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## Analysis of Lipid Composition and Biological Activities of Identical Extracts from Libyan *Allium orientale*

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### Abstract:

In this study, fatty acids were isolated and identified from the roots and leaves of *Allium orientale* (*Allium* spp.) using Soxhlet extraction GC/MS analysis of the extracts revealed a mixture of saturated and unsaturated fatty acids. Among the major constituents detected were oleic, linoleic, and palmitic acids, which were consistently present in high concentrations across all samples, indicating a similar fatty acid composition among the extracts.. Studying the biological activity of certain plant extracts from the roots and flowers against various pathogenic bacteria, we found that these extracts exhibit a significant inhibitory effect on bacterial growth. The hexane extract of the roots showed the highest antibacterial activity, followed by the methanol extract of the flowers. These findings demonstrate that *Allium orientale* is a rich source of bioactive fatty acids and that its extracts possess promising biological effects, suggesting potential applications in pharmaceutical or nutraceutical fields. in natural medicine and food preservation

**Keywords:** *Allium orientale*, lipid constituents, GC/MS, Antimicrobial activity.

## تحليل التركيب الدهني وتقييم الفعالية البيولوجية لبعض خلاصات الثوم الشرقي (*Allium orientale*) في ليبيا

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### الملخص:

في هذه الدراسة، تم استخلاص وتحديد الأحماض الدهنية من جذور وأوراق نبات الثوم الشرقي (*Allium orientale*) باستخدام استخلاص سوكسلت وتحليل GC/MS. أظهر التحليل وجود مزيج من الأحماض الدهنية المشبعة وغير المشبعة. ومن بين المكونات الرئيسية المكتشفة كانت أحماض الأوليك، اللينوليك، والبالمتيك، والتي وُجدت بتركيزات عالية بشكل مستمر في جميع العينات، مما يشير إلى تركيب دهني متشابه بين الخلاصات.

عند دراسة النشاط البيولوجي لبعض خلاصات النبات من الجذور والأزهار ضد عدة بكتيريا ممرضة، وجدنا أن هذه الخلاصات تظهر تأثيراً مثبطاً قوياً على نمو البكتيريا. أظهر مستخلص الهكسان من الجذور أعلى نشاط مضاد للبكتيريا، يليه مستخلص الميثانول من الأزهار.

تُظهر هذه النتائج أن نبات الثوم الشرقي مصدر غني بالأحماض الدهنية الفعالة حيويًا، وأن خلاصاته تمتلك تأثيرات بيولوجية واعدة، مما يشير إلى إمكانية تطبيقها في المجالات الصيدلانية أو الغذائية، وخاصة في الطب الطبيعي وحفظ الأغذية.

**الكلمات المفتاحية:** الثوم الشرقي (*Allium orientale*) ، المكونات الدهنية، التحليل باستخدام GC/MS ، النشاط المضاد للميكروبات.

### 1. Introduction

Allium species are a group of plants that have been extensively studied for their antioxidant properties. The leaves of certain wild Allium species, such as *Allium flavum* L. and *Allium ursinum* L., as well as cultivated Allium species like *Allium sativum* L., Allium

cepa L., *Allium vineale* L., *Allium fistulosum* L., and *Allium nutans* L., have been found to exhibit high antioxidant activities [1]. The natural habitats of *Allium grayi* and *Allium monanthum* are limited to specific altitude ranges, while *Allium thunbergii* and *Allium maximowiczii* have different habitat preferences [2]. The photosynthetic rates and other physiological characteristics of *Allium ochotense* and *Allium microdictyon* leaves suggest that they are adapted to different temperature conditions [3]. Studies have demonstrated that extracts from *Allium cepa*, *Allium ascalonicum*, and *Allium sativum* can prevent the oxidation of low-density lipoproteins (LDL), a critical process involved in the onset of atherosclerosis [4]. Karyotypic studies have revealed that *Allium cepa*, *Allium sativum*, and *Allium tuberosum* have different chromosome numbers and karyotype formulas. *Allium orientale*, commonly known as Oriental garlic or Eastern onion, is a flowering plant species belonging to the genus *Allium* in the Amaryllidaceae family. Native to parts of the Middle East and Eastern Mediterranean regions, this perennial herb has been valued for both its culinary applications and traditional medicinal properties throughout history [5].

