Mechanism of antimicrobial activity and antioxidant activities of the essential oil and the methanolic extract of *carum montanum* from Algeria

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ABSTRACT

The methanolic extract (ME) of *C. montanum* obtained by a hydo-alcoholic maceration and its polyphenol content was evaluated by Folin-Ciocalteu method. This extract and *C. montanum* essential oil (EO)were screened for antimicrobial activity against 21 microbial strains by agar diffusion method. MICs of the EO were determined by the broth micro dilution method. The action mechanism of EO was determined on the susceptible strains by the time kill assay and the lysis experience. Antioxidant properties were studied by both free DPPH radical scavenging and reducing power techniques. The TPC in the ME showed a high level of 101.50 ± 5.33 mg GAE /mg. *B. cereus* was the most sensitive strain with MIC of 55.5 µg/ml , then *K. pneumoniae* (111 µg/ml). A remarkable decrease in a survival rate as well as in the absorbance at 260 nm were recorded, which suggest that the cytoplasm membrane is one of the targets of the EO. Antioxidant effects concentration were dependent and IC_{50} values were 1.09 ± 0.37 µg/ml for the EO and 65.04 ± 0.00 µg/ml for the ME by DPPH method and a reducing power dose-dependent. In conclusion, *C. montanum* extracts showed potent which could be exploited in the food industry for food preservation.

Key words: *C. montanum*, Apiaceae, essential oils, antimicrobial activity, antioxidant activity, reducing power.

I. Introduction

The umbelliferae is large and taxonomically a complex family of flowering plants. They are relevant in a number of very different scientific fields and have many uses. They are also great interest to taxonomists, florists, ecologists and phytosociologists, phytochemists, horticulturists, as well as professionals involved in medicinal plants, aromatic, the food industry and gastronomy (**Reduron, 2004**). Common caraway is one of the oldest herbs known (**Fang et *al.*, 2010)** and the genus *Carum* was established since 1753 (**Zakharova *et al.*, 2014**). It is naturally found in Asia, Central Europe, North Africa (**Fang et *al.*, 2010),** Siberia and Turkey (**De Carvalho and da Fonseca, 2005**). The genus *Carum* is an important of the Apiaceae family and contains about 20-30 species. The best-known species of this genus is *Carum carvi L* (**Laribi et *al.*, 2013**). This herb was first used by the ancient Arabs and Pliny recommended it for hysterical complaints and pale complexions (**De Carvalho and da Fonseca, 2005**). Caraway seeds are used as a flavouring of bread, cheese, sauerkraut, candies, meat products and sauces and as a source of carvone for cosmetics, toothpaste, chewing gum and pharmaceutical preparations. The seeds have been used in alternative medicine as a laxative, in colic treatment and as a breath freshener. The seeds have been found to have antipasmodic, carminative, emmenagogue, expectorant, galactagogue, stimulant, stomachic and tonic properties (**De Carvalho and da Fonseca, 2005**). *C. montanum* (syn. *Selinopsis montana* Coss. et Dur.) grows wild in calcareous mountainous regions, such as the constantine Saharian and Tell Atlas, as well as in the Kabyle and Numidian areas. It is characterized by smooth stems (10–15 cm), white flowers and an oblong fruit (2–2.6 mm) (**Quezel and Santa, 1963**). *Carum carvi*, the generic type of this genus, has been widely studied regarding its chemical constituents and biological activities but there seems to be no report on *C. montanum*. Contrary to *C. carvi*, this species has neither medical nor culinary applications, but is commonly grazed by the livestock (**Laouer *et al.*, 2009**). In order to screen new bioactive chemicals from local wild plants, we have investigated antimicrobial and antioxidant properties of the essential oil and the methanolic extract of this species.

II. Materials and Methods

II.1. Plant material

The aerial parts of *C. montanum* were collected from the mountain Megress (located about twenty kilometers northwest of the capital of Sétif. Algeria) at an altitude of 1500 m above sea level. The plant was identified by Pr H. Laouer (Laboratory of Valorization of Natural Biological Resources, University of Sétif, Algeria) then they were freed of impurities and then dried in the shade at room temperature.

II.2. Microbial strains

The antimicrobial activity of the EO as well as the ME was evaluated on 11 strains of the American Type Culture Collection (ATCC). As for fungal strains, the activity was tested on *Candida albicans* ATCC 1024, *Aspergillus niger* 2CA936 and *Aspergillus flavus* NRRL 391 (Northern Regional Research Laboratory).

II.3. EO's extraction

To obtain the essential oil (EO), air-dried parts of the plant were cut into thin parts and were subject to a

hydrodistillation for 3 hours using a Clevenger-type apparatus. The oil was stored in a refrigerator (4°C), until use.

