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# ANTIMICROBIAL ACTIVITY OF SYMMETRICAL AZINES DERIVATIVES FROM ESSENTIAL OILS RICH IN ALDEHYDES

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**Keywords:**

*Ammodaucus leucotrichus; Cuminum cyminum L.; Essential oil; Azines; Antimicrobial activity*

**Abstract**

Symmetrical azines were prepared by the reaction of the hydrazine monohydrate with essential oils extracted from *Ammodaucus leucotrichus* and *Cuminum cyminum* L. containing respectively (perillaldehyde: 48.01%) and (cuminaldehyde: 21.60 %, phellandral: 0.10 % and 2-caren-10-al: 25.91 %). The conversion of aldehydes constituting essential oils was estimated by GC/MS, and their structures were confirmed by UV-vis, FTIR, and mass spectrometry analysis. The antimicrobial activity of azine derivatives from essential oils against three bacterial and one fungal strain showed an increase of inhibition zones and a decrease of minimal inhibition diameters (MIC) compared to native essential oils. The obtained azines characterized an important antifungal potential that can be isolated and extended, especially to other more resistant fungal strains.

## INTRODUCTION

The renewable natural products of plant origin were commonly used for the synthesis of new antibiotics and anticancer drugs [1-2] the main constituents of essential oils, which are originally mono or sesqui-terpenes and terpenoids; containing unsaturated levels and functional groups such as carbonyl function which can be used as raw material for the hemi-synthesis of bioactive molecules [3-5].

Symmetrical and unsymmetrical azines have received great attention due to their importance in organic and biological chemistry; they were usually synthesized by the reaction between two molecules of identical or different carbonyl compounds (ketones or aldehydes) and hydrazine [6-8]. These compounds constitute an important class of nitrogen products that have been widely used as starting materials in the synthesis of heterocyclic compounds. Azines, especially cyclic azines are known for their antimicrobial, antitumor activity and anticholinesterase activity [9, 8, 10]. They were also studied for their use as an antimalarial dye [11]. Recently, a new class of Triterpenicazines with selective cytotoxicity were synthesized [12]

In this context, the hemi-synthesis of some symmetrical azines from Algerian essential oils of *Cuminum cyminum* its richness in aldehydes knows L., especially cuminaldehyde [13], and that of *Ammodaucus leucotrichus* rich perillaldehyde [14, 15] was investigated. The chemical compositions of the native and modified essential oils were identified using the different spectroscopic methods, and their antimicrobial activity against bacterial and fungal strains was realized.

## MATERIALS

### Plant Material

The aerial parts of *Ammodaucus leucotrichus* (Hairy cumin) were harvested from Ghardaia (in the center of the northern part of the Algerian Sahara) in 2019 and *Cuminum cyminum* L. (Cumin seeds) were harvested in June 2018 from Djelfa (located in the highlands, 300 km south of Algiers), cumin seeds were finely ground into a powder with a Type A. 10 electric grinders (IKA-WERK) for use. The plant materials were identified at the botanical laboratory of the Department of Agronomy of Blida 1 University (Algeria).

**Microbial Strains**

Four bacterial strains, referenced as ATCC collected from the clinical bacteriology laboratory of the Franz-Fanon hospital in Blida (North, Algeria), were employed in the present study: *Staphylococcus aureus ATCC 25923* (Gram +), *Escherichia coli ATCC 25922* (Gram -), *Pseudomonas aeruginosa* ATCC *27812* (Gram -) and *Candida albicans ATCC 10231* (fungus).

**Chemicals**

Ethanol 98% and hydrazine monohydrate were purchased from Sigma-Aldrich.

**General Experimental Procedure**

The UV-vis spectra of the native and modified essential oils were obtained by Jasco UV-Vis apparatus after their solubilization in methanol. These essential oils were also analyzed by Bruker Fourier Transform Infra-Red (FTIR) spectrometer.

## METHODS

**Extraction of Essential Oils**

Essential oils of the ground cumin seeds and hairy cumin fruits were extracted by hydrodistillation with 200g of plant in 1L of water, using Clevenger apparatus for 3 hours. The obtained essential oils were dried over anhydrous sodium sulphate ($Na_2SO_4$) and stored at +4 °C.

**Azines Hemi-Synthesis**

Ten mmol of hydrazine monohydrate were drained to 20 ml of ethanolic *Ammodaucus leucotrichus* and *Cuminum cyminum* L. essential oils solutions (20 mmol of aldehyde calculated based on 48.01 and 47.61% mass, respectively), under constant agitation at ambient temperature for 2 hours. Once the reaction is complete, the solvent was evaporated using a rotary evaporator.

**GC/MS Analysis**

The constituents were identified by coupling a Bruker Scion SQ brand gas chromatograph equipped with a DB-5 type capillary column which has the following characteristics (length: 25 m, internal diameter: 0.220 mm, the thickness of film: 0.25 µm) to a single quadrupole mass spectrometer (SQ: single quadrupole). The temperature of the ion source is set at 280 ° C. The fragmentation was carried out in an electronic impact under a field of 70 eV, scanning 35-600 Da. The temperature of the column was programmed at 50 ° C. for 10 min after 50 at 250 ° C at a rate of 2 ° C / min then it is maintained at 250 ° C for 15 min. The temperature of the injector is set at 250 ° C. The injection mode is split (division ratio of 1: 100). The carrier gas flow (helium) is set at 1 mL/min. The volume of the injected sample is 0.2µL.

