



## GC-MS Characterization, Antioxidant, Antimicrobial and Insecticidal Potential of Moroccan *Cuminum Cuminum L.* Essential Oil

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### ABSTRACT

Human health has constantly been improved using medicinal plants. Notwithstanding synthetic chemistry advancements, medicinal plants remain widely used due to their effectiveness, reduced toxicity, and therapeutic use. This investigation examines the antioxidant, antimicrobial, and insecticidal properties of essential oil extracted from Moroccan cumin seeds (*Cuminum cyminum L.*). The essential oil was obtained through hydrodistillation from cumin seeds with a 3% yield. The EO was subjected to antioxidant, antimicrobial, and insecticidal activity screening using standard methods. The EO was investigated against a panel of pathogenic microorganisms using the disc diffusion method. The GC-MS analysis of the EO revealed the presence of 41 constituents. The major compounds identified were cuminaldehyde (21.94%),  $\gamma$ -terpene (18.20%), mentha-1,3-diene-7-al (14.93%), and  $\alpha$ -pinene (13.789%). The EO showed moderate antioxidant activity against DDPH free radicals with an IC<sub>50</sub> values of 273.409 mg/mL. The antibacterial screening results revealed that the EO of *Cuminum cyminum* possesses potent antibacterial activity against all tested bacterial strains, with MIC of 0.5  $\mu$ L/mL against *Acinetobacter baumannii* and 1  $\mu$ L/mL against *Proteus mirabilis*. The EO exhibited potent insecticidal activity against black bean aphids (*Aphis fabae* Scop.), with an 80% mortality rate. In conclusion, this study shows that *Cuminum cyminum L.* has interesting pharmacological effects and could be used to develop insecticidal and antimicrobial drug leads.

**Keywords:** *Cuminum cyminum L.*, essential oil, antioxidant activity, antibacterial effect, insecticidal activity, black bean aphid.

### Introduction

The misuse of antibiotics in an attempt to control bacterial infections has yielded several detrimental consequences. These include the emergence of bacterial resistance and adverse effects on human health, as highlighted by Jaber *et al.*<sup>1</sup> Additionally, the indiscriminate use of antibiotics has been associated with oxidative stress, a phenomenon that inflicts substantial cellular damage, accelerates the aging process, and contributes to the development of severe pathologies like cancer. Consequently, the current utilization of synthetic antioxidant compounds has come under scrutiny due to potential health risks.<sup>2</sup> In response to these concerns, scientific research endeavors have focused on identifying novel biomolecules possessing both antioxidant properties and antibacterial activity.

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Derivatives of these biomolecules, often found in extracts of medicinal and aromatic plants, are emerging as promising alternatives to synthetic antioxidants and antibiotics, as reported by Islam *et al.*<sup>3</sup> It is worth noting that the antimicrobial attributes of these plants have been recognized since ancient times. However, a comprehensive scientific investigation of their potential benefits did not commence until the early 20th century.<sup>4</sup> In the field of pest control, the use of pesticides, while intended to eliminate harmful organisms, presents a grave threat to human health. These chemicals have been classified as carcinogenic by the National Cancer Institute and are known to harm the nervous and endocrine systems.<sup>5</sup> Natural products of plant origin tend to degrade more rapidly in the environment, thereby reducing their harm to humans and animals and mitigating their adverse impact on the ecosystem.<sup>6</sup> Plants serve as a protective defense against the adverse effects of oxidative stress, which contribute to the development of severe medical conditions such as cancer, cardiovascular disease, diabetes, inflammation, and digestive disorders.<sup>7-11</sup> Furthermore, plants have been harnessed for centuries as natural remedies, providing a valuable source of bioactive compounds.<sup>12</sup> Among this diverse array of plants, *Cuminum cyminum L.* stands out. It is a small annual herbaceous plant belonging to the Apiaceae family (Umbelliferae). It is found in various ethno-medical systems across Northern Europe, the Mediterranean regions, Russia, Iran, Indonesia, and North America.<sup>13</sup> Moreover, the seeds of *Cuminum cyminum* have a rich history of use in traditional medicine, where they have been employed to alleviate conditions such

as flatulence, diarrhea, and various digestive ailments. Additionally, the species has demonstrated antimicrobial and insecticidal properties.<sup>14-16</sup> Research has shown that cumin seeds are used as a spice because of their unique aroma. They are widely used in traditional medicine to treat various health problems such as toothache, indigestion, diarrhea, and jaundice<sup>17,18</sup>. It is generally believed that these medicinal properties are due to the richness and powerful effects of active ingredients such as terpenes and polyphenols. There is a need to investigate the relationship between the chemical profile of cumin seed and its application in traditional medical practice and determine its antioxidant, antibacterial, and insecticidal potential to identify its bioactive principles.

