this solution was injected into the column. Chromatograms were plotted based on detection at 280 nm with the UV-Vis detector. The retention times and UV absorption spectra of the phenolic compounds were compared with those of pure standards. Gallic acid, 3, 4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, 2, 5-dihydroxybenzoic acid, chlorogenic acid, vanillic acid, caffeic acid, p-coumaric acid, ferulic acid, rutin, ellagic acid, naringin, and quercetin were used as standards. Peaks were identified by matching the retention times and UV spectra with those of the standards. The quantity of each phenolic compound was reported in μg per gram of the extract.

Isolation of essential oils
Essential oils were extracted from the air-dried and ground plants (100 g each) using the hydrodistillation method for 4 h in a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulfate and stored at 4 °C in the dark until further analysis.

GC-MS analysis
GC-EIMS analyses were accomplished with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness of 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. The following analytical specifications were used: injector and transfer line temperatures of 220 °C and 240 °C, respectively; oven temperature programmed from 60 °C to 240 °C at 3 °C/min; carrier gas of helium at 1 mL/min; injection volume of 0.2 μL (10% hexane solution); and split ratio of 1:30. For the identification of each compound, a comparison of the retention times with the standards’ retention times was performed. In addition, we evaluated the linear retention indices in relation to the retention times of a series of n-hydrocarbons and used computer-aided matching with commercially available mass spectra and mass spectra contained in an in-house library.

Antioxidant activities
Free radical-scavenging activity
The DPPH radical-scavenging capacity was measured as previously described by Boulanouar et al. [18]. In this study, 1 mL of either ethanolic extract or essential oil was added to 2 mL of a 60 μM DPPH methanol solution, and the reaction mixture was kept at room temperature and away from light for 30 min before the absorbance was recorded at 517 nm. The following equation was used to estimate the scavenging activity: scavenging effect (%) = [100*(A_c - A_s / A_c)], where A_c is the control sample absorbance and A_s is the test sample absorbance. The concentration of oil or extract at which 50% of the DPPH radicals (IC₅₀) were scavenged was calculated. Ascorbic acid was employed as a reference compound.

ABTS assay
The antioxidant capacity was estimated following the procedure described by Re et al. [20] with minor modifications. In brief, a 7 mM ABTS stock solution was reacted with 2.45 mM potassium persulfate at room temperature for 12–16 h while protected from light to produce the ABTS radical cation (ABTS⁺). The ABTS⁺ mixture (1450 μL) was diluted with methanol to reach an absorbance of 0.70 ± 0.02 at 734 nm, followed by the addition of 50 μL of either the test sample or Trolox standard and incubation at room temperature while protected from light for 6 min. The absorbance was read at 734 nm using a spectrophotometer (OPTIZEN 2120 UV). For each test sample, the percent inhibition of ABTS⁺ was computed using the following formula: (% inhibition) = [100*(A_c - A_s / A_c)], where A_c is the control sample absorbance and A_s is the test sample absorbance. The concentration of oil or extract that could scavenge 50% of the ABTS radicals (IC₅₀) was calculated. Trolox was used as a reference compound.

Reducing power assay
The method of Oyaizu 1986 was adopted to determine the reducing power of the examined EEs and EOs [21]. Extract solution (0.1 mL), phosphate buffer (2 mL, 0.2 M, pH 6.6) and potassium ferricyanide [K₃Fe(CN)₆] (2 mL, 1%) were mixed and then incubated at 50 °C for 20 min. Afterward, 2 mL of trichloroacetic acid (10%) was added to the solution to terminate the reaction. The mixture was then centrifuged at 3000 rpm for 10 min, and the
upper layer of the solution (2 mL) was collected. The collected layer was mixed with distilled water (2.5 mL) and FeCl₃ (0.5 mL, 0.1%) before the absorbance was measured at 700 nm. Greater reducing power was associated with increased absorbance of the reaction mixture. The results are expressed in terms of the ascorbic acid equivalent antioxidant capacity (AEAC).

