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Antimicrobial and antioxidant activity of *Ammi visnaga* (L) phenolic extracts and their effects on planktonic and biofilm growth of food spoilage *Bacillus cereus*

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Key words: *Ammi visnaga*, *Bacillus cereus*, Biofilm, Polyphenols, Flavonoids, Antioxidant

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Abstract

Ammi visnaga (L) is a species from *Apiaceae* family (Umbelliferae), it is widely used in Algeria. It is supposed to be an interesting source of phenolic compounds which can be used against biofilm growth of bacteria. *Bacillus cereus*, a crucial pathogenic bacterium that causes food poisoning, is known as a producer of gastrointestinal diseases. In the present work we used water, acetone, ethanol and methanol to extract phenolic compound from the plant *Ammi visnaga* (L). The extracts were evaluated for their antioxidant activity and their effects on planktonic cells, swarming motility and biofilm growth of *Bacillus cereus* isolates. The results indicate that 70% methanolic extract represent the highest amount of total phenols (176mg GAE/g), and the lowest amount was obtained with acetone extract (18, 66mg GAE/g). Flavonoids extractability was found to be highest with ethanolic extract (22mg QE/g). Among all the extracts of *A. visnaga* (L), methanolic extract 70% showed the most potent radical scavenging ability (IC 50: 1, 46mg/ml) and the highest reducing power values from 1,129 to 1,974 at 700nm. DPPH assay of plant extracts was well correlated with FRAP assay (R^2=0, 7018) and a good correlation was found between antioxidant activity (IC 50) and polyphenols content of different extracts (R^2=0, 8153). No correlation was found between total polyphenol and flavonoids contents (R^2=0, 4267). The obtained results show that *A. visnaga* (L) extracts might possess high antimicrobial activities and methanolic extract at 10mg/ml was more effective to swarming motility and biofilm formation in *Bacillus cereus* strains.

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Introduction

The ability of many pathogenic bacteria to adhere to surfaces and to form biofilms has major implications in a variety of industries including the food industry, where biofilms create a persistent source of contamination causing food spoilage. Microbial adhesion to surfaces and consequent biofilm formation is a survival strategy that has been studied and documented in recent decades (Watnick and Kolter, 2000). Plant secondary metabolites (phytochemicals) have demonstrated promising antimicrobial properties when applied against planktonic cells and biofilms. Natural antimicrobial products can be attractive to the food industry in that they control natural spoilage microorganisms (Tajkarimi *et al.,* 2010).

Therefore, new antimicrobial products need to be identified and their antimicrobial action against bacterial biofilms must be assessed. Previous studies (Cushnie and Lamb, 2005; Vaquero *et al.,* 2007; Simões *et al.,* 2009a) have demonstrated that phenolic substances, including simple phenols and phenolic acids, are a major class of phytochemicals that have already demonstrated significant antimicrobial properties. Phenolics of higher plants are ubiquitous low molecular compounds. They are the most widespread molecules among secondary plants metabolites, and are of high significance in plant development (Curir *et al.,* 1990).

A great number of medicinal plants containing flavonoids have been reported their antibacterial activity. The plant *Ammi visnaga (L)* Lam. known as Noukha (in Algeria) or Khella (in some parts of North Africa) is classified under the Apiaceae (Umbelliferae) family. It is found growing widely in North Africa, Asia and Europe (Hegnauer, 1973), were it is used in traditional medicines to treat gastrointestinal cramps. In Algeria *Ammi visnaga (L)* is largely used in traditional treatment of digestive diseases and culinary but not very well studied scientifically. The major components of the plant *Ammi visnaga* (L) are furanochromones and coumarins. Khellin and visnagin are the most biologically active of them. (Benigni *et al.,* 1962; Hegnauer, 1973).

Furthermore, *Bacillus cereus* is a spore former bacteria, responsible for the spoilage of different food products. It is widespread in nature and in many raw and processed foods. It can survive during the cooking process, resist to pasteurization and produce emetic toxins. (Granum and Lund, 1997; Finlay *et al.,* 2002).

The cooked food products when stored at room temperature, the spores can germinate, proliferate, and produce emetic and diarrhoeal toxins leading to poisoning. The adhesion of *B. cereus* to surfaces is mainly due to its high hydrophobicity, to spores surface charges, and to the long appendages covering its surfaces. (Andersson *et al.,* 1995). *B. cereus* cells can attach on stainless steel surfaces and form a biofilm which cause problems in several processes of food industry (Peng *et al.,* 2002). The bacterial biofilms are generally formed by cells clusters gathered with an extra-cellular material to colonize surfaces.

The plant *Ammi visnaga* (L) is widely used in Algerian culinary, but it has been rarely used for improving their antioxidant and antimicrobial effects. The main purpose of this study was to investigate the antioxidant activity and to evaluate the phenolic and flavonoids content of *A. visnaga* phenolic extracts, as well as their effects on planktonic cells and biofilms of *Bacillus cereus* isolates causing foodborne spoilage.

Materials and methods

Isolation of Bacillus cereus strains from different Food Sources

Several Samples from local commercial supermarket and home-made foods were collected. The used foods are mainly: fresh and raw ground meats, poultry, fish, dairy products and some cooked dishes. All food samples were transported in sterile plastic boxes. The isolation of *Bacillus* species was performed according to the conventional procedure by serial dilution in sterile phosphate buffered saline. 10g of food sample was added in 90 mL of phosphate buffered saline; the different solutions were heated at 85 C° for 10 min.

100µl from the appropriate dilutions were surface plated on LB agar, and on Mannitol egg-yolk polymyxin B (MYP) agar plates (Vanderzant and Splittstoesser, 1992). Incubation was carried at 30 °C for 48h. The characterization of the isolates was performed by studying colonies morphology, Gram stain, cell forms and biochemical tests, using API 20 E system and Bergey's Manual of Systematic Bacteriology. (Cappuccino and Sherman, 2004).

Screening of Bacillus cereus toxicity

Lecithinase activity: plates were prepared by adding egg yolk emulsion up to 5% (v/v) to nutritive agar. Each isolate was spotted on the medium and the plates were incubated at 30°C. Opaque zones around the colonies, caused by hydrolysis of lecithin indicated lecithinase production. (Guttmann and Ellar, 2000).

