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Original article

Study on the chemical composition, antioxidant and antimicrobial activities of essential oils of tunisian *Anethum graveolens* L. leaves

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Abstract. *Background and aim: Anethum graveolens* L. is a widespread aromatic and medicinal plant. It is used as a spice for culinary preparations and it has been used for several application in medicine and industry. Most of phytochemical and biological studies have focused on molecules produced by *A. graveolens* seeds and little reports were carried out particularly on essential oils (EOs) from leaves. Indeed, to our knowledge, no reports conducted on the essential oils produced by Tunisian *A. graveolens*. *Methods:* In the current study, EOs of *A. graveolens* leaves collected from two different origins were extracted by hydrodistillation. EOs were identified by using gas chromatography and mass spectrometry. In addition, the antimicrobial activity of EOs were evaluated against six pathogenic bacteria: *Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica, Staphylococcus aureus, Bacillus subtilis, Micrococcus luteus*, and two fungi strains: *Aspergillus niger* and *Candida albicans*. The antioxidant potential of tested EOs was evaluated by four different tests: Total antioxidant activity (TAA), DPPH, ABTS and reducing power assay (RPA). *Results:* GC analysis indicated the presence of twelve compounds and the most predominant compounds were *p*-cymene (41.51-62.07%), followed by limonene (27-28%) and α-phellandrene (1.43-21.45%). The two oils showed differences related to their provenance. Both oils have shown antioxidant potential. Likewise, very important and remarkable antimicrobial activities have been observed. *Conclusions: A. graveolens* essential oils leaves can be used for several applications due to their richness in biological molecules with antioxidant and antimicrobial potential.

Key words: *Anethum graveolens*, essential oils, antioxidant, antimicrobial activity

Introduction

In recent years, aromatic and medicinal plants have aroused great interest in several fields. Indeed, natural substances extracted from plants have allowed great advances due to their benefits in the preparation of many products, particularly in the agronomical, nutraceutical and pharmaceutical fields (1.2.3.4). Various factors also encourage scientists to innovate technologically and to improve the exploitation of these bioresources.

The *Apiaceae* (*Umbelliferae)* family or is one of the largest families of flowering plants, comprising more than 3000 species within more than 400 genera (5).

Apiaceae family was considered as a major source of raw materials used in pharmaceutical, cosmetic, flavor and perfume industries. This family is a source of active compounds (6). It is rich in fixed oils, proteins, fibers, carbohydrates and EOs and a significant diversity of chemical composition was detected depending on the seed varieties, genetic sources and environmental (6,7).

The EOs and extracts of species belonging to the *Apiaceae* family have been used in food preservation, pharmaceuticals, alternative medicine and natural therapies (6,8). Currently, it is necessary to investigate these plants scientifically for their importance as potential source of natural agrochemicals as well as their biological activities to improve the quality of healthcare (8,9).

Anethum graveolens L. (Dill) is an annual plant growing in the Mediterranean region, in central and south Asia. It is traditionally used as a popular aromatic herb and spice for several culinary preparations (10). Dill EOs can be extracted from the different plant parts, such as leaves, flowers and seeds (8). Variations in the profile of EOs have been attributed to different geographical origins, genetic variability, growth conditions, organ development, seasonal variations, treatments before extraction procedures (2, 6).

Numerous reports have been revealed that major compounds of *A. graveolens* EOs were carvone, limonene, α-phellandrene. It also contained furanocoumarin, furocoumarin, oxypeucedanin hydrate and falcarindiol (10, 11).

Due to it richness in biologically active compounds, the EOs of *A. graveolens* displayed various biological activities such as antioxidant activity: many authors reported that *A. graveolens* showed high antioxidant properties, it contains a wide variety of antioxidants that acted as an extracellular neutralizer of free radicals. (8,12,13). Other studies exhibited that *A. graveolens* EOs showed antibacterial activity (14). It was strongly effective against both Gram-positive bacteria such as *Enterococcus* sp and *Staphylococcus aureus* and Gram-negative bacteria such *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* (15). Many authors have mentioned that antimicrobial activities may be attributable to the chemical composition of *Anethum* and to it richness in furanocoumarin. (16,17). Biological activity can vary also according to plant organs (18).