II.4. Preparation of ME

To obtain the methanolic extract (ME), aerial parts were crushed and pulverized and then 20g were macerated in 100 ml of a water-alcohol mixture methanol/water (1-10 v/v) at room temperature for 72 hours. After filtration through filter paper several times, the methanol was removed from the filtrate by evaporation under reduced pressure in a rotary evaporator (BÜCHI) (**Athamena et *al.*, 2010; Sourabie et *al.*, 2010**).

II.5. Determination of total phenolic content (TPC) in the ME

The TPC was determined using Folin-Ciocalteu reagent assay (**Li *et al.*, 2007**). In brief, 200µl of the extract or the standard (prepared in methanol or distilled water) with suitable dilutions were mixed with 1 ml of the Folin-Ciocalteu reagent. 4 min later, 800 µl of sodium carbonate solution (75 mg/ml) was added to the mixture. After being kept for 2 hours, the absorbance at 765 nm was measured and the TPC was expressed in mg Gallic acid equivalents (GAE) /mg sample.

II.6. Antimicrobial activity assessment

II.6.1. Agar diffusion method

A preliminary antibacterial activity of the EO and the ME was determined by the agar diffusion method using the six mm diameter discs. Briefly, the Petri dishes were cultured by swabbing areas (**Rahal, 2008**), and then pre-incubated for 1/2 h at room temperature, allowing the complete diffusion of the EO and then incubated at 37°C for 24 h. The antimicrobial activity was determined by measuring of inhibition zone diameters (mm). Gentamicin was used as a positive control for bacterial strains and Miconazol as a positive control for fungal strains.

II.6.2. Determination of MICs by broth micro-dilution method

This method involves the use of small volumes of broth dispensed into sterile plastic micro-dilution trays. A two fold dilution of the EO volumetrically in broth was done. Then, it was dispensed into the wells so that each well contained 0.1 ml. A standardized inoculum of 5×10^5 CFU/ml was inoculated in each well. The inoculated micro-dilution trays were incubated at 35 ± 2°C for 24 h (**CLSI, 2012**).

II.6.3. Time kill assay

This method allows the characterization of the antibacterial EO activity over time. It assesses the decrease of bacteria, which are subject to a given EO concentration over several hours. A standardized suspension of 10^8 CFU/ml was diluted on 1/20. One ml of this inoculum was introduced into nine ml of Muller-Hinton (MHB)-Tween 80 (0.01%, v/v) in the absence (growth control) or in the presence of a concentration corresponding to the MIC of the EO in the liquid medium. The suspension obtained contained approximately $5x10^5$ UFC / ml and was maintained under stirring at 37 °C. 100 µl of the suspension were removed at different times (0, 2, 4, 6, 8 and 24 hours) to carry out a counting on MHA agar after incubation at 37 °C for 24 hours. The quantification of the number of bacterial colonies is limited to the value of 10^2 CFU / ml. The results were interpreted by a bactericidal curve representing the time intervals on the abscissa axis and the number of survivors on the ordinate axis (**Guinoiseau, 2010; Carson *et al.*, 2002**).

II.6.4. Bacterial lysis

This method (**Carson *et al.*, 2002**) determines whether a bacteriolytic action of EO by measuring the absorbance at 620 nm. Indeed, non-lysed bacteria absorb at 620 nm, so if there will be bacteriolysis absorbance at 620 nm over time decreases. A young bacterial suspension was standardized at 3×10^{10} CFU / ml (OD_{620} ~ 0.3), placed in a sterile tube in the absence (negative control) or in the presence of EO at two concentrations, one corresponding to the MIC and the other two times the MIC. Suspensions obtained were subjected to agitation. At time 0 s, 30 s, 30 min, 60 min, 90 min and 120 min are diluted to 1/100 and absorbance was measured at 620 nm. The results were expressed as the relative optical density (OD_{620}) in each time interval.

II.7. Antioxidant activity assessment

II.7.1. DPPH radical scavenging assay

The DPPH radical absorbs at 517 nm and the antioxidant activity can be determined by recording the decrease of the absorbance of the extracts. 50µl of each different EO dilution were mixed with 1250 µl of a methanol solution of DPPH (0.004 %). The absorbance was measured after 30 min of incubation in the dark. Synthetic antioxidant, BHT was used as a positive control. Thus the calibration curves representing the percentage of inhibition versus concentrations were performed using Graph-pad prism programs. The ability to scavenge DPPH radical is calculated as follows;

I % = [(Abs_{517} control–Abs_{517} sample) / Abs_{517}control] x 100

The IC_{50} values were estimated by a linear regression. The values are presented at least as the mean of triplicate measures (**Singh *et al.*, 2006**).