Hemolytic activity: The hemolytic activity was determined at 33°C on 5% sheep blood agar plates by surface inoculation as described by Pruss *et al.* (1999). The isolates were spotted on blooded nutritive agar medium. (Collins *et al.,* 2001). The strains were classified as α (partial), β (total), or non-hemolytic.

Amylase activity: The ability to hydrolyze starch was tested by inoculating on the starch agar medium; the zones were detected by adding lugol to the plate's surfaces. (Collins *et al.*, 2001).

Caseinase activity: Caseinase was identified according to the method of Gudmudsdo (1996) on milk agar medium. The isolated bacteria were streaked on the appropriate medium for 24 h at 37°C. A transparent zone around the colonies indicated caseinase activity.

Antibiogram pattern of the isolated strains

To select *Bacillus cereus* showing resistance to antibiotics, all isolates were tested for their sensitivity to antibiotics using following antibiotics: Penicillin G, Doxycycline (30µg), Erythromycin (15µg), Norfloxacine (10µg), Amoxicillin (30µg), Ampicillin (10µg), Cephalothin (30µg), Carbenicillin (100µg), Oxacillin (1µg), Piperacillin (30µg), Trimethoprim-Sulfamethoxazole (25µg), Tobramycin (30µg), Rifampin (5µg) and polymyxin B (30µg). The antibiogram was realized on Mueller– Hinton agar, using disc diffusion method as described by Bauer *et al.,* (1966). The Inocula were set to 0.5 McFarland or (OD= 0.08 to 0.1) at 620 nm, which corresponds to 108 CFU/ mL. The plates were incubated at 37°C for 24h. The inhibition zones around the disc was measured and interpreted as sensitive, moderate or resistant.

Plant material

The plant *Ammi visnaga* (L) was studied following its large use in Algerian culinary and its potential medicinal uses. The plant was collected during spring season from the region of Sidilakhdar (Mostaganem, Algeria), and identified by Microbiology and vegetal biology Laboratory at Mostaganem University. The plant was washed with distilled water and dried at room temperature under shade. The dried aerial parts were powdered by a blender and stored away from light for further studies.

Determination of moisture content

10 g of powdered aerial parts of *Ammi visnaga* (L) was weighed and put in an oven (70°C) until dryness. Moisture lost was determined by the difference between initial fresh weight and constant weight after drying. All samples were analyzed in triplicate and the result was expressed by percentage of moisture of the sample studied.

Extraction procedure

An absolute methanol, water and three 70% (v/v) organic solvents (Methanol, Acetone and ethanol) were used to extract phenolic compounds. 10% w/v of powdered plant material was extracted by 100ml of each solvent. The extraction was performed during 30 min. Centrifugation was done at 3000 rpm/ 20min, and vacuum filtration was used to separate the liquid extract. The filtrates were collected and the solvent was evaporated at 40°C using a rotary vacuum evaporator. The aqueous extract was obtained by soaked 10 g of powdered plant in 100 ml sterile water for 5 mi, the mixtures were centrifuged at 3000 rpm for 20 min. The supernatant was used for the determination of total phenolic content and antioxidant activity. (Oboh *et al.*, 2009).

Total phenolic content

Ammi visnaga (L) extracts phenolic content was determined by adding 5 ml of Folin-ciocalteau reagent (1:10) and 4 ml of 7.5% aqueous sodium carbonate to 0.5 ml of different extracts. The mixtures were kept in obscurity at room temperature for 15 min. The optical density was then measured at 765 nm using UV spectrophotometer. (Singleton and Rossi, 1965). The results were reported as Gallic acid equivalent (GAE) per gram of dry weight. Gallic acid concentrations were prepared as standard in methanol.

Flavonoid content

To determine the flavonoid content (TFC), the aluminum chloride colorimetric assay was used as described by Liu *et al.* (2008). A volume of 2 ml of diluted extract was allowed to 200µl of 0. 5% sodium nitrite and incubated for 5 min. Then, 200µl of aluminum chloride 10% was added to the mixture. 2 ml of sodium hydroxide 1M was added to the mixture after 6 min, and the absorbance wasreaded at 510 nm. Quercetin was used for the calibration curve. The results of flavonoids content were expressed as mg quercetin equivalents (QE) per g. All the samples were analyzed in tryplicate.

Antioxidant activity of Ammi visnaga (L) extracts

Free radical scavenging assay (DPPH): to determine the ability of scavenging of phenolic extracts on 1,1-diphenyl-2-picrylhydrazyl (DPPH) free-radicals the method of Shimada *et al.*, (1992) was used. 2ml of each plant extracts at different concentrations was added to 0.5 ml of 1 mM DPPH prepared in methanol. After shaking, the mixture was left at room temperature to stand for 30 min in the dark. The absorbance was measured at 517 nm against an aliquot blank. Methanol was used as a control. Scavenging ability (%) = $[A0–A1/A0] \times 100$. The higher values explain greater antioxidant activity of the tested sample, but higher IC 50 value indicates a weaker capacity to scavenge DPPH radicals.

Ferric reducing power (FRAP): The reducing power was determined by adding 1 mL of varying concentration of sample extracts to 2.5 mL phosphate buffer (0.2 M, pH 6.6), then the solution was mixed with 2.5 mL of potassium ferricyanide 1%.

After incubation at 50°C for 20 min, 2.5 mL of 10% acid trichloroacetic (w/v) was added to the mixture. After centrifugation at 3000 rpm for 10 min, the upper layer (2.5 mL) was mixed with 2.5 mL of deionized water. Finaly, 0.5 mL of 0.1% ferric chloride was added. The absorbance was measured at 700 nm. Disilled water was used as negative control. The assay was done in triplicate. (Oyaizu, 1986).

Antimicrobial activity of Ammi visnaga (L) extracts

Agar-well diffusion assay: The antibacterial activity of the different plant extracts was evaluated against five isolates of food spoilage *Bacillus cereus* and a reference strain *Bacillus cereus* ATCC 14579. The turbidity of the bacterial suspensions was adjusted to an equivalent to 0.5 McFarland. The agar-well diffusion assay was performed according to the recommended method of Valgas *et al.* (2007). A standardized bacterial inoculum was uniformly surface spread on a Mueller-Hinton agar. Next, 80 µL of each plant extract dissolved in distilled water (10mg /ml) was added into the wells of 6 mm in diameter, the plates were incubated at 30°C for 24h and the measure of the diameter of inhibition zones was done. The Ampicillin (30 µg) and well containing the solvent only were used as controls. All tests were done in triplicate.