## Materials and Methods

### Plant Collection and Identification

Cumin grains (*Cuminum cyminum L*) were harvested in May 2021 from the Alnif Region, a rural community situated in the province of Tinghir, Morocco (31° 06' 50.9" N 5° 09' 57.7" W). The plant was by Mr. Zidane Lahcen at Plant, Animal Productions and Agro-Industry Laboratory, Ibn Tofail University, Kenitra, Morocco.

### Preparation of plant material

All extraneous materials, including dust, straws, and stones, were manually removed from the harvested grains.

### Extraction of Essential Oils

The essential oil (EO) of cumin was obtained by hydrodistillation in a Clevenger apparatus as described by<sup>19</sup>. Distillation was done by boiling 250 g of ground grains with 1L water in a 2L flask for 4 hours. The resulting essential oil was carefully stored in an opaque glass container, maintained at a temperature of + 4 °C. The calculation of the essential oil yield percentage was conducted as follows:

$$\text{Yield \%} = (\text{Weight of EO in grams} / \text{Weight of dry matter in grams}) \times 100 \dots\dots\dots 1$$

### Chromatographic Analysis

The chromatographic analyses were conducted using CG/MS CLARUS 580SQ8S system from PERKIN ELMER. This system was equipped with a column measuring 40 meters long and 180 micrometers in diameter, utilizing Helium gas as the carrier. The injector was configured as a split-split injector and maintained at a temperature of 250 degrees Celsius. The temperature program for the chromatographic column commenced at 60 degrees Celsius and was gradually increased to 250 degrees Celsius over 10 minutes, with a heating rate of 4 degrees Celsius per minute. The identification of the chemical constituents present in the samples was accomplished by referencing the retention indices of the oil constituents and utilizing gas-phase chromatography coupled with mass spectrometry. The instrument was integrated with a computer system that managed a NIST mass spectrum library for precise compound identification.<sup>20</sup>

### Antioxidant Activity procedure

The assessment of the antioxidant activity in the EO was conducted via a methodology rooted in the reduction of 2,2-diphenyl-1-picrylhydrazyl (DPPH), as described by Haida and Kribii<sup>21</sup> and Zrouri<sup>22</sup>, with some modifications. Briefly, a solution of DPPH at a concentration of 76 µM (0.03 mg/mL) was prepared using ethanol. Subsequently, 0.1 mL of cumin essential oil (EO) of concentrations from 0-3 mg/mL, was mixed with 2 mL of DPPH solution. A solution of DPPH and methanol serves as blank control. These preparations were incubated in the dark for 30 minutes. The absorbance of the solutions was measured at 517 nm utilizing a conventional spectrophotometer (UV-2005, Selecta), with ascorbic acid serving as a reference standard for comparative purposes. The evaluation of antioxidant activity was derived from calculating the percentage inhibition of DPPH radicals, as shown in Equation 2.

$$\text{Inhibition (\%)} = \left( \frac{Ab - Ae}{Ab} \right) \times 100 \quad (2)$$

Ab: Absorbance of blank, Ae: Absorbance of the sample.

This assay was conducted in triplicate. The extract's half-maximal (IC<sub>50</sub>) concentration, i.e., the percentage of the antioxidant necessary to inhibit 50% of the DPPH free radicals, was computed from the linear regression curve (y = ax + b) plot shown in Equation 3.

$$IC_{50} = \left( \frac{50 - b}{a} \right) \times 100 \quad (3)$$

Where **a** is the slope of the regression line, and **b** signifies the y-intercept.

