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Research Article

Total Phenolic, Antioxidant, Antimicrobial Activities and Cytotoxicity Study of Wild *Anethum graveolens* L.

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ABSTRACT
The current study examine the antioxidant, antimicrobial and toxicity properties of hydromethanolic crude extract and its various fractions extracted from the aerial part of *Anethum graveolens*.L (dill). The total phenolic and flavonoid content were also determined for all extracts. The antioxidant activities were performed using 2,2-diphenyl-1-picrylhydrazyl (DPPH), reducing power and β-carotene–linoleic acid tests. Ethylacetate fraction exhibited a highest IC_{50} value 28.13±2,14 and 150.73±0.88µg/mL for the scavenging of free radical DPPH and reducing power tests respectively but a highest percentage of inhibition of b-carotene bleaching assay was in chloroform fraction. In vitro antimicrobial activity of extracts was tested against 4 Gram-negative bacteria, 2 Gram-positive bacteria and 1yeast. The MIC value of fractions extracts ranged from 0.19 to 12.5 mg/mL. The cytotoxicity test against brine shrimps showed that crude extract was toxic with LC_{50} value of 51.29 µg/mL. The obtained results demonstrated the considerable antioxidant and antibacterial activities, providing opportunities to explore dill extracts as biopreservatives.

Keywords: *Anethum graveolens,* total phenolic, total flavonoid, antioxidant, antimicrobial, cytotoxicity.

INTRODUCTION

Plants have always been used by humans to relieve and cure many diseases[1,2]. According to World Health Organization (WHO) traditional medicines are relied upon by 65–80% of the World's population for their primary health care needs[3]. Free radicals (FR) and reactive oxygen species (ROS) are produced through physiological and biochemical processes in the human body. ROS includes a number of chemically reactive molecules derived from oxygen such as hydrogen peroxide (H_2O_2), superoxide (O_2^-), hydroxyl radical (OH^-), etc. Overproduction of such free radicals might lead to oxidative damage of biomolecules in the body (e.g., lipids, proteins and DNA), that can initiate diseases such as atherosclerosis, diabetes, cancer and heart and neurodegenerative diseases[4].

Plants produce a wide range of secondary metabolites such as phenolic compounds (phenolic acids, flavonoids, quinines and coumarins), terpenoids, nitrogen compounds (alkaloids and amines), vitamins. These compounds can neutralize the free radicals and delay the progress of some chronic diseases associated with oxidative stress and reactive oxygen species[5,6,7]. Actually, the resistance to antibiotics in the pathogenic microorganisms become a health problem in the world, microorganisms have the ability to acquire resistance to antibiotics[8], plant derived antimicrobial compounds might inhibit bacteria though different mechanisms.

Exploration of natural products as antioxidants or antimicrobial agent is a research field with great potential, and is especially important in countries possessing vast biodiversity, like Algeria. Krimat et al reported that 20 extracts from Algerian medicinal herbs exhibited a great potential for production of bioactive compound[9].

In the present study, Algerian wild *Anethum graveolens* L. (Dill), belonging to the family Apiaceae were selected to assess their biological potential. The use of this plant for medicinal and consumption purposes have been recorded dating back to the Greek and Egyptian civilizations[7]. Some pharmacological effects have been reported, such as antimicrobial, antihyperlipidemic and antihyper-cholesterolemic activities. As a folk remedy, dill is considered to be the remedy for some gastrointestinal ailments such as flatulence, indigestion, stomachache and colic[10]. Literature demonstrates that *A. graveolens* flower extract showed higher antioxidant activity than the leaf and seed extracts[11]. It has been reported that the aqueous extract of *A. graveolens* has a broad-spectrum antibacterial activity[7]. To the best of our knowledge, the present study is the first positive report using different solvent extract of wild *A. graveolens* Algerian species. Thus, to provide a scientific justification for these traditional remedies. The aim of the current study was to investigate the antioxidant,

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antimicrobial and cytotoxicity potential of *A. graveolens* crude extracts and its fractions.

MATERIAL AND METHODS

Plant material

Fresh aerial parts of *Anethum graveolens* L. were collected in may 2012 from district of mghair area of El' Oued from north-east of the Algerian Sahara. The plant material was taxonomically identified by the Botanical survey and the voucher specimen (P40) was deposited in the herbarium of the institute of INA (Institut National d'Agronomie). The plant aerial parts were cleaned and air-dried at room temperature in the shade, and then crushed into fine particles.