Swarming behaviour assay: The effect of methanolic extract on swarming migration of *Bacillus cereus* food spoilage isolates was realized according to the method cited by Liaw *et al.,* (2000), bacterial suspensions were grown in Brain-Heart Infusion (BHI) broth and incubated overnight at 37 °C. After incubation, the cultures were adjusted to an OD =1 ± 0.05 at 620 nm. 5 µl of an overnight bacterial culture was spotted centrally onto the surface of dry PPGAS (phosphate-limited peptone–glucose– ammonium salt) agar plates (KCl 20 mM, NH_4Cl 20 mM, $MgSO_4$ 1.6 mM, Tris–HCl (pH 7.2) 120 mM, peptone 1.0%, glucose 0.5%) without or with 0, 5; 1, 5 or 10 mg/ ml of phenolic compounds, then incubated at 37°C for 24h. For monitoring swarming motility, glutamate 0.05% was used instead of NH_4Cl.

Crystal violet biofilm assay: Cell suspensions were prepared by inoculating 3 ml of TSB (tryptic soy broth) with bacterial *Bacillus cereus* strains and were cultured overnight at 37°C and then diluted in fresh media to an OD =0,06 at 620nm. The biofilm growth inhibition performed adopting method of biofilm inhibition spectrophotometric assay in 96 well microplates as described by Regev-Shoshani *et al.,* (2010). 100 µl of each prepared bacterial suspension of the tested isolates was added into 96 well microplates and 100µl of different concentration of methanolic extract of *Ammi visnaga* (L)was added and incubated at 37° C for 48h, 200 µl of 1% w/v aqueous solution of crystal violet was added after removing the liquid suspension. After 30 minutes, the wells were washed thoroughly after removing the dye and 200µl of ethanol at 95% was added and incubated for 15 minutes. The reaction solution was read in an ELISA reader at 595 nm. The TSB was used as a blank. After incubation, the MIC was defined as the lowest concentration of *A.visnaga (L)* extract that exhibit an inhibition of visible growth.

Reduction of biofilm biomass was calculated as following: % inhibition =OD in control-OD in treatment/OD in control* 100.

Statistical analysis

All the experiments were carried out in triplicate. The data were analyzed by ANOVA. The IC50 values were calculated from linear regression analysis. Correlations between variables were established by excel.

Results

Isolation and identification of the isolated Bacillus cereus strains

46 isolates were obtained by isolation from different food samples, 24 isolates were selected after screening of their ability of resistance to penicillin G and other antibiotics (Fig. 1). The Colonies of isolated bacteria were big waxy white or gray, surrounded by an opacity zone. 19 of them were unable to catabolize mannitol when cultured on MYP agar.

Table 1. Identification of isolates of food spoilage *Bacillus cereus.*

Isolate name	*Gram-endospore*	*Catalase*	*Oxydase*	*Mobilité*	*50°C*	*Indole*	*VP*	*Citrate*	*Urease*	*Nitrate*	*OF*	*7%NaCl*	*Man*
BCSV1	+	+	+	+	-	-	+	+	-	+	+	+	-
BLGM18	+	+	+	+	-	-	+	+	-	+	-	+	+
BVLY31	+	+	+	+	-	-	+	+	-	+	+	+	-
BVLY16	+	+	+	+	-	-	+	+	-	+	+	+	-
BVLY12	+	+	+	+	-	-	+	+	-	+	+	+	-
LLVC2	+	+	+	+	-	-	+	+	-	+	-	+	+
BVDE17	+	+	+	+	-	-	+	+	-	+	+	+	-
BRIS7	+	+	+	+	-	-	+	+	-	+	+	+	-
BYLG5	+	+	+	+	-	-	+	+	-	+	+	+	-
LLVB1	+	+	+	+	-	-	+	+	-	+	+	+	-
BPTS1	+	+	+	+	-	-	+	+	-	+	+	+	-
BCSV2	+	+	+	+	-	-	+	+	-	+	-	+	+
BPTS2	+	+	+	+	-	-	+	+	-	+	+	+	-
BVLY22	+	+	+	+	-	-	+	+	-	+	+	+	-
BLLP2	+	+	+	+	-	-	+	+	-	+	+	+	-
BYDN3	+	+	+	+	-	-	+	+	-	+	+	+	-
BEPC1	+	+	+	+	-	-	+	+	-	+	-	+	+
BSEM1	+	+	+	+	-	-	+	+	-	+	-	+	+
BLVC3	+	+	+	+	-	-	+	+	-	+	+	+	-
BFMG16	+	+	+	+	-	-	+	+	-	+	+	+	-
BSEM4	+	+	+	+	-	-	+	+	-	+	+	+	-
BRIS3	+	+	+	+	-	-	+	+	-	+	+	+	-
BFMG11	+	+	+	+	-	-	+	+	-	+	+	+	-
BFMG17	+	+	+	+	-	-	+	+	-	+	+	+	-

(+) : Positive result

(-) : Nigative result

The isolates were gram positive rods. Motile, and grow positively in 7% NaCl but could not grow at 50°C. Following results shown in table (1), 19 isolates were belonged to *Bacillus cereus* and 5 to *Bacillus subtilis*. The identification of the above species was confirmed by API 20E system as recommended by bergy's manual. All *Bacillus cereus* stains were beta-haemolytic producing phospholipase C, amylase and caseinase. The obtained results indicate that the studied *Bacillus cereus* strains are currying a level of toxicity and virulence.

Table 2. Antimicrobial activity of *Ammi visnaga* (L) extracts against isolates of *Bacillus cereus*.