**Antimicrobial Assay**

The evaluation of the antibacterial activity of the native and modified essential oils was evaluated by two methods.

***Disc Diffusion Method***

This technique consisted of the disposal of sterile Wattman paper disks of 6 mm in diameter soaked with 15 µL of the products tested at the surface of the agar medium poured into Petri dishes and previously seeded by the germ tested. After incubation at 37 ° C for 24 to 48 hours, the inhibition diameters were measured in millimeters.

Negative control with 15µL of DMSO and a positive control using antibiotic disks of Cephalexin and Metronidazole was released.

***Determination of Minimum Inhibitory Concentration (MIC)***

The tested essential oils were placed in sterile flasks containing hot Mueller Hinton agar medium, supplemented with Tween 80 (0.01%, v / v); cascade dilutions were carried out to obtain a concentration range between 20 mg. $mL^{-1}$ and 0.3 mg. $mL^{-1}$.

The mixture was homogenized and then put into Petri dishes seeded by 13 µL of bacterial inoculums, which were then incubated at 37 ° C for 24 hours. The minimum inhibitory concentration values were deduced from the first Petri dishes of the range devoid of bacterial growth.

## RESULTS AND DISCUSSION

**Yields and Chemical Composition of Essential Oils**

The hydrodistillation of *Cuminum cyminum* L. and *Ammodaucus leucotrichus* areal parts gave yellow oil with a yield of 2.15% and blue oil with a yield of 2.52% respectively. The chromatographic analyses of essential oils (Figures 1 and 2) represented in Table 1 identified a total of 22 compounds, of which 4 were present in both oils.