Extraction procedure

Powdered plant material (10g) was extracted for 48h with 100 mL of methanol–water (70%–30%) at room temperature. The solvent was then removed by filtration throw disks of Watman paper n°1 and fresh solvent was then added to the residue. The extraction process was third repeated. The combined filtrates were then concentrated under reduced pressure at 40°C using vaccum rotary evaporator to obtain dry extract (3.5 g). The hydromethanolic crude extract was subjected to fractionation using different solvents. The crude extract was first suspended with hot distillated water (100 mL) and kept at room temperature for 12 hours. Then the suspension was defatted using hexane (50 mL, three times) and then successively fractionated with equal volumes of chloroform, diethyl ether, ethyl acetate and n-butanol (50 mL, three times). These fractions were dried over anhydrous sodium sulfate, filtered and concentrated to dryness under vacuum using rotary evaporator. The yields of these fractions were 80 mg, 20.5 mg, 75 mg and 875 mg respectively. The fractions were then redissolved in methanol at a concentration of 10 mg/mL. All extracts obtained were kept in the dark at +4°C prior to use.

Total phenolic contents

The total phenolic contents of extracts were determined spectrophotometrically, using the Folin–Ciocalteu assay[12]. Briefly, an aqueous aliquot (0.25 mL) of the extract was added to 3.75 mL of distilled water in a test tube, followed by 0.25 mL of Folin-Ciocalteu's reagent. After 3 min, 0.75 mL of 20% sodium carbonate was added. Tube contents were vortexed and heated at 40°C for 40 min, the blue coloration was read at 760 nm. The concentrations of phenolic compounds were calculated according to the following equation that was obtained from the standard gallic acid graph: Absorbance = 0.1035 gallic acid (µg/mL)+0.1046 (R^2:0.98).

Total flavonoid contents

The total flavonoid contents in the extracts were determined by a colorimetric method[13]. 1.5 mL of 2% $AlCl_3 6H_2O$ dissolved in methanol was added to equal volumes of the diluted extract. The mixture was shaken and the absorbance was read at 440 nm after 10 min incubation at room temperature. The concentrations of flavonoid compounds were calculated according to the following equation that was obtained from the standard quercetin graph: Absorbance = 0.2829 quercitin (µg/ml) – 0.1155 (R^2:0.99).

Antioxidant Activity

DPPH Radical Scavenging Activity Assay

The method of Braca et al[14] was used for determination of scavenging activity of DPPH free radical. Different methanolic dilutions of extracts (5 µg/mL to 1000 µg/mL) were mixed with equal volumes of freshly prepared DPPH methanol solution (0.004% w/v). The reaction mixture was vortexed and incubated in the dark for 30 min, the absorbance was read at λ=517 nm using a blank containing the same concentration of extracts without DPPH. Ascorbic acid and α-tocopherol were taken as standards. Inhibition of the DPPH free radical in percent (I%) was calculated based on control reading, which contain equal volumes of DPPH solution and methanol without any test compound using the following equation:

% inhibition = $[(A_C - A_S)/A_C] \times 100$

Where A_C is the absorbance of control reaction, and A_S is the absorbance of the sample. The extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph of scavenging effect percentage against extract concentration.

Reducing Power Assay

The reducing antioxidant power of plant extracts was determined[15]. Different concentrations of plant extracts in 1 ml of distilled water were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide [$K_3 Fe(CN)_6$] (2.5 mL, 1%). The mixture was incubated at 50°C for 20 min. then, 2.5 mL of trichloroacetic acid (10%) was added to mixture, which was then centrifuged for 10 min at 3000 rpm. The upper layer of solution (2.5 mL) was mixed with distilled water (2.5 mL) and $FeCl_3$ (0.5 mL, 0.1%). The absorbance was measured at 700 nm against a blank using UV-Vis spectrophotometer. Phosphate buffer (pH 6.6) was used as blank solution. Ascorbic acid and α-tocopherol were used as standards. Increased absorbance of the reaction mixture indicates increase in reducing power. IC_{50} value ($\mu g.mL^{-1}$) is the effective concentration at which the absorbance was 0.5 for reducing power.