Plant extracts (solvent)	BVDE17	BPTS3	BCSV1	BRIS3	BSEM4	ATCC 14579
Acetone 70%	6+0,81	9+0,91	8+0,03	7+0,02	9+0,05	8,33+0,057
AQ (water)	8,33+0,85	9,33+1,07	11,33+0,037	9,33+0,025	9,33+0,057	9,33+0,057
MeOH 70%	21+1,05	15,33+1,15	15,66+0,05	15,33+0,057	15,33+1	12,66+0,057
MeOH 100%	14+1	12,66+1,09	13+0,047	12,66+0,047	12,66+0,047	11,33+0,057
EtOH 70%	11,33+0,87	12,66+1,09	12,33+0,057	11+0,037	11,66+0,87	10,33+0,057

Antibiotic sensitivity

The profile of antibiotic resistance of the tested strains to different antibiotics was performed. All *Bacillus cereus* strains showed high resistance towards Penicilline G (100%), but found variably resistant to the other antibiotics tested (Fig. 1). All the strains showed high sensitivity towards Norfloxacine (89, 13%), Doxycycline (84, 78%), Erythromycin (78, 26%), but were found less sensitive to Amoxicillin (21, 73%) Oxacillin (8, 69%) and Ampicillin (4, 34%).

Among the sample studied, the aerial part of the plant *Ammi visnaga* (L) showed high moisture content which showed a value of 90.46 ± 0.09%.

Effect of concentration and solvent type on total phenols yield

The results in figure (2) show the extraction yield of total phenols and flavonoids using different solvents. The phenolic content of all extracts ranged from 18, 66 to 172, 66 mg/g. The highest values was found in the aqueous methanol extract (172, 66 mg/g) which was significantly higher than ethanol extract 38 mg/g and acetone extract which represent the lowest yield with 18, 66 mg/g. The total flavonoid contents in *Ammi visnaga* (L) ranged from 3, 3 to 22 mg EQ/g. Ethanolic extract showed the highest value, while the lowest value was obtained by the pure (100%) methanol extract. (Fig.2). In general, considering all the solvents used in this study, aqueous methanol at 70% was found to be the most effective solvent to extract total phenols from plants. To improve the effect of the concentration of extraction solvent, we have used absolute, aqueous methanol and water extract. We also found that the yield of extraction of total phenols obtained by aqueous methanol was higher (172, 66 mg/g) than absolute methanol and water extract with 96 mg/g and 21, 66 mg/g respectively.

DPPH radical scavenging activity assay

In terms of antioxidant activities, and in comparison with ascorbic acid (AA) as positive control, it was found that methanolic extracts represented the highest DPPH scavenging activity (89, 21%) at concentration of 3mg/ ml, this value was significantly different from ethanol and acetone extracts at the same concentration. (Fig. 3). These values were lower than those obtained by ascorbic acid (positive control) which represent 91, 79%. Regardless the type of solvent, extracts with concentrations 70% of solvent used presented higher DPPH radical scavenging capability than those with their respective absolute methanol and water extracts. This result was similar to thus observed by total polyphenol content. The Figure 4 represents the IC 50 values obtained for the investigated *Ammi visnaga* (L) extracts. Methanolic extract was found to be 1, 46 mg/ml while the acetonic extract was 3,1. The results indicate that the scavenging ability of methanolic extract of *Ammi visnaga* (L) on DPPH radical was strong.

Figure 5 represent the results of correlation between antioxidant activities (DPPH and FRAP methods) and correlation between total polyphenol and flavonoid content.

Free Reducing power

The results of the reducing power of *Ammi visnaga* (L) extracts at different concentration used showed a range of absorbance values from 0.4 to 2.57 at 700 nm (Fig. 6). It was also found that high concentration of each extract increase the reducing power.

Table 3. Minimum Inhibitory Concentration (MIC) and % biofilm inhibition by *Ammi visnaga* (L) methanolic extract for *Bacillus cereus* isolates.

MIC mg/ml	BRIS3		BVDE17		BPTS2		BSEM4		BCSV1	
	OD_{595nm}	%Inhibition	OD_{595nm}	%Inhibition	OD_{595nm}	%Inhibition	OD_{595nm}	%Inhibition	OD_{595nm}	%Inhibition
C	$0,18+0,01^{B}$	0	$0,19+0,01^{A}$	0	$0,11+0,02^{J}$	0	$0,15+0,01^{E}$	0	$0,17+0,01^{C}$	0
0,5	$0,15+0,02^{D}$	15,78	$0,112+0,01^{J}$	41,05	$0,091+0,01^{N}$	17,27	$0,139+0,01^{F}$	7,33	$0,123+0,01^{H}$	27,64
1	$0,11+0,01^{I}$	38,88	$0,097+0,02^{L}$	48,94	$0,086+0,01^{O}$	21,81	$0,126+0,01^{G}$	16	$0,104+0,01^{K}$	38,82
5	$0,096+0,01^{L}$	46,66	$0,058+0,01^{S}$	69,47	$0,063+0,01^{R}$	42,72	$0,095+0,01^{M}$	36,66	$0,076+0,01^{P}$	55,29
10	$0,066+0,01^{Q}$	63,33	$0,04+ 0,01^{U}$	78,94	$0,028+0,01^{W}$	74,54	$0,055+0,01^{T}$	63,33	$0,032+0,01^{V}$	81,17

Absolute and aqueous methanolic extracts exhibited a high reducing power in comparison with the other plant extracts. Methanolic extract at 70% represent the highest value of 1.974±0.14 at 3mg/ml that might attribute to the collective antioxidant effects of phenolics and flavonoids.

Antimicrobial activities of Ammi visnaga (L) extracts

Agar-well diffusion assay: The results of antibacterial activity against strains of *Bacillus cereus* are illustrated in table 2.

The inhibition zones of bacterial strains obtained by the methanolic extract were in the range of 12, 66 ± 0.5 to 21 ± 1 mm, which was significantly different from those obtained by ethanolic extract and acetonic extract with 10,33+0,05 to 12,66+0,05 and 7+0,05 to 9+0,05, respectively. It can be observed that the extracts of *A. visnaga* (L) possessed an inhibitory effect on all tested strains, depending on solvent of extraction and amounts of phenolic compounds.