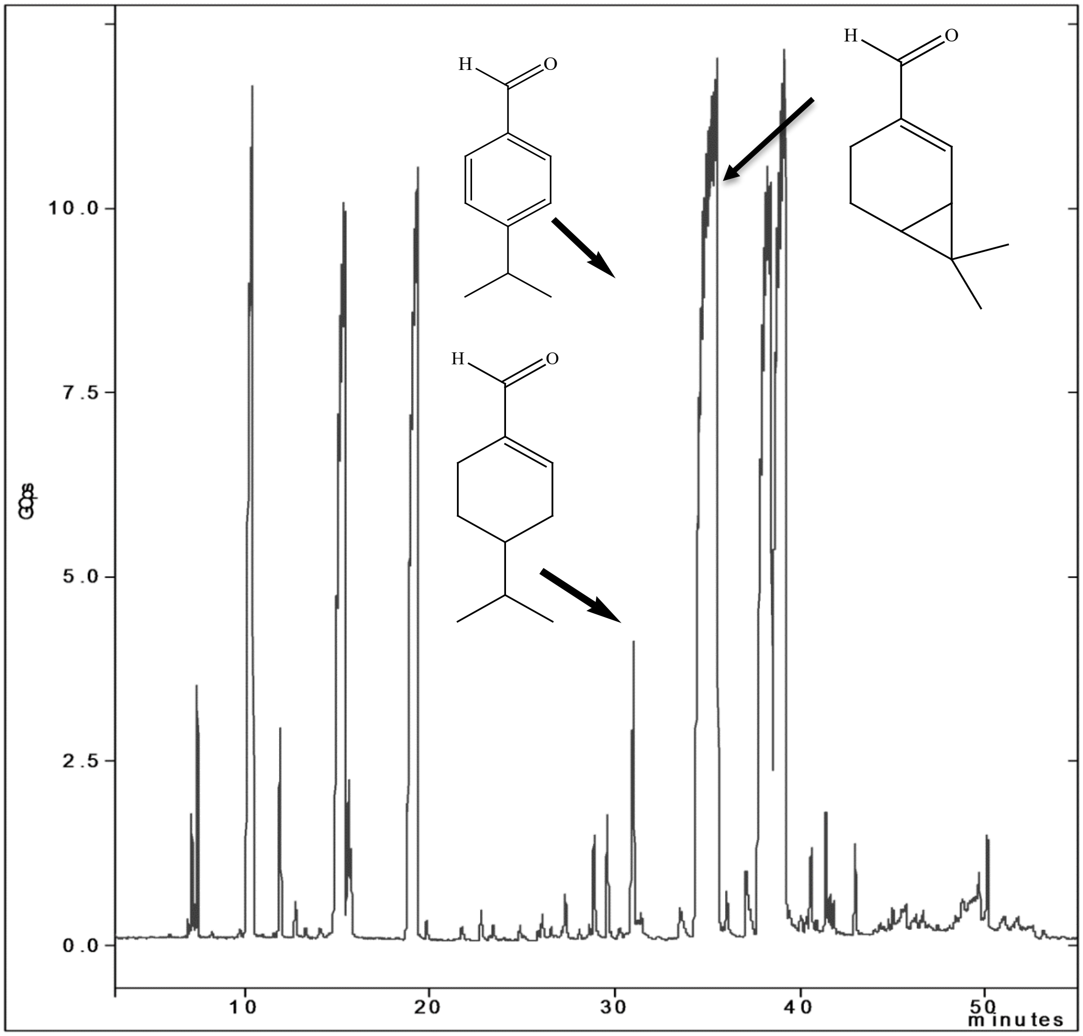


**Figure 1.** Chromatogram of the essential oil of *Cuminum cyminum* L.

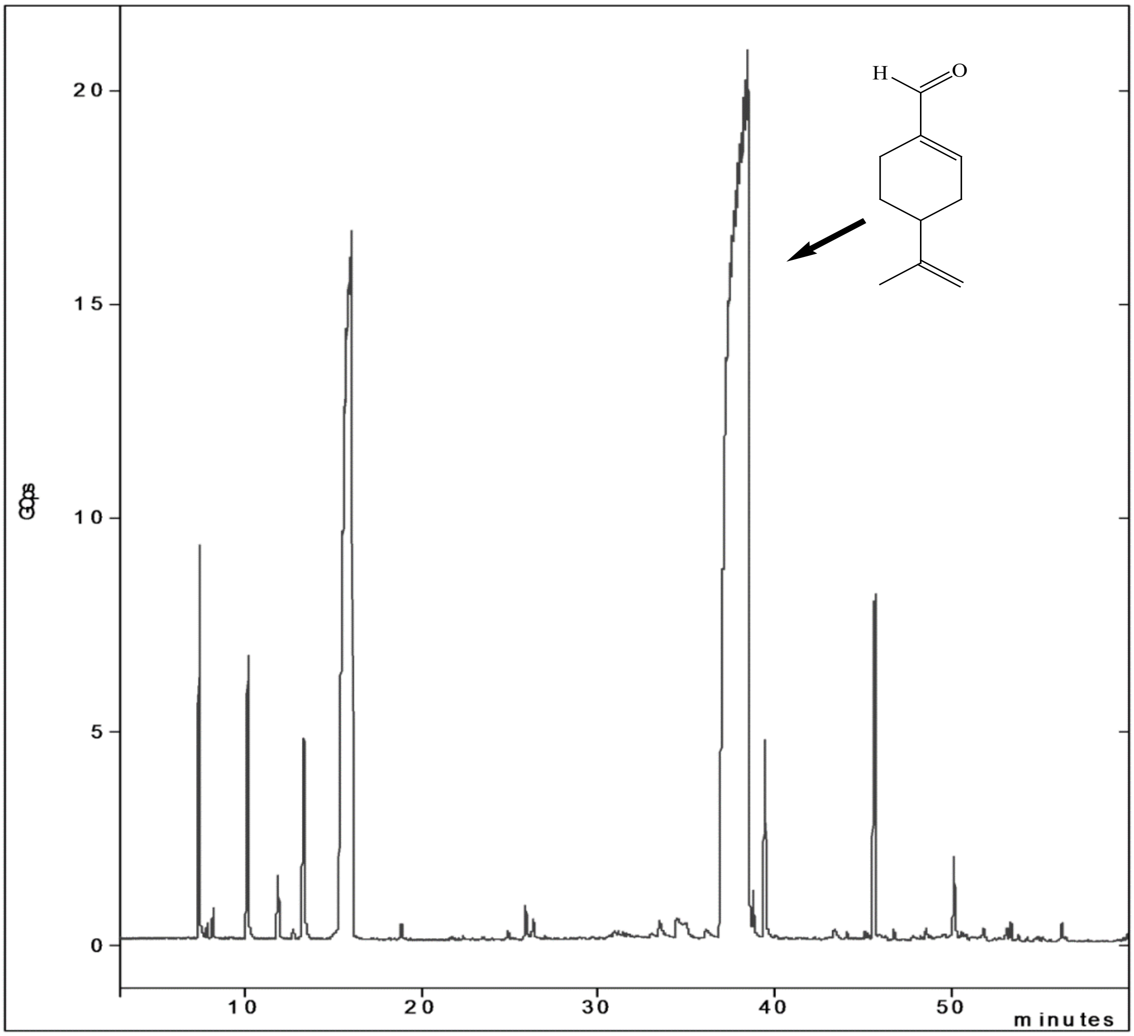


**Figure 2.** Chromatogram of the essential oil of *Ammodaucus leucotrichus*

**Table 1.**Chemical composition of essential oils of *Ammodaucus leucotrichus* and *Cuminum cyminum* L.

| Component | RT (min) | *A. leucotrichus* | *C. cyminum* L. |
|---|---|---|---|
| α-Thujene | 7.14 | - | 0.44 |
| α-Pinene | 7.48 | 5.96 | 1.06 |
| β- Pinene | 10.22 | 5.72 | 2.93 |
| Camphene | 11.94 | 1.48 | 1.31 |
| α-Phellandrene | 13.33 | 5.46 | - |
| p-Cymene | 15.41 | - | 6.48 |
| D-Limonene | 15.74 | 19.62 | 0.91 |
| γ- Terpinene | 19.00 | - | 7.88 |
| 4-Carene | 22.77 | - | 0.20 |
| cis-p-Mentha-1(7),8-dien-2-ol | 25.9 | 0.59 | - |
| trans-2,4-Nonadienal | 27.31 | - | 0.35 |
| (cis)-p-Mentha-2,8-diene-1-ol | 28.87 | - | 0.73 |
| Terpinene-4-ol | 29.58 | - | 0.86 |
| (cis)-p-Mentha-1,5-diene-1-ol | 31.01 | - | 2.67 |
| Cuminaldehyde | 34.48 | - | 21.60 |
| o-Cumenol | 36.03 | - | 0.26 |