β-Carotene/Linoleic Acid Bleaching Assay

This test was carried out according to a described procedure[16], based on the aptitude of various extracts to decrease the oxidative discoloration of β-carotene in an emulsion. 2 mg of β-carotene was dissolved in 10 mL of chloroform (HPLC grade). 1 mL of this solution was pipetted into a round-bottom flask containing 20 mg of linoleic acid and 200 mg of Tween 40. Chloroform was completely evaporated using a vacuum evaporator. Then, 50 mL of distilled water was added slowly to the residue and the solution was vigorously agitated to form a stable emulsion. 4.8 mL of the obtained emulsion were transferred into different test tubes containing 0.2 mL of extract (2 mg/mL). The mixture was then gently mixed and placed in a water bath at 50°C for 120 min. Absorbance at 470 nm was measured every 30 min for 120 min. Blank solution was prepared in a similar way except that addition of β-carotene was omitted. Ascorbic acid and BHT were

used as standards. The bleaching rate (R) of β-carotene was calculated according to first-order kinetics[17]:

$R=In\ (A_{t=0}\ /A_{t=t})/t$

Where, ln = natural log, t is the time in minutes, A t=0 is the initial absorbance of the emulsion immediately after sample preparation (t = 0 min) and A t=t is the absorbance at time t (30, 60, 90, and 120 min). The percent of antioxidant activity (AA) was calculated using the equation:

$AA= (R_{control} - R_{sample})/ R_{control} \times 100$

Where, R control and R sample are average bleaching rates of the negative control and the antioxidant (plant extract, ascorbic acid or BHT), respectively.

Antimicrobial Activity

Microbial strains

The hydro-methanolic crude extract and its fractions were individually tested against pathogenic microbes including two gram positive bacteria (*Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (CIP 7625), five gram negative bacteria (*Escherichia coli* (ATCC 10536), *Pseudomonas aeruginosa* (CIP A22), *Enterobacter cloacae* (E13), *Klebseilla pneumonia* (CIP 82.91), *Agrobacterium tumefaciens* (2410); and one yeast (*Candida albicans* (IPA 200)). All microorganisms were obtained from The Microbiological laboratory, Department of Biology, ENS, Algiers, Algeria. Bacterial strains were cultured in Muller–Hinton agar (Institut Pasteur, Algeria) and yeasts were cultured in Sabouraud dextrose agar (Institut Pasteur, Algeria). All microbial strains were incubated for 24 h at 37°C.

Disc Diffusion Assay

Antimicrobial tests were carried out using the disk diffusion method. The microbial cultures were harvested and then suspended in sterile saline (0.9% NaCl) and the cell density was adjusted to 0.5 McFarland. Sterile 5.5 mm paper discs, impregnated with 10 µL of the extracts solutions (100 mg/mL) were placed on the inoculated surface. Before incubation, all Petri dishes were stored in the dark at +4°C for 1 hour, to allow the diffusion of the extracts from disc to medium without microbial growth. At the end of incubation time (18-24h at 37°C), the diameter of the zones of inhibition around each disc (in millimeters, diameter of the disc included) were used as a measure of antimicrobial activity. Levofloxacin (10 µg/disc) was used as positive control for bacteria and nystatin (10 µg/disc) for yeast.

Agar Dilution Method

MIC determination of extracts was carried out by the agar dilution method[18]. Appropriate amounts of the extract were added aseptically to sterile medium to produce the concentration range of 25–0.097 mg extract/mL medium. The resulting agar solutions were immediately mixed and poured into Petri plates. The plates were spot inoculated with 1 µL of microorganism. At the end of incubation period, the plates were evaluated for the presence or absence of growth. The MIC was defined as the lowest concentration of the extract needed to inhibit the growth of microorganisms.

Cytotoxicity Analysis

Table 1: Total phenol and flavonoid content of *A.graveolens* crude extract and soluble fractions.

