



## CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITY OF *ALLIUM SATIVUM* L. ESSENTIAL OIL AGAINST MDR BACTERIA ISOLATED FROM BUCCAL CAVITY AFFECTED BY CARIES

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Natural remedies have proven to be very effective and have fewer side effects than commercial antibiotics. The objectives of this work were to determine the chemical compounds and biological (antibacterial and antioxidant) activities of *Allium sativum* L. essential oil against three multi-drug strains of bacteria isolated from buccal cavity affected by caries namely *Aerococcus viridans*, *Staphylococcus epidermidis*, and *S. xylosus*. The essential oil was extracted by hydrodistillation, chemical compounds were quantified by gas chromatography-mass spectrometry and gas chromatography with flame ionization detection. 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay and ferric reducing power were used to study antioxidant activity. Antibacterial activity was carried out by the disk diffusion method. The average yield of the essential oil was 0.173±0.009% (w/w). All tested strains were resistant to three different classes of antibiotics. Diallyl disulfide (39.22%) and diallyl trisulfide (34.85%) were the main components of *Allium sativum* L. essential oils. DPPH radical scavenging assay showed the median inhibitory concentration (IC<sub>50</sub>) value of 51.12±11.77 mg/ml, while the ferric reducing power assay recorded the median effective concentration (EC<sub>50</sub>) value of 6.54 ± 0.63 mg/ml. The results showed that all multidrug-resistant bacteria strains tested are sensitive to essential oils. The results indicate that *Allium sativum* L. essential oil exercises good in-vitro antibacterial and weak to moderate in-vitro antioxidant activities.

**Keywords:** *Allium sativum* L., essential oil, antibacterial activity, antioxidant activity, multidrug-resistant bacteria

### INTRODUCTION

Nowadays, natural remedies have proven to be very effective and have fewer side effects than commercial antibiotics. These natural products have various therapeutic activities such as antioxidant, anti-inflammatory, antibacterial, antifungal, etc.<sup>1</sup> as well as their efficacies in the treatment of various oral diseases such as pulpitis, periodontitis, gingivitis, stomatitis, herpes labialis, oral candidiasis, dental plaque,

and oral cancers<sup>2</sup>. Some potential sources of natural compounds are essential oils, which exert an antimicrobial, antiseptic, anti-inflammatory, and antioxidant activity. These aromatic compounds are described as a combination of volatile constituents produced as secondary metabolites by aromatic plants<sup>3</sup>.

One of the best plants used since ancient times in folk medicine, traditional or modern, is garlic (*Allium sativum* L.)<sup>4-8</sup>. It is belonging to the family of the Amaryllidaceae<sup>9-10</sup> and it has

been widely used as a raw vegetable for culinary purposes<sup>6</sup>.

Garlic essential oil has potent antibacterial activity against Gram-positive and Gram-negative bacteria such as *E. coli*, *Salmonella* spp., *Klebsiella* spp., *Staphylococcus aureus*, *Helicobacter pylori*, *Shigella sonnei*, *Pseudomonas aeruginosa*, *Streptococcus* spp., and *Proteus mirabilis*<sup>11</sup> and fungi<sup>12</sup>. Nazzaro et al.<sup>13</sup> reported that the antioxidant and antibacterial activities of garlic are due to its bioactive chemical compound allicin (diallyl thiosulfinate). It is an unstable compound found only in fresh crushed garlic<sup>11,14</sup>, and easily broken down into allyl sulfides, allyl disulfides, and allyl trisulfides, which are the main compounds of garlic essential oils<sup>15</sup>.

