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ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF THE ESSENTIAL OIL OF CUMINUM CYMINUM SEEDS

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ABSTRACT

Cuminum cyminum (in arabic: Kemoun) is an important source of therapeutic, cosmetic, bio-food and technological agents. Ancient civilizations used this plant for therapeutic purposes. Extracts from this plant are nowadays strongly used in the industrial and research fields, particularly for the medicinal, pharmacological and cosmetological purposes. Bioactive molecules extracted from *Cuminum cyminum* may express biocidal activities and prove to be good candidates for new antioxidants. The objective of the present work is to evaluate the biological properties of this plant, including antibacterial and antioxidant effects. The seeds of cumin are harvested at the wilaya of Biskra, and stored in a dry place until their use. They were identified at Hassiba Benbouali University of Chlef. Essential oil is obtained by hydro-distillation using a Clivenger type device (AFNOR, 2000). The obtained sample is stored in sealed bottles at low temperature (4°C) and away from light. The antibacterial effect is assessed by the disc method. The minimum inhibitory concentration (MIC) is determined by standard methods. The antioxidant activity is evaluated by the DPPH free radical trapping method of the methanolic extracts. The essential oil of *C. cyminum* shows a better activity against Gram+ strains compared to Gram- strains. The determination of MICs leads to the conclusion that its activity can be triggered at a very low concentration. The reaction with DPPH gives an interesting IC50. This testifies to the ability of this essential oil to reduce free radicals. Thanks to the antibacterial activities that we have highlighted, cumin can be considered as a palliative that could replace certain antibiotics. Moreover, its antioxidant activity allows us to use it as a natural food additive.

Keywords: *Cuminum cyminum, antibacterial and antioxidant activities, Minimum inhibitory concentration, IC50.*

INTRODUCTION

Plants have therapeutic properties and valuable characteristics that have been passed down from generation to generation or are kept in old scrolls. The phytotherapeutic, antimicrobial and antioxidant properties are triggers for intense industrial and research activity (Mohammedi, 2006).

Cuminum cyminum (in Arabic: Kemoun) is an important source of therapeutic, cosmetic, bio-food and technological agents. Ethnopharmacological data show that ancient civilizations used this plant for therapeutic purposes. Extracts of this plant are nowadays widely used in the industrial, and research fields and, in particular, in the medicinal, pharmacological and cosmetological fields. Bioactive molecules extracted from *Cuminum cyminum* may express biocidal activities and are good candidates for new antioxidants (Gachkar et al., 2007).

The objective of this work is to assess the biological properties, in particular the antibacterial and antioxidant properties of the essential oil of this plant.

MATERIAL AND METHODS

Seed harvest. The seeds are collected from the wilaya of Biskra (south of Algeria), and stored in a dry place until they are used. They were identified at Hassiba Benbouali University in Chlef.

Obtaining essential oil. The choice of the process for obtaining essential oil (EO) is generally limited by the standards linked to its use. The technique of exploiting the plant material can have a noticeable influence on the final composition of the essential oil (Piochon, 2008). In our study, essential oil is obtained by hydro-distillation using a Clivenger type device (AFNOR, 2000). The obtained sample is stored in tightly closed bottles at low temperature (4° C) and protected from light.

Evaluation of the "in vitro" antibacterial effect. The disc method is used to examine the antibacterial effect, which is measured by the diameter of the area of inhibition (Piochon, 2008). The paper discs are impregnated with the substance to be tested, then they are placed on the surface of an agar uniformly seeded with suspensions of five frequent strains (*E. coli*, *K. pneumoniae, S. aureus*, *P. aeruginosa*, *S. typhi*), maintained by subculturing on nutritive agar. The bacteria inhibition phenomenon results in clear areas around the discs.

Evaluation of the minimum inhibitory concentration (MIC). The method described by Remmal et al. (1993) and Satrani et al. (2001) makes it possible to determine the minimum inhibitory concentrations (MIC) of essential oils. To induce an effective germ/compound contact, a 0.2% agar solution with the dilutions: 1/10, 1/25, 1/50, 1/100, 1/200, 1/300 and 1/500, is used and poured into test tubes each containing 9 ml of agar medium sterilized in an autoclave (20 min at 121° C) and cooled to 45° C. Then 1 ml of these dilutions is added in order to obtain the following concentrations: 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000, 1/5000 (v/v). Controls are prepared with the same agar solution alone. The seeding is done by streaks using a calibrated platinum handle. Incubation is carried out at a temperature of 37° C and away from any light source for 24 hours. In order to minimize the experimental error, each test is repeated three times.