II.7.2. Reducing power assay

It is a technique that measures the reduction of Fe^{3+} (ferric iron) to Fe^{2+} (ferrous iron) in the presence of the extract tested. The presence of reducers in plant extracts causes the reduction of Fe^{3+} in a complex of ferricyanid to form ferrous iron Fe^{2+}. Therefore, Fe^{2+} can be assessed by measuring the increase of the

density of the green color in the reaction medium at 700 nm (**Bougandoura and Bendimerad, 2013**). In a test tube containing 1 ml of EO, was added 2.5 ml of phosphate buffer (0.2M, pH 6.6) and 2.5 ml of potassium hexacyanoferrate [K_3Fe $(CN)_6$] (10g/l). The whole is heated to 50 °C in water bath for 20 minutes. A volume of 2.5 ml of trichloroacetic acid (TCA) (100 g/l) was then added to stop the reaction. Finally, 2.5 ml of the supernatant was mixed with 2.5 ml of distilled water and 0.5 ml of ferric chloride [$FeCl_3$] (1g/l). A blank sample is prepared in the same conditions. The absorbance was read at 700 nm. BHT is used for the positive control. An increase in absorbance corresponds to an increase of reducing power of the extract tested (**Bourkhiss *et al.*, 2010; Liu *et al.*, 2011; Narasimhan *et al.*, 2013**). The values are presented as the means of triplicate measures.

II.7. Statistical analysis

All experiments were done in triplicate and results were reported as mean ± SD. The results of various tests were analyzed by the student t test for single comparisons and ANOVA followed by Tukey and Dunnett's test for multiple comparisons and for thre determination of significance level. The analyze was done through "Graphpad Prism" version 5.0 software.

III. Results and Discussion

III. 1. TPC dosage

The hydo-alcoholic maceration gave a dark brown extract, soluble in DMSO and methanol with a yield of 3.77%. The TPC is estimated to 101.50 ± 5.33 mg GAE /mg. This level is close to that found by **Zahin *et al.* (2010)**, in the fruit extract of *C. copticum*; 119.2 mg/g but very high compared to most species of the same genus; *C. carvi* from Australia 5 µg/mg **(Polovka and Suhaj, 2010)**, *C. carvi* from Himalayas to 45.5 µg/mg **(Xavier *et al.*, 2011)** and 50.2 µg/mg in the extract of *C. carvi* from India **(Thippeswamy *et al.*, 2013)**. *C. nigrum* extract contained only 3,065µg/mg of polyphenols **(Padmashree *et al.*, 2007)**. Previous studies have shown that the amount of phenolic compounds in plants depends on biological factors (genotype and species), edaphic, and environmental (temperature, salinity, water stress and light intensity) **(Ksouri *et al.* 2008).**

III. 2. Antimicrobial activity

III. 2.1. Disc diffusion assay

The activity exhibited by the EO and the ME was not selective for Gram positive and Grab negative **(Table 1**). This can be attributed to the huge variety in composition of extracts which does not define any particular spectrum at each extract or even to classify them by adopting the conventional activity test $Gram^+$ and $Gram^-$. The susceptibility of different bacteria toward *Carum*'s EO has been reported by several authors; *C. carvi* (**De Martino *et al.*, 2009; Fang *et al.*, 2010; Seidler-Lozykowska *et al,* 2013**) and *C. copticum* (**Rahmatabadi-Dashti *et al.*, 2007; Rezaei Kahkha *et al,* 2014**). *K. pneumoniae* and *B. cereus* were most susceptible, which was the same with the EO of *C. carvi*. (**De Martino *et al.*, 2009**), but *S. aureus* sensitive to this oil was moderately sensitive to *C. carvi* EO, from 17 different regions **(Seidler-Lozykowska *et al.*, 2013)**. The chemical composition of this EO was previously reported by **Laouer *et al.*, (2009)**, it was mainly composed of phénylpropanoids (71.9%) and sesquiterpen hydrocarbon (15.4%). The α-pinene, limonene, β-pinene, the thymol, γ-terpinene, the myrcene, the phellandrene, the geranial, the dillapiole and the cymene are present in this oil and considered as potential antimicrobials **(Duke, 2015).** Generally, phenylpropanoids, may be the cause of the antibacterial activity because they have been previously isolated from a species known to exert a protective action against phyto-pathogens (bacteria and fungi) (**Da Silveira *et al.*, 2014)**. It is to note that activities observed by the EO were bacteriostatic. The EO was completely inactive on *A. flavus*, while *A. niger* was susceptible at first reading after 48 hours. However, it was found that the spores germinated from the 15th to 20th day, resulting in the appearance of numerous fungal colonies. This result is similar to that observed by **Basilico and Basilico (1999)**, when studying the effects of Basil EO on *A. ochraceus*, they found that it was initially completely effective, but after 7 days, the EO became inactive and allowed the growth of mycelium. Oregano EO was fully effective until the 14th day, and then the inhibition of fungal growth has become partial after the 21st day. Similarly, the extract of oregano tested by **Koci Tanackov *et al.*, (2012)**, caused a lack or delay in germination of spores of *Aspergillus* species. The retarding effect on the EO on *A. niger* can be explained by the fact that this EO has shown fungistatic activity, which prevented spore germination. Beyond the 20^{th} day, the EO has evaporated due to its volatile nature, allowing the spores to germinate and give new mycelium. The antifungal activity of *Carum* EO was reported earlier by *C. carvi* and *C. copticum* **(Fang *et al.*, 2010; Rezaei Kahkha *et al.*, 2014)**. Growth dermatophytes and development of their spores are easily inhibited by the EO rich in phenylpropanoids and monocyclic sesquiterpene alcohols. The ME was less active than the EO and antibiotics. According to the literature, the crude extracts have in general a significant antimicrobial activity, for example, phenolic extract of *C. carvi* tested on *E. coli*, *B. cereus*, *S. aureus* and *S. typhimurium* and inhibited of bacterial growth (**Thippeswamy *et al.*, 2013**). Sites and number of hydroxyl groups on the phenol group can be linked to their toxicity to microorganisms. Some authors found that more phenols are oxidized more inhibitory activity increases. Mechanisms responsible for the toxicity to microorganisms of phenolic compounds include inhibition of enzymes **(Cowan, 1999).**