| | | | |
|---|---|---|---|
| Phellandral | 37.04 | - | 0.10 |
| Perillaldehyde | 37.21 | 48.01 | - |
| 2-Caren-10-al | 38.54 | - | 25.91 |
| α-2-Propenyl-benzenemethanol | 39.14 | 4.75 | 26.32 |
| Perillalcool | 39.44 | 8.42 | - |
| Methyl perillate | 45.64 | | - |

The characterization of chemical classes of cumin seed and hairy cumin essential oils showed the prevalence of aldehydes (47.61-48.01 %); cuminaldehyde, 2-caren-10-al and phellandral were detected at high contents (21.60, 25.91 and 0.1 %) in the seeds cumin essential oil, followed by alcohols (30.84%). Perillaldehyde was detected as the principal constituent (48.01%) of the hairy cumin essential oil and terpenic hydrocarbons formed the second main class of this oil (38.24 %) represented mainly by D-limonene (19.62), α and β-pinene, camphene and α-phellandrene. Alcohols, ketones, and esters, formed the minor essential oil chemical classes.

These results are in agreement with those reported in the literature, where previous studies have found similar chemical compositions; cuminaldehyde has been detected as the main component of the essential oil of *Cuminum cyminum* L. from Tunisia (39.48 %) and China (36.3 %) [16, 17]; it has also been reported the predominance of perillaldehyde in the *Ammodaucus leucotrichus* essential oil originating from Algeria and Morocco with high contents (72-87%) [18,19].

**Hemi-synthesis and Identification of Hydrazones**

Essential oils of *Cuminum cyminum* L. and *Ammodaucus leucotrichus,* consisting mainly of aldehydes, were used as substrates to synthesize several symmetrical azine derivatives. The reaction of hydrazine monohydrate with aldehydes constituting the essential oils in ethanol forms hydrazones, aldehydes in excess was condensed with their hydrazones formed to obtain symmetrical azines. The reaction of aldehyde hydrazones with a second aldehyde molecule is faster than the reaction with hydrazine itself (Figure 3).

The conversion of aldehydes constituting essential oils to their analogs aldazine (Figure 4) was estimated by GC/MS, and their structures were confirmed by UV-vis, FTIR, and mass spectrometry analysis.

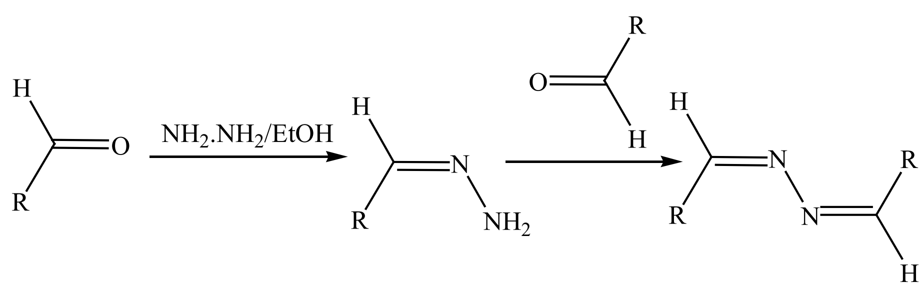


**Figure 3.** Formation of Azines by the Reaction of Hydrazine with aldehydes

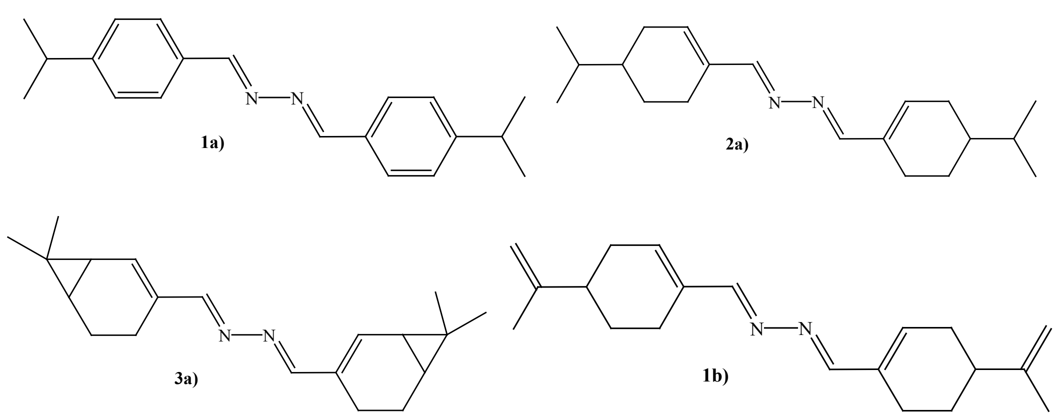


**Figure 4.** Chemical structure of the obtained symmetrical azines

The symmetrical azines molecules tend to have a large conjugated system; in this case, the delocalization of the π electrons will lower the energy and shift the spectrum to the long wavelengths, which explains the intense colors of the modified essential oils. The UV-visible spectra of both modified essential oils dissolved in methanol represented in Figure 5 showed that the symmetrical azines derivatives from the essential oil of cumin seeds revealed a band at 214 nm, a second one at 238 nm and a large band at 260-383 nm; on the other hand, perillaldazine, synthesized from the essential oil of hairy cumin is characterized by a large band at 224-289 nm.

