Molecules responsible for the antimicrobial effect of the essential oil of *Cuminum cyminum*

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Abstract

The extraction of the essential oil (EO) of cumin is performed by a technique of distillation (or drive) to the steam. The isolation of volatile substances in the oil is carried out by gas chromatography on a capillary column and the identification of major compounds is achieved mainly through further analysis of mass spectrometry. The antimicrobial activity of EO of cumin is very interesting, especially with *Staphylococcus aureus.* We also obtain varying diameters with other bacteria studied.

I. Introduction

Since immemorial time, man has used plants to alleviate and even cure certain troubles. In contemporary societies, this herbal medicine, known as traditional, is experiencing a resurgence of interest in allying with a new "green" consciousness.
The World Health Organization estimates that 80% of the world's population rely on traditional medicine (Fleurentin and Pelt, 1990).

This plant medicine relies on two approaches, plants" soft" and" hard" plants (Fleurentin and Pelt, 1990).

Soft plants that are devoid of toxicity and which, taken as extracts or infusion, relieve some discomfort with all of their components. Hard plants, in turn, contain an active chemical principle that we can isolate and synthesize if possible. This is the case of all alkaloid plants, generally toxic (eg plants of genus Taxus).

The EO extracted from plants are traditionally used for food preparation industries (lemon oil), cosmetics industries (pink, geranium, lavender), pharmaceutical and medicinal industries (eucalyptus, garlic). Homeopathy and aromatherapy are common examples of use of EO in alternative medicine, hence their popularity that has grown in a big way in recent years.

The EO is found in all plant parts (bark, roots, leaves, flowers and fruits) and in all climatic regions (Guenther, 1948). Environmental factors such as temperature, irradiance and photoperiod can play a vital role in the quality and quantity of the essential oil (Yamaura et al, 1989). Nutrients essential for plant growth rations, water, minerals and nitrogen also play on the chemical composition and quality of the HE (Rajeswar et al, 1990).
In general, as long as the appropriate chemical variety increases, the maximum yield of EO can be achieved by adjusting nutrients to optimize biomass production, harvesting of plant material that can coincide with the maximum concentration of a target compound in plant (Hay et al, 1993).

II. Materials

II.1 Biological materials

The strains used for this work are:
-Three strains of *Staphylococcus aureus, Echerechia coli, Klebsiella pneumoniae,*
-Yeast *Candida albicans*
- A fungus *Aspergillus Niger*

II.2 Culture media

-Midfield enrichment: nutrient medium Sabouraud and PDA
-Midfield interaction: Muller Hinton, PDA and Sabouraud

-Specific environments: Hecktoen, Mc conkey, Chapman, PDA and Sabouraud

II.3 Plant material

Cumin (*Cuminum cyminum*). This plant was chosen for the frequency of its use for culinary and therapeutic purposes.

III. Methods

The extraction method is steam distillation. It consists in boiling water and a mixture of seeds contained in a flask. The vapors are condensed in a condenser and collected in a flask. The key steps in our experiment are summarized in Figure 1.