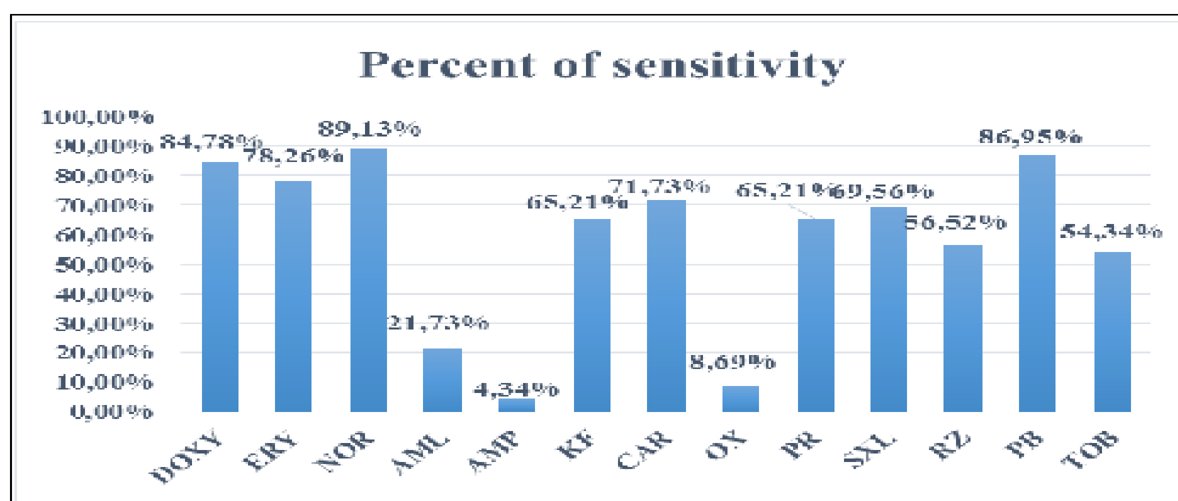

Fig. 1. *Bacillus cereus* isolates sensitivity towards antibiotics studied.

Swarming motility assay: The obtained results of motility revealed that methanolic extract possess a significant effect on swarming motility of strains tested.

The diameter of swarming zones was highly correlated to the increasing of phenolic concentrations and the highest zone diameter was obtained by the concentration of 10 mg/ml. (Fig. 7).

Biofilm inhibition assay: In biofilm quantification assay, a significant decrease was observed when bacterial strains grown in the presence of the methanolic extract. The apppropriate concentrations of methanolic extract (0, 5; 1; 5 and 10 mg/ml) provides a decrease in biofilm growth. The reduction in biofilm biomass of the isolates tested range from 63, 33 to 81, 17%. (Table 3).

The biomass of biofilm formed by the different strains of *Bacillus cereus* decrease with the increase of the concentration of the methanolic extract and this reduction differ from stain to another. These results supposed that the methanolic extract of *A. visnaga* (L) has a strong effect against biofilm of *Bacillus cereus* due to their total phenolic content.

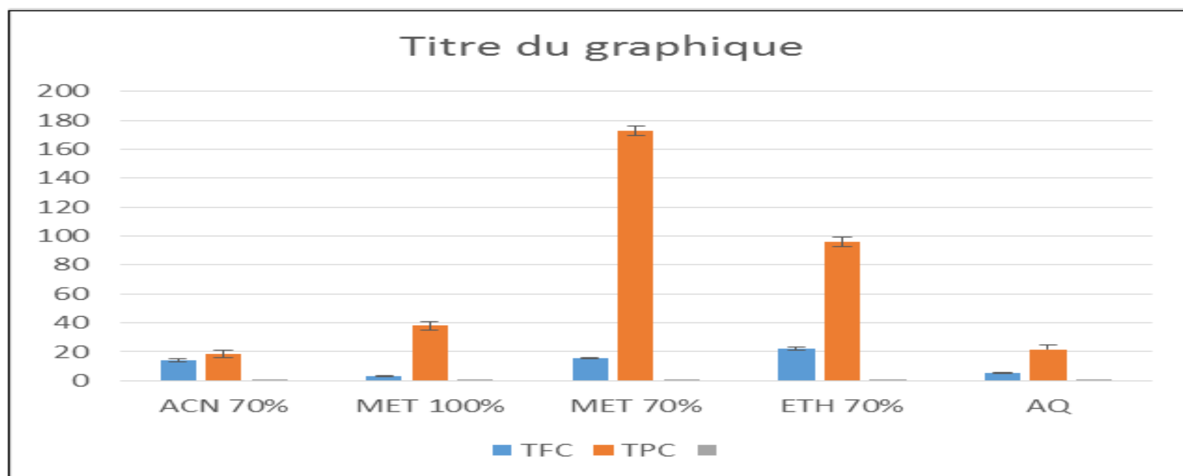

Fig. 2. Polyphenol and flavonoids content of *Ammi visnaga* (L) extracts.

Discussion

Although, substantial amount of data are available about the incidence of *B. cereus* in various types of food and food poisoning cases worldwide. (Stenfors *et al.,* 2008). Currently, the selective MYP plating medium is recommended by food authorities as standards for the detection of *B. cereus*.

The main identification feature of this medium is the lecithinase activity, resulting in opaque precipitation zones around suspect colonies. (Szabo *et al.,* 1984). The opaque zone might be due to the fact that the egg yolk tellurite emulsion used is a lipoprotein basically composed of lecithin, and lecithinase breaks down lecithin to produce an insoluble precipitate, resulting in opaque zones around the colonies. (Hussain *et al.,* 2011).

The obtained results designate that the studied *Bacillus cereus* strains are currying a level of toxicity and virulence, as cited by Oh (2006), Kashid and Ghosh (2010) which reported that the major features of *B. cereus* on plating media, produced caseinase, lecithinase with hemolytic activity. In this regard, Awny *et al.* (2010) reported that haemolysin, lecithinase and caseinase of *B. cereus* and *Staphylococcus aureus* from food samples are considered as virulence and toxicity factors. Our results are supported by the broad picture of antibiotic susceptibility patterns of *B. cereus* as shown by Fenselau *et al.* (2008).

Precedent works showed that antimicrobial susceptibility of *Bacillus cereus* was highly susceptible to Erythromycin, Ciprofloxacin, Streptomycin, Chloramphenicol, and less sensitive to Ampicillin, Cloxacillin, Ampiclox, and Cotrimazole, (Umar *et al.,* 2006). Our finding can prove that actually antibiotics are widely used in foods, consequently they can be an important factor to transfer resistant antibiotic foodborne pathogens which can be very harmful to human health. (Khan *et al.,* 2000).