**Phosphomolybdenum assay**
The Prieto et al. method was adopted to evaluate the reducing capacity of the EEs and EOs from the two assessed plants [22]. In summary, 0.3 mL of each diluted extract was added to 3 mL of a phosphomolybdic reagent solution containing ammonium molybdate (4 M), sodium phosphate (28 mM) and sulfuric acid (0.6 M). The mixture was placed in a water bath at a temperature of 95 °C for 90 min. After cooling to room temperature, the absorbance was measured at 695 nm against a blank solution containing 0.3 mL of ethanol mixed with 3 mL of phosphomolybdic reagent. The results are expressed in terms of the ascorbic acid equivalent antioxidant capacity (AEAC).

**Antimicrobial activity**

**Microorganisms**
Six bacterial and two fungal species were acquired from the Microbial Culture Collection Center of the Medical School at the University of Jordan, Jordan. The species used were *Staphylococcus epidermidis* ATCC12228 (gram-positive bacterium), *Staphylococcus aureus* ATCC25923 (gram-positive bacterium), *Bacillus subtilis* ATCC11562 (gram-positive bacterium), *Escherichia coli* ATCC29245 (gram-negative bacterium), *Pseudomonas aeruginosa* ATCC15442 (gram-negative bacterium), *Klebsiella pneumoniae* ATCC43816 (gram-negative bacterium), *Candida glabrata* ATCC22553 (fungus), and *Candida albicans* ATCC10231 (fungus). The eight microorganisms represent predominant food pathogens that are frequently encountered [23–25].

**MIC determination**
Measurements of the minimum inhibitory concentration (MIC), defined as the minimum concentration at which more than 80% of the microbial growth is restrained, were performed in 96 flat-bottom microtiter plates (TPP, Switzerland) in accordance with the microdilution method, as previously reported by Bardaweel et al. [23]. An inoculum volume of 1 × 10⁵ CFU mL⁻¹ for each microorganism was used in each microtiter plate well. Ampicillin and amphotericin B (positive controls), as well as media (negative control), were employed under comparable experimental conditions. Plates containing bacteria were placed in a shaker incubator for 48 h at 37 °C, whereas plates containing *Candida* were incubated in the shaker for 48 h at 33 °C. To assess microbial growth, optical densities were read at 600 nm (OD₆₀₀) using a microplate reader (Palo Alto, CA, USA).

**Anticancer activity**

**Cell lines and cell viability**
All cancer cell lines used in this study (MCF7, MDA-MB-231 HeLa, PC3, and K562) were acquired from the American Type Culture Collection (Rockville, MD, USA). All cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% foetal bovine serum, 100 U/mL penicillin, and 100 μg/mL streptomycin at 37 °C with 5% CO₂. The count of viable cells was determined using the trypan blue method [24].

**MTT assay**
The anticancer activity of the phenolic extracts and essential oils were studied in 96-well round-bottom microplates using the MTT (3-[4, 5-dimethylthiazole-2-yl]-2, 5-diphenyl-tetrazolium bromide) assay (Sigma-Aldrich, USA) as previously described by Bouziane et al. [25]. In summary, cells were seeded in 96-well plates at a cell density of 1 × 10⁴ cells/mL and incubated for 24 h to permit attachment. Each well was treated with different concentrations (0.001–1000 μg/mL) of the prepared extracts in triplicate and incubated for 48 h. Subsequently, 10 μL of 0.5 mg/mL MTT was added to each well and further incubated for 4 h before measuring the absorbance at 570 nm. Growth inhibition was determined based on the following equation: inhibition (%) = 100- (mean Abs of test sample - mean Abs of negative control) ×100/(mean Abs of positive control-mean Abs of negative control). Graph Pad Prism 7 software (GraphPad Software, Inc., La Jolla, CA, USA) was used for data analysis to determine the inhibition percentage, and the results are presented as the LD₅₀ value, described as the concentration that produces 50% growth suppression. The positive control doxorubicin was employed under the same experimental conditions.

**Data analysis**
For all experiments, the analyses were performed in triplicate, and the values are reported as the mean ± standard deviation (SD). The results were analysed via Student’s t-test, with α = 0.05. The analyses were carried out using IBM SPSS Statistics for Windows, version 22.0. (IBM Corp., Armonk, New York, USA).

**Results and discussion**

**Total phenolic and flavonoid contents**
The total phenol contents of extracts estimated by the Folin-Ciocalteu procedure and the total flavonoid contents estimated by the AlCl₃ method are presented in Table 1. The ethanolic extract of *O. basilicum* had the
highest phenolic compound content and flavonoid content (226 mg/g, gallic acid equivalents, and 213 mg/g, rutin equivalents).