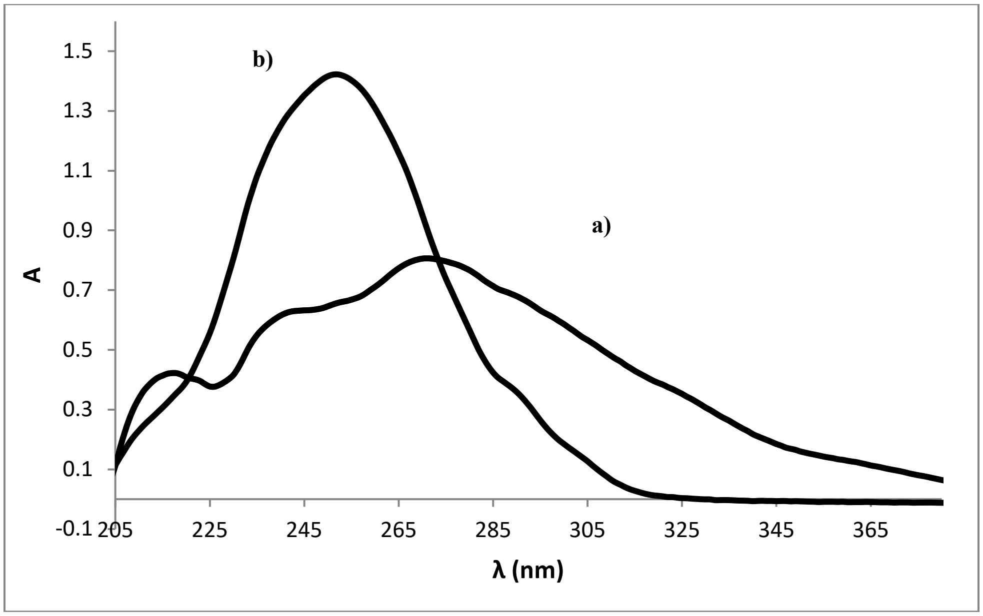


**Figure 5.** UV-visible spectra of azines obtained from essentials oil: **a)** *Cuminum cyminum* L. and **b)** *Ammodaucus leucotrichus*

An examination of the electronic absorption spectral data for Benzaldazine, Salicylaldazine, m-hydroxybenzaldazine, and p –hydroxybenzaldazine understudy in various solvents reveals that three band systems appearing at 200-225 nm, 289- 307 nm and 352-375 nm, influenced by both the position of the hydroxyl substituent in the benzene ring and the solvent [20].

The conversion of the aldehydes constituting the essential oils has led to the disappearance of the characteristic peak of the carbonyl function represented in the FTIR spectra of the cumin seeds and hairy cumin essential oils represented in Figure 6 (a and b) at 1671-1700 and 1682 $cm^{-1}$. The FTIR spectrum of the azines derivatives from the essential oil of cumin seeds, represented in Figure 6 is characterized by relative picks of (=$CH_{arom}$ and C=C) bonds at 3050 and 1611$cm^{-1}$ respectively, the appearance of the absorption band of the C=N group was observed at 11637 $cm^{-1}$; characteristic picks of $NH_2$ groups of remaining hydrazones were detected at 3379 and 3199 $cm^{-1}$. On the other hand, the characteristic picks of (=$CH_{arom}$ and C=C) bonds of the hemi-synthesized azines from the essential oil of hairy cumin were detected at 3079 and 1599$cm^{-1}$, the pick of the C=N group at 1643$cm^{-1}$and those of $NH_2$ at 3379 and 3193 $cm^{-1}$(Figure 6, c and d).