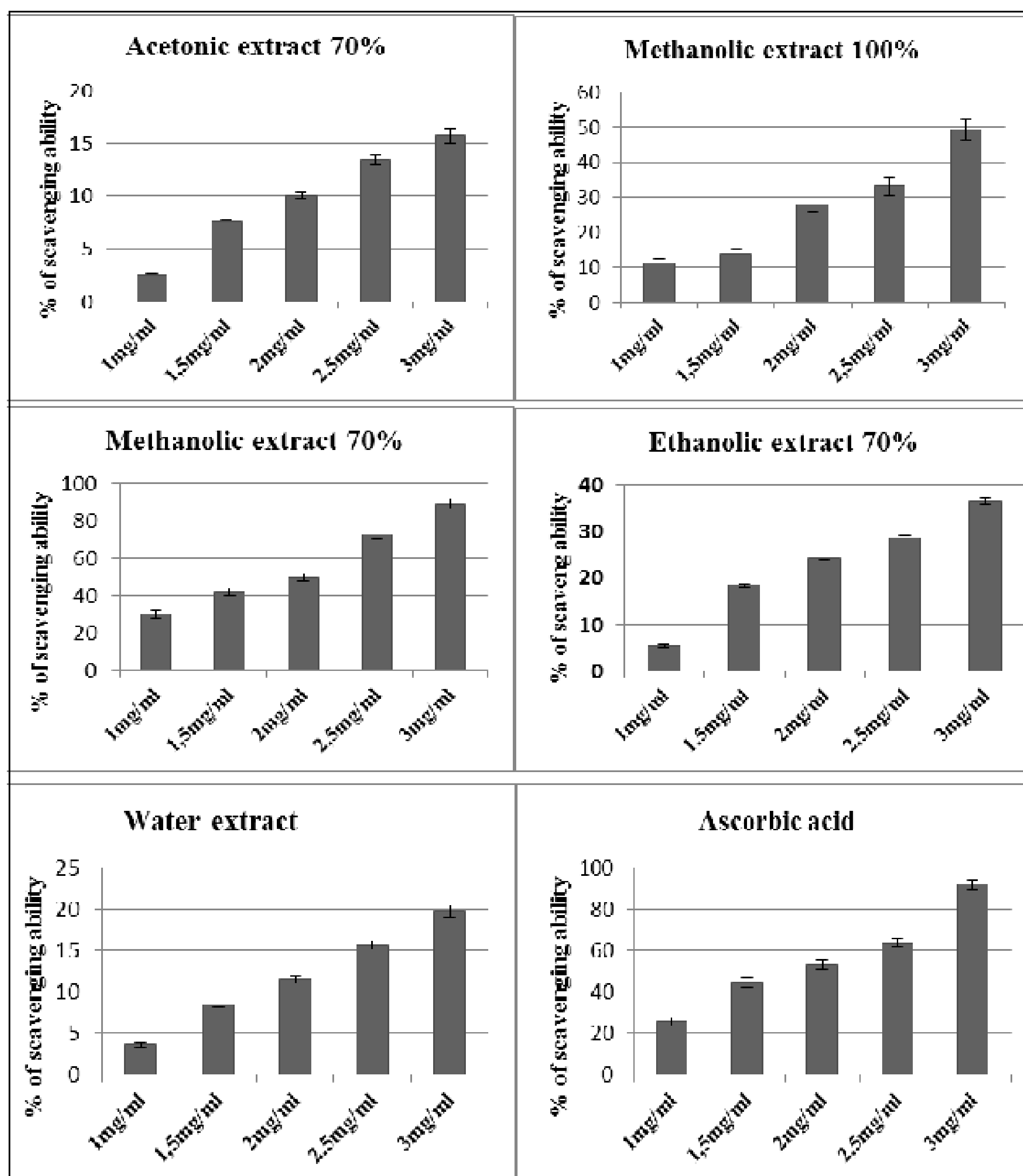

Fig. 3. Scavenging ability of different extracts of *Ammi visnaga (*L).

The difference in the yield of total phenols obtained from different plant extracts was mainly due to the difference in the nature of phenolic compounds obtained by each solvent used. (Ignat *et al.,* 2011). In addition, Horax *et al.* (2005) highlighted the importance of solvents effect that significantly influenced the quantity of phenolic compounds extracted. Following Ross *et al.* (2009), a great number of phenolic compounds can be obtained by methanol extraction such as phenolic acids, flavanons, flavanols, anthocyanins, catechins, and procyanidins. These results highlight that the polarity of the extraction solvent used can influence the yield of extracted phenolic compounds. This result is in line with those obtained by Zhao *et al.* (2006), the solubility and the extraction yield of chemical constituents of a sample can be affected by the difference in polarity of the solvent used for extraction.

The quantification of the total phenolic content in the extracts showed that the aqueous mixtures at 70% solvent and in comparison with pure solvents, were more effective to extract the phenolic compounds. (Rødtjer *et al.* 2006) Lapornik *et al.* (2005) suggest that ethanol and methanol extracts (70%) represent higher values of total polyphenols than water extracts.

The results of free radical scavenging ability (DPPH assay) agreed with the results reported by Zhang *et al.* (2013). DPPH scavenging is significantly influenced by solvents used for extraction of polyphenols. Phenolic compounds have been investigated to be a strong hydrogen donators to the DPPH radical. (Von Gadow, Joubert, &Hansmann, 1997).

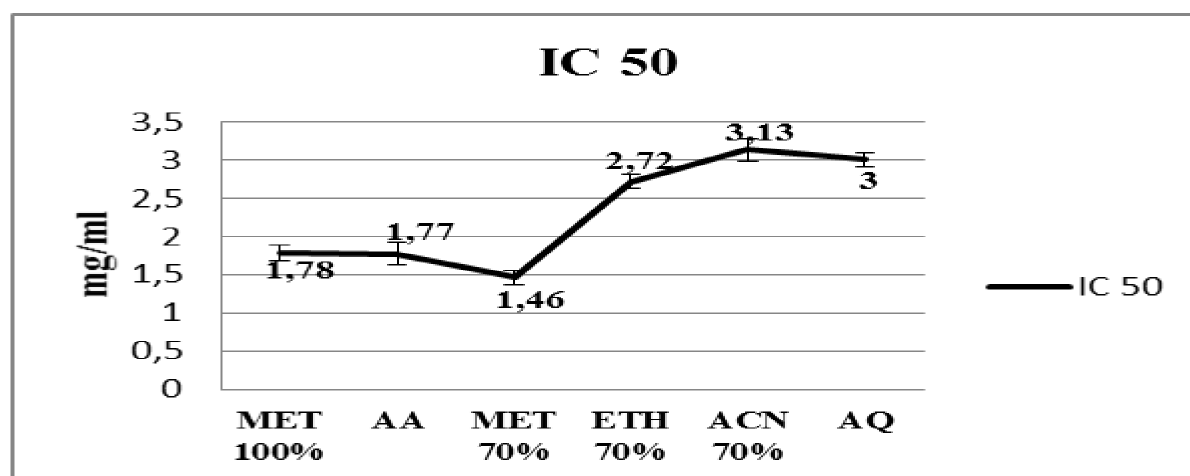

Fig. 4. IC 50 of different extracts of *Ammi visnaga* (L).