Solvents	Content (mg/g of dried extract)	
	Phenols	Flavonoids
Hydromethanolic crude	5.23±0.91[a]	2.31±0.02[a]
Chloroform	40±0.69[b]	2.32±0.04[a]
Diethylether	245±2.18[e]	14.21±0.2[d]
Ethylacetate	150.28±1.36[d]	5.97±0.05[b]
n-butanol	82.6±4.81[c]	6.72±0.04[c]

Cytotoxicity of the plant extract was determined by brine shrimp lethality bioassay[19]. Brine shrimp (*Artemia salina* Leach) eggs were obtained from CNRDPA, Algeria. Seawater was prepared by dissolving 36 g of sea salt in 1L of distilled water and put in a shallow rectangular plastic container. Oxygen was supplied and 60-W lamp was positioned near the container to provide direct light and heat (~27-/28°C). About 1 g of shrimp eggs were placed in 1L of sea water. After 10-12h, eggs began hatching. Two days was allowed for the shrimp to mature as nauplii (shrimp can be used 48-72h after the initiation of hatching). After 72h they were discarded. Nauplii were harvested by turning off the aeration and letting the culture settle for about 10 min. Hatched, empty eggs floated on the surface and unhatched eggs sank to the bottom. Newly hatched nauplii concentrated just above the unhatched eggs on the bottom. Since the nauplii are positively phototropic (attracted to light), shining a light in the middle of the container and shading the container at the bottom helped direct them to an area where they can be easily harvested by siphoning or draining. Stock solutions of extract were prepared by suspending dried extract in saltwater to prepare a 10 000 µg/mL solution. The suspension was mixed for 5 min; then, 1000, 100, 10, 1 µg/mL solutions were prepared by dilution. A suspension of nauplii was removed and 10 nauplii were placed into each of the test tubes and 2.5 mL of appropriate concentration of extract/salt mixture was added. Uncovered tubes were incubated for 24h at room temperature under illumination. Three replicates were prepared for each concentration. The same saline solution used to prepare the stock test sample solution was used as a negative control. After 24h, the surviving nauplii were counted with the aid of a 3x magnifying glass, and the percentage of deaths was determined. The median lethal concentration, LC_{50} value of the plant extract was determined.

Statistical Analysis

All experiments were carried out in triplicate. Data were expressed as means ± S.D. Differences were evaluated by one-way analysis of variance (ANOVA) test completed by Tukey's multiple range tests. *P* values < 0.05 were regarded as significant. The correlations between methods were quantified in terms of the correlation factor. LC_{50} value was obtained by a plot of percentage of dead shrimps against the logarithm of the sample concentration. The statistical analysis was done by Microsoft Excel with XLSTAT complement.

Table 2: Antioxidant activities of various extracts from A. *graveolens* and standards measured by different assays.

Extracts	DPPH[1,2]	Reducing power[1,3]	β-Carotene / linoleic acid (%)[1]
Hydromethanolic crude	135.22±0.22^{f}	1409.87±26.41^{g}	34.56±9.18^{ab}
Chloroform	183.80±3.72^{g}	866.77±6.51^{e}	70.22±1.34^{d}
Diethylether	34.11±2.14^{d}	447.51±4.53^{c}	64.72±2.75^{cd}
Ethylacetate	28.13±2.14^{c}	150.73±0.88^{b}	55.77±1.44^{c}
n-butanol	130.10±0.58^{e}	1161.847±6.07^{f}	37.10±3.23^{b}
Ascorbic acid	4±0.1^{a}	47±0.28^{a}	17.21±0.96^{a}
α- tocopherol	9.55±0.07^{b}	507±4.16^{d}	-
BHT	-	-	93.21±0.29^{e}

[1]Values (mean ± SD, n = 3) in the same column followed by a different letter are significantly different ($p < 0.05$). [2]IC_{50} in µg/mL; [3]Concentration at which the absorbance was 0.5

RESULTS AND DISCUSSION

Total phenolic contents (TPC)

Many plant extracts have been reported to have multiple biological effects, including antioxidant properties due to their phytoconstituents including phenolics. The antioxidant activity of phenolics is mainly due to their redox properties[20]. Determination of total phenolic content of different extracts from *A. graveolens* was done by using folin-ciocalteu colorimetric method. Phenolics including phenolic acids and flavonoids form a blue colour complex with maximum absorbance at 760 nm. The results are given in Table 1. The TPC varied significantly ($p<0.05$) in crude extract and its different fractions and ranged from 5.23 to 245 mg GAE/g, the fraction with the highest TPC was diethylether (245 mg GAE/g extract). The TPC were in the following order: diethylether>ethylacetate> n-butanol> chloroform >hydromethanolic crude Results are the mean±SD of three parallel measurements. The values bearing different letters are significantly different ($P < 0.05$).