About 700 species of bacteria naturally colonize the mouth<sup>16</sup>, which exhibits commensalism, symbiosis, and pathogenic relationships with the host<sup>17</sup>. The composition of the oral microbiota can be modified following a modification of homeostasis by several factors such as chemical interactions with enzymes or microorganisms, reduction in salivary flow, reduction in the production of immunoglobulins, and the presence of proteases and neuraminidase in the oral cavity<sup>17</sup> and/or in people with different systemic deficiencies, e.g., diabetes mellitus, neutropenia, agranulocytosis, and AIDS<sup>18</sup>. These modifications will allow the colonization of the oral cavity by Gram-negative microorganisms and strains resistant to multiple antimicrobials<sup>17</sup>. In addition to the emergence and spread of drug resistance, dysbiosis of the microbiome as a result of antibiotic overuse is increasingly associated with a wide range of oral diseases<sup>19-21</sup>.

The objectives of this work were to determine the chemical compounds and biological (antibacterial and antioxidant) activities of *Allium sativum* L. essential oil against three multi-drug strains of bacteria isolated from the buccal cavity with caries (*Staphylococcus epidermidis*, *Staphylococcus xylosus* and *Aerococcus viridians*).

## MATERIALS AND METHODS

### Extraction of essential oil

The bulbs of garlic (*Allium sativum* L.) were freshly purchased from the local market in Tiaret City (Western Algeria). The plant parts were peeled, cleaned, and prepared for extraction. One hundred and fifty grams of dried

garlic bulbs were crushed and macerated for 1h with 500 ml of distilled water. The extraction of essential oil was performed by hydrodistillation for 1h 30' of the mixture. The obtained essential oil was dehydrated with anhydrous sodium sulfate (Na<sub>2</sub>SO<sub>4</sub>)<sup>22</sup> weighted and stored at 4 °C in sealed brown flasks until use. The yield was calculated using the following equation:

$$\text{Yield of essential oil (\%)} = \left[ \frac{\text{essential oil weight (g)}}{\text{sample weight (g)}} \right] \times 100$$

### Analysis of essential oil

Gas chromatography (GC-FID)/gas chromatography-mass spectrometry (GC/MS) of *Allium sativum* essential oil was carried out method previously described by Selles et al.<sup>23</sup> by Pyrenessences Analysis Sarl according to ISO 11024.

### Antimicrobial study

#### Ethics statement

This study was approved and validated by the scientific committee of the Faculty of Life and Nature Sciences, University Ibn Khaldoun, Tiaret, Algeria, and registered under the number 516/VRPG/2018.

### Microorganisms

Three clinical strains of bacteria (*Aerococcus viridans*, *Staphylococcus epidermidis*, and *Staphylococcus xylosus*) provided by the Microbiology Laboratory of Veterinary Institute (University of Tiaret, Algeria) were used for the assessment of *in-vitro* antimicrobial activity of the *Allium sativum* essential oils.

The strains were isolated from patients suffering from dental caries and before use, bacteria were inoculated in enriched blood agar [Columbia agar base supplemented with 5% blood] for 24 h at 37°C under 5% CO<sub>2</sub> condition for *Aerococcus viridans* and in Chapman agar media for *Staphylococcus xylosus* and *Staphylococcus epidermidis* for 24hrs at 37°C.

The bacterial suspension was then diluted in sterile saline water (0.85% NaCl) to provide initial cell counts of about 1–5 × 10<sup>8</sup> CFU/ml.

### Disk diffusion method

The agar disc diffusion method was performed to assess the antimicrobial susceptibility of bacteria and the antibacterial

potential of the essential oil, based on the measurement of the zone of inhibition after incubation (disc diameter included) using a ruler<sup>24</sup>.

The plates were inoculated with the suspension adjusted on Mueller-Hinton agar for *Staphylococcus xylosus* and *Staphylococcus epidermidis* and Mueller-Hinton agar supplemented with 5% blood for *Aerococcus viridans* by a cotton swab.

After drying, the sterile filter paper disc (diameter 6 mm/Whatman No. 40) pre-impregnated with 5µL of essential oil was placed on their surface. The plates were incubated for 24 hours at 37°C under 5% CO<sub>2</sub> for *Aerococcus viridans* and 24 hours at 37°C for the other bacteria.