Evaluation of the antioxidant effect of essential oils. The antioxidant activity is evaluated by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical trapping method of methanolic extracts (Brand-Williams et al., 1995). To the primary solution, 50 µl of each methanolic solution of the EO is added at different concentrations **(**from 1 to 5 mg/ml**)** and 5 ml of the methanolic solution of DPPH (0.004%). In parallel, a negative control is prepared by mixing 50 µl of methanol with 5 ml of the methanoic solution of DPPH. The positive controls are butyl-hydroxy-toluene (BHT) as well as ascorbic acid. The absorbance was read by spectrophotometry against a blank prepared for each concentration at 517 nm after 30 minutes of incubation in the dark and at room temperature. The antioxidant activity is evaluated according to the following equation:

$$\%\ \text{antioxidant activity} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{sample}}{\text{Abs}_{\text{control}}} \times 100$$

where Abs means Absorbance. By plotting the percentage of inhibition as a function of the concentration of essential oil, the inhibitory concentration of 50% of DPPH (or IC50) is calculated therefrom (Braca et al., 2001).

RESULTS AND DISCUSSION

Antibacterial effect. The appearance of zones of inhibition around the discs reflects the activity of the essential oil on the pathogenic strains tested. The diameters are variable depending on the strain tested (Remmal et al., 1993). Research of Oussalah et al., (2007) proved that essential oils have a specific and variable activity depending on the strain. Figure 1 shows the results of the activities of two antibiotics (nalidixic acid (NA) and co-trimoxazole (Co)) compared to those of the essential oil of *Cuminum cyminum*.

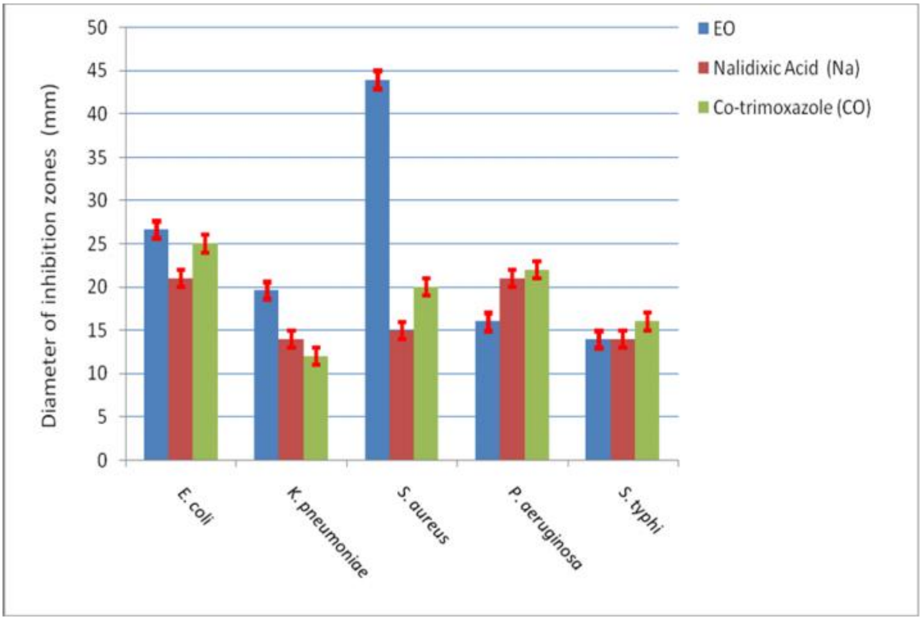

Figure 1. Average diameters of the inhibition zones of the bacterial strains with respect to the EO of *C. cyminum* and antibiotics.

Figure 2. Effect of the EO of *C. cyminum* on *S. aureus.*

Figure 3. Effect of the EO of *C. cyminum* on *E. coli.*

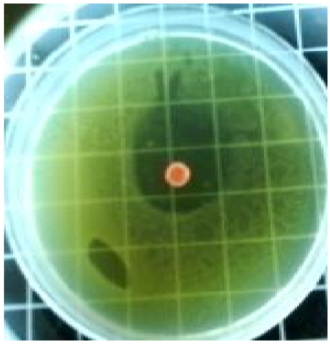

Figure 4. Effect of the EO of *C. cyminum* on *K. pneumoniae.*

Table 1. shows the different minimum inhibitory concentrations of *C. Cyminum* essential oil on the different strains tested.

Table 1. Minimum inhibitory concentration of the essential oil of *Cuminum cyminum*

Dilutions (v/v) Strains	Wiitness	1/100	1/250	1/500	1/1000	1/2000	1/3000	1/5000
E. coli	+	-	-	-	-	+	+	+
S. aureus	+	-	-	-	-	-	-	-
K. pneumoniae	+	-	-	+	+	+	+	+
P. aeruginosa	+	-	-	-	+	+	+	+
S. typhi	+	-	-	+	+	+	+	+

Growth +, Inhibition -, v /v: volume ratio of EO to agar solution.