The antioxidant activity of the majority of plants is mostly due to the presence of phenolic compounds. It is known as free radical scavengers. (Skerget *et al.,* 2005). The antioxidant activity estimation and the ability of solvent to dissolve a selected group of antioxidant compounds can be influenced by the change in solvent polarity. (Zhou & Yu, 2004). The correlation between antioxidant activity and total phenol contents has been largely studied in different food products. (Kiselova *et al.,* 2006).

Our results of antioxidant activity obtained by the reducing power assay showed that the methanolic extract had the highest antioxidant activity values at 700 nm, this result was similar to those obtained from the yield of total phenolic compounds. Previous researches and reports in the literature agree with our results, phenolic compounds are the major phytochemicals responsible for the antioxidant capacity of natural extracts (Cevallos-Casals *et al.,* 2006), maybe due to their redox properties, which permit them to act as reducing agents, hydrogen donors, and singlet oxygen quenchers. (Chang *et al.,* 2001).

The antibacterial effect can be explained by the relationship between the number of hydroxyl groups and their position on the phenol group and their relative toxicity to microorganisms, the increase in hydroxylation induce an increase of antimicrobial activity. (Marjorie, 1999). The *Ammi visnaga* (L) or Noukha has been shown to be effective against microorganisms. (Abroush *et al.,* 2001). Jaradat *et al.* (2015) found that the plant extracts of *Ammi visnaga* (L) obtained by using organic solvents have shown a strong antimicrobial activity than the aqueous extract and variations among species were obvious. Baydar *et al.,* (2004) have suggest that the antibacterial activity of plant extracts is proportional to the amount of phenolic compounds in investigated plant.

Some researchers suggested that tannic acid and tannin are the main phenolic acids which can stopped *P. aeruginosa* swarming motility without reducing their growth capacity (Omay and Tufenkji, 2011). Proteins can have a strong effect on the mechanism of inhibition of swarming motility that is propably due to binding and precipitation of phenolic compounds to them. (Pratt and Kloter, 1998).

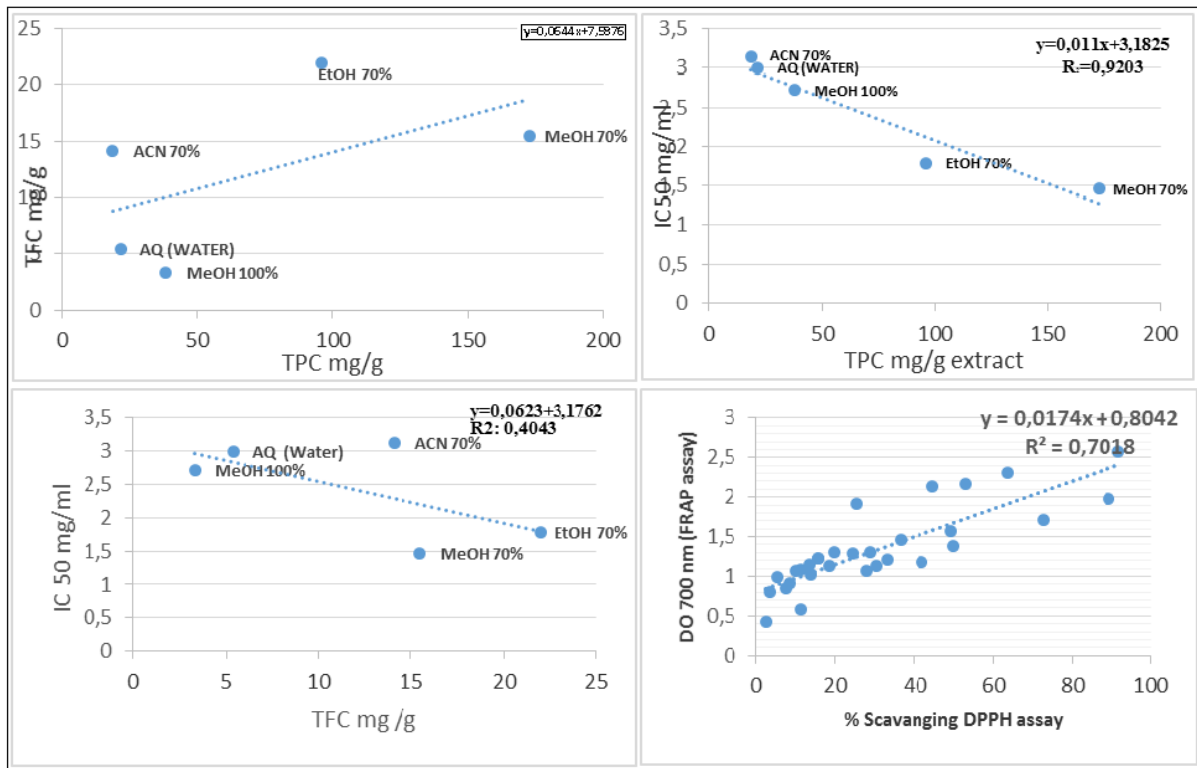

Fig. 5. Correlation between polyphenol and flavonoid content (a) and between antioxidant activity (IC 50) and polyphenol content of extracts of *Ammi visnaga* (L) (b). (c) Represent correlation between IC 50 and flavonoid content, and (d) correlation between antioxidant activities DPPH and FRAP methods.