Several studies have been conducted on Dill seeds and flowers. However, and according to our knowledge, little researches on the volatile oils produced by the leaves of *A. graveolens* have been investigated. Therefore, the aims of this study were to identify for the first time, the chemical composition of volatile oils extracted from Tunisian *A. graveolens* leaves and to assess their antioxidant and antimicrobial activities for their applications in pharmaceutical and food purposes.

Material and methods

Plant material

Leaves of *A. graveolens* were collected from Oued Safsaf and Oued Saaden: two regions in the government of Nabeul (Tunisia). Dill leaves were collected in Mars during the pre-flowering stage.

Plant material was identified by Pr Abdelhamid Khaldi (Laboratory of Management and Valorisation of Forest Resources, National Institute of Researches on Rural Engineering, Water, and Forests, Tunisia) and a voucher specimens AGS181 and AGF182 were deposited in the herbarium of the institute.

Extraction process

200 grams of dried leaves were hydrodistilled in the Clevenger type apparatus for 3 hours according to the European Pharmacopoeia (19). EOs were dried over anhydrous Na_2SO_4 and then stored in opaque bottles at 4°C for analysis.

EOs yields extraction were calculated based on the dried weight.

$$\% \text{ yield of EOs} = \text{Weight of EOs} / \text{Weight of dried leaves} \times 100.$$

EOs analysis by Gas Chromatography/ Mass Spectrometer (GC/MS)

EOs analysis was carried out by a GC/MS-QP2010 Shimadzu apparatus equipped with a

ZB-5MS capillary column (Dimensions: 30meter x 0.25 mm internal diameter x 0.25 μm film thickness) and a QP2010 mass selective detector.

The injector temperature was kept at 230°C. The column temperature was initially kept at 60°C, then gradually increased at a rate of 3°C/min until reaching to 240°C, and held constant for 5 minutes. For GC/MS, ion source and interface temperatures were set respectively at 200°C and 245°C. The mass scan ranged between 35 to 400 m/z at ionization energy 70 eV. Helium was the carrier gas at a flow of 1.03mL/min. For each analysed sample, Diluted samples of 1 μL (1/100 in hexane, v/v) were injected in splitless mode. Components identification was confirmed by comparison of their retention indices data calculated from a series of alkanes retention times (relative to C9–C25) obtained on a ZB-5 MS capillary column and those of standards or with data described by Adams (2007) (20). Further confirmation was done by comparison of their mass spectra with those of Wiley (21) and NIST library data (22).

Antioxidant activity

In the current study, Dill EOs were tested for their antioxidant potential by four different methods as follow: Total antioxidant activity (TAA), DPPH radical scavenging assay, ABTS-free radical scavenging activity and Reducing power assay (RPA).

Total antioxidant activity (TAA)

TAA was evaluated according to the method described by Singh HP et al., (2012) (23) with minor modification. 100 μL of EOs was mixed with 1 mL of reagent solution composed of: [0.6 M H_2SO_4, 28 mM sodium phosphate and 4 mM ammonium molybdate]. Samples were then incubated at 95 °C for 90 min and the absorbance was read at 695 nm. The antioxidant capacity was expressed as mg gallic acid equivalent per gram dry weight (mg GAE/g DW).

DPPH assay

DPPH free-radical scavenging activity of Dill EOs was estimated using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical test according the method described by Khammassi et al. (2022) (6). Dill Eos and the positive control Butylated hydroxytoluene (BHT) at various concentrations were added to 2mL of the DPPH methanolic solution (0.1mM). The mixtures were vortexed, incubated at room temperature for 30 min in the dark and their absorbance was measured at 517 nm. The free-radical scavenging activity IC50 was defined as the concentration required to scavenge 50% of free radicals present in the test solution.

The percentage of inhibition was calculated against blank according to the following equation:

$$\% \text{ inhibition} = (A_{control} - A_{sample}/A_{control}) \times 100$$

where, A_{sample} is the absorbance value of the sample and $A_{control}$ is the absorbance of the control reaction.

ABTS assay

$ABTS^+$ assay was carried out using the method of Ud-Daula et al., (2016) (24). ABTS radical cations ($ABTS^+$) was generated from an equal solution of: 7 mM ABTS and 2.45 mM potassium persulfate. The mixture was kept in the dark for 16h and the $ABTS^+$ solution was then diluted to an absorbance of 0.70 ± 0.02 at 734 nm.