Phenolic compounds disrupt the cell membrane resulting in inhibition of the functional properties of the cell, and possibly cause leakage of the contents of the cell. Mechanisms of action may be related to the ability of phenolic compounds to modify cellular permeability, damage cell membranes, interfering with the production of cellular energy (ATP), and disrupting the driving force of protons. The disturbed permeability of the cytoplasmic membrane can result in cell death **(Calo *et al.*, 2015).**

Table 1. Inhibition diameters in mm of *C. montanum* extracts

Microbial strains	**10 µl EO in DMSO (50 %)**	**ME 70 mg/ml**	**T+**
Pseudomonas aeruginosa (ATCC 27853)	-	7 ± 00	08g
Escherichia coli (ATCC 25922)	08 ± 0.00	-	22g
Salmonella typhimurium (ATCC 13311)	11 ± 0.00	-	20g
Acinetobacter baumanii (ATCC 19606)	11 ± 0.00	-	20g
Staphylococcus aureus (ATCC 25923)	13 ± 0.00	-	31g
Klebsiella pneumoniae (ATCC 700603)	20 ± 0.00	-	25g
Bacillus cereus (ATCC 10876)	16 ± 0.00	7± 00	19g
Enterococcus faecalis (ATCC 49452)	12 ± 0.00	-	20g
Lysteria monocytogenes (ATCC 15313)	-	8 ± 00	19g
Citrobacter freundii (ATCC 8090)	-	7± 00	18g
Proteus mirabilis (ATCC 35659)	8 ± 2.8	-	26g
Aspergillus niger 2CA936	++	nt	10mcz
Aspergilus flavus NRRL 391	-	8 ± 00	30mcz
Candida albicans ATCC 1024	37 ± 3.35	-	26mcz

g :gentamicin, mcz : miconazol, EO : essential oil, ME : methanolic extract, nt : not tested, ++ : complete inhibition : - no activity.

III. 2.2. Determination of MICs

MICs of the EO were evaluated on the strains which gave inhibition diameters equal to or greater than 15mm; *K. pneumoniae* and *B. cereus*. The inhibition in agar occurred in the highest concentration tested (35.05 µg/ml). Indeed, the MIC value cannot be determined exactly because of the spaced concentrations. *B. cereus* was the most sensitive strain to the EO with MIC of 55.5 µg/ml broth micro-dilution method. Then *K. pneumoniae* (111 µg/ml). However, we can say that the MIC is in the range where the minimum value is greater than the highest concentration where there was the bacterial development and lower than or equal to the lowest concentration where there was a complete absence of bacterial growth. The observed differences in MICs values can be due to changes usually known and observed in these techniques, even using standardized methods the in difference species. By testing the sensitivity of several strains overlooked antibiotics, **Luber *et al.*, (2003)** concluded that the method of micro-dilution seems to be a simple and reliable method for the determination of MICs and it can offer an interesting alternative to the agar dilution method. It is also economic as well in material as extracts but delicate.

