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Anethum graveolens from the Algerian Sahara: Chemical composition and antibacterial Potential of essential oils from stems

Saidani Abdelmadjid*[1], Berreghioua Abdelaziz[2], Belboukhari Nasser[1], Sekkoum khaled[1]

[1]Bioactive Molecules & Chiral Separation Laboratory, Faculty of Exact Sciences , University Tahri Mohamed of Bechar, , Istiklal Street, PO 417, Bechar 08000, Algeria.
[2]Faculty of Technology, University Tahri Mohamed of Bechar, Istiklal Street, PO 417, Bechar 08000, Algeria.
Algeria university de bechar
+213660159979
Personal email : majid.saidani92@gmail.com
Professional email : saidani.abdelmadjid@univ-bechar.dz

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Abstract:
Anethum graveolens "dill plant" or "chibitt" in Arabic is a plant belonging to the Umbelliferae family, found in the south-west of Algeria specifically at Taghit in Bechar city, region of this work, with different biological benefits of medicinal interest. In this research paper, we discussed the potential application of the essential oils of this plant as antibacterial agent. Chemical compositions of *Anethum graveolens* essential oil was analyzed via chromatographic and spectral methods by GC-MS. In total, thirty four compounds were identified representing the majority of the EO, with Apiol (35.21 %) being the major compound, followed by (E)-3-(3,4,5-tri-methoxyphenyl)-2-propenoic acid methyl ester (23.55 %), β-Pinene (6.47 %), Falcarinol (6.23 %), and Myristicin (5.79 %). In addition, antibacterial effect was evaluated against 04 pathogenic bacterial strains. The results of antibacterial activity of *Anethum graveolens* essential oil was showed an important activity with inhibition ranging diameters from 10 to 17 mm for the Gram-positive and Gram-negative bacteria with MICs variable from 1 to 9 µL/mL.

Keywords: *Anethum graveolens*, GC-MS, Essential oil, Antibacterial, MICs.

Introduction:

Plants have formed the basis of traditional systems of medicine including rural populations in developing countries among others. Searching for news molecules, nowadays, has taken a clear growth where the science of ethnobotany and ethno pharmacognosy is used as a guide to lead researchers towards different sources and classes of compounds. (Singh 2015) Throughout the ages, it has been used by humans in various journals, including flavors, perfumes and beauty. To some extent, it continues to provide people with new treatments. It has relied on them through experimentation and the codification of all its knowledge.(Gurib-Fakim 2006) In modern medicine, plants have been used in the treatment of various diseases. Their essential oils have been used in the treatment of some infectious diseases and in the problems of the digestive tract, urinary tract, dermatological diseases, etc.(Rios and Recio 2005)

Anethum graveolens is an annual aromatic herb belonging to the Umbelliferae family This plant has been used for more than 5,000 years in the treatment of wounds, convulsions, indigestion and increased appetite grows in the Mediterranean regions in regions of Asia and is also grown in regions of the world(Al-Snafi and Sciences 2014) Most of this plant's essential oil is produced in seeds and is widely used to add flavors in drinks and foods. Its leaves also contain oil(Callan, Johnson et al. 2007) essentials oils have an ancient history. Arabs, Egyptians and ancient Greeks knowing that these oils are extracted from various plants and used, the oils contain volatile compounds that give the oil its flavor and distinctive smell(Schiller and Schiller 1994, Sharopov, Wink et al. 2013) These oils are obtained by hydro-distillation or steam(Bakkali, Averbeck et al. 2008) The use of these oils has evolved from conventional to modern use. They are used in the medical field as disinfectant(Bouaziz, Yangui et al. 2009) and treatment of wounds and also as anti-inflammatory(Pérez G, Zavala S et al. 2011), bacteria(Kalemba and Kunicka 2003) and fungi.(Nazzaro, Fratianni et al. 2017) Calming and palliative to the pain, and its use is still being explored today through scientific studies.

Our study was undertaken to assess, the chemical composition of Algerian essential oil extracted from *Anethum graveolens* (Dill). Then, the characterized *Anethum graveolens* (Dill) essential oil was evaluated for its in vitro antibacterial effect against some bacterial pathogens. The expected results were then used for the valorization of *Anethum graveolens* (Dill) essential oil as a new bio agent for several pharmaceutical or food applications.