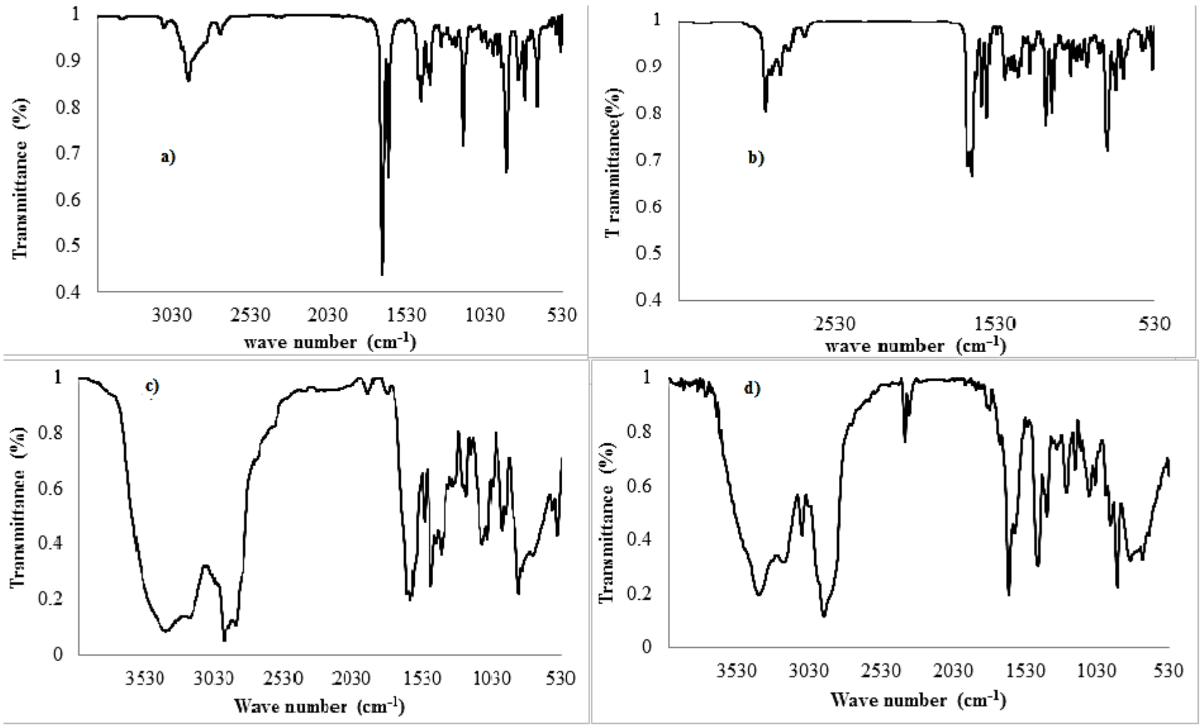


**Figure 6.** FTIR spectra of essentials oil: **a)** *Cuminum cyminum* L., **b)** *Ammodaucus leucotrichus*, azines obtained from essentials oil **c)** *Cuminum cyminum* L. and **d)** *Ammodaucus leucotrichus*.

These results were confirmed by GC/MS analysis, where a disappearance of the characteristic picks of aldehydes (cuminaldehyde, phellandral, and 2-caren-10-al) with the appearance of new peaks in the chromatograms of the modified cumin seed essential oil was observed at about 100-103 min (Figure 7), which means that aldehydes were almost totally converted to their symmetrical azines identified by their mass spectra; the remaining hydrazones were detected with low contents; on the other hand, the conversion of perillaldehyde to his azine analog was also confirmed by the disappearance of his characteristic pick and the appearance of new pick at 101.98 min (Figure 8) characterized by a molecular weight of 296 g; low contents of hydrazones that have not been reacted were also detected at 49.09 min. The conversion rate of aldehydes to azines and the exact contents of the obtained compounds are shown in Tables 2 and 3.

**Table 2.** Estimated conversions of aldehydes components of the essential oils

| Component | RT (min) | Total conversion (%) |
|---|---|---|
| Cuminaldehyde | 34.48 | 63.75 |
| Phellandral | 37.04 | 100 |
| Perillaldehyde | 37.21 | 89.04 |
| 2-Caren-10-al | 38.54 | 93.94 |

**Table 3.** Chemical composition of the modified (azines derivatives) essential oils of *Ammodaucus leucotrichus* and *Cuminum cyminum* L.

| Essential oil of *Cuminum cyminum* L. | | | |
|---|---|---|---|
| **Product** | **Molecular mass** | **RT (min)** | **Composition (%)** |
| Cuminaldehydehydrazone | 162 | 50.055 | 0.99 |
| 2-caren-10-alhydrazone | 164 | 47.185 | 3.60 |
| Cuminaldazine (1a) | 292 | 100.808 | 12.78 |
| 2-caren-10-alazine(3a) | 294 | 102.183 | 20.74 |
| Phellandralazine (2a) | 294 | 103.404 | 0.10 |
| Essential oil of *Ammodaucus leucotrichus* | | | |
| Perillaldehydehydrazone | 164 | 49.090 | 18.71 |
| Perillaldazine (1b) | 294 | 102.080 | 24.04 |