In clinical and foodborne pathogenesis biofilm associated infection is known as a trigger to chronic diseases, food spoilage. Even dairy and refrigerated food spoilage was also created by the bacterial biofilm. (Teh *et al.,* 2014; Mizan *et al.,* 2015). Some previous works revealed that the enzymatic activity of glucosyltransferase which permit the colonization of bacteria and their adherence can be affected by the phenolic compounds. (Yanagida *et al.,* 2000, Gregoire *et al.,* 2007). Biofilm formation can be inhibited by the action of phenolics due to lack of iron, some phenolics have intermediate property of iron chelating. (Devosset *al.,* 1999).

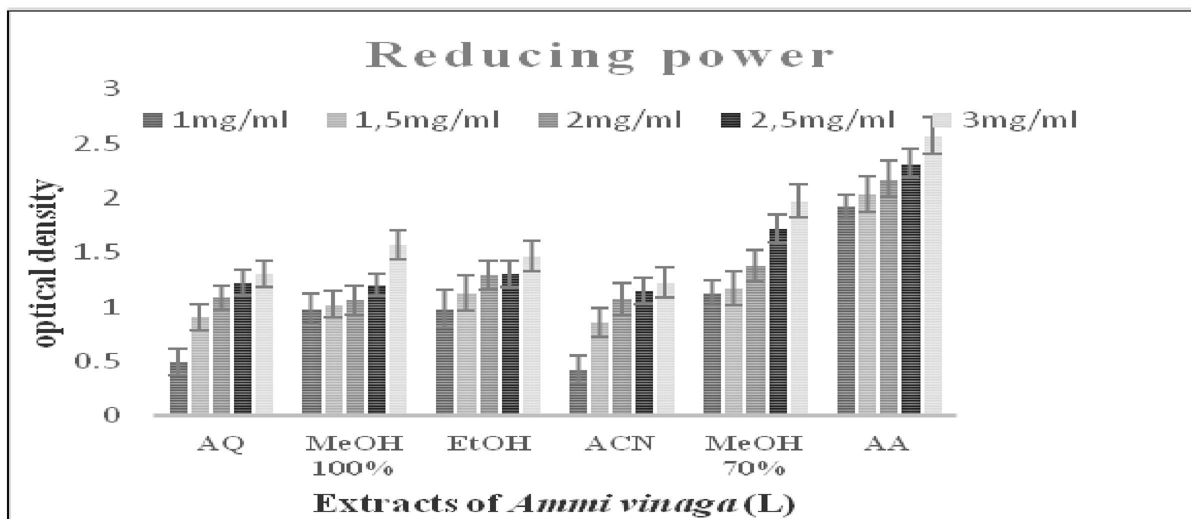

Fig. 6. Reducing power of *Ammi visnaga* (L) extracts.

The peptide's capability to cover either the surface of biomaterial or the bacterium itself, reduce the adherence of microorganisms on surface and decrease the biofilm growth. (Segev-Zarko *et al.,* 2015) Developed studies are demonstrating that there is a biological rationale between quorum sensing and biofilm which work on a coordinate manner leading to spoilage. (Bai and Vittal, 2014).) Bacterial AHLs can be imitate by substances secreted by several plants and affect the regulated behaviors of quorum-sensing plant-associated bacteria, respectively. (Teplitski *et al.,* 2000).

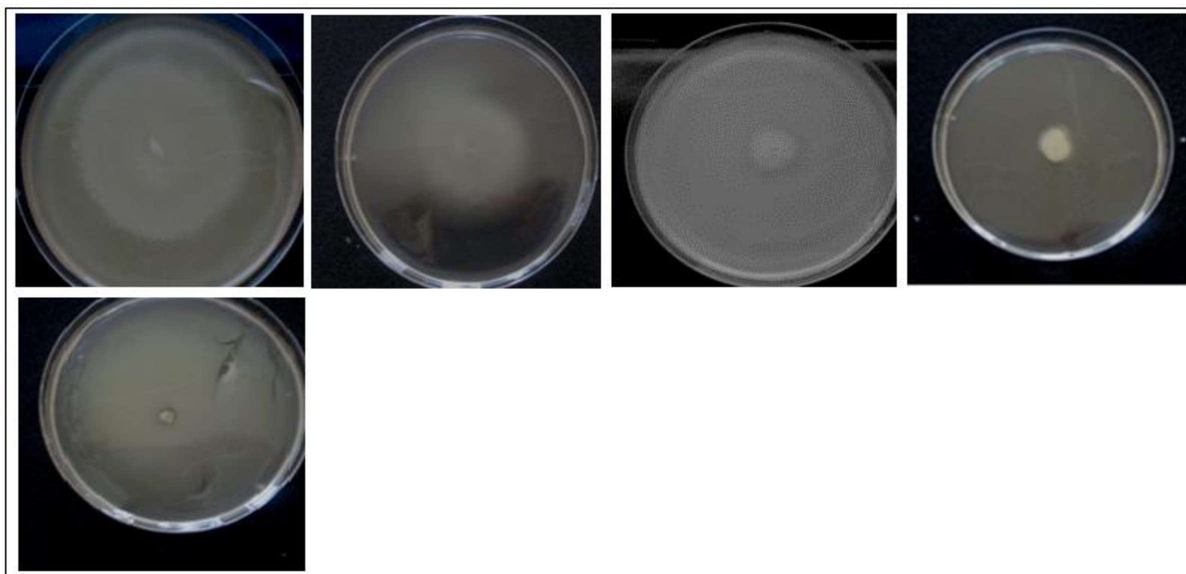

Fig. 7. Effect of *Ammi visnaga* (L) methanolic extract on swarming motility of *Bacillus cereus*.

Conclusion

In conclusion, the extracts of *Ammi visnaga* (L) obtained by using solvents with less polarity were strong radical scavengers than those obtained with high polarity solvents. The selection of the appropriate solvent is a important step to optimize the extraction of total polyphenol, flavonoid and antioxidant activities of the investigated plant extracts. Methanol was recommended as the best solvent for the production of antioxidant compounds from the aerial parts of the plant of *A. visnaga* (L). It also possess a strong antibacterial and antibiofilm activity against *Bacillus cereus* strains responsible for food spoilage. For this, it can be used in food industries as a natural preservative. However, further studies are needed to explore the individual or major polyphenolic groups and other bioactive compounds in the extracts of *A. visnaga* (L) and their contribution to health care and biotherapy. In addition, in vivo assay of antioxidant are recommended to confirm the strong potentiel of *Ammi visnaga* (L) to treat diseases caused by food spoilage.

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