Total flavonoid content (TFC)

Flavonoids comprise the most widespread and diverse group of polyphenolic plant secondary metabolites. The quantitative analysis TFC of crude extract and its various fractions of *A. graveolens* revealed that this content varied from 2.31 to 14.21 mg QE/ g extract (table 1). The In agreement with our findings, shyu et al[11] report a same value of IC_{50} in ethylacetate fraction of flower extract from Taiwan *A. graveolens* and this fraction showed a highest antioxidant activity than ethanol and hexane soluble fractions. Previous studies[23] by also showed that methanolic extracts of *Anethum graveolens* had a good free radical scavenging activity with IC_{50} value of 22.3 µg/mL. Results of this study suggest that the plant extract contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage.

Reducing Power Assay

For the measurements of the reductive ability it has been investigated from the Fe^{3+} to Fe^{2+} transformation in the presence of extract samples. The reducing powers of the various solvent extracts from *A. graveolens* are shown in (Figure 2). Different extracts exhibited different degrees of electron donating capacities in a concentration-dependent manner, where by ethylacetate fraction was the most outstanding at the various concentrations.

diethylether fraction showed highest amount of TFC followed by n-butanol and ethylacetate Antioxidant Activity

DPPH Radical Scavenging Activity Assay

DPPH has been widely used for free radical-scavenging assessments due to its ease and convenience[21]. DPPH accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction capability of DPPH radical was determined by the decrease in absorbance induced by plant antioxidants[22]. In the present study, all extracts were found to be effective scavengers against DPPH radical and their activities increased in a concentration dependent manner (Figure 1)

(Table 2) shows DPPH radical scavenging activity of crude extract and its different fractions from *A.graveolens.* The ethylacetate fraction was showed highest DPPH scavenging activity and compared with ascorbic acid, α-tocopherol as standards. Their IC 50 value is 4µg/ml, 9.55 µg/ml respectively while IC_{50} value is 28.13µg/ml for ethylacetate fraction. Antioxidant activity of *A.graveolens* extracts were showed in following order with significant difference ($P<0.05$): Ascorbic acid (4 µg/ml) > α-tocopherol (9.55 µg/ml) > ethylacetate fraction (28.13 µg/ml) > diethylether fraction (34.11 µg/ml) > n-butanol fraction (130.1 µg/ml) >hydromethanolic crude (135.22 µg/ml) > chloroform fraction(183.8 µg/ml).

The reducing capacities expressed in IC_{50} of crude extract, different fractions and references molecules exhibited the following order with significant difference ($P<0.05$): Ascorbic acid > ethylacetate fraction > diethyletherfraction > α- tocopherol > chloroform fraction > n-butanol fraction > hydromethanolic crude .The reducing properties are generally associated with the presence of reductones, which have been shown to exert antioxidant action by breaking the free radical chain or by donating a hydrogen atom[20]. Our results are in accordance with other published data showing the high reductive capability of various extract extracted from different part of *A. graveolens*[11].

β-Carotene/Linoleic Acid Bleaching Assay

In this model, b-carotene undergoes rapid discoloration in the absence of an antioxidant. The presence of an antioxidant such as phenolics can hinder the extent of b-carotene destruction by ‘‘neutralizing” the linoleate free radical and any other free radicals formed within the system[22]. (Figure 3) depicts the inhibition of b-carotene bleaching by the crude extracts and its fractions of