III. 2.3. Time kill assay

Bacterial growth susceptible strains exposed to EO tested is measured over a period of 24 hours. The bactericidal rate generated is determined by measuring the bacterial decay during the same period. Cell death curves obtained for EO concentrations equal to the MIC and the control populations' growth curve are shown in **Figure1**. Control strains have a regular growth curve with an exponential phase during the first 10 minutes. The stationary phase occurs in one hour for *B. cereus*.

For strains treated with equivalent MICs, their numbers fall continuously. However, the detection limit (10^2 CFU / ml) is not reached. First, the number of CFU slowly decreased; until the fifth hour, then the decrease continues until the 24^{th} hour. The effect of the EO on *K. pneumoniae* is not observed throughout the first 5 hours, this effect was observed after 15 hours where there was been a decrease in the number of CFU relative to the initial number.

It is noted that the activity of the EO endures over time. This activity slowly begins to the fifth hour, and then there is a rapid decline leading to low concentrations, without arriving at the total absence of viable forms of bacteria. This can be explained the bacteriostatic effect previously reported. Despite the continuous agitation of the reaction medium, there is always the problem of miscibility, which shares the middle giving rise to bacterial growth away from EO's micelles.

Indeed, the activity of EO differs depending on the growth stage; the action is lower during the stationary phase where bacteria are in a state of rest, in decline phase this activity increases. These results showed that the treatment time and the concentration of the EO had a large influence on the antibacterial effect.

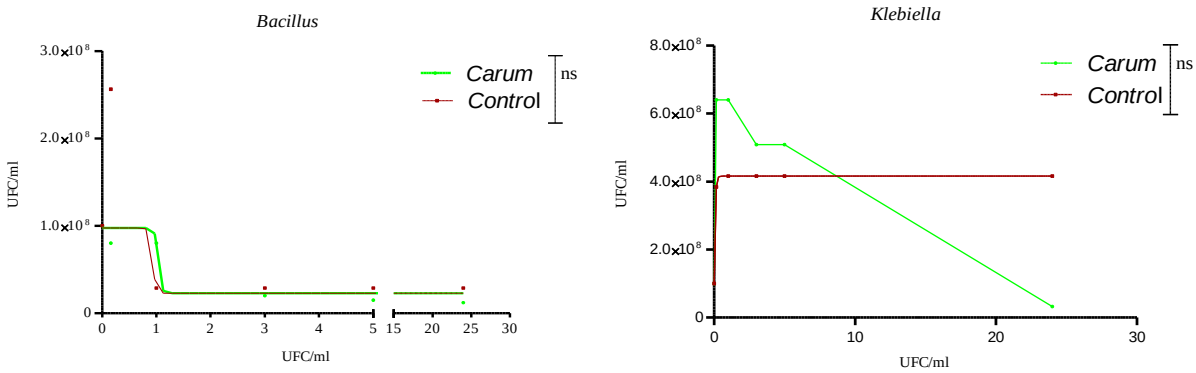

Figure 1: Time kill curves of *B. cereus* and *K. pneumoniae* exposed to *C. montanum* essential oil .
Values are the average of 3 measures ± SD.
Comparison was realized against the control; $p \leq 0.1$, ns: not significant.

III. 2.4. Lysis experience

This experience informs us if the EO acts on the cell membrane and induce lysis. This is a frequent mechanism caused by antimicrobial agents. The obtained results are represented by curves showing the relative percentage of the absorbance of the bacterial suspensions as a function of time (**Figure 2**). The decrease in absorbance is explained by cell lysis rate since only the live cells absorb at the wavelength of 620 nm, which is not the case for lysed cells. Absorbance measurements are performed for 2 hours at different intervals. Control strains represent an increase in absorbance reflecting the exponential phase. Then, an absorbance drop appears along the stationary phase. Each strain has a different absorbance after one and half hour of incubation, it depends on the life cycle of each strain. Upon exposure of the bacteria to the MIC, values of the relative absorbance was decreased to 100% at lower but different values; 66.7% (*B. cereus*) and 12.5% (*K. pneumoniae*). Incubation of strains with corresponding 4 times the MIC, gave no significant differences ($P \leq 0.01$) in percentages of absorbencies. Monoterpenes or sesquiterpen hydrocarbons and oxygenated derivatives exhibit potential antimicrobial activity (**Bajpai *et al.*, 2013**). Interactions with hydrophobic structures of the bacteria have a key role in the antimicrobial effect of these molecules (**Carson *et al.*, 2002**). The bacteria are less sensitive in the stationary phase than in exponential phase. As antimicrobial agents who act on the synthesis process often have small effects in the stationary phase, these results suggest that the main target of the EO is not the synthesis of macromolecules (**Carson *et al.*, 2002**). Regrowth observed with certain strains (*Bacillus*) may be due to the labile nature of the compounds of the EO (**Petersen et *al.*, 2007**).