Identification of phenolic compounds by HPLC

In this study, 15 phenolic compounds in the ethanolic extracts of *O. basilicum* and *T. algeriensis* were investigated by HPLC. The concentrations of the identified polyphenolic compounds in all analysed samples are presented in Table 2 (shown in the order of their retention time). The data in Table 2 revealed that the ethanolic extract of *O. basilicum* had high amounts of rutin, epicatechin and vanillic acid: 476.28 μg/g, 225.01 μg/g, and 138.24 μg/g, respectively. Two polyphenolic compounds, quercetin (0.36 μg/g) and caffeic acid (6.48 μg/g), were found in low concentrations. In the *T. algeriensis* ethanolic extract, six compounds were detected in notably high concentrations: epicatechin (major compound; 824.79 μg/g), 2, 5-dihydroxybenzoic acid (778.76 μg/g), ellagic acid (374.58 μg/g), rutin (280.39 μg/g), vanillic acid (182.67 μg/g), and naringin (120.67 μg/g). Epicatechin was found to be the major compound in the *Thymus algeriensis* ethanolic extract (824.79 μg/g), and 3, 4-dihydroxybenzoic acid was detected in the lowest concentration (1.42 μg/g). Nonetheless, to the best of our knowledge, HPLC identification of phenolic compounds from *Thymus algeriensis* has never been previously reported.

**Table 1** Total phenolic and flavonoids contents in the ethanolic extracts of *Ocimum basilicum* and *Thymus algeriensis*

| Plant                  | phenolic content (mg/g extract) | flavonoid content (mg/g extract) |
|------------------------|---------------------------------|----------------------------------|
| *Ocimum basilicum*      | 226 ± 2                          | 213 ± 3                          |
| *Thymus algeriensis*    | 125 ± 1                          | 118 ± 1                          |

**Table 2** HPLC analysis of ethanolic extracts for phenolic compound contents

| Phenolic standard compounds | Standard retention time RT (min) | *Ocimum basilicum* (μg/g) | *Thymus algeriensis* (μg/g) |
|-----------------------------|----------------------------------|---------------------------|-----------------------------|
| Gallic acid                 | 6.8                              | 17.61                     | 10.49                       |
| 3,4-dihydroxy benzoic acid  | 10.7                             | 21.56                     | 1.42                        |
| 4-hydroxy benzoic acid      | 15.7                             | 46.29                     | 10.03                       |
| 2,5 dihydroxybenzoic acid   | 17.3                             | 52.14                     | 778.76                      |
| Chlorogenic acid            | 18.2                             | 25.11                     | 22.68                       |
| Vanillic acid               | 19.2                             | 138.24                    | 182.67                      |
| Epicatechin                 | 21.3                             | 225.01                    | 824.79                      |
| Caffeic acid                | 22.7                             | 6.48                      | 33.30                       |
| p-coumaric acid             | 26.1                             | 12.83                     | 83.80                       |
| Ferulic acid                | 30.1                             | 25.00                     | 34.30                       |
| Rutin                       | 45.6                             | 476.28                    | 280.39                      |
| Ellagic acid                | 47.7                             | 27.64                     | 374.58                      |
| Naringin                    | 49.7                             | 21.02                     | 120.67                      |
| Quercetin                   | 70.4                             | 0.36                      | 2.84                        |
| Cinnamic acid               | 71.1                             | 54.68                     | 20.51                       |

**Essential oil yield and chemical composition**

The yields of the essential oils under study were 1.05 and 0.51% for *O. basilicum* and *T. algeriensis*, respectively. Chromatographic analyses resulted in the identification of 61 compounds, representing 98.3% (*O. basilicum*) and 96.5% (*T. algeriensis*) of the essential oils (Table 3). These compounds were grouped into six chemical classes: monoterpenes hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, oxygenated sesquiterpenes, apocarotenones, and non-terpene derivatives.