2. Materials and methods

2.1 plant material

Anethum graveolens (stems parts) was collected from Taghit region near the province of Bechar, Algeria in September and October 2021. The plant material was dried for a 7 days and then distilled 100 grams of the sample into the Clevenger device by hydrodistillation for 3 hours. The essential oil obtained from distillation was separated by the addition of sulfate du sodium anhydre (Na_2SO_4) and kept at 4 ° C.

2.2 Gas chromatographic –mass spectral analysis

The chemical analysis of essential oils was carried out in the research platform physico-chemical analyzes in Laghouat (PTAPC-CRAPC) and research laboratory in analytical chemistry at the University of Laghouat. Once the essential oil extract is obtained, the analysis makes it possible to identify and quantify the components. Advances in analytical methods make it possible to identify quickly a very large number of constituents. The most appropriate method today is gas chromatography (GC).

The analysis of the samples was carried out using a SHIMADZU Instrument GCMSQP 2020, equipped with an Rxi®-5ms fused capillary column (Phase: Crossbond® 5% diphenyl/95% dimethyl polysiloxane). Its dimensions are: 30 m × 0.25 mm and 0.25 µm film thickness, this column has a phase similar to the following columns: HP-1ms, HP-1msUI, DB-1ms, DB-5ms, DB- 1msUI, Ultra-1, VF-1ms, ZB-1, ZB-1ms, and also considered equivalent to USP phases G1, G2, G38. A volume of 0.5 µL of solution prepared at 10% volume of the dilution of the sample in n-hexane, was injected in split mode (30:1).

The injector and detector temperatures were maintained at 250°C and 310°C, respectively the column temperature was programmed at: 60°C fixed for 3min then raised to 310°C with an increment of 2°C/min, then maintained at 310°C for 10 min. The carrier gas used was helium (99.995% pure) with a flow rate of 1mL/min. THE mass spectrometer conditions were as follows: ionization voltage 70 eV, ion source temperature 200 °C and electron ionization mass spectra were acquired over the mass range of 45 to 600 m/z.

2.3.1 Antimicrobial screening

The strains used are reference strains according to the international standard, the American Type Culture Collection (ATCC). These strains were provided by the laboratory Sustainable management of natural resources in arid and semi-arid areas, Department of SNV, Institute of Science and Technology, Salhi Ahmed University Center Nâama. They are frequently involved in the pathogenicity and spoilage of foodstuffs.

Anethum graveolens (stems parts) essential oil against antibacterial activity was examined on three bacteries Gram positive: *Bacillus subtilis* (ATCC 21332), *Bacillus cereus* (ATCC 10876), *Staphylococcus Aureus* ATC 25923) Gram-positive and one bacteria Gram negative: *Escherichia coli* (ATCC 25922).

2.3.2 Confirmation of the purity and authentication of the bacterial strains tested

Confirmation of the purity of the strains selected for this test was based on macroscopic observation of the appearance of colonies, microscopic observation of cells after Gram staining, then biochemical tests. After sowing the bacteria on their selective media, Gram staining was carried out after 24 hours incubation at 370 C, which allowed observation of the shape of the bacterial cells and their methods of grouping. Also, the two biochemical tests for catalase and oxidase were carried out.

2.3.3 Culture medium

To evaluate the antibacterial activity, we used Mueller Hinton agar. In fact, it is a non-selective medium, which contains ions promoting good diffusion of bioactive molecules. Sterile Muller Hinton agar was poured into Petri dishes of 90 mm in diameter; the thickness of the agar must be 2 mm, distributed then dried for 30 min at room temperature before use. Before solidification of agar, the plates were placed on a flat surface to ensure good standardization of the agar surface (Hussain et al., 2010).

2.3.4 Antibacterial Activity

The disk diffusion method was used to test the antibacterial activity of EO as previously described by CLSI (1997) with slight modifications. Briefly, the sterile blotting paper discs (6 mm) were soaked with 15 µL of *Anethum graveolens* EO and deposited on the surface of Muller hinton medium previously seeded by the prepared bacterial suspensions. The Petri dishes were incubated for 2 h at 40°C to promote diffusion, then stored at 37°C for 24 hours. After incubation, the areas inhibition observed were measured (mm). Each test has was carried out in triplicate.