This sensitivity was classified according to Ponce et al.<sup>25</sup> as follows: Resistant for a diameter less than 8 mm; sensitive for a diameter of 9–14 mm; very sensitive for a diameter of 15–19 mm and extremely sensitive for a diameter larger than 20 mm. All tests were carried out in triplicate and the results were expressed as mean values ± standard deviation (SD).

The susceptibility of isolates to a panel of antimicrobial agents was determined by using commercial antimicrobial discs according to the recommendations of the Standardization of Antimicrobial Susceptibility Testing in Human and Veterinary Medicine<sup>26</sup>. The following antimicrobial agents, loaded on the disks, were tested: Gentamicin (10µg), chloramphenicol (30µg), tetracycline (10µg), sulfamethoxazole (25µg), ofloxacin (5µg), erythromycin (15µg), cefoxitin (30µg), Spiramycine (100 µg), Oxacilline (1µg), and vancomycin (30µg).

#### **Antioxidant activity 2, 2-diphenyl -1-picrylhydrazyl (DPPH) Radical Scavenging Assay**

The DPPH radical scavenging assay was assessed according to the method described previously<sup>27</sup>. Two ml of final concentrations of garlic essential oil (10.84mg/ml to 54.2 mg/ml) were added to 0.4 mL of 0.5mM ethanol solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 517 nm. Inhibition of free radical DPPH in percent (DPPH I%) was calculated as follows:

$$\text{DPPH I\%} = \left[ \frac{(\text{A blanc} - \text{A sample})}{\text{A blanc}} \right] \times 100$$

Synthetic antioxidants (quercetin, gallic acid, and ascorbic acid) were used as a positive control. The antiradical activity was expressed as IC<sub>50</sub> (mg/ml). IC<sub>50</sub> was calculated from the plotted graph of scavenging activity against the sample and standard concentration. All tests were carried out in triplicate and the results were expressed as mean values ± standard deviation (SD).

#### **Ferric Reducing power**

The reducing power of the essential oil and standard (Quercetin and acid ascorbic) was determined according to the method of Yen and Duh<sup>28</sup> with some modifications. A mixture containing an equal volume (2.5 ml) of final concentrations of the essential oil (0.1084 to 1.084 mg/ml) was mixed with phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferricyanide. After incubation for 20 min at 50 °C. Trichloroacetic acid (2.5ml, 10%) was added to the mixture followed by centrifugation at 3000 rpm for 10 min. One milliliter of supernatant was recovered and mixed with 1 ml of distilled water and 0.5 ml of ferric chloride (0.1%).

Finally, the absorbance was measured at 700 nm. Reducing power assay of the sample and standards were expressed by EC<sub>50</sub> value. The EC<sub>50</sub> was obtained by interpolation from regression analysis. All tests were carried out in triplicate and the results were expressed as mean values ± standard deviation (SD).

#### **Statistical analysis**

One-way analysis of variance (ANOVA) followed by Tukey's post hoc test of honestly significant difference (HSD) (p<0.05 was used to calculate significant differences using R software (version 3.3.0/2016- 05-03).

## **RESULTS AND DISCUSSION**

### **Results**

#### **Yield and chemical composition of *Allium sativum* L. essential oil**

The yield of *Allium sativum* L. essential oil obtained in our study was 0.173 ± 0.009% (w/w). A similar result was reported by Mnayer et al.<sup>29</sup>, Johnson et al.<sup>30</sup>, and Haciseferogülları et al.<sup>31</sup> with a rate of 1300 to 2000 g/t, 0.16%, and 0.14%, respectively. Nevertheless, Douiri et al.<sup>32</sup> and Lawrence et al.<sup>33</sup> recorded a higher yield of 0.32% and 0.4% of the plant collected from Moroccan and Indian, respectively. Likewise, Moumene et al.<sup>34</sup> and Casella et al.<sup>35</sup> cited a

higher garlic essential oil yield of plants collected in west Algeria and that from Italy with a rate of 0.28 %; and 0.30 (w/w) respectively, by using the same mean fresh weight in each extraction procedure. Higher yields using Clevenger equipment, for different drying methods and varying extraction times, have been reported by Herrera-Calderon et al.<sup>36</sup> with 0.78% (v/dry weight of garlic) for 2 hrs as well as Dziri et al.<sup>1</sup> with rates 0.5% and 0.6% (w/w) for oven-dried and freeze-dried samples, respectively for 3 hrs.

