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ORIGINAL RESEARCH PAPER

ANTIBACTERIAL ACTIVITY EVALUATION OF *ALLIUM SATIVUM* ESSENTIAL OIL COMPARED TO DIFFERENT *PSEUDOMONAS AERUGINOSA* STRAINS IN EASTERN ALGERIA

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Abstract: The chemical composition of essential oil extracted from *Allium sativum* bulbs harvested in the region of El Harrouch in Skikda was studied by gas chromatography coupled with mass spectrometry; six compounds were identified which predominant materials are: methyl allyl trisulfide (34.61%) and diallyl disulfide (31.65%). The antibacterial activity was tested on 52 clinical strains and one reference strain of *Pseudomonas aeruginosa* by both variants of the dilution method, the dilution method in liquid medium and the dilution method in solid medium. The results showed that the essential oil of *Allium sativum* has an inhibitory activity of growth compared to over 50% of strains tested with MICs relatively averages between 32 and 128 $\mu g \cdot mL^{-1}$.

Keywords: *antibacterial activity, Allium Sativum, essential oil, medicinal plants, Pseudomonas aeruginosa*

INTRODUCTION

The treatment of bacterial infections is based primarily on the use of antibiotics, including widespread prescription and/or sometimes inappropriate for these antibacterial agents can result in the selection of multiresistant bacterial strains from which the importance of directing research towards medicinal plants as a source of new molecules with antibacterial activity to limit the emergence of the phenomenon of multidrug resistance [1, 2].
Medicinal plants are able to naturally produce biologically active metabolites. Indeed, next to the primary metabolites, medicinal aromatic plants accumulate secondary metabolites known among them, essential oils which are volatile substances with characteristic odor, is beginning to have much interest as a potential source of natural bio-molecules active in particular treatment of infectious diseases [2, 3].
In recent decades, researchers have published over 2000 scientific works [4] on the therapeutic potential of garlic (*Allium sativum*), one of the most used plants in traditional medicine and the wider cited in the literature for its medicinal properties. The essential oil of *Allium sativum* extract is best known primarily for its antibacterial activity which is attributed to particular compounds that contain sulfides [5, 6].
In order to enhance the aromatic plant *Allium sativum* in our country we considered important to study in the context of this work the chemical composition of essential oil of this plant and its antibacterial activity compared to different strains of *Pseudomonas aeruginosa*; a bacterium that often causes problems for its intrinsic resistance to several antibacterial agents and its capacity to acquire resistance during antibiotic therapy.

MATERIALS AND METHODS

Extraction and chemical analysis of the essential oil of *Allium sativum*

Extraction

Our study focused on the bulbs of *Allium sativum* harvested in the region of El Harrouch in Skikda (Algeria). After harvesting the bulbs are kept in the shade in a dry and ventilated place. The extraction of essential oil was carried by the hydrodistillation method using the technique described by Benkeblia [7].
The essential oil obtained was dried with anhydrous sodium sulfate and then stored at 4 °C in tubes under protection from light until use. The essential oil yield is the ratio of the amount of oil recovered after hydrodistillation to the quantity of the plant to treat expressed in percentage [8].

Analysis of chemical composition

The essential oil was analyzed on a chromatograph coupled to a mass spectrometer (MS) type Shimadzu 2010 equipped with a capillary column OV17X. The operating conditions are:

- Gauze: helium
- Injection temperature: 220 °C

- Programming of temperature: 60 to 250 °C at 3 °C/min
- Injection mode: Split

The identification of different components is achieved from their mass spectra compared with those of standard compounds of the computerized data base NIST 98.

Microbiological analysis

Bacterial strains tested

- 52 strains of *Pseudomonas aeruginosa* clinical origins were tested *in vitro*. They have been isolated from various pathological specimens from different hospital departments;
- a reference strain of *Pseudomonas aeruginosa* ATCC 27853.