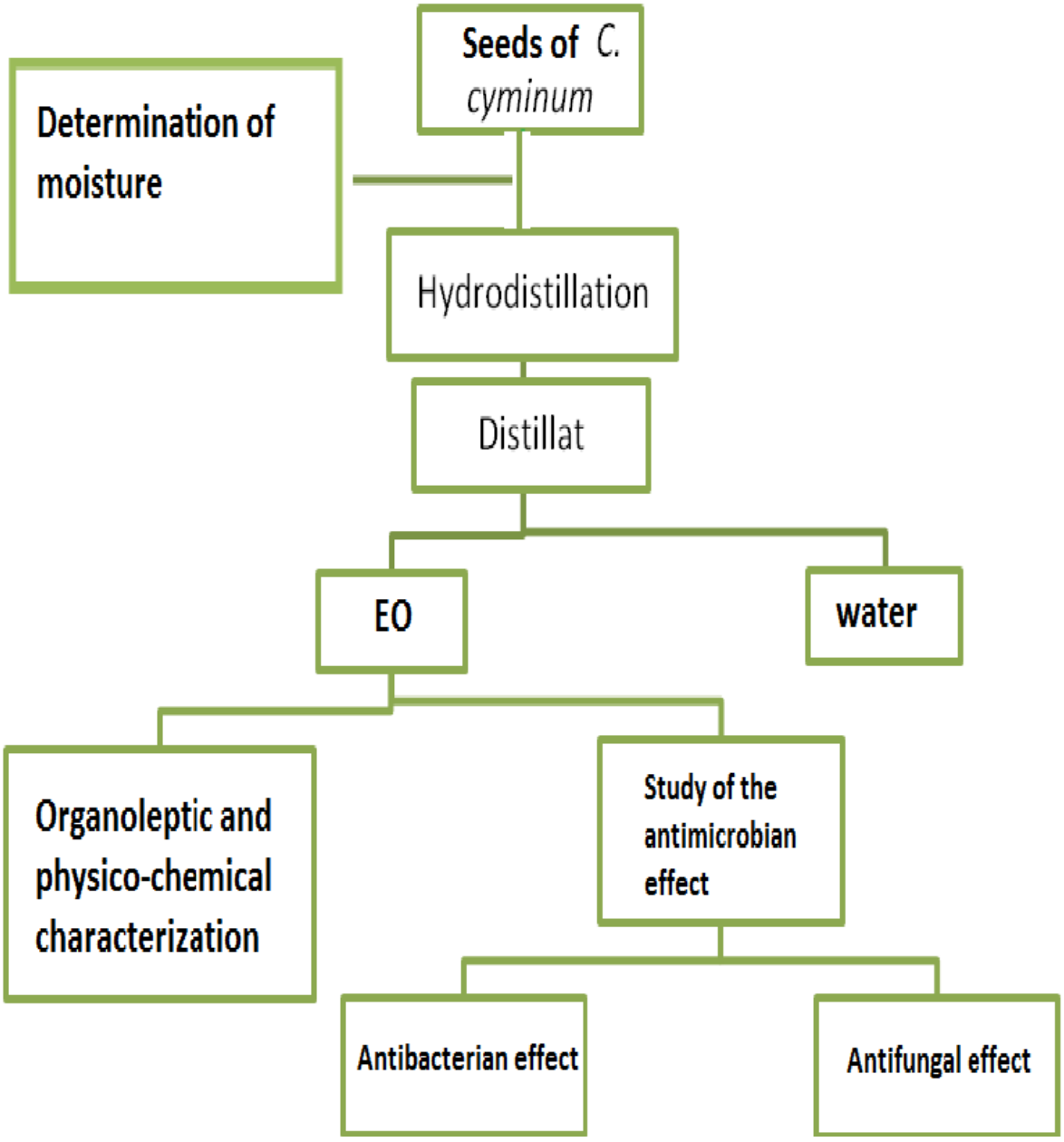

Figure 1: Diagram showing the main steps of the experimental protocol

III.1 Characterization of essential oils

To assess the quality and composition of the essential oils in this study, the analysis (shown schematically in Figure 2) were performed to determine the organoleptic and physico-chemical properties.