300μL of EOs at different concentrations, or of the control (methanol) was added to 3 mL of the ABTS radical solution. The mixture was then kept in the dark for 5 min and the absorbance was measured at 734 nm. Trolox (6 -hydrox y -2,5,7, 8 - tetramethylchroman - 2 -carboxylic acid) was used as standards.

The inhibition percentage was calculated as follow:

$$\% \text{ of scavenging} = (A_c - A_s/A_c) \times 100$$

Where: A_c is the absorbance of the control and A_s is the absorbance of the sample

ABTS scavenging capacity was expressed as an IC_{50} value (μg/mL).

Reducing power assay

The reducing power was conducted by the method described by Oyaizu(1986) (25) with slight

modifications. 1 mL of different concentrations of EOs was added to 1 mL of phosphate buffer (0.2 M, pH=6.6) and 1 mL of potassium ferricyanide [$K_3Fe(CN)_6$] (1%). The mixture was incubated at 50°C for 20 min. 1 mL of Trichloroacetic acid (TCA) (10%) was then added to the mixture and was centrifuged for 10 min at 3000 rpm. The supernatant was mixed with distilled water (1.5 mL) and 0.1% of $FeCl_3$ (150 µL). The absorbance was read at 700 nm and the reducing power was expressed as the effective concentration EC_{50} (µg/mL) at which 0.5 absorbance was 50% for reducing capacity. BHT was used as standard.

Antibacterial activity

Tested microorganisms

The antibacterial activity was tested against three Gram-positive bacteria (*Staphylococcus aureus* CIP 53156, *Bacillus subtilis* CIP 5262, and *Micrococcus luteus* CIP 5345), three Gram-negative strains (*Pseudomonas aeruginosa* CIP 82118, *Salmonella enterica* CIP 8039, *Escherichia coli* CIP 53126) and two fungi (*Aspergillus niger*, ATCC 16404 and *Candida albicans* ATCC 10231). Bacterial species were cultured on tryptone soy agar BK047HA (TSA) while fungi were cultured on potato dextrose agar BK095HA (PDA). TSA and PDA were purchased from BIOKAR diagnostics (France).

Determination of inhibition zones

The antimicrobial activity of Dill leaves oils was evaluated by the disc diffusion method. 100 µL of each microorganism's suspension (10^7 CFU mL^{-1}) was spread on the appropriate agar medium plates. Sterile filter paper discs (6 mm) were placed in the inoculated petri dishes and 10 µL of EOs was dripped on the paper. The plates were then incubated at 37°C for 24 h for bacteria, 30°C for 48 h for yeast and 25°C for 48 h for fungi. Each experiment was carried out in triplicate.

Antimicrobial activity was evaluated by measuring the growth inhibition zone diameter (mm).

Ampicillin (10 µg/disc) and fluconazole (10 µg/disc) were used as positive standard for bacteria and fungi, respectively.

Minimum inhibitory, minimum bactericide and minimum fungicidal concentrations of EOs

The broth microdilution method was used to determine minimum inhibitory concentration (MIC), minimum bactericide, and minimum fungicidal concentrations (MFB) as described by Roby et al., (2013) (26). Different concentrations of EOs (1.25–10 mg/mL) were added to 5 mL of appropriate broth tubes containing 10^7 CFU/mL of live cells. To disperse the oil throughout the broth, the tubes were incubated on an incubator shaker and examined for evidence of the growth. Tube containing inoculated broth with only DMSO was used as negative control.

The MIC was determined as the lowest concentration of the EOs showing no visible growth after incubation. About 20 µL from all tubes showing no visible growth was subcultured on appropriate nutrient agar plates. The plates were then incubated for 24–48 h. MBC and MFC were the lowest concentrations of the EOs that showed no visible growth after this subculturing. Each experiment was repeated in triplicate.

Statistical analysis

Results are expressed as the mean values, ± standard mean of error (SME). Data was analyzed by using analysis of variance (ANOVA) and differences among the means was determined using the Student –Newman–Keuls (SNK) test with SPSS Statistics, version 21. All statistical tests were performed at a 5% significance level.