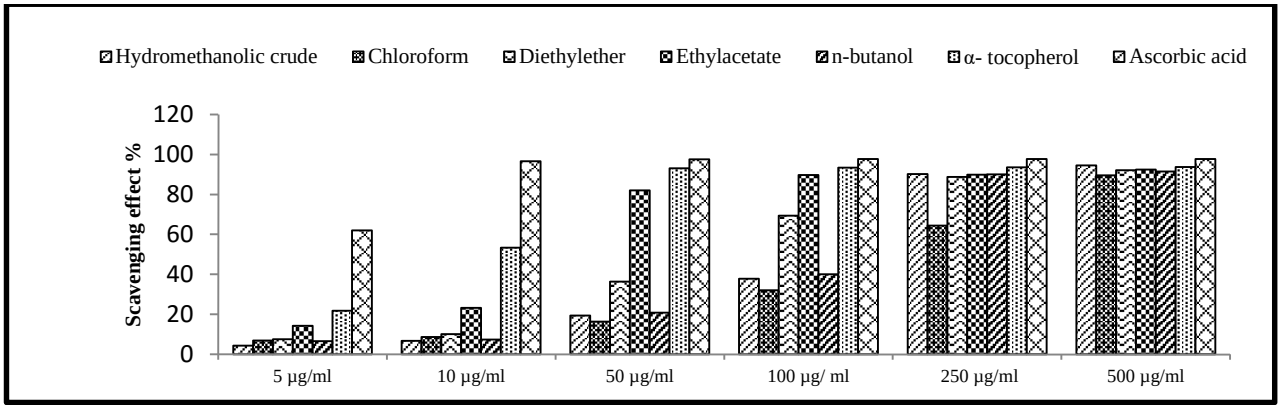

Figure 1: The DPPH radical scavenging activities of ascorbic acid, α-tocopherol and extracts of *A. graveolens.* Each value is expressed as mean ± SD (n = 3).

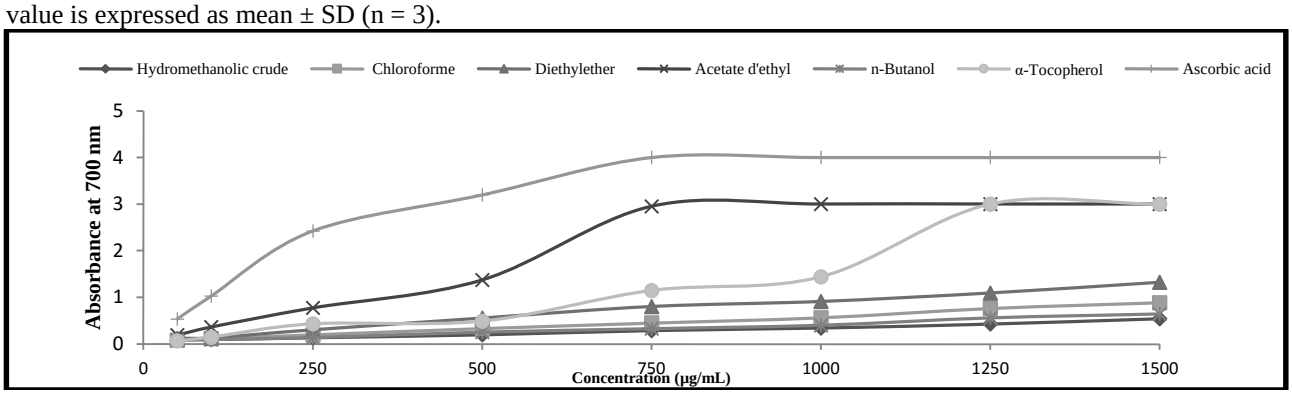

Figure 2: Reducing power of *A.graveolens* extracts, ascorbic acid, and α-tocopherol. Results are means ± SD of three parallel measurements.

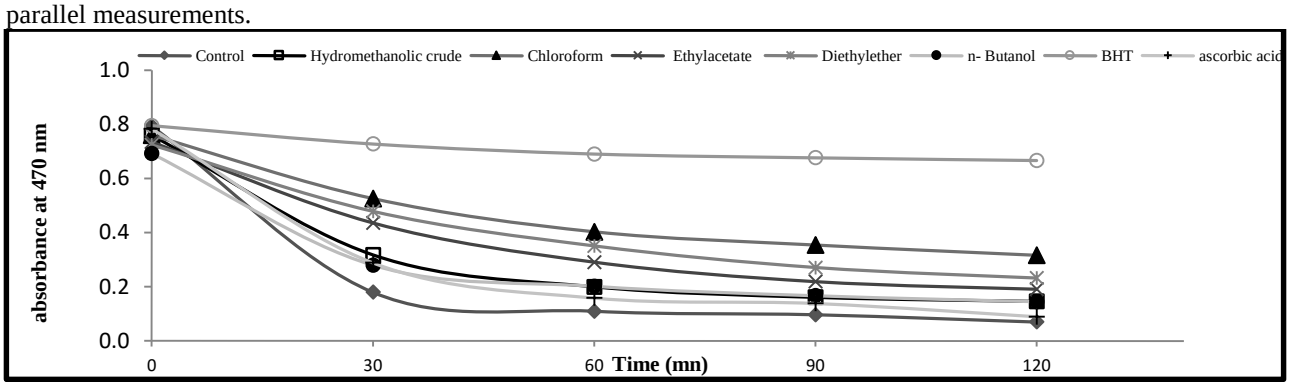

Figure 3: Degradation rate of different extracts of *A.graveolens* assayed by β-carotene bleaching assay. Values are expressed as mean ± standard deviation (n=3).