Furthermore, variable yields ranging from 0.18% to 0.22% were obtained by using various devices: Clevenger apparatus, industrial hydrodistillation, and steam distillation for an extraction period respectively of 3hrs, 4hrs, and 5hrs<sup>37</sup>. However, the lowest yields of 0.09% and 0.1% (w/w) essential oil were reported by Khadri et al.<sup>38</sup> for *Allium sativum* L. bulbs harvested in the Skikda region (Algeria) and Dziri et al.<sup>1</sup> using Clevenger equipment for air-dried bulbs for 3 hrs extraction, respectively.

The difference in yield can be influenced by water temperature, distillation time and particle sizes<sup>39-40</sup>, and drying of plant material<sup>41</sup>.

The composition of *Allium sativum* L. essential oil is presented in **Table 1**. Forty-two components represented 100% of the chemical composition of *Allium sativum* L. essential oil. Diallyl disulfide Mw=146 were the main constituents (39.22%) followed by diallyl trisulfide Mw=178 (34.85%), diallyl disulfide isomer Mw=146 (5.45%), allyl methyl disulfide Mw=120 (5.20%), allyl methyl trisulfide Mw=152 (3.75) and diallyl sulfide Mw=114 (3.70%).

Several previous studies report similarities and differences in the major compounds of garlic essential oils. Mnayer et al.<sup>29</sup> reported that the main compounds of garlic essential oil were diallyl disulfide (37.90%), diallyl trisulfide (28.06%), allyl methyl trisulfide (7.26%), diallyl sulfide (6.59%), diallyl tetrasulfide (4.14%) and allyl methyl disulfide (3.69%) purchased from a local supermarket in Avignon province (France). Likewise, Sommano et al.<sup>42</sup> showed that the major compounds of essential oil of *Allium*

*sativum* L. from Mexico are: diallyl disulfide (42.46%), allyl methyl disulfide (15.25%), diallyl trisulfide (12.52%), allyl methyl trisulfide (10.36%), diallyl sulfide (6.96%), Methyl allyl sulfide (3.15%) and diallyl tetrasulfide (1.09%). Whereas Khadri et al.<sup>38</sup> found that allyl methyl trisulfide (34.61%), diallyl disulfide (31.65%), allyl methyl disulfide (9.27%), diallyl sulfide (6.8%), diallyl trisulfide (1.47%) and diallyl tetrasulfide (4.92%) are the major compounds of *Allium sativum* L. essential oil harvested in the region of El Harrouch in Skikda (Algeria). Garlic essential oils from China, Tunisia, and Egypt were characterized by a chromatographic profile dominated by diallyl trisulfide (35.30% to 50.92%) and diallyl disulfide (27.47% to 29.10%)<sup>42</sup>. The same authors reported that the major compounds of garlic essential oil harvested from Morocco were: Trisulfide, di-2-propenyl (46.52%), disulfide, di-2-propenyl (14.30%), trisulfide, methyl 2-propenyl (10.88%) while this oil contains little diallyl disulfide (0.46%<sup>o</sup>) and no diallyl trisulfide<sup>42</sup>.

Moumene et al.<sup>34</sup> noted that allyl trisulfide and diallyl disulfide are the main components of red and white garlic essential oil grown at the technical institute for market gardening and industrial crops (ITCMI) in the wilaya of Sidi Bel Abbes with varying percentages for each plant.