Results and discussion

The yields of *A. graveolens* leaves EOs from Oued safsaf and Oued saaden were of 1.2 and 1.3% respectively.

Oils components percentages, retention indices, formula and subclasses of each compound are listed as shown in figure 1 and table 1.

GC/MS analysis revealed the identification of twelve compounds, representing more than 98% of the total oils. For Oued safsaf, the major components identified were *p*-cymene (62.07%), followed

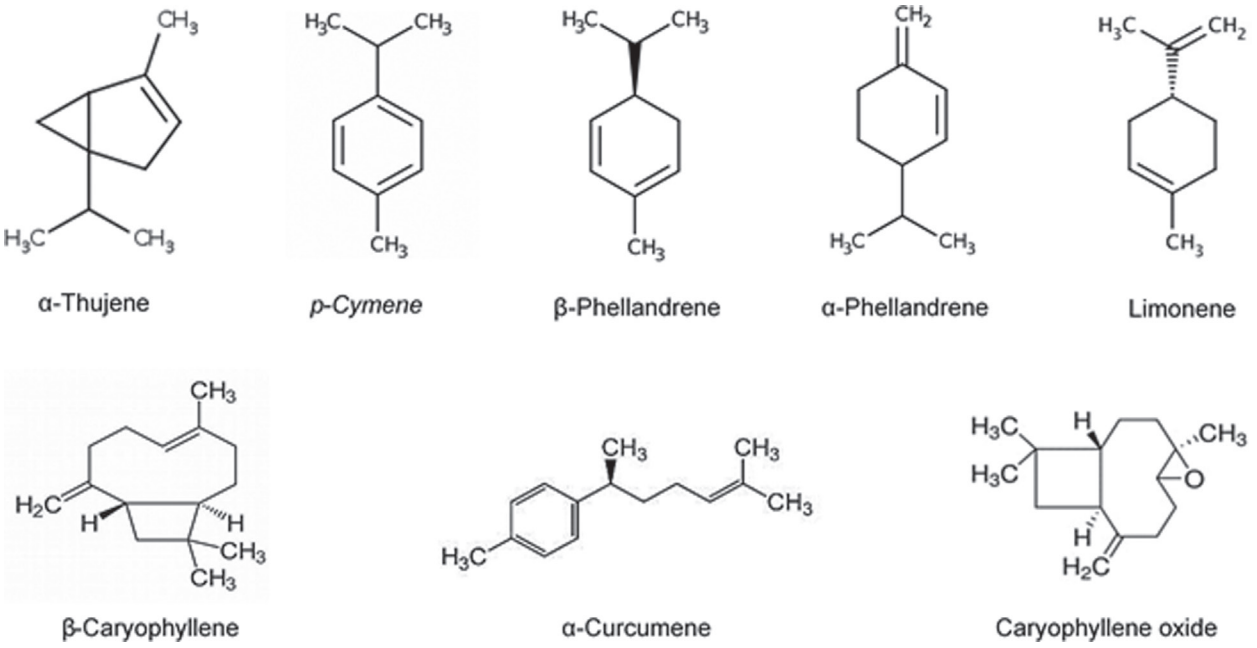

Figure 1. Major compounds of *Anethum graveolens* EOs.

Table 1. Chemical composition of *Anethum graveolens* L. leaves EOs.

Peaks	Compounds	LRI	RI	Formula	Oued safsaf	Oued saaden
1	Tricyclene	932	927	$C_{10}H_{16}$	0.0±0.0	0.43±0.0
2	α-Thujene	935	929	$C_{10}H_{16}$	0.0±0.0	3.79±0.01
3	α-Pinene	943	938	$C_{10}H_{16}$	0.0±0.0	0.43±0.01
4	Sabinene	975	971	$C_{10}H_{16}$	0.0±0.0	0.67±0.0
5	β-Myrcene	991	986	$C_{10}H_{16}$	0.0±0.0	1.7±0.0
6	α-Phellandrene	1007	1003	$C_{10}H_{16}$	1.43±2.02	**21.45±0.03**
7	*p*-Cymene	1026	1022	$C_{10}H_{16}$	**62.07±0.16**	**41.51±0.11**
8	Limonene	1029	1024	$C_{10}H_{16}$	**27±0.12**	**28±0.08**
9	β-Phellandrene	1034	1031	$C_{10}H_{16}$	0.0±0.0	1.97±0.0
10	(*Z*)-β-Caryophyllene	1419	1416	$C_{15}H_{24}$	3.75±0.01	0.0±0.0
11	α-Curcumene	1517	1518	$C_{15}H_{22}$	1.84±0.0	0.0±0.0
12	Caryophyllene oxide	1582	1580	$C_{15}H_{24}O$	2.43±0.01	0.0±0.0
Total identification					98.52	99.95
Monoterpeneshydrocarbons					90.5	99.95
Sesquiterpeneshydrocarbons					5.59	0
Oxygenatedsesquiterpenes					2.43	0