3. Results and discussion

Table1. Chemical composition of the *Anethum graveolens* stems essential oil

Peak	Rt	Index	Area %	Name	
1	8.55	927	0.13	α-Thujene	(Singh, Maurya et al. 2005)
2	8.80	933	3.69	α-Pinene	(Singh, Maurya et al. 2005)
3	9.38	948	0.09	Camphene	(Ksouri, Krimat et al. 2020)
4	10.38	973	0.11	Sabinene	(Kazemi 2015)
5	10.50	976	6.47	β-Pinene	(Vokk, Lõugas et al. 2011)
6	11.13	992	0.68	Myrcene	(Singh, Maurya et al. 2005)
7	11.62	1004	0.27	Octanal	
8	11.69	1006	0.18	α-Phellandrene	(Roomiani, Soltani et al. 2013)
9	11.93	1011	0.15	delta-3-Carene	
10	12.55	1025	0.50	o-Cymene	
11	12.74	1029	3.72	Limonene	(Yili, Aisa et al. 2009, Al-Snafi and Sciences 2014)
12	13.19	1039	0.32	trans- β- Ocimene	
13	14.11	1059	0.23	γ-Terpinene	(Jianu, Misca et al. 2012)
14	19.57	1178	0.53	Terpinen-4-ol	

15	20.19	1191	0.89	α-Terpineol	
16	25.28	1303	0.15	Carvacrol	(Huopalahti, Linko et al. 1983)
17	30.62	1428	0.24	Cubenene	
18	31.07	1438	0.10	cis- α-Bergamotene	
19	31.93	1459	1.46	cis- β-Farnesene	
20	32.98	1485	0.41	α-Curcumene	
21	33.51	1498	0.19	α-Zingiberene	
22	34.05	1512	0.10	α-Bisabolene	
23	34.52	1524	5.79	Myristicin	(Babri, Khokhar et al. 2012, Ruangamnart, Buranaphalin et al. 2015)
24	34.65	1527	0.35	γ-Cadinene	
25	35.90	1559	1.21	Elemicin	
26	36.17	1566	0.24	Nerolidol	
27	36.58	1576	0.22	Sesquisabinene hydrate	
28	36.74	1580	2.36	Spathulenol	(Ksouri, Krimat et al. 2020)
29	38.57	1629	35.21	Apiol	(Babri, Khokhar et al. 2012, Ozliman, Yaldiz et al. 2021)
30	39.50	1654	3.23	β-Eudesmol	(Ksouri, Krimat et al. 2020)
31	39.62	1657	0.85	γ -Eudesmol	

32	40.80	1689	0.15	β-Nootkatol
33	44.01	1779	23.55	(E)-3-(3,4,5-tri-methoxyphenyl)-2-propenoic acid methyl ester
34	52.43	2037	6.23	Falcarinol