In the essential oil of *O. basilicum*, 26 compounds were identified, accounting for 98.3% of the oil. Interestingly, the content of oxygenated monoterpenes (92.0%) was 23 times greater than that of monoterpene hydrocarbons (4.0%). Linalool (52.1%) and linalyl acetate (19.1%) were detected as the major compounds in the oil. The composition of the essential oil of *O. basilicum* is extremely variable because of the existence of several chemotypes, i.e., linalool, methyl chavicol, eugenol, methyl eugenol and neral are the main compounds [26]. The essential oil obtained in the present study can be classified as a linalool-linalyl acetate chemotype, a characteristic chemotype of European basil [26].

In the *T. algeriensis* essential oil, 29 compounds were identified, of which 74.7% were oxygenated monoterpenes and 15.6% were monoterpenic hydrocarbons. Among the minor compounds, oxygenated sesquiterpene (4.4%) was characterized. The major compounds in *T. algeriensis* EO were α-terpinyl acetate (47.4%), neryl acetate (9.6%), and α-pinene (6.8%). The minor compounds in essential oil from *T. algeriensis* from different geographical areas have been frequently reported to include camphor and/or α-pinene, while thymol and linalool have been occasionally reported as the main compounds [27]. Interestingly, the chemical composition of the *T. algeriensis* essential oil reported in this study appears to be unique, as α-terpinyl acetate and neryl acetate were detected as the major compounds in the oil but thymol was not detected at all.

**Antioxidant activities**

**DPPH assay**

DPPH is a stable radical that appears purple in solution and has an absorbance maximum at 515 nm. Upon reduction, its colour changes into yellow with a concomitant decrease in absorbance at 515 nm. The colour change is
| Constituents                  | LRI | O. basilicum (%) | T. algeriensis (%) | Method of identification |Remarks |
|------------------------------|-----|------------------|-------------------|--------------------------|--------|
| α-pinene                     | 941 | 0.3              | 6.8               | MS; LRI; RC              |        |
| camphene                     | 955 | - a              | 0.8               | MS; LRI; RC              |        |
| sabinene                     | 977 | 0.4              | 0.7               | MS; LRI; RC              |        |
| β-pinene                     | 982 | 0.6              | 1.2               | MS; LRI; RC              |        |
| myrcene                      | 993 | 1.3              | 1.2               | MS; LRI; RC              |        |
| 3-octanol                    | 994 | - a              | - a               | MS; LRI; RC              |        |
| α-terpinene                  | 1020| - a              | - a               | MS; LRI; RC              |        |
| p-cymene                     | 1028| - a              | 0.3               | MS; LRI; RC              |        |
| limonene                     | 1032| - a              | 2.7               | MS; LRI; RC              |        |
| 1,8-cineole                  | 1034| 9.2              | 4.1               | MS; LRI; RC              |        |
| (Z)-β-ocimene                | 1042| 0.7              | - a               | MS; LRI; RC              |        |
| (E)-β-ocimene                | 1052| 0.5              | 1.5               | MS; LRI; RC              |        |
| γ-terpinene                  | 1063| - a              | - a               | MS; LRI; RC              |        |
| cis-sabinene hydrate         | 1070| - a              | - a               | MS; LRI; RC              |        |
| terpinolene                  | 1090| 0.2              | 0.4               | MS; LRI; RC              |        |
| linalool                     | 1101| 52.1             | 12.2              | MS; LRI; RC              |        |
| nonanal                      | 1104| - a              | - a               | MS; LRI; RC              |        |
| pentylisovalerate            | 1108| 0.1              | - a               | MS; LRI; RC              |        |
| 1-octen-3-yl acetate         | 1111| 0.1              | 0.4               | MS; LRI; RC              |        |
| cis-p-menth-2-en-1-ol        | 1123| - a              | - a               | MS; LRI; RC              |        |
| 3-octyl acetate              | 1126| 0.2              | - a               | MS; LRI; RC              |        |
| α-campholenal                | 1127| - a              | 0.5               | MS; LRI; RC              |        |
| camphor                      | 1145| - a              | 1.1               | MS; LRI; RC              |        |
| trans-verbenol               | 1147| - a              | 0.5               | MS; LRI; RC              |        |
| δ-terpineol                  | 1172| 0.2              | - a               | MS; LRI; RC              |        |
| 4-terpineol                  | 1179| 0.1              | 0.5               | MS; LRI; RC              |        |
| α-terpineol                  | 1191| 5.7              | 4.9               | MS; LRI; RC              |        |
| cis-dihydrocarvone           | 1195| - a              | - a               | MS; LRI; RC              |        |
| trans-carveol                | 1219| - a              | 0.4               | MS; LRI; RC              |        |
| (Z)-3-hexenyl isovalerate    | 1238| - a              | - a               | MS; LRI; RC              |        |
| pulegone                     | 1239| 0.2              | - a               | MS; LRI; RC              |        |
| neral                        | 1242| - a              | 0.2               | MS; LRI; RC              |        |
| carvone                      | 1244| - a              | 0.3               | MS; LRI; RC              |        |
| linalyl acetate              | 1259| 19.1             | - a               | MS; LRI; RC              |        |
| bornyl acetate               | 1287| - a              | 3.1               | MS; LRI; RC              |        |
| dihydroedulan IA             | 1292| - a              | - a               | MS; LRI; RC              |        |
| isodihydrocarvyl acetate     | 1329| - a              | - a               | MS; LRI; RC              |        |
| α-terpinyl acetate           | 1352| - a              | 47.4              | MS; LRI; RC              |        |
| cis-caryl acetate            | 1364| - a              | - a               | MS; LRI; RC              |        |
| neryl acetate                | 1365| 1.8              | 9.6               | MS; LRI; RC              |        |
| geranyl acetate              | 1383| 3.6              | 0.9               | MS; LRI; RC              |        |
| β-elemene                    | 1392| - a              | - a               | MS; LRI; RC              |        |
| (Z)-jasmone                  | 1395| - a              | - a               | MS; LRI; RC              |        |
monitored spectrophotometrically and is utilized for the determination of antioxidant capacity \[28, 29\]. To evaluate the different antioxidant potentials of the phenolic extracts and essential oils, their IC\(_{50}\) values, as determined by the DPPH assay, were compared to the IC\(_{50}\) of the reference compound ascorbic acid (vitamin C) in mg/mL. The results are summarized in Table 4. The ethanolic extracts demonstrated excellent DPPH radical-scavenging activities. Generally, the activity associated with the \(O.\) basilicum extract was stronger than that associated with the \(T.\) algeriensis extract, and both extracts had less activity than ascorbic acid (IC\(_{50}\) = 0.002±3.826×10\(^{-6}\) mg/mL). For the essential oils, the strongest activity was associated with the \(T.\) algeriensis oil, with an IC\(_{50}\) value of 1.437 mg/mL, followed by BHA (IC\(_{50}\) of 1.437 mg/mL) and the \(O.\) basilicum oil (IC\(_{50}\) of 16.296 mg/mL). However, all examined samples were less active than the standard antioxidant ascorbic acid.