The penetrations of active compounds in the plant cell membrane have a profound effect on the physical property of the phospholipid bilayer. This change could interfere with trans-membrane transport processes which result in changes in the secretion of proteins associated with bacterial virulence in the surrounding medium (**De Souza *et al.*, 2010**). Some antimicrobial agents cause large alterations in the plasma membrane causing complete lysis of the cell. Although self lytic enzyme activation may be responsible for this effect, lysis may also be due to the weakening of the cell wall and subsequent rupture of the cytoplasmic membrane due to the osmotic pressure (rather than a specific action on the membrane) (**Carson *et al.*, 2002**).

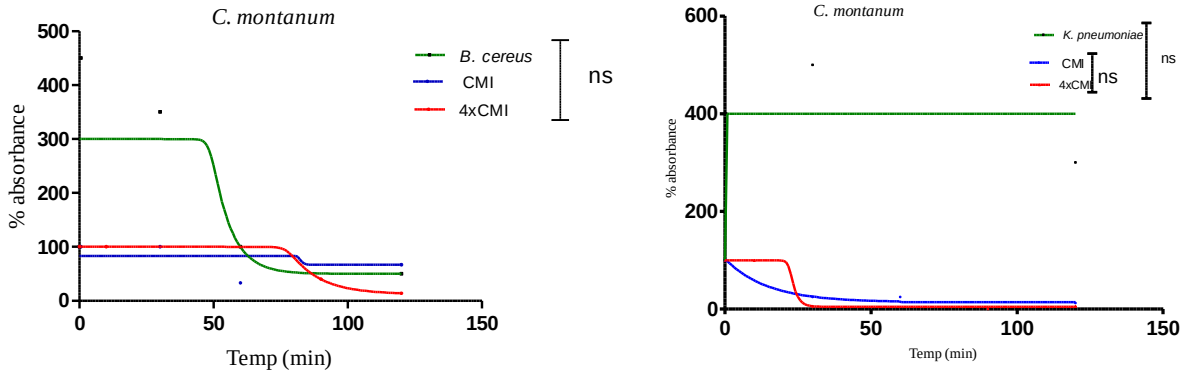

Figure 2: Curves of bacteriolysis of *B. cereus* and *K. pneumoniae* exposed to the essential oil of *C. montanum.* Comparison was realized against the control; $p \leq 0.01$, ns: not significant.

III. 3. Antioxidant activity

III. 3. 1. Scavenging ability on DPPH radicals

It is clear that the scavenging activity of the EO is dose-dependent (**Figure 3**). The IC_{50} value 1.09 ± 0.37 µg/ml showed that this EO was more active than the BHT (87.26 ± 0.001 µg/ml), that was the same with *C. bulbocastanum* **(Kapoor *et al.*, 2010)**. Despite these values, the difference is statistically not significant at $p \leq 0.05$**.** Many studies on the antioxidant activities of EOs of a wide variety of aromatic plants show that these properties are related to their chemical composition, it is difficult to attribute these activities to one compound but that is the result of a synergistic effect between the various compounds. Some EOs has the potential to preserve food. Oregano EO, rich in Thymol and Carvacrol, had a significant antioxidant effects on the oxidation process **(Kulisic *et al.*, 2004).** However, **Kapoor *et al.*, (2010)** attribute the activity of the EO of *C. bulbocastanum* to the Nothoapiole which is the main component in *C. montanum* EO **(Laouer *et al.*, 2009)**.

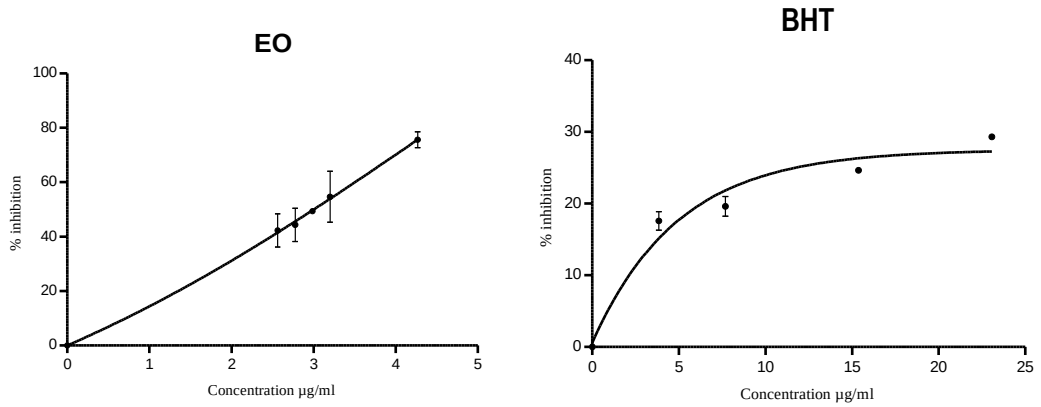

Figure 3: DPPH scavenging effect of *C. montanum* EO and this of BHT. Each value represents the mean ± SD (n= 3)