The plant typically grows to a height of 30-60 cm and features linear leaves and spherical umbels of white or pinkish flowers. Like other members of the *Allium* genus, it contains organosulfur compounds that contribute to its distinctive aroma and potential health benefits. These compounds have been associated with antimicrobial, antioxidant, and anti-inflammatory properties [6]. *Allium orientale* has been used in traditional medicine systems across its native range for various ailments, including respiratory issues, digestive disorders, and as a general tonic. In culinary applications, various parts of the plant, including the bulbs and sometimes the leaves, are used to add flavor to a variety of regional dishes [7]. The chemical constituents of *Allium* species include: Sulfur compounds, steroidal saponins, flavonoids, and polysaccharides [8].

*Allium* species contain cysteine sulfoxides and volatile secondary metabolites, with the distribution of these compounds being determined primarily by the paternal wild species [9]. *Allium macrostemon* Bunge contains various chemical constituents, such as steroid saponin, nitrogen compounds, volatile oil, acidity constituents, polysaccharides, and more [10]. Compounds identified in *Allium fistulosum* (L.) var. *giganteum* include  $\beta$ -sitosterol, daucosterol, quercetin, rutin, kaempferol, baicalin, and glucose.

*Allium ursinum* (ramson) contains sulfur constituents, such as Sulfur-containing compounds such as S-alk(en)yl-L-cysteine sulfoxide (alliin) and its bioactive derivative allicin, which have been found to have antifungal activities [11]. Studies on *Allium* species have revealed important insights into their lipid-related properties. Methanol extracts from *Allium senescens* L. have been shown to reduce the production of reactive oxygen species (ROS) and inhibit lipid accumulation during the formation of fat cells (adipogenesis) in 3T3-L1 cells<sup>1</sup>. These extracts suppressed the expression of pro-oxidant enzymes such as glucose-6-phosphate dehydrogenase (G6PDH) and decreased the mRNA levels of key adipogenic transcription factors, including SREBP1c, PPAR $\gamma$ , and C/EBP $\alpha$ . This indicates that *Allium senescens* L. possesses anti-adipogenic properties by inhibiting adipogenic gene expression through ROS suppression[12,13].

The *Allium* genus, rich in bioactive compounds like allicin and flavonoids, exhibits diverse therapeutic effects including antimicrobial, antioxidant, and anticancer activities through modulation of key cellular pathways. This review emphasizes their health potential, species-specific differences, and the need for further clinical validation to bridge traditional use with modern medicine[14]. A 2024 study analyzed garlic essential oil (GEO) and oleoresin (GOL), revealing key bioactive compounds such as allyl sulfides and fatty acids using GC-MS. These components, including allyl methyl trisulfide and hexadecanoic acid, showed potential as natural flavoring and preservative agents in food and pharmaceutical applications[15].

In 2013, a study was conducted, a comparative analysis of the phytochemical profiles of three *Allium* species, highlighting both shared and distinct bioactive compounds among them. Allicin was found in all three species, with higher concentrations in *A. schoenoprasum* and *A. obliquum*, whereas alliin was limited to *A. obliquum* and *A. senescens* subsp. *montanum*. All species exhibited similar phenolic acid patterns, particularly the presence of p-coumaric and ferulic acids. The flavonoid composition varied: isoquercitrin was identified in *A. obliquum* and *A. schoenoprasum*, while rutin appeared in *A. senescens* subsp. *montanum* and *A. schoenoprasum*. Notably, luteolin and apigenin were exclusive to *A. obliquum*. Kaempferol and quercetin glycosides were consistently detected across all species. Regarding sterol content, all three species contained  $\beta$ -sitosterol and campesterol, with *A. obliquum*