Satyral et al.<sup>37</sup> gave the chemical composition of *Allium sativum* L. essential oil collected from Spain and extracted by three different methods namely Clevenger-type hydrodistillation, industrial steam distillation, and industrial hydrodistillation. These authors noted that the chromatographic profile of this oil was dominated by diallyl trisulfide, diallyl disulfide, allyl methyl trisulfide, and allyl methyl disulfide for the Clevenger-type hydrodistillation and industrial hydrodistillation technique with varying concentrations when diallyl disulfide followed by allyl methyl trisulfide, diallyl trisulfide, and allyl methyl disulfide are the main compounds of this essential oil extracted by industrial steam distillation.

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

Peak	RT (min)	Compounds	%
1	5.49	Allyl mercaptan mw=74	0.13
2	5.95	Ethanol	0.02
3	6.12	Methyl-thiirane mw=74	0.07
4	6.55	Sulfure de methyl-allyle mw=88	0.71
5	8.04	Alpha-pinene	0.01
6	8.94	2-Methyl-4-Pental	0.06
7	9.37	Camphene	0.01
8	9.59	Disulfure de dimethyle mw=94	0.19
9	10.59	2-propen-1-ol	0.03
10	10.67	Composé soufré	0.10
11	10.86	Sulfure de propyl-allyle mw=116	0.03
12	12.51	<b>Sulfure de diallyle mw=114</b>	<b>3.70</b>
13	14.32	Sulfure de diallyle isomere mw=114	0.05
14	14.76	Sulfure de diallyle isomere mw=114	0.05
15	15.41	1.8-Cineole	0.03
16	16.76	Disulfure de methyl-propyle mw=122	0.03
17	17.92	2.4-Dimethyl-Thiophene mw=112	0.01
18	18.55	Disulfure de methyl-allyle isomere mw=120	0.13
19	19.55	<b>Disulfure de methyl-allyle mw=120</b>	<b>5.28</b>
20	20.01	Disulfure de methyl-allyle isomere mw=120	0.44
21	25.58	Trisulfure de dimethyle mw=126	2.34
22	28.78	Tisulfure de propyl-allyle isomere mw=148	0.17
23	29.29	Tisulfure de propyl-allyle isomere mw=148	0.01
24	29.32	Isochrysanthenone	0.02
25	31.04	Disulfure de diallyle isomere mw=146	1.28
26	32.22	<b>Disulfure de diallyle mw=146</b>	<b>39.22</b>
27	32.49	<b>Disulfure de diallyle isomere mw=146</b>	<b>5.45</b>
28	34.10	Camphre	0.05
29	34.62	Composé thiourée	0.99
30	35.02	Trisulfure de methyl-propyle mw=154	0.03
31	38.93	<b>Trisulfure de methyl-allyle mw=152</b>	<b>3.75</b>
32	47.29	Composé mw=180	0.03
33	47.55	3-Vinyl-1.2-Dithiacyclohex-4-ene mw=144	0.31
34	51.02	<b>Trisulfure de diallyle mw=178</b>	<b>34.85</b>
35	53.70	3-Vinyl-1.2-Dithiacyclohex-5-ene mw=144	0.31
36	55.04	Composé mw=166	0.03
37	70.67	Composé alcool	0.01
38	73.93	Composé aromatique	0.01
39	75.29	Eugenol	0.01
40	81.27	Composé soufré	0.02
41	85.62	Composé aromatique	0.01
42	102.97	Acide palmitique	0.04
		<b>Total</b>	<b>100.00</b>

### Antioxidant activity

In our study, the antioxidant activity of garlic essential oil was evaluated by reducing power and DPPH radical scavenging capacity. The reducing power is based on the capacity of antioxidants to reduce ferric ions ( $\text{Fe}^{3+}$ ) to ferrous ions ( $\text{Fe}^{2+}$ ) despite the DPPH assay is based on the scavenging of the stable DPPH• by an antioxidant. **Table 2** summarized the  $\text{EC}_{50}$  and the  $\text{IC}_{50}$  values of reducing power and DPPH scavenging activity of *Allium sativum* L. essential oil.