For the ME, a direct proportional relationship was found between concentrations and the antioxidant activity, which is also observed by standards (**Figure 4**). Unlike the EO, the IC_{50} value (65.04 ± 0.00 µg/ml) was higher than that of references (Quercetin; 2.56 ± 0.00, Rutin; 5.82 ± 0.00)**.** This value is very good compared to that obtained by **Thippeswamy *et al.*, (2013)** for the phenolic extract of *C. carvi* (IC_{50}: 2.7 mg/ml) and the ethanol extract of *C. nigrum* (IC_{50}: 14 mg/ml), where antioxidant activity was also dose-dependent **(Padmashree *et al.*, 2007, Fang *et al.*, 2010).** However, the ME of *C. carvi* from the Himalayas presented a higher activity than that of our extract with an IC_{50} of 6.9 µg/ml **(Xavier *et al.*, 2011).** The antioxidant effect is may be according to **Kulisic *et al.*, (2004)**. to the presence of hydroxyl groups in their chemical structures. Several non-volatile compounds such as Carnosol, Quercétine, Caffeic acid and Rosmarinic acid are well known to be good free radical scavengers.

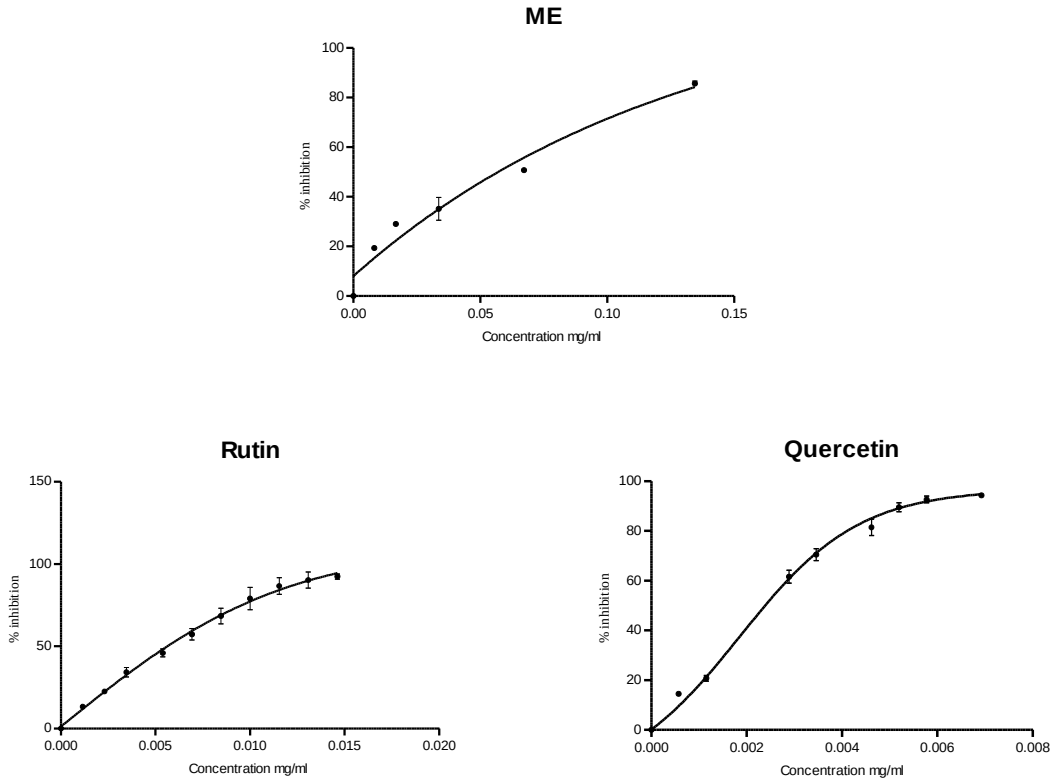

Figure 4: DPPH scavenging effect of *C. montanum* ME and this of standards. Each value represents the mean ± SD (n= 3).