showing the highest  $\beta$ -sitosterol concentration and *A. senescens* subsp. *Montanum* has the highest level of campesterol[16]. The compounds found in *A. triquetrum* bulbs include fatty acids (such as hexadecanoic acid, the major component), hydrocarbons (like n-tetradecane, n-octadecane, n-tricosane, and 1-hexadecene), sulfur compounds (such as trans-propenyl-methyl disulfide, 1,3-dithiane, and methyl-trans-propenyl disulfide), and other substances (including phenol, 2,4-bis(1,1-dimethylethyl), and neophytadiene). These components contribute to the plant's chemical profile [17]. In this study, we analyzed the lipid compounds of *Allium orientale* using GC-MS and evaluated the biological activity of six different extracts obtained from the leaves and roots of the plant.

## 2. MATERIALS

### 2.1. Plant Material

The plant *Allium orientale* was collected for chemical analysis in February from the city of Sirte, Libya. The plant was identified by Dr. Chbob T from the University of Tripoli. The collected samples were prepared following standard procedures for phytochemical studies.

### 2.2. Chemicals

- Hexane (40-60), purity  $\geq 95\%$ , Sigma-Aldrich
- Methanol (HPLC grade), purity  $\geq 99.9\%$ , Merck
- Sulfuric acid (concentrated, 98%), Sigma-Aldrich
- Sodium bicarbonate (analytical grade), Merck
- Diethyl ether (anhydrous,  $\geq 99.5\%$ ), Fisher Scientific
- Sodium sulfate (anhydrous,  $\geq 99\%$ ), Sigma-Aldrich
- Magnesium sulfate (anhydrous,  $\geq 99\%$ ), Merck

## 3. METHODS

### 3.1. Lipid Extraction from *Allium orientale* Using Soxhlet Apparatus and Hexane

Lipids were extracted from the *A. orientale* using the Soxhlet extraction technique, focusing on both the roots and leaves as sources of plant material. The roots and leaves were dried at a low temperature to preserve active compounds, and then ground into a fine powder [18]. Each powdered sample was placed in a paper thimble and inserted separately into the Soxhlet apparatus to conduct independent extraction processes for each plant part. hexane was used as the organic solvent due to its high efficiency in dissolving non-polar lipids, such as vegetable oils and saturated fats. The extraction process lasted for several hours (typically 6–8 hours),

allowing the hot hexane to pass repeatedly through the plant material to ensure maximum lipid recovery. After extraction, hexane was evaporated using a rotary evaporator to obtain the crude lipid extract from both the roots and leaves separately. This approach facilitates further comparative analysis of lipid composition between the two plant parts.



Figure 1. Soxhlet Apparatus for lipid Extraction from *Allium orientale*

### 3.2. Conversion to Ester:

To prepare the extracted lipid for GC/MS analysis, an esterification reaction is often performed. This is because the ester form of compounds is generally more volatile and easier to analyze via GC/MS [19].

#### General Esterification Reaction:

The essential lipid is reacted with an alcohol—typically methanol or ethanol—in the presence of a strong acid catalyst like hydrochloric acid (HCl) or sulfuric acid (H<sub>2</sub>SO<sub>4</sub>). This transesterification process converts the fatty acids into alkyl esters, such as methyl esters when methanol is used. Steps for Esterification:

1. In a round-bottom flask, add the essential lipid and an excess of methanol (or the alcohol of choice).
2. Add a few drops of sulfuric acid or another strong acid catalyst.

3. Heat the mixture gently under reflux conditions for about 1-2 hours.
4. After the reaction is complete, neutralize the solution by adding a sodium bicarbonate solution to neutralize the acid.
5. Separate the ester from the mixture using extraction with diethyl ether or another suitable organic solvent
6. Remove residual moisture from the organic phase by drying it over anhydrous sodium sulfate or magnesium sulfate.
7. Concentrate the esterified product by evaporating the solvent, leaving the esterified lipid.