Antibacterial tests

In our work, studying the antibacterial activity of essential oil of *Allium sativum* compared to different strains of *Pseudomonas aeruginosa* was performed by the two variants of the method of dilution:

- **Dilution in liquid medium:** The essential oil is first diluted in a minimum amount of ethyl alcohol 95 %, 2/10 v/v, which is added an aqueous solution containing 0.5 % v/v Tween 80. This is followed by successive dilution of geometric progression with ratio 2, so as to obtain successive dilutions: 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, 1/128 [9]. From a culture of 18 hours we realized a bacterial suspension to a turbidity of approximately 10^6 CFU·mL^{-1}. This solution represents the bacterial inoculum to be used throughout the study. In another series of tubes are introduced respectively 1mL of the solution of essential oil and 9 mL of the bacterial inoculum prepared. The mixture is incubated at 30 °C for 24 hours [10, 11]. The MIC (minimum inhibitory concentration) is the lowest concentration of essential oil at which visible growth in is not observed [12].

- **Dilution in solid medium:** The antimicrobial test was conducted according to the method reported by Koba and coworkers [13]. In the same way that the dilution method in liquid medium, the oil is first diluted in ethanol and Tween 80 to obtain a homogeneous mixture. It is incorporated into the agar medium during cooling to obtain dilutions from 1 µg·mL^{-1} to 128 µg·mL^{-1}. The seeding was done in the form of parallel stripes with a bacterial suspension of 10^6 CFU·mL^{-1}. The duration and incubation temperature were 24 h at 30 °C. The MIC (minimum inhibitory concentration) corresponds to zero growth for the incubation period passed.

Note: All tests, both for the dilution method in liquid medium than in solid medium dilution were done in triplicate. Witnesses with and without ethanol were made.

Survey statistics

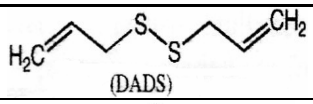
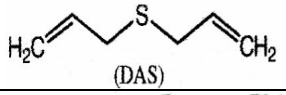
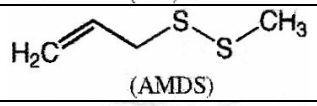
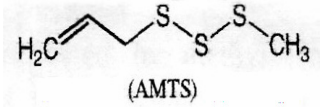
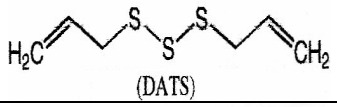
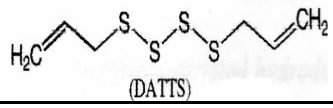
Our statistical study focused on the test "T" for Student associates sample pairs to compare the two methods of dilution. This study was conducted by the statistical package Minitab version 13 whose difference was considered significant at $p < 0.05$.

RESULTS

Yield and chemical composition

The average yield of essential oil of *Allium sativum* bulbs is 0.09 %. The results of chemical analysis of the essential oil are contained in Table 1. Six compounds were identified consisting of almost 100% composed of sulfides with different percentages with allyl methyl trisulfide (34.61%) and diallyl disulfide (31.65%) are the major components of this essential oil while the other components are identified in a relatively small percentage: allyl methyl disulfide (9.27%), diallyl sulfide (6.8%), diallyl trisulfide (1.47%) and diallyl tetrasulfide (4.92%).

Table 1. *Chemical composition of essential oil of* ***Allium sativum***

Compound	Percentage	Chemical structure
Diallyl disulfide	31.65	 (DADS)
Diallyl sulfide	6.80	 (DAS)
Allyl methyl disulfide	9.27	 (AMDS)
Allyl methyl trisulfide	34.61	 (AMTS)
Diallyl trisulfide	1.47	 (DATS)
Diallyl tetrasulfide	4.92	 (DATTS)

Antibacterial activity of essential oil

The results of antibacterial activity of essential oil of *Allium sativum* compared to different strains of *Pseudomonas aeruginosa* are summarized in Table 2. We note that both dilution methods showed that the essential oil of *Allium sativum* shows antibacterial activity compared to the 30 tested strains of *Pseudomonas aeruginosa* with MICs relatively average between 32 $\mu g \cdot mL^{-1}$ and 128 $\mu g \cdot mL^{-1}$. Moreover, the observed results illustrate that the MIC values obtained by the dilution method in solid medium

(64 µg·mL^{-1} and 128 µg·mL^{-1}) are higher compared to those obtained by the dilution method in liquid medium (32 µg·mL^{-1} and 64 µg·mL^{-1}).