### Antibacterial Activity

The antimicrobial efficacy of cumin essential oil (EO) was determined by the disk diffusion method, as described.<sup>23-26</sup> This investigation utilized ten clinical isolates of pathogenic bacterial, namely Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*, *Proteus mirabilis*, *Acinetobacter baumannii*, *Salmonella sp.*, *Klebsiella pneumonia*, *Citrobacter freundii*, *Enterobacter cloacae* and *Pseudomonas aeruginosa*). In this assay, microbial suspensions of the bacteria strains were inoculated onto the Mueller Hinton (MH) agar medium surface in Petri dishes. Sterile discs, measuring 6 mm in diameter, were then impregnated with 10 µL of cumin EO and gently placed onto the surface of the MH agar medium. The Petri dishes were subsequently incubated at 37 degrees Celsius for 24 hours. This entire process was performed in triplicate. The EO antimicrobial activity was assessed by measuring the diameters of the inhibition zones (in millimeters) surrounding the impregnated discs. Cefazolin and penicillin were employed as standard control agents.

The Minimal Inhibitory Concentration (MIC) is the lowest concentration of essential oil (EO) at which no observable growth occurs compared to a control.<sup>1,27,28</sup> The MIC of the EO was determined by incorporating different concentrations (100 µL/mL, 40 µL/mL, 20 µL/mL, 10 µL/mL, 5 µL/mL, 3.3 µL/mL, and 2 µL/mL) of the oil in a 0.2% agar solution to ensure a uniform dispersion. Each dilution was introduced into test tubes containing 13.5 mL of Muller Hinton medium, which had been sterilized by autoclaving at 121°C for 20 minutes and cooled to 45°C. Following this, 1.5 mL of each dilution was added to achieve final concentrations of 10 µL/mL, 4 µL/mL, 2 µL/mL, 1 µL/mL, 0.5 µL/mL, 0.33 µL/mL, and 0.2 µL/mL, according to an established.<sup>29</sup> The medium was thoroughly mixed to ensure uniform dispersion of the EO in the growth medium, and each tube's contents were transferred separately to a sterile Petri dish. With a platinum handle, the test organisms were streaked on the plates and incubated at 37°C for 24 hours. Control samples were concurrently prepared, consisting of the culture medium and 0.2% agar solution. A triplicate determination was carried out.

### Insecticidal Activity

The protocol described by Rashedi *et al.* was adopted for this assay.<sup>30</sup> In this assay, Black aphids (*Aphis fabae*) were grown in a controlled laboratory environment with a temperature of 25.2°C, relative humidity of 75%, and a photoperiod of 14 hours of light and 10 hours of darkness in entomological cages. An emulsion of the EO was prepared by mixing a dispersion of the oil in water with 0.01% Triton X-100. Five concentrations (0.01%, 0.02%, 0.03%, 0.05%, and 0.10%) of the emulsified cumin essential oil were administered by direct contact using a 30 mL sprayer. For each concentration, five adult aphids were introduced into a glass petri dish equipped with Whatman No.1 filter paper and a fresh bean leaf for subsistence. Mortality assessments were performed at 1, 2, 3, 24, and 48 hours after contact by observation with a binocular microscope (Motic, Germany). Aphid's mortality was determined by gently probing each adult insect with a brush; those displaying immobility of their legs were classified as dead.<sup>31</sup> The observed mortality rate was adjusted using the Abbot formula, using a corrected method by Khandekar and Yadu<sup>32</sup>, as shown in Equation 4.

$$M(\%) = \left( \frac{Mi - Mt}{100 - Mt} \right) \times 100 \quad (4)$$

Where:

M % represents the percentage of mortality.

Mi signifies the observed mortality in the insect population.

Mt represents the observed mortality in the control group.

### Statistical Analysis

Statistical analysis of the data was done by the analysis of variance (ANOVA) with Excel (Microsoft Suite, USA). In some cases, results were presented as mean $\pm$ SD.