The  $\text{EC}_{50}$  and  $\text{IC}_{50}$  values of *Allium sativum* L. essential oil were  $6.54 \pm 0.63$  mg/ml and  $51.12 \pm 11.77$  mg/ml, respectively. These values were very high compared to the standard (**Table. 2**). A very highly significant difference between the standard molecules and the essential oil was observed for Ferric Reducing power ( $P > 0.001$ ) while a highly significant difference was observed between the standard molecules and the essential oil for DPPH ( $P > 0.01$ ).

Ndoye Foe et al.<sup>43</sup> reported that the  $\text{EC}_{50}$  of *Allium sativum* L. essential oil was  $5.33 \pm 0.01$   $\mu\text{g}$  AAE/mg this value is 1000-fold lower than that registered in this study.

Most studies have demonstrated low  $\text{IC}_{50}$  compared to that obtained in the current study, ranging from 0.5 mg/ml to 7.67 mg/ml<sup>29,33,36</sup>. Nevertheless, Akinyemi et al.<sup>44</sup> observed a significant difference in DPPH free radical scavenging capacity between garlic essential oil ( $\text{IC}_{50} = 24.8$   $\mu\text{g}/\text{ml}$ ) and ascorbic acid ( $\text{IC}_{50} = 61.5$   $\mu\text{g}/\text{ml}$ ).

The antioxidant activity of garlic is due to its richness in sulfur compounds that represent the main constituents of this essential oils<sup>29</sup>. In the current study, the main compound of *Allium sativum* L. essential oil was diallyl disulfide (39.22%) followed by diallyl trisulfide (34.85%). However, diallyl disulfide isomer,

allyl methyl disulfide, allyl methyl trisulfide, and diallyl sulfide were present in smaller amounts. Although the presence of diallyl disulfide and diallyl trisulfide in garlic oil breaks the chain of free radicals through the donation of hydrogen atoms neutralizing DPPH radicals and consequently increases antioxidant activity<sup>45</sup>. Amorati et al.<sup>46-47</sup> reported that diallyl disulfide and allyl methyl sulfide have no antioxidant activity as inhibitors of the controlled auto-oxidation of isopropylbenzene or styrene. These same authors have suggested that these compounds are oxidized with the oxidizable substrate. Nevertheless, water-soluble garlic has higher antioxidant activity than garlic oil because water-soluble garlic is made up of N-acetylcysteine, a derivative of the amino acid L-cysteine that has the highest antioxidant activity among organosulfur compounds. Additionally, previous studies have shown that garlic has stable organosulfur compounds, flavonoids, and polyphenols and that phenolic compounds are more effective antioxidants than non-phenolic compounds such as allyl sulfide<sup>45</sup>.

### Antibacterial activity

In the present study, all strains tested are resistant to at least one class of antibiotic. However, *Staphylococcus epidermidis* and *Staphylococcus xylosus* were resistant to three class strains. While *Aerococcus viridans* was resistant to four classes of antibiotics (**Table 3**).

Based on the most frequent definition of multi-drug resistance proposed by Magiorakos et al.<sup>48</sup> for Gram-positive and Gram-negative bacteria are "resistant to three or more classes of antimicrobials", all strains used in this study were considered multi-resistant, namely: *Aerococcus viridans*; *Staphylococcus epidermidis* and *Staphylococcus xylosus*.

**Table 2:** Antioxidant activity of *Allium sativum* essential oil in reducing power and DPPH assays.

	Reducing power ( $\text{EC}_{50}$ ) mg/ml	DPPH ( $\text{IC}_{50}$ ) mg/ml
Quercetin	$24.62 \pm 1.95 \times 10^{-3}$ <sup>a</sup>	$8.52 \pm 0.52 \times 10^{-3}$ <sup>a</sup>
Gallic acid	-	$12.86 \pm 3.37 \times 10^{-3}$ <sup>a</sup>
Ascorbic acid	$53.31 \pm 0.21 \times 10^{-3}$ <sup>a</sup>	$8.39 \pm 0.24 \times 10^{-3}$ <sup>a</sup>
<i>Allium sativum</i> essential oil	$6.54 \pm 0.63$ <sup>b</sup>	$51.12 \pm 11.77$ <sup>b</sup>

Each value in the table is represented as mean  $\pm$  SD ( $n = 3$ ). Means not sharing the same letter are significantly different (LSD) at  $P < 0.05$  probability level in each column.