LRI: Literature retention index, RI: Calculated retention index, MH: Monoterpenes hydrocarbons, SH: Sesquiterpenes hydrocarbons, OS: Oxygenated sesquiterpenes. All values of compounds pourcentage are mean ± standard mean of errors.

by limonene (27%). *p*-cymene (41.5%) limonene (28%) and α-phellandrene (21.45%) were found in Oued Saaden. The oils were predominated by hydrocarbon monoterpenes representing about 90.5% in Oued Safsaf and 99.95% in Oued Saaden (total oil). However, other subclasses were detected only in Oued

Safsaf such hydrocarbon sesquiterpenes (5.59%) presented by β-caryophyllene (3.75%) and α-curcumene (1.84%), and oxygenated sesquiterpenes (2.43%) representing by caryophyllene oxide. *A. graveolens* oils showed remarkable variability in their chemical composition among location.

A. graveolens from Oued Safsaf is distinguished by the presence of β-caryophyllene, α-curcumene and caryophyllene oxide whereas the sample of Oued Saaden is distinguished by a high percentage of α-phellandrene and the presence of α-thujene (3.79%) and β-phellandrene (1.97%) which explains its richness in hydrocarbon monoterpenes. Previous reports conducted on the secondary metabolites of various species from different locations revealed differences in their chemical composition relative to the location (2, 6, 27).

The two tested samples are characterized by their richness in *p*-cymene, a phenolic compound known to have multiple biological properties. This compound is also an important industrial intermediate used in the synthesis of fungicides, pesticides, perfumes, fragrances and in the production of some precursors of standard antioxidants such as *p*-cresol, *p*-cymene has shown a variety of pharmacological properties (28, 29).

According to the literature, several studies on EOs of *A. graveolens* have been reported. However, no studies carried out on Tunisian *A. graveolens* oils. In agreement with the current study, the main components of *A. graveolens* EOs from Egypt, obtained by hydrodistillation from leaves at vegetative stage were α-phellandrene (46.33%), limonene (13.72%), β-phellandrene (11.01%) and *p*-cymene (17.88%) (30).

A study conducted by Rana and Blazquez (2014) (31) indicate that the volatile oil of Indian *A. graveolens* aerial parts at flowering stage was characterized by their richness in monoterpenes and the main components were α-phellandrene (31.8 %), apiole (15.3 %), dill ether (13.2 %), limonene (11.8 %), geraniol (10.6 %) and *p*-cymene (5.3 %) (31). Similarly, EOs of *A. graveolens* from Iran, have been reported to be rich in *p*-cymene (20.8%) and α-phellandrene (20.7%) as major compounds (32), which is in agreement with obtained results. Whereas, in another study reported by Kazemi et al (2015) (33), the major identified components of EOs obtained from the aerial parts at flowering stage in Iran were: α-phellandrene (19.1%), limonene (26.3%), dill ether (15.2%) and sabinene (11.3%) (33). Whereas, for Saudi *A. graveolens*, the main components in the EOs obtained by headspace solid-phase microextraction in flowering stage were: dill apiole (16.0%), carvone (14.1%), dill ether (7.3%), α-phellandrene (6.1%), neophytadiene (6.1%) and limonene (4.9%) (34).

The current study is in agreement with the literature; indeed, the majority of studies exhibited the presence of *p*-cymene, limonene and α-phellandrene in *A. graveolens* oils, with difference in the percentages of major compounds and often of others compounds such as: carvone, dill ether, sabinene and dill apiole (33,34). These differences are related to several factors able to influence the EOs production, in particular: the season of collection of the plant material, the part of the plant used for the extraction (leaves, flowers, seeds, stems), the extraction method, the drying method, the origin of plant and also the genetic diversity (1,2, 6,27,35).