Table 2. *Values of MICs of the essential oil obtained by two variations of the dilution method (in mg·mL^{-1})*

Strains tested	CMI in liquid medium	CMI in solid medium
Strain 1	64	128
Strain 2	64	128
Strain 3	64	128
Strain 4	64	64
Strain 5	32	64
Strain 6	32	64
Strain 7	64	128
Strain 8	32	64
Strain 9	64	128
Strain 10	64	128
Strain 11	64	128
Strain 12	32	64
Strain 13	32	64
Strain 14	64	128
Strain 15	64	128
Strain 16	64	128
Strain 17	64	128
Strain 18	64	128
Strain 19	32	64
Strain 20	64	128
Strain 21	64	64
Strain 22	64	64
Strain 23	64	128
Strain 24	64	128
Strain 25	64	128
Strain 26	32	64
Strain 27	64	128
Strain 28	64	128
Strain 29	64	128
Strain 30	64	128

DISCUSSION

In our study, chemical analysis performed by GC/MS revealed that essential oil of *Allium sativum* is characterized by the presence of two major compounds, which are: methyl allyl trisulfide (34.61%) and diallyl disulfide (31.65%) together with other constituents at relatively low levels. This essential oil is substantially similar in chemical composition to that found by O'Gara *et al.* [14] for a plant of the same species from India whose oil essential is mainly composed by diallyl disulfide (53.00%) and diallyl trisulfide (11.5%) while the profile found by Pyun and Shin [15] with a plant native to Korea, however, is significantly different with certain compounds that are not detected in our study as: *N,N*-dimethylthiourea (1.46%), 3-vinyl-4H-1,2-dithiine (1.99%), 3,3-thio bis-1-propene (0.87%).

The difference in composition found on the essential oils investigated is likely to be related to abiotic factors such as climate-specific regions of origin of samples, geographical factors such as altitude and soil type. In recognition of the best authors, diallyl disulfude often constituted the dominant component of essential oil of *Allium sativum*. Allyl methyl trisulfide has never been reported as a major constituent of this plant and, we can assume that the plant *Allium sativum* from El Harrouch presumably represents a new chemotype typical of eastern Algeria.

Regarding the antibacterial activity of essential oil of *Allium sativum* compared to *Pseudomonas aeruginosa*, the MIC values obtained appear to be quite in agreement with those obtained by Tsao and Yin in 2001 [16] that tested antibacterial activity of essential oil of *Allium sativum* originating from China on a variety of strains of *Pseudomonas aeruginosa* by microdilution method in liquid medium: their results yielded MICs vary between 16 $\mu g \cdot mL^{-1}$ and 64 $\mu g \cdot mL^{-1}$. O'Gara *et al*. [14] tested the antibacterial activity of the same essential oil of a plant native to India compared to *Helicobacter pylori* revealing MICs between 8 $\mu g \cdot mL^{-1}$ and 32 $\mu g \cdot mL^{-1}$. In this case the low activity of the essential oil used in our study may be explained partly by its low-profile chemical diallyl trisulfide (DATS) and diallyl tetrasulfide (DATTS) with concentrations of 1.47% and 4.92% respectively, slightly lower than that of China which has shown strong antibacterial activity (11.5% of DATS and 53% DATTS); these two components are demonstrated by several authors [14, 16] as the most active components of the essential oil of *Allium sativum*. On the other hand, the variability of the CMI is due to the difference in the sensitivity of the bacterial species tested.

The value of the MIC may also be influenced by the amount of emulsifier used, however, the addition of an emulsifier in the study of antibacterial activity may limit the contact between the oil and bacteria test. This has been noticed when using a high amount of emulsifier. Similarly, the MIC may be affected by the dilution method used: for awareness that the MIC values obtained by the dilution method in liquid medium are lower than those obtained in solid medium, this same finding was reported by Hammer *et al.* [17] when studying the antibacterial activity of essential oils of 20 medicinal plants. This highly significant difference of MICs between the two methods of dilution can be explained by the influence of certain factors that vary with the test environment. These include: the difference in the exposure of bacteria to the essential oil, the difference in the solubility of essential oil and particularly its active compounds and its steaming.

CONCLUSIONS

This study confirmed the antibacterial properties *in vitro* towards *Pseudomonas aeruginosa* of the essential oil of *Allium sativum*; therefore, that oil could find a possible application in the treatment of various infections caused by *Pseudomonas aeruginosa*. Of course these results obtained *in vitro* are only a first step in finding new antibacterial products offering natural and human medicine. Additional tests are necessary before the confirmation of the highlighted performances. Furthermore, the toxicity of some tested essential oils has to be supplemented by toxicity tests and allergenicity tests.

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