### ABTS assay

The results of this assay are shown in Table 4. Based on the results, the EOs appeared to possess higher anti-free radical activity than the ethanolic extracts. The ethanolic extract of \(O.\) basilicum demonstrated higher antiradical activity than the ethanolic extract of \(T.\) algeriensis, which may be attributed to its high phenolic content (rutin, vanillic acid, epicatechin); such compounds are responsible for trapping the cation radical ABTS\(^{+}\) by providing H\(^+\).

### Ferric-reducing antioxidant power test

Table 4 shows the AEAC values for the ethanolic extracts and essential oils obtained from the studied plants, together with that of BHA, which was chosen as the standard antioxidant. Considerable attention has been focused on the determination of total antioxidant capacity using the antioxidant activity of ascorbic acid in terms of equivalence and reflected in the AEAC, defined...
as the concentration of ascorbic acid (\(\mu g/mL\)) that gives an antioxidant power equivalent to that of a concentration of 1 mg/mL of the EO and EE. A high AEAC indicates a notable antioxidant capacity [30]. Relative to that of the positive control, the EE of \(O. \text{ basilicum}\) had the most potent activity (IC\(_{50}\) of 3.657 ± 0.009 \(\mu g/mL\)), followed by the EO of \(T. \text{ algeriensis}\) (IC\(_{50}\) of 1.387 ± 0.265 \(\mu g/mL\)). However, the EE of \(T. \text{ algeriensis}\) demonstrated a lower antioxidant potential, with an IC\(_{50}\) of 0.897 ± 0.064 \(\mu g/mL\). Notably, the EOs from both plants demonstrated poor Fe\(^{2+}\) reducing potential relative to the standard antioxidant BHA.