III. 3.2. Reducing power

The reducing power of the EO **(Figure 5)** was in the range of 49.47 µg/ml to 84,57µg ml, it increased only slightly. However, the power of BHT is much higher but statistically not significant at $p \leq 0.05$. Other species tested of *Carum* have also presented interesting reducing powers; The EO of *C. nigrum*, *C. bulbocastanum* showed a very high reducing power than BHT **(Kapoor *et al.*, 2010; Singh *et al.*, 2006)**. Although we cannot attribute this activity to a specific substance, some are known to be antioxidant such as γ-Terpinene and Myrcene **(Duke, 2015).** Mostly, the antioxidant potential of the EOs may be due to the presence of various types of compounds. In addition, the antioxidant activity could be a result of synergistic effects of two or more compounds and most compounds natural antioxidants work in synergistic with each other to produce a wide range of antioxidant properties that create a defense effect system against free radicals **(Kapoor *et al.*, 2010)**.

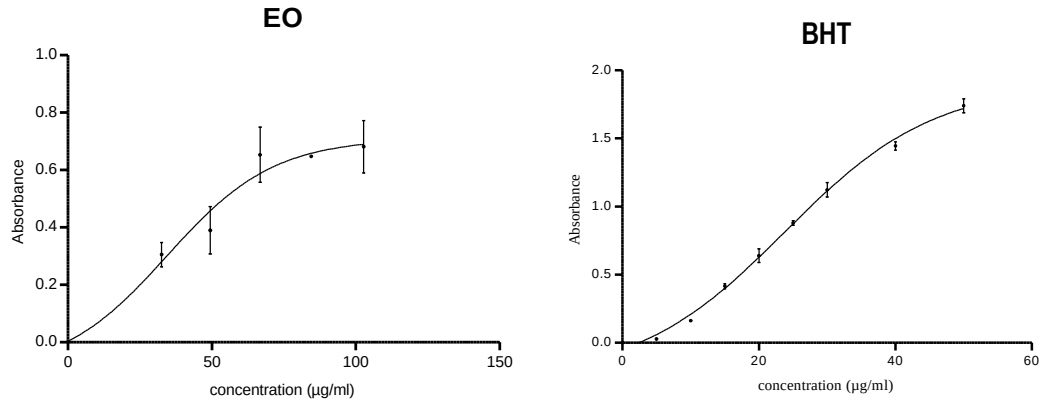

Figure 5: Reducing power of *C. montanum* EO and this of BHT. Each value represents the mean ± SD (n= 3).

Unlike the EO, the extract exhibited a most important reducing power than the standard (BHT) (the difference is statistically not significant at $p \leq 0.05$) **(Figure 6).** These results are in accordance with those of **Thippeswamy *et al.*, (2013)** where the phenolic extract of *C. carvi* showed a great power than the BHT. However, **Xavier *et al.* (2011)** found that the extract of *C. carvi* from the Himalayas was the less active among several species tested compared to ascorbic acid which used as a reference. The components with a reducing power are electron donors and can reduce the oxidized intermediates of the process of lipid peroxidation reaction, so they can act either as primary or secondary antioxidants **(Narasimhan *et al.*, 2013).**

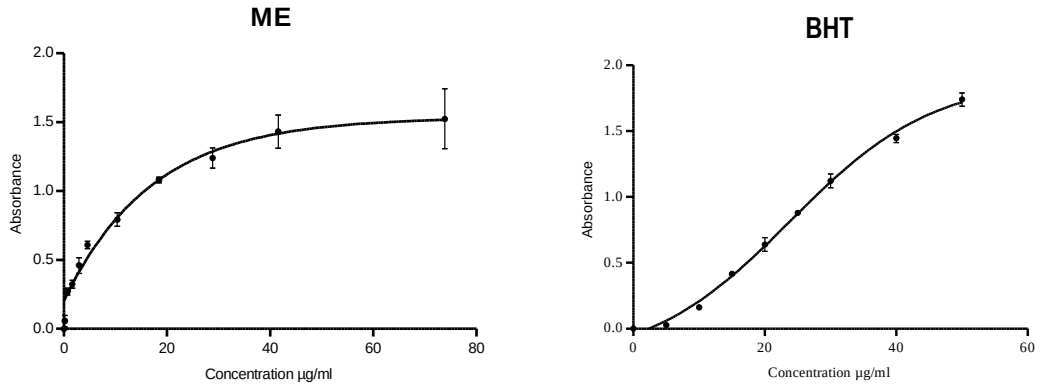

Figure 6: Reducing power of *C. montanum* ME and this of BHT. Each value represents the mean ± SD (n= 3).

IV. Conclusion

In conclusion, we can say that the essential oil of *C. montanum* and its methanolic extract exhibited very interesting antimicrobial activities with MICs which differ depending on the technique used. Also they showed antioxidant activities in the following order; EO > Quercetine > Rutine > ME > BHT. These activities can be used in food preservation after being tested for an eventual cytotoxicity.

Acknowledgements

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Conflicts of Interest

The authors declare no conflict of interest.

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