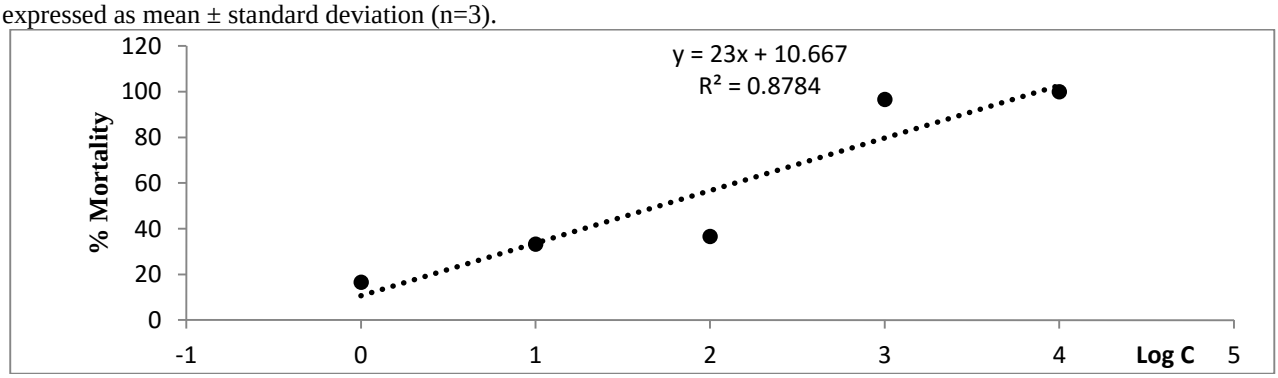

Figure 4: Determination of LC_{50} of hydromethanolic crude extract of *Anethum graveolens* against brine shrimp nauplii.

A.graveolens, and by the two positive controls (BHT and ascorbic acid). Chloroform fraction exhibited a marked antioxidant activity (70.22 %) some close to that of BHT (93.21%), while others extracts were less active. Ramadan et al[7] found that aqueous extract from dry fruits of *A.graveolens* at highest concentration of 400 µg/ml a percentage of inhibition was 87.5%, at the same concentration shyu et al[11] report a same value with a

Table 3: antimicrobial activity in terms of inhibition zones and minimum inhibitory concentration of dill hydromethanolic crude extract and its factions against the selected strains of bacteria and yeast.

	Hydromethanolic Crude extract		Chlorofom		Diethyl-ether		Ethylacetate		n-Butanol		Standards[c]	
Test microoganisms	IZ[a]	MIC[b]	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC	IZ	MIC
Gram- bacteria												
Escherichia coli	-	-	-	-	12.67±0.58	1.56	-	-	-	-	29±1	0.024
Pseudomonas aeruginosa	-	-	-	-	-	-	9.33±2,31	-	13.67±3.21	0.39	24.16±0.76	0.024
Enterobacter cloacae	-	-	-	-	13.67±0.58	-	12.33±0,58	6.25	-	-	20±00	0.048
Agrobacterium tumefaciens	-	-	-	-	11.67±1.53	1.56	9±2	3.12	8.67±0,58	-	ND	
Gram+ bacteria												
Staphylococcus aureus	-	-	10.02±1.53	12.5	11.67±1.53	1.56	12.33±1,15	6.25	-	-	32±1	0.012
Bacillus subtilis	-	-	-	-	13±1.73	0.19	9.33±1.15	1.56	11±2.65	0.39	36±1	0.006
Yeast												
Candida albicans	-	-	-	-	-	-	-	-	10±1	0.39	33	0.125

A dash (–) indicate no antimicrobial activity, (ND) not determined.
a: Inhibition zone in diameter (mm) around the impregnated discs and each value is presented as mean ± SD (n = 3).
b: Minimal inhibition concentrations; values given as mg/ml.
c: : standards :levofloxacin for bacteria, nystatin for yeast.

percentage of inhibition of 88.92 % for ethylacetate fraction. This data are partially in agreement with our results.