### Results and Discussion

The yield of *C. cyminum* essential oil (EO) was 3.5% based on the dry material weight. This result closely aligns with Petretto's findings<sup>33</sup>. Still, it is slightly lower than the yield reported for Indian cummin grains at 4.5%<sup>34</sup> and higher than that observed for Algerian EO (1.08%) reported by Ben Miri and Djenane.<sup>35</sup> These variations in yield underscore the influence of the cummin grain's origin and the prevailing climatic conditions. The analysis of *C. cyminum* EO using Gas Chromatography-Mass Spectrometry (GC-MS) revealed the presence of 41 constituents, representing approximately 98.04% of the plant's composition (Table 1, Figure 1). The major constituents in the EO from Alnif cummin include cuminaldehyde (21.94%),  $\gamma$ -terpinene (18.20%), mentha-1,3-dien-7-al (14.93%),  $\alpha$ -pinene (13.789%), and cymene (7.80%). These findings are consistent with some prior studies<sup>43</sup>, which identified cuminaldehyde (29.1%),  $\gamma$ -terpinene (10.0%),  $\alpha$ -pinene (7.1%), and p-cymene (6.1%) as primary components.<sup>40</sup> Variations in EO composition were observed in cummin from different sources, including Chinese cummin, where cuminaldehyde was the major constituent (36.6%), accompanied by  $\gamma$ -terpinene (11.14%), p-cymene (9.85%), and  $\alpha$ -pinene (7.75%), as reported.<sup>36,37</sup> Cuminaldehyde was also the predominant constituent in Algerian *C. cyminum* EO, constituting 65.98% of its composition.<sup>35</sup> These disparities underscore the impact of factors such as sample variation, geographical origin, soil conditions, climate, and extraction methodologies on the nature and content of essential oil compounds.<sup>26</sup> Figure 2 illustrates the variation in percentage inhibition as determined through the DPPH reduction assay. The figure shows that the inhibition is dose-dependent, indicating that the tested essential oil (EO) can counteract the aggressive DPPH radical as the concentration of the antioxidant increases. This activity is quantified by the IC<sub>50</sub> values, as presented in Table 2, where ascorbic acid exhibits robust antioxidant potency with an IC<sub>50</sub> of 0.090 mg/mL. In contrast, the EO derived from cummin records a moderate IC<sub>50</sub> value of 273.409 mg/mL. It is essential to note that a higher IC<sub>50</sub> value correlates with reduced antioxidant activity, while a lower value signifies potent antioxidant efficacy.<sup>38</sup> Notably, the EO of cummin demonstrates lower antioxidant activity than ascorbic acid and falls below the levels reported in other studies.<sup>39,40</sup> The disparity in antioxidant activity could be attributed to various factors, including genetic variances, harvest stage, geographical origin, and environmental conditions.<sup>41</sup> Furthermore, the chemical composition of cummin EO predominantly comprises monoterpenes, namely  $\gamma$ -terpinene and  $\alpha$ -pinene. Several research endeavors have suggested that higher monoterpenes content may be less effective in generating the desired antioxidant effects.<sup>34,42</sup>

The outcomes of the antibacterial assessment and antibiotic susceptibility determined through the disc diffusion method are presented in Table 3. The result showed that cummin essential oil exhibits antibacterial activity against all tested bacterial strains except *Pseudomonas aeruginosa*, which displayed resistance with an inhibition zone of up to 11 mm. Conversely, a significant portion of the bacterial strains in our study exhibited resistance to Cefazolin and penicillin. These results agree with the findings of Hajib *et al.*<sup>43</sup>, who also assessed the susceptibility of these strains. In contrast, the research conducted by Athamena *et al.*<sup>44</sup> indicated that *C. cyminum* EO exclusively inhibited *Staphylococcus aureus*. The results align with numerous studies demonstrating the antibacterial activity of *C. cyminum* EO against both Gram-positive and Gram-negative bacteria.<sup>34</sup> Table 4 illustrates the minimum inhibitory concentrations (MIC) ranging from 0.5  $\mu$ L/mL to 4  $\mu$ L/mL. Notably, a concentration as low as 0.5  $\mu$ L/mL is sufficient to impede the growth of *A. baumannii*. *Salmonella sp* exhibits the highest level of resistance, necessitating a concentration of 4  $\mu$ L/mL for inhibition.