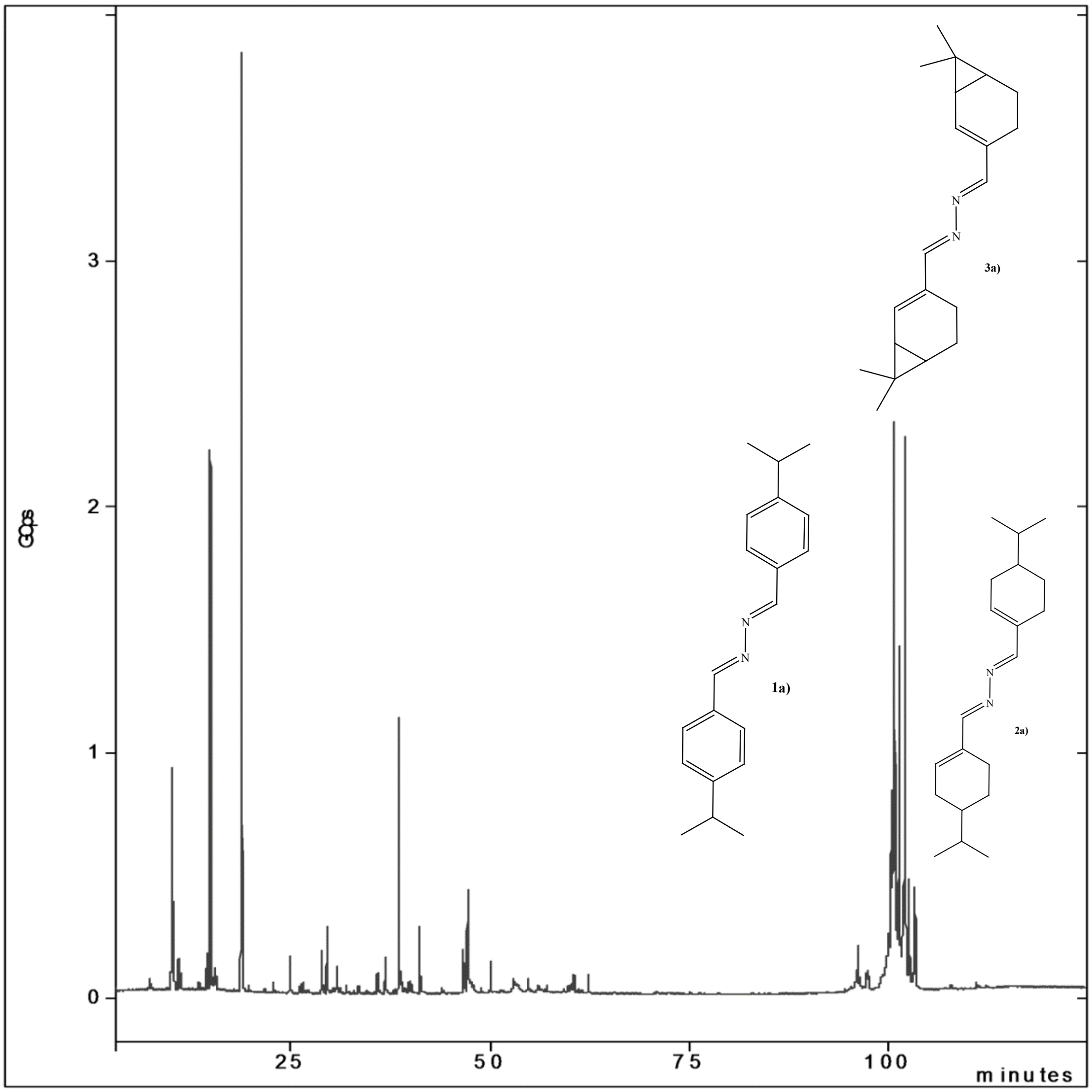


**Figure 7.** Chromatogram of azines obtained from the essential oil of *Cuminum cyminum* L.

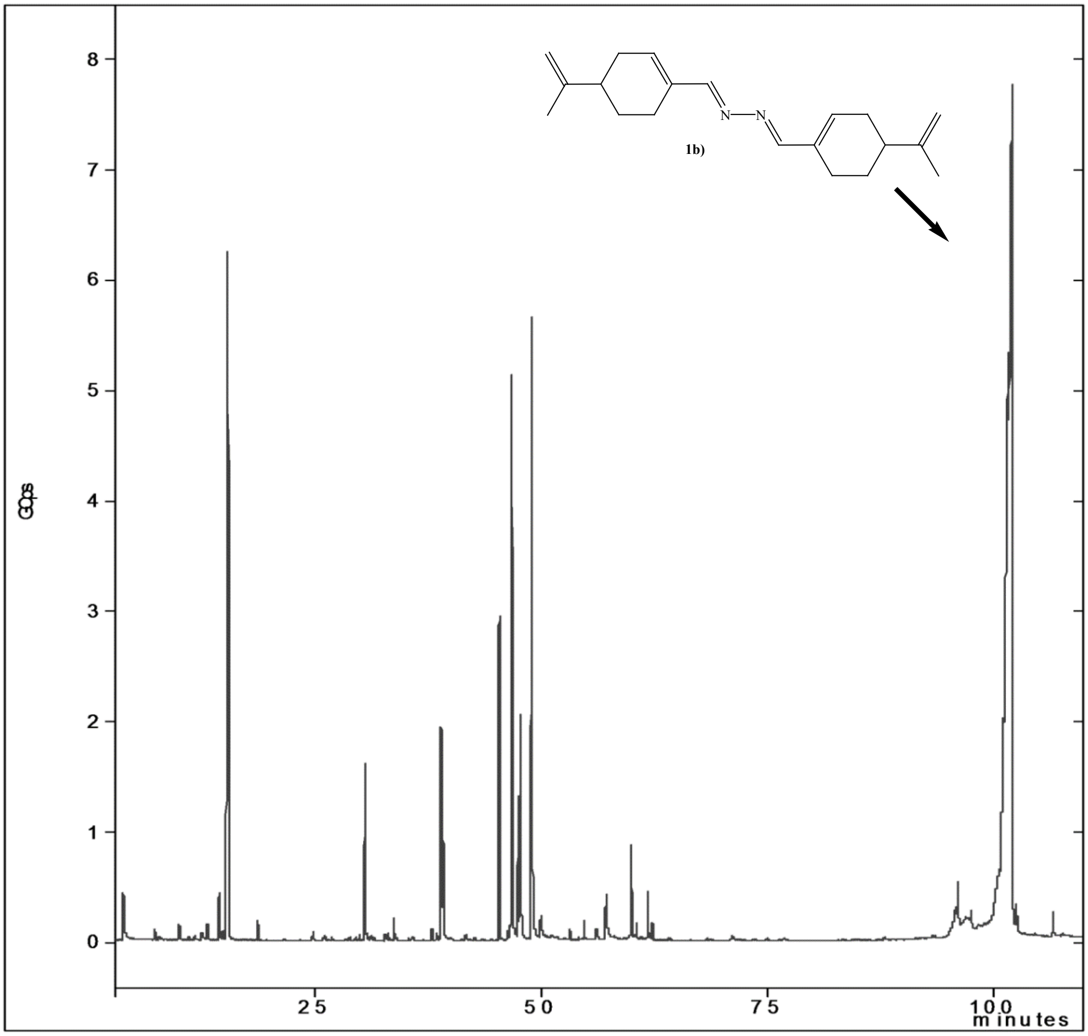


**Figure 8.** Chromatogram of azines obtained from the essential oil of *Ammodaucus leucotrichus*

**Antimicrobial Activity Assay**

The results of the antimicrobial activity of the *Cuminum cyminum.* and *Ammodaucus leucotrichus* L. essential oils pure and diluted in dimethylsulfoxid against tree microbial strains and one fungus reported in Table 4 show the efficiency of these oils as antibacterial agents against all tested bacterial strains, with moderate diameters of inhibition due to their richness in aldehydes known by their antibacterial activity [21, 22]. On the other hand, the fungi *Candida albicans* were resistant to both essential oils.

The conversion of aldehydes constituting these essential oils into their analogs azines has led to a considerable increase of the moderate inhibition zones and a decrease of the minimum inhibitory concentration values, especially against Gram-negative bacteria; where zones of inhibitions >40 mm were observed against all strains using pure oils; zones of (15-17 mm) using 50% of cumin seeds essential oil, and (13-29 mm) using 50% of hairy cumin essential oil was reported; these diameters were more important than those provided by Cephalexin and Metronidazole.

On the other hand, these modified essential oils provided an important antifungal activity against the fungi *Candida albicans* which was resistant against the native essential oils.

Several studies resonate with the importance of azine functional group (>C=N-N=C) in the antimicrobial compounds [23]. The antimicrobial activity of a series of symmetrical acyclic aromatic aldazines against 10 bacterial and 3 fungal species showed the efficiency of eleven compounds as an antibacterial agent. At the same time, only four bicyclic azines possessed significant antifungal activities [24].