Antioxidant activity

The antioxidant activity of EOs was evaluated by total antioxidant activity (TAC), DPPH test, ABTS test and reducing power assay (RPA). Dill EOs showed significant and variable antioxidant activities depending on their chemical composition (Table 2).

The two oils showed a total antioxidant activity of 7.66 and 8.4 mg EAG. g-1DW for Oued Saaden and Oued safsaf, respectively.

Both oils showed remarkable DPPH radical scavenging activity. EO from Oued Saaden exhibited the highest activity (IC_{50}=4.3μg mL^{-1}). However, the observed results were lower than BHT (IC_{50}=3.1 μg mL^{-1}). The same sample showed the highest antiradical scavenging activity by ABTS assay with an IC_{50} values of 4.36 μg mL^{-1}. The tested oils present lower potential that the synthetic antioxidant (IC_{50}=3.1 μg mL^{-1}).

A. graveolens EOs were tested for their reducing power (RPA). The highest iron reducing capacity was found in Oued Safsaf (EC_{50}=52.26 μg mL^{-1}), lower than BHT standard (EC_{50}: 22.4 μg mL^{-1}).

Plants belonging to Apiaceae family produce various components with several biological activities (6, 36). Several studies on the antioxidant activity of *A. graveolens* seeds have been reported. However, little

Table 2. Total antioxidant capacity (TAC) (mg EAG. g^{-1}MS), DPPH (IC_{50} in µg/mL), ABTS (IC_{50} µg mL^{-1}) and reducing power (EC_{50} in µg/mL) of *Anethum graveolens* EOs.

	TAC (mg EAG. g^{-1}DW)	DPPH (IC_{50} µg mL^{-1})	ABTS (IC_{50} µg mL^{-1})	RPA (EC_{50} µg mL^{-1})
Oued safsaf	8.4±0.2[b]	6.1±0.16[a]	68.16±0.7[a]	33.46±0.3[b]
Oued saaden	7.66±0.2[c]	4.36±0.15[b]	42.13±0.35[b]	52.26±0.36[a]
BHT	9.13±0.14[a]	3.1±0.1[c]	-	22.45±0.3[c]
Trolox	-	-	22.06±0.25[c]	-

All the values are expressed as mean ± standard mean of errors; means followed by different letters within the same column are significantly different ($p \leq .05$)

are the studies conducted on the antioxidant activity of EOs extracted from Dill leaves.

Antioxydant potential of EOs leaves of Iranian *A. graveolens* and their major components particularly limonene were tested by the inhibition of β-carotene bleaching and reducing power methods. Obtained data showed that Dill oil and limonene significantly reduce reactive oxygen species production and thus exhibited an antioxydant properties (33), which is in agreement with results in this current study.

The antioxidant potential of *A. graveolens* leaves essential oils could be attributed to the presence of the phytochemicals compounds in the oils (Table 1).

Several EOs characterized by their richness in p-cymene, were reported to have antioxidant activities. In fact, the high free radical scavenging ability of some oils can be ascribed also to the presence of p-cymene at a high level that has conjugated double bonding and known to have high antioxidant activity (37, 38). Moreover, De Oliveira and Coworkers (2015) demonstrated that p-cymene possesses high antioxidant properties (39). In addition, foods treatment with p-cymene have been reported as a protective agent against lipid peroxidation (40). The EOs are a complex mixture of compounds and their antioxidant potential depends on their major compounds and the synergism and antagonisms among oil components.

EOs of *A. graveolens* have shown a richness in Monoterpenes Hydrocarbons (90.5-99.95%). This fraction is represented mainly by limonene and p-cymene that their DPPH, ABTS radical-scavenging effects and reducing power capacities were confirmed by several studies (38, 41, 42). According to these studies, the antioxidante activity of these compounds exceeds that of α-tocopherol (42). Others studies reported that the antioxidant activity shown by *A. graveolens* oils can be related to their richness in phenylpropanoids derivates represented only by p-cymene (36.15-67.37%) (43). In fact, Sharopov (2015) reported the antioxidant activity of this compound (41). This is in agreement with the results recorded with leaves oils in this present study.