**Table 1:** The Chemical Composition of *Cuminum cyminum* L Essential Oil

number	Compound	Retention time	%
1	$\alpha$ -Thujene	11.242	0.340
2	1-isopropylcyclohexene	11.38	0.058
3	D- $\alpha$ -Pinene	11.58	0.829
4	Sabinene	12.980	0.651
5	$\alpha$ -Pinene	13.234	13.789
6	$\alpha$ -Myrcene	13.370	0.675
7	$\alpha$ -Phellandrene	14.114	0.388
8	Carene	14.364	0.050
9	$\alpha$ - Terpinolen	14.551	0.129
10	Cymene	14.851	7.801
11	4-ethenyl-1,4-dimethylcyclohexene	15.025	0.284
12	$\alpha$ -Sabine	15.112	0.192
13	Eucalyptol	15.208	0.161
14	$\gamma$ - Terpinene	16.139	18.205
15	2-Carene	17.286	0.059
16	2-Pentadecen-4-yne, (Z)-	19.261	0.046
17	Trans-Pinocarveol	19.424	0.042
18	Cyclohexene, 1-butyl-	19.681	0,150
19	2-Isopropylidene-3-methylhexa-3,5-dienal	20.312	0,155
20	Terpinenol-4	20.749	0,289
21	Terpineol	21.182	0,146
22	3- Mentha-7-al	21.322	1.075
23	Cuminaldehyde	23.133	21,947
24	Menth - 2-in-7- ol,trans	23.874	0,051
25	Phellandral	24.431	0,201
26	Mentha-1,3-dien-7-al	24.768	14.931
28	Mentha-1.4-dien-7-al	24.915	14.149
29	1.4cyclohexanediol	25.452	0.043
30	4-(2-Methyl-3oxocyclohexyl)butanal	25.858	0.123
31	1.4 -Menthandien-7-ol	26.055	0.247
32	2 -Caren-10-al	26.636	0.067
33	Daucene	28.017	0.115
34	Caryophyllene	29.547	0.069
35	Trans- $\alpha$ - Bergamotene	29.697	0.042
36	Farnesene	30.044	0.145
37	Cis- $\beta$ -Copaene	31.209	0.052
38	3.11-Acoradian	31.285	0.172
39	Acorenol	32.142	0.059
40	Caryophyllene oxide	34.834	0.042
41	Caratol	35.184	0.217

**Table 2;** Comparative antioxidant activity results of cumin essential oil and ascorbic acid, quantified by IC<sub>50</sub> values derived from the DPPH radical scavenging assay

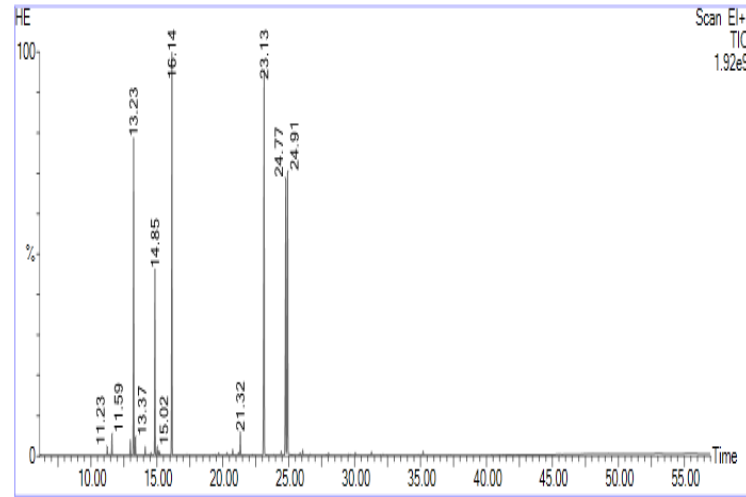
Assays	Cumin Essential oil	Ascorbic acid
DPPH (IC <sub>50</sub> mg/mL)	273.409 ± 10.735	0.090 ± 0.008