Antioxidant effect. Table 2 presents the IC50 values of this essential oil compared to ascorbic acid and BHT.

Table 2. IC50 values for the essential oil of *Cuminum cyminum*, BHT and ascorbic acid.

Witness IC50 (mg/ml)		**Sample**
BHT	Ascorbic acid	EO of *Cuminum Cyminum*
0,788 ± 0.1	4,424 ± 0.1	0,318 ± 0.1

Figures 1 to 4 show that the essential oil of *C. cyminum* expressed a very good antibacterial activity, with diameters of the inhibition zone ranging from 11.66 to 44mm. Furthermore, it is observed that the inhibitory effect is greater on *S. aureus* (Gram +) than on the other two bacteria (Gram -).

According to Oussalah et al., (2007), the efficiency threshold or minimum inhibitory concentration (MIC) is defined as the lowest oil concentration capable of inhibiting microbial growth. Therefore, the possibility of using a lower concentration of essential oils is desirable not only to avoid possible toxic effects, but also to benefit from their antimicrobial effects while reducing operating costs since their extraction costs are relatively high.

The essential oil of *C. cyminum* expressed an important inhibitory activity against bacterial strains (Table 1). Thus, it has been shown to be very effective against *S. aureus* at a concentration of 1/5000. *E. coli* was inhibited at a concentration of 1/1000 and *K. pneumoniae* at 1/250.

Furthermore, it is observed that *S. aureus* (Figure 1 and Figure 2) is the most sensitive among the bacterial strains with an average diameter of 44mm, followed by *E. coli* (Figure 3) and *K. pneumoniae* (Figure 4) with average diameters of 26.66mm and 19.66mm respectively.

According to Chami, (2005), the highest inhibitory concentration recorded in vivo by the EO of cumin is 1/250 and is therefore much lower than the toxic concentration (1/200 v/v). According to Hammer, (1999) and Dorman et al., (2000), the membrane permeability of Gram + and Gram- bacteria is a determining factor which explains the action of these oils.

As for the antioxidant activity of the EO of cumin, it is expressed in IC50. This EO has a capacity to reduce the free radical. The concentration required for neutralization and stability of 50% of the concentration of DPPH is 0.318 g/l. From this result, it can be said that the essential oil of cumin has a very important antioxidant activity and superior to that of ascorbic acid and BHT (Table 2).

The comparison of the IC50 of the EO of *Cuminum Cyminum*, *Citrus ladaniferus* [948.06 µg/ml] and *Lavandulastoechas* [1852µg/ml]) (Mohammedi, 2006), showed that the former has a higher antioxidant activity than that of the other two plants. Gachkar et al. (2007), noted that the antioxidant activity differs depending on the test used. Rosemary essential oil is better than cumin in the DPPH free radical scan, while cumin is better when the β-carotene bleaching test is used. The antioxidant activity of EO is attributed to certain alcohols, ethers and ketones (Piochon, 2008). EOs which contain phenolic compounds have remarkable antioxidant properties (Brand-Williams et al. 1995).

Finally, it should be noted that there are several published studies indicating a relationship between the long-term intake of synthetic antioxidants and some health issues, such as skin allergies, gastrointestinal tract problems and in some cases increased the risk of cancer (Lourenço et al., 2019). Plants are potential sources of natural antioxidants such as ascorbic acid, tocopherol, carotenoids, flavonoids and phenolic acids (Hatami et al., 2014). This explains the enthusiasm of manufacturers to improve techniques for extracting essential oils from plants (Stratakos et al. 2016, Arslan et al. 2017, Zhenfeng et al. 2019).

CONCLUSIONS

The study that we conducted through the extraction and analysis of the essential oil from seeds of *Cuminum cyminum* allowed us to establish a certain number of conclusions:

* The essential oil of *C. cyminum* showed excellent activity with respect to Gram + strains compared to those with Gram-.

* The determination of MIC allowed us to deduce that the studied antibacterial activity of essential oil can be triggered at a very low concentration.

* The reaction with DPPH yielded an IC50 = 318.47 μg/ml. This essential oil has ability to reduce free radicals.

For this purpose, the essential oil extracted from cumin can be considered as a medicine (which can replace, at least partially, antibiotics) but must obey a strict medical prescription in order to benefit from its therapeutic contribution. It can be used as a preservative additive in foodstuffs without altering its organoleptic properties due to the low MICs. Another virtue, no less negligible, is their volatility which allows them, as sanitation and preservation of air quality products in the processing and storage of food products, to reduce the risk posed by chemicals commonly used.

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