**Table 3:** Antibacterial susceptibility of tested strains.

Class	Antibiotics	A	B	C
Sulfonamides	STX	S	R	-
Aminosides	GN	S	S	-
Fluoroquinolones	OFX	S	S	-
Phenicols	C	S	S	S
Tetracyclines	TE	R	R	R
Macrolides	E	R	R	-
	SR	-	-	R
Betalactams	OXA	R	R	-
	FOX	-	-	R
Glycopeptides	VAC	-	-	R

A : *Staphylococcus epidermidis* ; B : *Staphylococcus xylosus* ; C : *Aerococcus viridans*.

sulfamethoxazole ; GN : Gentamicine ; OFX : Ofloxacin ; C : Chloramphenicol ; TE : tetracycline ; E : erythromycine ; SR : Streptomycine ; OXA : Oxacilline ; Fox : Cifoxitin ; VAC : Vancomycine.

**Table 4** summarizes the results of the Antibacterial activity of *Allium sativum* essential oil towards strains studied. The inhibition diameter of *Allium sativum* L. essential oil against tested strains varies from 9mm to 15.66 mm.

In our study, *Allium sativum* L. essential oil exhibited good *in-vitro* antibacterial activity towards strains tested. Based on strain sensitivity to essential oils classified by Ponce et al.<sup>25</sup> *Staphylococcus epidermidis* and *Aerococcus viridans* were classified as sensitive to the antibacterial activity of *Allium sativum* essential oil. Whereas, *Staphylococcus xylosus* have been classified as very sensitive to the antibacterial activity of *Allium sativum* L. essential oil (**Table 4**).

**Table4:** Antibacterial activity of *Allium sativum* essential oil.

Isolats	Inhibition Diameter (mm)*
<i>Aerococcus viridans</i>	9 ± 1.63
<i>Staphylococcus epidermidis</i>	14.33 ± 1.15
<i>Staphylococcus xylosus</i>	15.66 ± 0.58

\* Inhibition Diameter (mm) including disk diameter of 6.0 mm.

Several studies report a higher activity of *Allium sativum* L. essential oils against *Staphylococcus aureus* with a ranging inhibition diameter from 18 to 20mm<sup>29,49</sup>. Nevertheless, the

study conducted by Zouari Chekki et al.<sup>50</sup> reports a low activity of *Allium sativum* L. essential oils against *Staphylococcus aureus* (12 mm). Whereas, inhibition diameters of 10.2mm, 11mm, and 11.5mm were registered for concentrations of 5%, 10%, and 15% of *Allium sativum* L. essential oil respectively against *Staphylococcus aureus* by Pratama and Perangin-angin<sup>51</sup>. Evenly, Moumene et al.<sup>34</sup> reports that *Allium sativum* L. essential oils exhibited a sensitive *in vitro* antibacterial activity towards *Staphylococcus aureus* (inhibition diameters vary from 9mm to 11mm). Likewise, weak inhibition ranging from 6.3mm to 9.3 mm was shown at various concentrations of *Allium sativum* L. essential oils varying from 50ml/l to 500ml/l against *Staphylococcus aureus*<sup>52</sup>. Nonetheless, a complete inhibition (90 mm) against *Staphylococcus aureus* exerted by the essential oil *Allium sativum* L. was noted by Torpol et al.<sup>7</sup>

This good antibacterial activity of the essential oil of garlic is due to its richness in organosulfide compounds. Bhatwalkar et al.<sup>12</sup> reports « two main mechanisms of action of garlic organosulfur compounds emerged from the reported studies: (1) the reaction of garlic compounds to the free sulfhydryl group on the proteins and/or enzymes to inactivate them, and (2) the disruption of composition and integrity of bacterial cell membrane and/or cell wall. Besides, some work also suggests that garlic compounds could also have a global effect on DNA, RNA, and protein synthesis ».