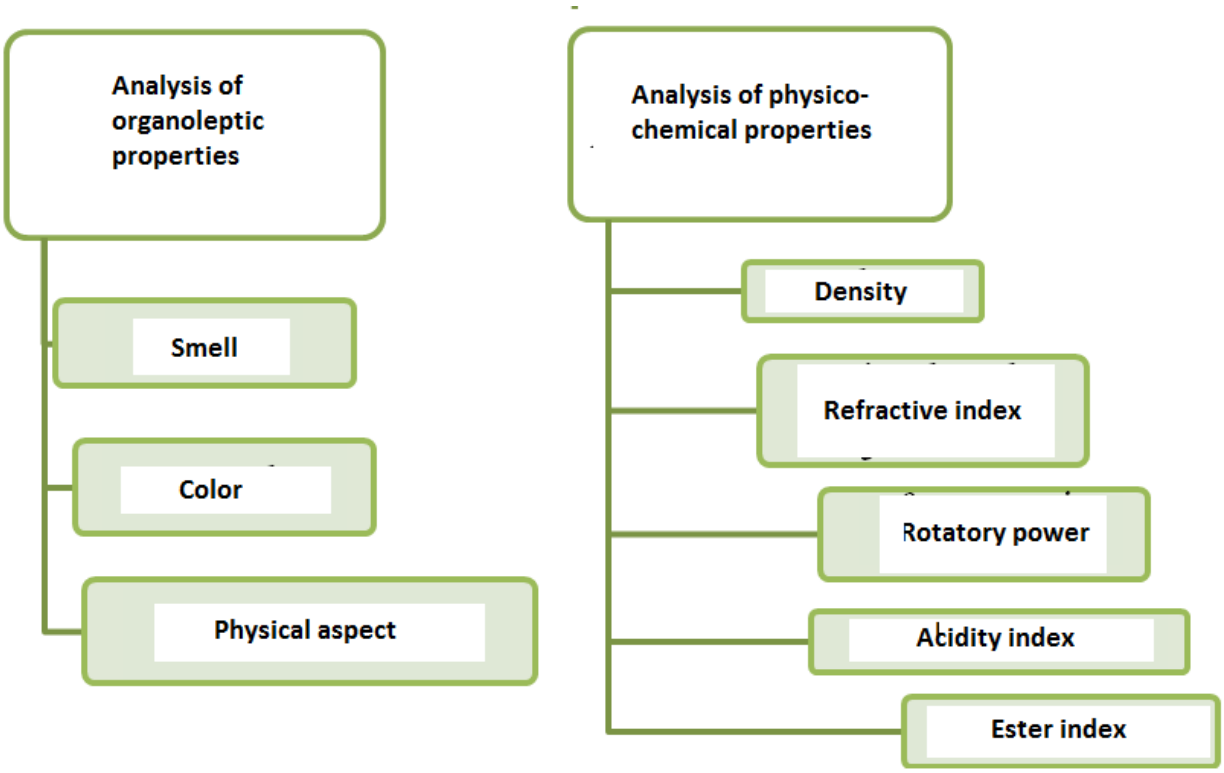

Figure 2: Organoleptic and physico-chemical analysis of the extracted EO

III.2 Chemical Analysis

In order to identify their active ingredients, the cumin seeds were studied by photochemistry which revealed that they are richer in secondary metabolits of which the content varies according to the geographical and climatic conditions and also on the research methods (extraction and detection).

Our essential oil was analyzed on a gas chromatograph Hewlett-Packard 6890N type Agilant controlled by ChemStation (NIST 98) and equipped with a capillary column (HP5MS 30m x 0.25 mm x 0.25 µm) coupled to a mass spectrometer (MS) type Agilant 5973. The analytical conditions are as follows:

- Injection of 0.5 gl mode split 1/50
- Injection temperature of 250°C
- HP5MS capillary column (30m x 0.25 mm x 0.25 µ m)
- Programming the temperature: 50°C for 0 min, 4°C/min to 250°C for 30 min.
- Flow of carrier gas: helium (1 ml / min), mass spectrometer model 5973 Agilant
- Temperatures: interface (280°C), strains (230°C), quadrupole (150°C).

Identification of components: The individual components of the essential oil were identified by comparing their mass spectra with those of the WILLET NIRST 98 databases, the mass spectrometer GC / MS and those of Adams spectral databases. The identification of molecules was confirmed by comparison of their retention indices with those known in the literature (Adams 2001). Retention indices of the compounds were calculated using the retention time of a series of n-alkanes using a linear interpolation.

III.3 Microbiological tests

An antibiograam test was performed for the bacterial strains and the antimicrobial power of essential oils was evaluated on various microorganisms. These are prepared before use.

Microbiological techniques

Strain sensitivity against antibiotics: The antibiotic selection was made according to availability, as well as their actions cited in literature. The antibiotics selected are:

- Nalidixic acid (NA 30) (Bioanalysis): 30 µg
- Co-trimoxazole (CO25) (Humedia): 25 µg

Study of the antimicrobial activity (bacteria and yeast)

In order to test the antimicrobial potency of EO, we have chosen the disk diffusion method (aromatogram) and the wells method.

Study of the antifungal activity and determination of the MIC

The method used is reported in REMMEL et *al.* (1993) et SATRANI et *al.* (2001). It allows for the study of the inhibitory effect of the EO against filamentous fungi. It also allows for the measurement of the minimal inhibitory concentrations against fungi (*A. niger* and *C. albicans*), and bacteria.

Reading the results: The MIC is defined as the lowest concentration where no growth is visible. For *Aspergillus niger*, the diameters of colonies were measured after incubation for each concentration. Moreover, we calculate the antifungal index (percentage of inhibition) which is determined by the formula:

Antifungal index = (Db - Da) / Db x 100 (Wang et al, 2005.) With:

→ Da: diameter of the growth zone of the test.

→ Db: Diameter of the growth area of the control.

For the others, the onset (+) or not (-) colonies (water) was noted.

IV. Results and discussions

IV.1 Physico-chemical properties

The physico-chemical properties of the EO of *C. cyminum* are listed in table 1, and compared to those given by the AFNOR database.