Aromatic compounds characterized by the presence of benzene rings with hydroxyl groups, gives an important stability to the hydrogen bond donor or to the electron donor compounds. Cyclical compounds containing double bonds and hydroxyl groups show a significant free radical scavening potential, making the elimination of free radicals faster (44).

The antioxidant capacity of the EOs can be attributed in the presence of several classes of terpenes that provide redox properties to EOs and then antioxidant properties (quenching singlet and triplet oxygen, neutralizing free radicals, decomposing peroxides and chelating transition metal) (45). Several researches reported that most natural antioxidative compounds (terpenes, phenols, flavonoides, alkaloides) work synergistically with each other to produce a broad spectrum of antioxidative potentialities that create an effective defense system against free radicals (46, 47).

Antibacterial activity

The study of the antimicrobial activity of *A. graveolens* oils against bacteria and fungi strains was carried out by the agar disc-diffusion method based on the determination of the inhibition zones (IZ)., Ampicillin and fluconazole were used as a positive control. The main results obtained are presented in Table 3.

Table 3. Antimicrobial activity (ZI in mm) of *A. graveolens* EOs.

		Oued safsaf	Oued saaden	Ampicilin	Fluconazole
Bacteria Gram -	*E.coli*	15.16±0.28[b]	16. 2±0.26[b]	19±1[a]	-
	P. aeruginosa	16.16±0.73[b]	15.83±0.28[b]	21.66±0.57[a]	-
	S. enterica	16.9±0.79[b]	15.9±0.17[b]	26.66±0.57[a]	-
Bacteria Gram +	*S. aureus*	16.5±0.86[b]	15.33±0.28[c]	23.16±0.28[a]	-
	B. subtilis	15.5±5[a]	14.16±0.28[b]	15.6±0.52[a]	-
	M. luteus	17.1±0.17[b]	15.33±0.28[c]	21.86±0.23[a]	-
Yeast	*C. albicans*	14.16±0.28[b]	14.93±0.6[b]	-	16.16±0.28[a]
Fungi	*A. niger*	15.43±51[b]	15.3±0.26 [b]	-	20.16±0.28[a]

Mean values ± standard mean of error (n = 3) in the same line with different letter(s) are significantly different (p 0.05) based on Student Newman Keuls test

Table 4. MIC, MBC, and MFC (mg/mL) of *Anethum graveolens* Eos.

		Essential oils	
Strains		**Oued saaden**	**Oued safsaf**
E. coli	MIC	7.5	7.5
	MBC	10	10
P. aeruginosa	MIC	7.5	7.5
	MBC	>10	>10
S. enterica	MIC	7.5	5
	MBC	>10	>10
S. aureus	MIC	2.5	5
	MBC	5	7.5
B. subtilis	MIC	7.5	7.5
	MBC	10	10
M. luteus	MIC	2.5	2.5
	MBC	5	5
C. albicans	MIC	7.5	7.5
	MBC	>10	>10
A. niger	MIC	7.5	5
	MBC	10	7.5

All the tested strains showed sensitivity to *A. graveolens* essential oils between the tested stains (Table 4). Statistical analysis showed that the two tested oils act similarly in fungi and Gram-negative bacteria. However, Gram-positive bacteria were most sensitive to dill essential oils and Oued safsaf oil revealed highest inhibitory effect than Oued saaden sample.

Results exibited that *A.graveolens* EOs were less effective against the tested strains in comparison with antibiotic and fungicide. Except, Oued Safsaf oil that act similarly as the antibiotic against *B. Subtilis,* a significant difference between the effect of the EOs and the antibiotic and the fungicide on the same bacterial strain was observed.

A. graveolens EOs from the two regions exhibited the highest effect against *B. subtilis* (MIC=7.5 mg/ml), *S. aureus* (MIC of 2.5 mg/ mL for Oued Saaden and 5 mg/ml for Oued safsaf) and *M. luteus* (MIC = 2.5 mg/mL) and a MBC value ranging from 2.5 mg/mL to 10 mg/ml for all Gram positive bacteria.The higher sensitivity of Gram-positive bacteria leaves EOs can be due to their outer peptidoglycan layer which was not an effective permeability barrier (Nostro et al., 2000).