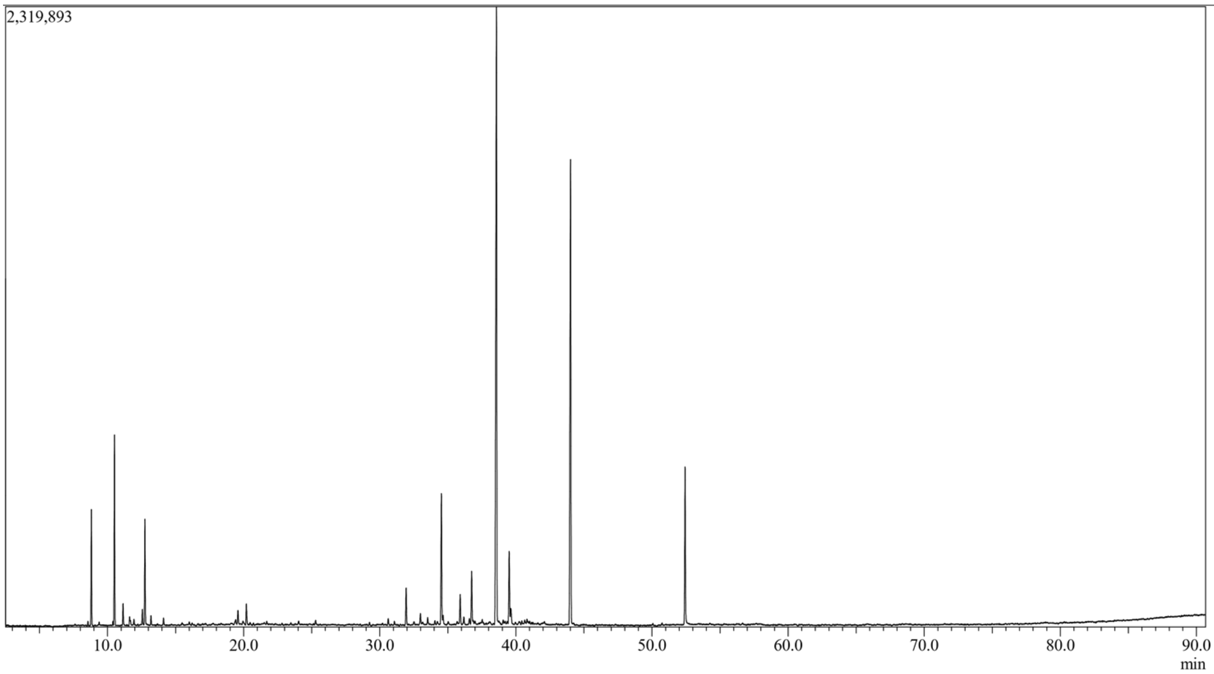

Figure 1: Spectrum of stems parts of *Anethum graveolens* EO.

Hydrodistillation of stems parts of *Anethum graveolens* has given an essential oil of yellow color.Yields, calculated w/w versus dry material, ranged between 0.9 – 1%.These obtained EO yields are greater than those found for some plants of the same species with an EO yield value of 0.8 % (Khaoula et al., 2022) and (0.007-0.045%) (Said and Omer, 2016), but is lower than those found by (Lidija et al., 2024). Indeed, the yield of an EO depends on many factors (growth stage, pedoclimatic conditions, extraction technique, etc.).

The detailed analysis of a bulk sample was carried out by a combination of chromatographic and spectroscopic techniques. One oil sample was analyzed by GC(MS) (Table 1).The essential oil has allowed the identification of 34 chemical components, corresponding the majority of the total essential oil (Table 1, Fig. 1).

The chemical composition of the essential oil of *A. graveolens* is dominated by Apiol (35.21 %) that being the major compound, followed by (E)-3-(3,4,5-tri-methoxyphenyl)-2-propenoic acid methyl ester (23.55 %), β-Pinene (6.47 %), Falcarinol (6.23 %), and Myristicin (5.79 %).

Other compounds like Limonene (3.72%), α-Pinene (3.69%), β-Eudesmol (3.23%) and Spathulenol (2.36%) were identified in low percentage.

According to our results, the composition of the essential oil isolated from Algerian *A. graveolens* differs in rate, but similar by some compounds from those of other *Anethum graveolens*, dominated by carvone with a rate of 34.33%, followed by α-phellandrene (22.03%), dill ether (18.84%), limonene (6.93%) and dill apiol (5.01%) (Khaoula et al., 2022). (Lidija et al., 2024) showed that *Anethum graveolens* EO cultivated in Moravac in south Serbia was presented by: Carvone (49.8%), Limonene (37.8%), trans-Dihydrocarvone (8.4%), α-Phellandrene (1.2%). The results obtained in the *Anethum graveolens* EO cultivated in Egypt by (Said and Omer, 2016) showed that six major compounds exist in eight cultivars, but with different percentages. A phellandrene>limonene>dill apiol (*Anethum graveolens* cv. Local, cv. Compatto, cv. Common and cv. Bouquet); α-phellandrene>limonene>myristcin (Anethum graveolens cv. Tetra, cv. Vierling, cv. Dukat and cv. Elephant) were the major components in the first harvest (α-phellandrene chemotype). α-phellandrene>limonene>dill ether (*Anethum graveolens* cv. Local, cv. Tetra, cv. Vierling, cv. Ducat and cv. Common); α phellandrene>limonene>p-cymene>dill ether (*Anethum graveolens* cv. Compatto, cv. Bouquet and cv. Elephant) were the major components in the second harvest(α-phellandrene and limonene chemotype).