**Phosphomolybdenum assay**

The different extracts of \(O. \text{ basilicum}\) and \(T. \text{ algeriensis}\) were also used to determine their antioxidant capacities from the formation of the green phosphomolybdenum complex. The antioxidant activities of the extracts were referenced to that of ascorbic acid in terms of equivalence by calculating the AEAC value. This method is based on the reduction of molybdenum from an oxidation state of VI to an oxidation state of V in the presence of an antioxidant. This reduction is materialized by the formation of a greenish complex detectable in the visible region at 695 nm at an acidic pH [31]. The results indicate that under the applied experimental conditions, the \(O. \text{ basilicum}\) and \(T. \text{ algeriensis}\) EOs are potent antioxidants, while their phenolic extracts have the lowest potential to reduce molybdenum. The \(O. \text{ basilicum}\) oil recorded the highest AEAC, which surpassed vitamin E's AEAC under the same experimental conditions. This result may be attributed to the chemical composition of the \(O. \text{ basilicum}\) oil, which mainly contained 92% oxygenated monoterpenes, with linalool as the predominant compound [32].

**Antimicrobial activity**

The ethanolic extracts of \(O. \text{ basilicum}\) and \(T. \text{ algeriensis}\) as well as their essential oils were evaluated in terms of potential antimicrobial activity against a group of pathogenic microorganisms, comprising gram-positive and gram-negative bacteria and a fungus (Table 5). Superior antimicrobial activity was observed against the gram-positive bacteria examined in this study, while both plant extracts and essential oils demonstrated moderate activities against gram-negative bacteria. Interestingly, the antimicrobial activities observed after essential oil treatment appeared to be generally more potent than those associated with the ethanolic extracts of \(O. \text{ basilicum}\) and \(T. \text{ algeriensis}\). As shown in Table 5, moderate antifungal activity was associated with both the ethanolic extracts and essential oils from \(O. \text{ basilicum}\) and \(T. \text{ algeriensis}\) when examined against \(Candida\) \(g\text{labrata}\) and \(Candida\) \(a\text{l}bicans\). Numerous literature studies have provided support for the antimicrobial activities of several of the compounds in the \(O. \text{ basilicum}\) and \(T. \text{ algeriensis}\) ethanolic extracts and essential oils. For instance, quercetin has been reported as a very efficient antimicrobial compound against several pathogenic gram-positive and gram-negative bacteria [23–25]. In addition, it has been shown that the antibacterial activities of flavonoids, such as quercetin, were effectively enhanced in the presence of rutin, a major component of the ethanolic extracts of both plants, although rutin did not show activity on its own [33].

**Anticancer activity**

The in vitro antiproliferative effects of the ethanolic extracts and essential oils from \(O. \text{ basilicum}\) and \(T. \text{ algeriensis}\) were assessed on five human cancer cell lines, namely human breast adenocarcinoma MCF-7 and MDA-MB-231 cell lines, the human adenocarcinoma HeLa cell line, the human prostate cancer PC3 cell line, and the human leukaemia K562 cell line. The observed antiproliferative activities

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**Table 4** IC\(_{50}\) (DPPH), IC\(_{50}\) (ABTS), AEAC (FRAP assay), AEAC (Phosphomolybdenum assay) of the extracts, ascorbic acid, and BHA