**Table 4.** Results of the antimicrobial activity of the native and modified essential oils

| Essential oils | *Staphylococcus aureus* (G+) | | | *Escherichia coli* (G-) | | *Pseudomonas aeruginosa* (G-) | | *Candida albicans* | |
|---|---|---|---|---|---|---|---|---|---|
| | Conc. (%) | ID (mm) | MIC (%) | ID (mm) | MIC (%) | ID (mm) | MIC (%) | ID (mm) | MIC (%) |
| *Ammodaucus leucotrichus* | 100<br>50 | 25<br>13 | 13.30 | 20<br>19 | 5.00 | 25<br>13 | 9 | -<br>- | 13.00 |
| *Cuminum cyminum* L. | 100<br>50 | 35<br>12 | 14.00 | 20<br>15 | 5.00 | 35<br>12 | 10 | -<br>- | 10.00 |
| Azines derivatives | Conc. (%) | ID (mm) | MIC (%) | ID (mm) | MIC (%) | ID (mm) | MIC (%) | ID (mm) | MIC (%) |
| *Ammodaucus leucotrichus* | 100<br>50 | 33<br>17 | 10.6 | > 40<br>13 | 2.3 | > 40<br>29 | 6.3 | 30<br>17 | 15 |
| *Cuminum cyminum* L. | 100<br>50 | >40<br>19 | 8.30 | > 40<br>17 | 1.6 | > 40<br>15 | 5 | 30<br>12 | 3.30 |
| Cephalexin | | 09 | - | 11 | - | 16 | - | 15 | - |
| Metronidazole | | 12 | - | 11 | - | 07 | - | 09 | - |

Conc.: concentration (%), ID: inhibition zone diameter (mm) and MIC, minimum inhibitory concentration (%)

## CONCLUSION

A series of mixed symmetrical azines from essential oils of plants rich in cyclic aldehydes were synthesized and evaluated for their antimicrobial activity. The azines were obtained in good yields by a simple and rapid procedure from the reaction of hydrazine monohydrate with the essential oils of Cuminum cyminum. L and Ammodaucus leucotrichus, extracted by hydrodistillation and consisting mainly of (cuminaldehyde: 21.60%, phellandral: 0.1% and 2-caren-10-al: 25.91%) and (perillaldehyde: 48.01%). The resulting azine-rich essential oil caused a remarkable improvement of the native essential oils tested against three microbial and one fungal strain. It is desirable to extend the antimicrobial activity to other strains and mainly to resistant fungal strains in addition to the isolation and purification of these new azines.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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