Table 1: Physico-chemical properties of the extracted EO

Properties	EO of *C. cyminum*	
	Results	AFNOR
Density	0,929	0,905-0,930
Refraction index	1,448	1,490-1,506
Rotatory power (°)	-0,75	+1 à +8
Acidity index (mg/g of EO)	1,68	/
Ester index (mg KOH/g HE)	12.34	/

IV.2 Results of the chemical analysis

Table 2: Main chemical components of the EO of cumin

Component	Retention time (sec)	(%)
P-xylen	6.27	0.31
Alpha-pinem	8.30	0.61

Beta-phellandren	9.70	1.29
Beta-pinem	10.08	6.55
Beta-myrcen	10.41	0.80
D-limonem	12.13	0.53
1,4-cyclohexadine	13.58	14.16
Alpha-tujenal	22.59	3.69
Caryophyllene	25.86	0.20
Duditetradecanone	32.77	0.13

The antimicrobial activity of EO's is based on their chemical composition (Oussalah et al, 2007). The essential oil of cumin is rich in hydrocarbons aldehydes terpéniquess (β-pinème and β-myrcene, cumin aldehyde, β-phellandrene, ...). According to Valnet (2005), the antimicrobial activity is related to its chemical composition and to the synergetic effects between its components.

According to Lahlou (2004), minority compounds act synergistically with the major compounds. For example, the β-pinem present in small quantities in the EO of cumin has antifungal properties (Hammer et al, 2003) and antiseptic effects on the oral bacterial flora (Chat et al, 2007) and on many gram + and gram (Martins et al, 2003).

The action of EO's is their ability to prevent the growth of bacteria, their sporulation and synthesis of their toxins. As for the yeast, they act on the biomass and production of the pseudo mycelium, sporulation and toxin production in molds (Oussalah et al, 2007; Caillet and Lacroix, 2008).

IV.3 Study of the antimicrobian effect of the extracted EO's.

The diameters of the inhibition of pathogenic strains obtained against the essential oil of *Cuminum cyminum*, after 24 hours of incubation at 37°C, by two methods: disks and wells are grouped in Table 3.

Table 3: Averages of inhibition zone diamters of bacterial strains of of *C. albicans* tested by two methods (disks and wells) against the EO of *C. cyminum*

Method	Test	Tested strains			
		E. coli	*K. pneumoniae*	*S. aureus*	*C. albicans*
Disk method	1st test			39	10.5
	2nd test			45	11.5
	3rd test			48	11.5
	Avergage	**26.66**	**19.66**	**44**	**11.16**
Well method	1st test	13	12	21	21
	2nd test	15	16	21	23
	3rd test	16	23	22	23
	Avergage	**14.66**	**17**	**21.33**	**22.33**

We note that with the disks method, *S. aureus* is the most sensible sensible among the bacterial strains, with a diameter of 44 mm, followed by *E. coli* and *K. pneumoniae* with averge diameters of 26,66 mm and 19.66 mm respectively.
Compared to bacterial strains, the yeast of, with a diameter of 11,16 mm is the most resistant pathogenic strainof the EO of *C. cyminum.*
On the contrary, by the wells method, *C. albicans* is the most sensible with a diameter of 22,33 mm. We note that the average inhibition zone diameters are reduced compared to those obtained by the disks method.. Their values are respectively 21,33 mm, 14,66 mm and 17 mm for *S. aureus*, *E. coli* and *K. pneumoniae*. Figure 3 shows the difference between the results of these two methods.