These findings underscore the pronounced antibacterial efficacy of cumin oil. Research on Cumin Essential Oil in Morocco has previously demonstrated its ability to inhibit the growth of *Salmonella sp* at low concentrations while failing to exhibit significant effects against the bacterium *Proteus mirabilis*, even at high concentrations, as reported by Hajib *et al.*<sup>43</sup> The essential oil derived from *C. cyminum* demonstrates a bactericidal effect, as evidenced by the minimal inhibitory concentration (MIC) being equivalent to the minimum bactericidal concentration (CMB), against a panel of bacterial strains including *S. aureus*, *S. epidermidis*, *E. cloacae*, *E. coli*, and *Salmonella sp* (Table 4). Conversely, with regards to *C. freundii*, *K. pneumoniae*, *P. mirabilis*, and *A. baumannii*, the EO of *C. cyminum* exerts a bacteriostatic influence. The observed potency of cumin EO can be attributed to its chemical compounds, notably  $\gamma$ -Terpinene, which acts synergistically with sabinene against pathogens, as reported in some studies.<sup>45,46</sup> Additionally,  $\alpha$ -pinene has been shown to induce inhibition of pathogenic bacteria by exerting toxic effects on cell membrane structure and function, thereby compromising cellular integrity and hindering both respiration and ion transport processes, ultimately leading to alterations in cellular permeability.<sup>47</sup> Furthermore, the antibacterial activity of  $\beta$ -pinene and cymene, recognized for their antibacterial efficacy, further contributes to the overall antimicrobial potency of cumin EO.<sup>34</sup>

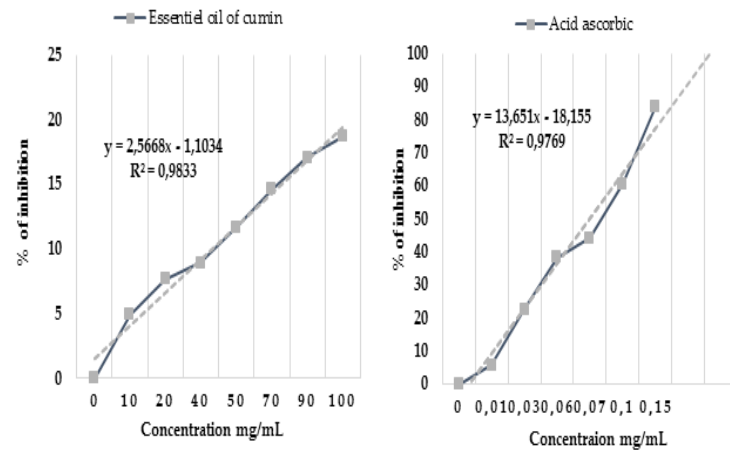
In the insecticidal activity screening, the adjusted mortality rates observed following the treatment of *Aphis fabae* populations with varying concentrations of *C. cyminum* essential oil over time and dosage are presented in Figure 3. The EO of *C. cyminum* exhibited a potent toxic effect on black aphids infesting bean plants. It induced mortality rates as high as 13% after just 2 hours of contact at a concentration of 0.1%, rising to an impressive 80% mortality within 48 hours of contact. The results indicate that Cumin Essential Oil achieves a 50% mortality rate against black aphids within 24 hours of contact, suggesting a positive correlation between repulsion percentage and time and dosage. Our findings align with the research conducted by Aouina in 2017, who reported an 84.17% repulsion rate, demonstrating the high toxicity of *C. cyminum* essential oil against pests.<sup>44</sup> It showed better insecticidal activity when compared to *Rosmarinus officinalis*, which exhibits relatively lower activity against *Aphis fabae*, with a mortality rate of 30% after 48 hours of initial treatment.<sup>48</sup> This elevated mortality rate can be attributed to the constituents of cumin essential oil, particularly  $\gamma$ -Terpinene and  $\alpha$ -Pinene, which significantly impact insects, as corroborated by some studies.<sup>49,50</sup> The substantial variations in repulsive activities can be attributed to multiple factors, encompassing the chemical composition of these essential oils, the part of our aromatic plant tested, oil extraction methods, the insect's sensitivity levels, application conditions, treatment procedures, as well as time, dosage, and the sampling season.<sup>51</sup>