## Conclusions

This study found that diallyl groups were the major active compounds in *Allium sativum* L. essential oils. These groups include diallyl disulfide and diallyl trisulfide. However, this essential oil exhibited good *in-vitro* antimicrobial activity against all tested microorganisms and a weak to moderate *in-vitro* antioxidant. Nevertheless, further and expanded investigations will be needed to confirm and justify the potential use of this oil as an antibacterial agent against multi-drug strains of bacteria isolated from buccal cavity affected by caries

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## Conflict of interest

The authors declare that there is no conflict of interest in this study.

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## نشرة العلوم الصيدلانية جامعة أسيوط



### عنوان التركيب الكيميائي والنشاط البيولوجي للزيت الأساسي من نبات الثوم ضد بكتيريا ذات المقاومة المتعددة للأدوية معزولة من تجويف الفم المتأثر بالتسوس

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أثبتت العلاجات الطبيعية أنها فعالة جدًا ولها آثار جانبية أقل من المضادات الحيوية التجارية. كانت أهداف هذا العمل تحديد المركبات الكيميائية والأدوية البيولوجية (المضادة للبكتيريا ومضادات الأكسدة). للزيت العطري *Allium sativum L* تجاه ثلاث سلالات من البكتيريا موجبة الجرام معزولة من تجويف الفم المصابة بالتسوس وهي *Aerococcus viridans* و *Staphylococcus epidermidis* و *Staphylococcus xylosum*. استخلصت الزيت الأساسي بطريقة التقطير المائي. تم تحديد التركيب الكيميائي لهذه الزيت بواسطة كروماتوغرافيا الغاز المدمج مع المطيافية الكتلية و كروماتوغرافيا الغاز المدمج مع كاشف التآين باللهب بينما تم دراسة النشاط المضاد للأكسدة بواسطة القوة الاختزالية وطريقة DPPH (1-diphenyl-2-picrylhydrazyl) تم إجراء اختبار الحساسية لمضادات الميكروبات والنشاط المضاد للميكروبات للزيت الأساسي للبكتيريا المختبرة بطريقة نشر القرص. بلغ مردود استخلاص الزيت الأساسي  $0,173 \pm 0,009\%$  (وزن/وزن). كانت جميع السلالات المختبرة مقاومة لثلاث فئات مختلف من المضادات الحيوية. اثنان وأربعون مكونا تم تحديدها وقياسها كميًا. كان ثنائي كبريتيد ثنائي الأليل وم<sup>١٤٦</sup> = هو المركب الرئيسي للزيت الأساسي الذي تمت دراسته (٣٩,٢٢%) يليه ثلاثي كبريتيد ثنائي الأليل وم<sup>(٣٤,٨٥%)</sup> = ١٧٨. أظهر نشاط مضادات الأكسدة بواسطة مقايضة DPPH قيمة متوسطة التركيز المثبط (IC50) تبلغ  $51,12 \pm 11,77$  مجم / مل، بينما أعطى اختبار قدرة الاختزال الحديدي قيمة متوسطة التركيز الفعال (EC50) تبلغ  $6,54 \pm 0,63$  مجم / مل. أظهرت النتائج أن جميع السلالات البكتيرية متعددة المقاومة التي تم اختبارها حساسة للزيوت الأساسية. تشير النتائج إلى أن الزيت العطري *Allium sativum L* يمارس نشاطًا جيدًا مضادًا للبكتيريا في المختبر بينما كان ضعيفًا إلى متوسط بالنسبة للنشاط المضاد للأكسدة في المختبر.