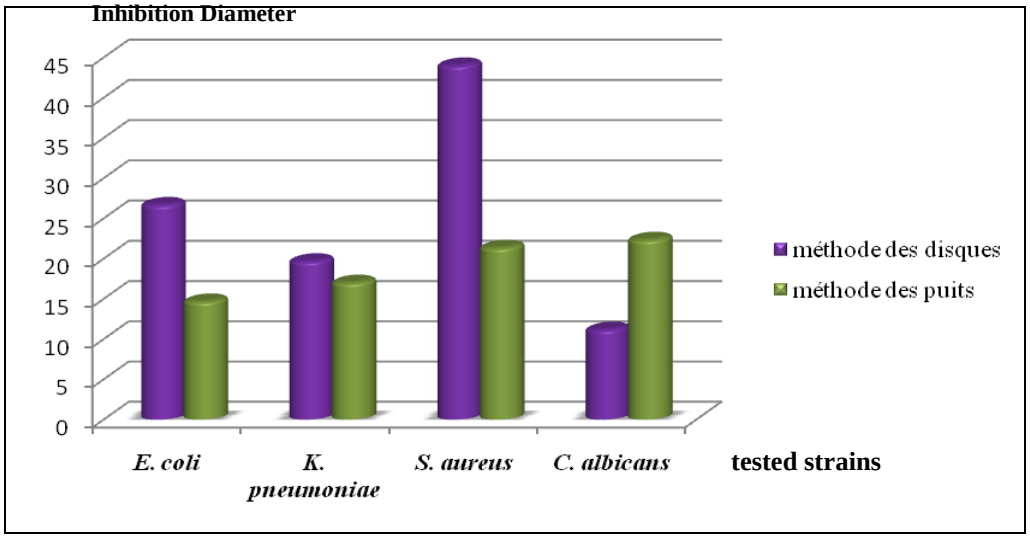

Figure 3: Average inhibition zone diameters of the bacterian strains of *C. albicans* tested by two methods (disks and wells) against the EO of *C. cyminum*

According to these results, we can safely say that the EO of *C. cyminum* has exerced an excellent antimicrobian activity, the diameters varying between 11,66 mm and 44 mm. In fact, this EO presents a more efficient effect on *S. aureus* which is a Gram + bacteria, than the two other bacteria which are Gram -. This is probably due to the difference between the structure of the bacterian membrane. LEE et *al.* (2003) confirm this hypothesis. They think that the presence of lipopolysaccharides in the membrane structure of negative gram bacteria hinder the penetration of essential oils.
The techniques for determining the antimicrobial power of EO's have a great influence on the results, which explains the existing differences between the results obtained by the two methods. According to FERHAT et al. (2009), the practical difficulties come from the insoluble constituents of EO in water and then the culture medium, their volatility and problems of standardization of methods. According to Thomson et al. (1996), our EO have bactericidal activity and yeasticidal (C. albicans), because even after a long incubation period, the growth of target strains was not resumed and the inhibition zones retained their diameters.
The mean diameters of the zones of inhibition of bacterial strains by EO (by both methods) and those with antibiotics tested representing a better action are compared in Table 4.

Table 4: Average nhibition zone diameters of EO and antibiotics against bacterian strains

Bacteria	**Average inhibition zone diameters (mm)**		
	Disks method	Wells method	Antibiotics

E. coli	26,66	14,66	25 (Co-trimoxazole)
S. aureus	44	21,33	14 (: Acide nalidixique)
K. pneumoniae	19,66	17	20 (Co-trimoxazole)

The diameters of the zones of inhibition obtained with the two essential oils by the method of disc, which is inspired by antibiogram, are more important than the best diameters obtained with the two antibiotics. However, they are less important by the wells method. This can be explained by the difference existing between the two methods, and issues related to the spread of oil in the mass media culture

After the study of the inhibitory power of the EO of *C. cyminum*, we can infer that it has a microbicidal effect (lethal) against the bacterial strains and the yeast *C. albicans*. The thresholds of efficiency in this case is essential

IV.4 Determination of the minimal inhibitory concentration (MIC)

The efficiency and minimum inhibitory concentration (MIC) threshold is defined as the lowest concentration of oil capable of inhibiting microbial growth (Oussalah et al., 2007). Because of irritability manifested by the pure and the high cost of extraction of EO's, a lower concentration avoids toxic effects and benefit from their antimicrobial effects and to save them.

Table 5: EO MOC

Dilutions / strains	witness	1/100 v/v	1/250 v/v	1/500 v/v	1/1000 v/v	1/2000 v/v	1/3000 v/v	1/5000 v/v
E. coli	+	-	-	-	-	+	+	+
S. aureus	+	-	-	-	-	-	-	-
K.pneumoniae	+	-	-	+	+	+	+	+
C. albicans	+	-	-	-	-	+	+	+

The essential oil of *C. cyminum* has a significant inhibitory activity against the bacterial strains and *C. albicans* (Table 5). Thus, it has been very effective against *S. aureus* even at a concentration of 1/5000. *E. coli* was inhibited at a concentration of 1/1000, and *K. pneumoniae* at 1/250. For *C. albicans*, the minimum inhibitory concentration of essential oils of *C. cyminum* is 1/1000. The determination of the minimum inhibitory concentration has allowed us not only to confirm, quantify and compare the activities, but also to characterize the nature of the effect revealed by the EO studied the microorganisms tested, which is a lethal (bactericidal and fungicidal) as we have already seen the two previous methods. According Chami et al. (2005), concentrations of EO for performing tests in vivo in animal models should be less than a 200 V / V concentration of 1 /200 (toxic). The highest inhibitory concentration recorded by our EO was 1/250 (below toxic concentrations).