Correlation between Antioxidant Capacity and Total Phenolic Contents

Numerous studies have been conducted on the relationship between the antioxidant activity and content of phenolic compounds in plant extracts. Very often the results of these studies are contradictive. Some researchers have found a correlation between the concentration of polyphenol and antioxidant activity, while others have reported quite the contrary[10].

Pearson correlation showed there was a positive correlation relationship between phenol content and antioxidant activity assayed by DPPH radical scavenging assay (r=0.72) but a lower correlation was found between total phenol content and reducing power assay (r=0.60). Conversely, no correlation was found for antioxidant activity assayed by β-carotene bleaching test with phenolic content (r=0.20). Our findings were consistent with the study conducted by Othman et al[24] who showed that there was no correlation between antioxidant activity based on β-carotene bleaching assay and total phenolic content for 4 malaysian herbal plants. But the results of Zhang et al[25] are shown a correlation between the structure of compounds and antioxidant activity.

Antimicrobial activity

The in vitro antimicrobial activities of *A. graveolens* extracts against the microorganism employed and their potentials were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and the zones diameters, MIC values being showed in Table 2. It showed that Diethyl-ether fraction and ethyl acetate fraction have a better activity against Gram positive than Gram negative bacteria. Diethyl-ether fraction was the most active. It was active against 5 from 7 strains tested, and maximum inhibition zone diameter was obtained in *E. cloacae*, *B. subtilis* with diameter 13,67±0,58 mm, 13±1,73 mm respectively. Similarly, Ethyl-acetate fraction showed maximum inhibition zone with diameter of 12,33±0,58 mm in *E. cloacae*, and 12,33±1,15 mm for *S. aureus* respectively. The n-Butanol fraction (8-13 mm) showed restrained activity while the chloroform fraction was only effective against *S. aureus*, but there is no activity of the hydromethanolic crude extract against all microorganisms tested. Concerning antifungal tests, both fractions extracts failed to show any activity against *Candida albicans* excepting n-Butanol fraction which showed moderate activity.

The Diethyl-ether fraction showed MIC ranged between 0,19-1,56 mg/mL and it exhibited a higher degree of antimicrobial activity (0,19 mg/mL) against *B. subtilis* as compared with the other fractions. These results suggest that Diethyl-ether fraction were more efficient to inhibit bacterial growth, probably in relation to their active molecules. Several studies attributed the inhibitory effect of plant extracts against bacterial pathogens to their phenolic composition[22,26]. The inhibitory effect of these phenolics could be explained by adsorption to cell membranes, interaction with enzymes, substrate and metal ion deprivation[22].

The findings in this study support the observations of some other researchers which confirm that methanolic extract of *A. graveolens* has not been reported to possess any

antibacterial activity[23,27] while its ethanolic extract in another study showed reasonable antibacterial potential[3]. However, it is difficult to compare the data with the literature because several variables influence the results, such as the environmental and climatic conditions of the plant and the choice of the extraction method and antimicrobial test[28]***Toxicity*** *against brine shrimps*

The hydromethanolic crude extract of *A. graveolens* showed positive results indicating that the test sample is biologically active. Plotting of log of concentration (log C) versus percent mortality (% Mortality) for test sample in (figure 4) showed a good correlation (R^2 =0.88). The degree of lethality was directly proportional to the concentration of the extract. Maximum mortalities (100%) were observed at a concentration of 1000 µg/mL. From the graphs (figure 4), the median lethal concentration (LC_{50}, the concentration at which 50% mortality of brine shrimp nauplii occurred) of this extract was 51.29 µg/ml. Based on the results, the brine shrimp lethality of the hydromethanolic crude extract was found to be concentration-dependent. The observed lethality of this extract to brine shrimps indicated the presence of potent cytotoxic and probably antitumor components of this plant. According to Meyer et al[29] crude plant extract is toxic (active) if it has an LC50 value of less than 1000 µg/mL while non-toxic (inactive) if it is greater than 1000 µg/mL.

CONCLUSION

In conclusion, most extracts from *Anethum graveolens* possessed a good antioxidant, antimicrobial and cytotoxic activities. Which confirm their traditional use as a folk remedy. Higher levels of total phenolics in this plant might be responsible for their therapeutic effects. Thorough chemical analysis of this plant species should be directed to carry out in vivo studies of its medicinal active components in order to prepare a natural pharmaceutical product of high value.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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