## Conclusion

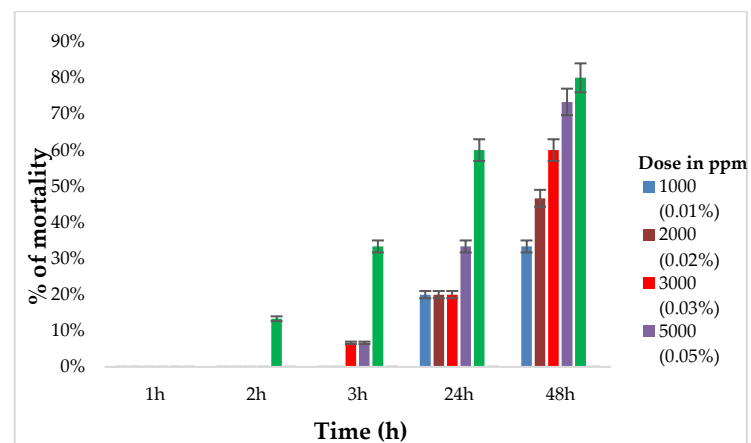
The EO of *C. cyminum* was characterised by GC-MS, which revealed the presence of some terpenes and other potent plant secondary metabolites with significant insecticidal, antibacterial, and antioxidant activities. This plant can be used to develop alternative drugs to treat infectious diseases caused by pathogenic organisms, as well as a bioagent for use as an insecticide against the black aphid and the food grains insects. There is, however, room for further studies to highlight the exact phytoconstituents in the plant implicated in the observed biological activity and its mechanism of action. This study highlights cumin essential oil's multiple applications in various fields, from food preservation to pest management and pharmaceuticals.



**Figure 1:** Chromatographic chromatogram of *Cuminum L* essential oil, analyzed by GC-MS



**Figure 2:** Linear representation of percent inhibition variation of essential oil relative to ascorbic acid in the DPPH radical scavenging assay.



**Figure 3:** Mortality rate following the essential oil treatment of *C. cyminum* depending on time and dose.

**Table 3:** The antibacterial activity of *Cuminum cyminum* L. essential oil by the diffusion method on disc.

Microorganisms	Inhibition diameter (mm)		
	Essential oil	Antibiotic	
	<i>Cuminum cyminum</i> L	Cefazolin	Penicillin
<b>Gram +</b>			
<i>Staphylococcus aureus</i>	08 ± 1.15	12 ± 0.00	00 ± 0.00
<i>Staphylococcus epidermidis</i>	08 ± 1.00	00 ± 0.00	00 ± 0.00
<b>Gram -</b>			
<i>Escherichia coli</i>	10 ± 1.05	00 ± 0.00	00 ± 0.00
<i>Acinetobacter baumannii</i>	11 ± 1.52	00 ± 0.00	00 ± 0.00
<i>Proteus mirabilis</i>	08 ± 0.57	00 ± 0.00	00 ± 0.00
<i>Salmonella sp</i>	10 ± 1.02	00 ± 0.00	00 ± 0.00
<i>Klebsiella pneumoniae</i>	08 ± 0.57	00 ± 0.00	00 ± 0.00
<i>Citrobacter freundii</i>	08 ± 1.15	00 ± 0.00	00 ± 0.00
<i>Enterobacter cloacae</i>	06 ± 1.00	00 ± 0.00	00 ± 0.00
<i>Pseudomonas aeruginosa</i>	00 ± 0.00	00 ± 0.00	00 ± 0.00

Diameter of the inhibition zone, including the disc diameter of 6 mm, by the agar disc diffusion method at a concentration of 15 µl of oil/disc and a concentration of 30 et 5 µg / disc of Cefazolin, respectively.

**Table 4:** *Cuminum cyminum* L. minimal inhibition concentration (MIC) and bactericidal concentration (MIB)

Microorganisms	Essential Oil	
	MIC	MIB
<b>Gram +</b>		
<i>Staphylococcus aureus</i>	4 µL/mL	4 µL/mL
<i>Staphylococcus epidermidis</i>	1 µL/mL	1 µL/mL
<b>Gram -</b>		
<i>Acinetobacter baumannii</i>	0.5 µL/mL	1 µL/mL
<i>Escherichia coli</i>	4 µL/mL	4 µL/mL
<i>Enterobacter cloacae</i>	4 µL/mL	4 µL/mL
<i>Proteus mirabilis</i>	1 µL/mL	10 µL/mL
<i>Klebsiella pneumoniae</i>	2 µL/mL	4 µL/mL
<i>Citrobacter freundii</i>	4 µL/mL	10 µL/mL
<i>Salmonella sp</i>	10 µL/mL	10 µL/mL

### Conflict of Interest

The authors declare no conflict of interest.

### Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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