IV.5 Antifungal effect (against *Aspergillus niger*) of the extracted EO's

The method used is that of microdilution (direct contact) which consists in aseptically adding a mixture of EO and agar solution at 0.2%, to obtain a stable and homogeneous dispersion of EO in the culture medium. The measurements of Proliferation diameters of colonies were made after 7 days of incubation at 25 °C. For each concentration, the antifungal index (percentage of inhibition) was calculated by the formula:

Antifungal index = (Db - Da) / Db x 100 (Wang et al, 2005).

→ Da: diameter of the growth zone of the test.

→ Db: Diameter of the growth area of the control.

The average proliferation diameters of colonies Niger (mm) for each dilution and the control, eliminating the disc diameter mycelial filed which is 6 mm, and the antifungal indices are reported in Tables 6 and 7 respectively.

Table 6: Proliferation diameters of colonies of *A. niger* (in contact with EO) (mm)

Dilutions / EO	1/100 v/v	1/250 v/v	1/500 v/v	1/1000 v/v	1/2000 v/v	1/3000 v/v	1/5000 v/v	**Witness**
C. cyminum	0	0	0	0	0	0	0	84

Table 7: Percentage of inhibition (%) of *A. niger* by the EO of *C. cyminum*

Dilutions / EO	1/100 v/v	1/250 v/v	1/500 v/v	1/1000 v/v	1/2000 v/v	1/3000 v/v	1/5000 v/v
C. cyminum	100	100	100	100	100	100	100

The essential oil of *C. cyminum* exerted an excellent antifungal activity against A. Niger, where there has been a total absence of mycelial growth, excluding the mycelial disk filed even at a concentration of 1/5000 v / v.

The calculation of the antifungal index by Wang formula, allowed us to assess the percentage of inhibition of *A. Niger* for the EO of cumin. The inhibition percentage of *A. Niger* by the EO of *C. cyminum* was very important for all dilutions. A concentration of 1/5000 has the same effect of fungicide concentration that 1/100, so all added to the first volume has no effect. The minimum inhibitory concentration of *A. Niger* is 1/5000 of the EO of *C. cyminum*. *A. Niger* is known as a widespread contaminant of foodstuffs (wheat, onion,....). During storage, it can produce mycotoxins (ochratoxin A) (ABARCA 1994; KLICH, 2002). Because of its significant inhibitory effect and its volatility, the EO of *C. cyminum* can be used as a preservative for the control of air hygiene in local storage of food, to fight against this organism, including economic damage and health are very serious. However, it is ranked among the promising EO because of its effect on fungi (Caillet and Lacroix, 2008).

The antimicrobial activity of EO's is based on their chemical composition (Oussalah et al., 2007). The biochemical composition of EO of *C. cyminum* reported in the literature (Gilly, 2005), and ISO 6465 and 7386, shows that:

The compounds are aldehydes (the cuminaldehyde (49%), 1,3-menthadiene-7-al (12.3%) and other aldehydes (16.4%)) and terpene hydrocarbons δ-terpinene (7 , 5 to 23%), β-pinene (3-19%), and p-cymene (12%))

Conclusions

Essential oils commonly known species are volatile odoriferous substances in plants. They are mixtures of more or fewer components which are at the origin of the biological properties of EO's. The study we conducted through the extraction and analysis of the essential oil of *Cuminum cyminum* has enabled us to establish a number of conclusions:

♠ The results obtained show that the extraction performance is affected by:

- The mass of seeds used;
- The shape of the seeds (crushed or not);

- The extraction time

The yield obtained was 5.19%, which gives the EO of interest for industrial exploitation. ♠ The examination of the organoleptic properties of the EO shows that it is relatively similar to those cited in the AFNOR standard, and physico-chemical examination shows that it is more or less stable.

As for the antibacterial and antifungal activity of the EO, the results show that it is microbicide. Indeed, the EO *C. cyminum* showed excellent activity against the Gram+ strains (area of 44mm in diameter) compared to Gram- (zones of 19.66 and 26.66 mm in diameter)

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