| Plant/Control | Extract  | IC\(_{50}\) (DPPH) (mg/mL) | IC\(_{50}\) (ABTS) (mg/mL) | AEAC-FRAP assay (\(\mu g/mL\)) | AEAC-Phosphomolybdenum assay (mg/mL) |
|--------------|----------|-----------------------------|-----------------------------|-----------------------------|--------------------------------------|
| \(O. \text{ basilicum}\) | EE       | 0.679 ± 0.0383\(^*\)        | 0.970 ± 0.022\(^a\)         | 3.657 ± 0.009\(^a\)          | 0.005 ± 0.0003\(^a\)                  |
|              | EO       | 16.296 ± 0.394\(^c\)        | 0.6870 ± 0.0203\(^a\)       | 0.003 ± 0.0007\(^c\)         | 0.760 ± 0.001\(^c\)                  |
| \(T. \text{ algeriensis}\) | EE       | 1.560 ± 0.010\(^a\)         | 1.743 ± 0.195\(^a\)         | 0.897 ± 0.064\(^d\)          | 0.007 ± 0.0006\(^a\)                  |
|              | EO       | 1.437 ± 4.51 E-05\(^d\)     | 0.8960 ± 0.203\(^a\)        | 1.387 ± 0.265\(^d\)          | 0.432 ± 0.001\(^c\)                  |
| Ascorbic acid|          | 0.002 ± 3.826 E-06\(^b\)    | 0.001 ± 5.13E-05\(^b\)      | ND                          | ND                                   |
| Vitamin E    |          | ND                           | ND                          | ND                          | 0.674 ± 0.057\(^b\)                  |
| Trolox       |          | ND                           | ND                          | ND                          | 1.685 ± 0.658\(^b\)                  |
| BHA          |          | 1.685 ± 0.658\(^b\)         | ND                          | 1000 ± 0.047\(^b\)          | ND                                   |

\(EE\) Ethanol extract

\(EO\) Essential oil

\(ND\) Not determined

Each value in the table is represented as mean ± SD (n = 3). In the same column, means not sharing the same letter are significantly different at \(P < 0.05\) probability level in each column.
on the examined cell lines are presented in Table 6 as LD$_{50}$ values, referring to the concentration at which cancer cell survival was reduced by 50%. The dose-dependent curves obtained by nonlinear regression were used to calculate the LD$_{50}$ values. Doxorubicin, a well-known anticancer agent, was used as a positive control and demonstrated LD$_{50}$ values ranging from 1-20 μg/mL against the different cancer cell lines used in this study. Generally, the essential oils from both plants were much more effective in suppressing cancer cell growth than the ethanolic extracts.

The phytochemical constituents of the essential oils under investigation include many of the most active naturally existing anticancer phytochemicals, such as 1, 8-cineole and linalool [23–25]. Similarly, polyphenols, such as quercetin, are often reported to exert anticancer activities against various cancer cell lines [25]. Remarkably, the numerous phytochemical constituents in essential oils and their potency to prevent cancer cell growth at fairly low concentrations may be attributed to the additive or synergistic effects of the different components.

### Conclusion

In summary, the chemical composition of ethanolic extracts and essential oils from Ocimum basilicum and Thymus algeriensis cultivated in the Algerian Saharan Atlas was reported for the first time in this study. A rich flavonoid content of the EEs of both plants was revealed by HPLC analysis of the phenolic compounds. GC-MS analysis demonstrated that linalool (52.1%) and linalyl acetate (19.1%) are the major compounds in the essential oil from O. basilicum, while α-terpinyl acetate (47.4%), neryl acetate (9.6%), and α-pinene (6.8%) were identified as the major compounds in the essential oil from T. algeriensis. Additionally, diverse bioactivities of the ethanolic extracts and essential oils from both plants were observed against a range of clinically relevant strains of microorganisms, with enhanced activities against gram-positive bacteria. The essential oils from both plants were much more potent in inhibiting cancer cell growth than the ethanolic extracts. Moderate antioxidant activities were elucidated, suggesting their potential for use in pharmaceutical industries and food production technologies.

### Abbreviations

- Ac: Absorbance of the control reaction; AEAC: Ascorbic acid equivalent antioxidant capacity; A$_2$: Absorbance of the test sample; EE: Ethanolic extract; EO: Essential oil; GAE: Gallic acid equivalents; GC-EIMS: Gas chromatography with electron impact mass spectrometry; LD: Lethal dose; MIC: Minimum inhibitory concentration; RE: Rutin equivalents

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### Authors’ contributions

MR and SB designed and performed the experiments, interpreted the results, drafted the manuscript and revised it. BB and AG collected the plants, isolated the essential oils, characterized the antioxidant activities of the oils, drafted the manuscript and participated in the design of the study. AR and FG prepared and evaluated the results and participated in writing the manuscript. OK and RM conducted the chemical composition analysis, performed the statistical analysis and participated in drafting the manuscript. All authors read and approved the final manuscript.

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### Availability of data and materials

The data that support the findings of this study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

Not applicable because the research did not involve human participants.
Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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