A. niger was also sensitive to dill oils especially Oued safsaf sample that displayed fungicidal effect with MFC value of 5 mg/ml. *S. enterica, P. aeruginosa* and *C. albicans* were more resistant and exhibited less effects with MIC value of 7.5 mg/mL.

The antimicrobial activity of *A. graveolens* EOs extracted from flowers and seeds have been studied (38, 49, 50). Recent studies of *A. graveolens* rich in *p*-cymene and limonene as found in our study have been tested for their antimicrobial activity. The antibacterial activity of dill EOs was tested against 30 clinical cariogenic bacteria and remarkable activity was obtained against *Streptococcus mitis*, *Streptococcus oralis*, *Streptococcus mutans*, *Streptococcus constellatus* and *Gemella haemolysans (*51).

In the study conducted by Weisany et al., (2016) (52), The contents of limonene and *p*-cymene were enhanced in seed volatile oils of plants colonized by *Funneliformis mosseae* : arbuscular mycorrhizal fungi. This explains that these compounds found in *A. graveolens* leaves oil in our study, are involved in the plant's defense mechanism particularly against fungi, which could explain the antimicrobial potential of tested oils and it allows us to attribute the antimicrobial properties of *Aneth* EOs from this study to their major compounds: limonene and *p*-cymene.

In fact, the antibacterial activity of *p*-cymene was studied against several strains of bacteria and their MIC and MBCs against *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia* coli have been determined (53-55).

The study of the effect of *p*-cymene on the growth of *Candida lusitaniae* showed that this monoterpene completely inhibited the yeast growth (56). These results are in agreement with other studies showing the efficiency of *p*-cymene in inhibiting *Candida krusei*, *Candida albicans* and *Candida tropicalis* (57).

In addition, the antimicrobial properties of limonene were tested against *Trichophyton rubrum* by two methods: microdilution and vapor. Obtained result revealed a very remarkable antimicrobial potential due to the presence of this compound. Indeed, limonene vapor significantly inhibited the growth of *Trichophyton rubrum*. For the microdilution method: limonene showed a remarkable fungicidal effect (58). This could explain the antimicrobial activity of *A. graveolens* EOs in the current study, since the *A. graveolens* leaves oils in the current study showed a specific richness in this compound.

Regarding the mode of action of EOs as an antimicrobial agent, in the literature there is sufficient data to understand how EOs act in bacteria and fungi. In fact, EOs were characterized by their non-polar nature, and as a result, they were endowed with a penetrating potential, in particular through the membranes. For this, several studies have shown that the oils exert their antimicrobial effect through the membrane. A recent study has shown that the EOs of hop cones and their compounds are able to penetrate into lipid monolayers and bilayers and increase membrane fluidity (59) and subsequently loss of membrane integrity which results remarkable leakage from bacterial and fungi cells and exit of several crucial molecules that lead to death (60). Bajpai and Coworkers (2013) showed that terpenes induce alteration of membrane integrity thus causing to the leakage of various molecules like amino acids, ATP, nucleic acids and ions (61), which play a crucial role in the vital functions of microorganisms, namely the regulation of cytoplasmic and cellular pH, damage of the cytoplasmic membrane and eventually the exposure of the vital intracellular material (62).

Conclusion

Anethum graveolens is a widespread aromatic plant known by its culinary and medicinal uses. In Tunisia, there are no studies conducted on the chemical composition and biological activities of it leaves essential oil. Therefore, the aims of this study is to identify, for first time, the chemical composition and to evaluate the antioxidant and antimicrobial activities of *A. graveolens* leaves EOs. Chemical analysis showed that EOs extracted from dill leaves from two regions, were rich in *p*-cymene, a phenolic compound known by its diverse properties. In addition, the EOs of this species have shown substantial antioxidant activity related their chemical composition. Data revealed also an important antimicrobial potential of dill EOs especially against Gram-positive bacteria which may provide data for suitable conditions for cultivation of the best population and it exploitation.

This study is a significant contribution to the valorization of wild aromatic and medicinal plants and such results encourage the use of *A. graveolens* leaves as natural antioxidant and antimicrobial for their potential use in food and pharmaceutical applications

Conflict of Interest: authors declare that there is no conflict of interest in connection with the submitted article

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