Generally, the difference in chemical composition of our essential oil comparing to others from different cultivated areas of *Anethum graveolens* plants could be attributed to many factors such as the part of the plant from which was extracted the essential oil, the genetic factors as well as the physiological, environmental and geographic variations (Dridi et al., 2020).

Antibacterial Activity

Table 2: Antibacterial activity of *A. graveolens* essential oil (Inhibition zones and MICs).

		Inhibition zones (mm)		
Bacteria	The ATCC code	Gram	Essential oil 10µL	MIC of *Anethum* essential oil (µL/mL)
Bacillus cereus	10876	+	-	-
Bacillus subtilis	21332	+	10.13±0.00	1
Escherichia coli	25922	-	17.5±0.00	9
Enterococcus faecalis	49452	+	16.5±0.00	6

The antibacterial activity of *Anethum graveolens* EO was assayed against four bacteria using the agar disc diffusion method and measuring the minimum inhibitory concentration (MIC) (Table 2).The results showed that the EO had substantial antibacterial activity. In fact, it was effective against *Enterococcus faecalis* and *Escherichia coli* with inhibition zone diameters ranging from 16.5±00 and 17.5±00 mm and MIC values of 6-9µL/mL. *B. subtilis* were less sensitive with diameter of the zone of inhibition: 10.13±0.00 and MIC: 1 µL/mL. Our results is showed that no activity againt *Bacillus cereus*. According to these founds, it could be observed that the sensitivity of the bacterial strains to our *Anethum graveolens* EO was not correlated to the bacterial types (Gram-positive or Gram-negative). Hence, the studied essential oil could affect both of the bacterial membranes.

Generally, the action mechanisms of EOs and their selectivity towards certain bacteria have so far remained poorly understood (Hammer et al., 1999, Bagamboula et al.,2004).

In the whole, as it was previously reported by many researchers that the antimicrobial activity of the EOs depends on their chemical composition, the obtained antimicrobial activity of our *Anethum graveolens* EO towards the tested bacteria could be attributed to one or more of its major biological components: Apiol (35.21 %), (E)-3-(3,4,5-tri-methoxyphenyl)-2-propenoic acid methyl ester (23.55 %), β-Pinene (6.47 %), Falcarinol (6.23 %), and Myristicin (5.79 %). However, α and β-Pinene have been reported to exhibit slight activity against a range of microorganisms with MIC values ranged from 7.5 to 20.0 mg/mL against *E. coli*, *S. aureus* and *E. faecalis* (Dorman and Deans, 2000; Sonboli et al., 2006; Leite et al., 2007; Jung, 2009; Runyoro et al., 2010). Indeed, it is important to notice that synergistic interactions could occur between major or minor components of the EO thus playing a key effect on its antimicrobial activity (Dridi et al., 2020). By understanding the antibacterial activity of the EO extracted from some other cultivated species of *Anethum graveolens*, showed a weak antibacterial effect against Escherichia coli (inhibition zone of 15–18 mm in diameter) with a concentration of 10 µL, while no increased effect against *Bacillus subtilis* (Lidija et al., 2024). (Khaoula et al., 2022) shows that *E. coli*, *B. cereus* are extremely sensitive to pure *Anethum graveolens* essential oil with inhibition diameters varying between 20.33 ± 1.52 mm and 29.66 ± 0.57 mm. *P. aeruginosa, S. aureus* and *E. faecalis* bacteria are moderately sensitive to the essential oil.

4. Conclusions

The present study investigated, the chemical composition of *Anethum graveolens* essential oil endemic of Algeria. The studied essential oil is dominated by Monoterpenoids especially (E) -3- (3,4,5-tri-methoxyphenyl) -2-propenoic acid methyl ester, Falcarinol, Apiol and Limonene Myristicin. Furthermore, our results showed that the essential oil of *Anethum graveolens* possessed interesting antimicrobial properties which potentially suggest its exploitation as a new natural antimicrobial agent in pharmaceutical applications for treating or preventing human infectious diseases and/or in food industries.

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