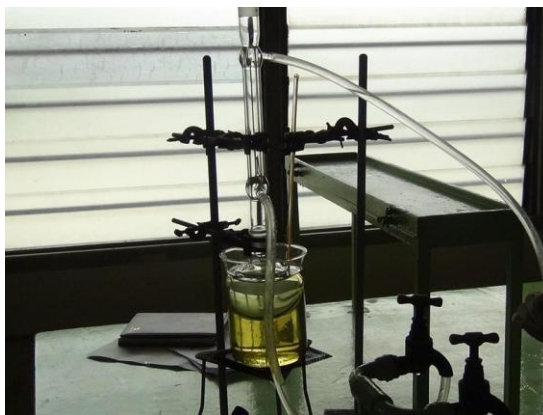


Figure 2. Esterification Process of Eastern *A. orientale* Lipids

### 3.3. GC/MS Analysis of Lipid Compounds

The analysis of Fatty Acid Methyl Esters (FAMES) was conducted using a Shimadzu GC/MS–QP 5050 A system, controlled by Class 5000 software. Chromatographic separation was achieved using a DB-5 capillary column (30 meters in length, 0.53 mm internal diameter, and a film thickness of 1.5  $\mu$ m). Helium served as the carrier gas, flowing at a rate of 1 mL/min. Ionization was carried out through electron impact (EI) at an energy of 70 eV.

The oven temperature was initially set to 30°C and held for 2 minutes, then increased at a rate of 2°C per minute to reach 100°C, followed by a further rise to 150°C, which was maintained for 7.5 minutes. The injector and detector temperatures were both held constant at 150°C. Data acquisition was performed in both full scan and selected ion monitoring (SIM) modes.

Under these analytical conditions, allicin was detected with a retention time of 0.9 minutes. The purity of each compound was verified by evaluating the ratio of its primary identifying ion to a secondary characteristic ion throughout the run.



### 3.4. Biological activity

#### Preparation of different extracts for biology

A total of 30 grams of finely ground plant material, consisting of both seeds and flowers, was used for the extraction process. The plant material was subjected to successive solvent extractions using three different solvents: n-hexane, methanol, and acetone, to obtain a broad range of phytochemical constituents based on their polarity. Each extraction was performed separately to yield six distinct extracts in total—three extracts from the seeds (one per solvent) and three from the flowers (one per solvent). The extraction procedure involved macerating the powdered plant material in each solvent for a specific period under continuous agitation at room temperature. Following extraction, the mixtures were passed through Whatman No. 1 filter paper to separate the solid materials [20]. The resulting filtrates were subsequently concentrated under reduced pressure using a rotary evaporator, with the temperature carefully adjusted for each solvent to prevent heat-induced degradation of the bioactive constituents.

#### Biological experiments.

Antimicrobial activity was assessed through the microdilution technique to establish the minimum inhibitory concentration (MIC). The extracts were evaluated against a range of Gram-positive and Gram-negative bacterial strains, in accordance with the methodology described by Koeth [21],

#### Cultures:

bacteria culture used for *Escherichia coli*, *Bacillus subtilis* and *Candida albicans*

#### Maintenance of the microorganism

The bacterial species used in this study were maintained on nutrient agar medium at 37°C and on blood agar (PDH). After 12 hours of activation, bacterial suspensions were prepared, and the turbidity was standardized by measuring the optical density using a UV-VIS spectrophotometer (Model 1610, Shimadzu, Tokyo, Japan). The final bacterial concentrations were adjusted to  $1 \times 10^6$  cells/mL and  $1 \times 10^5$  cells/mL, respectively [22]. The microorganisms used in the current study were obtained from Emhammed Al-Magariaf Hospital Laboratory – Ajdabiya, Libya."

#### Bioassay experiment.

The antimicrobial properties of the extracts were tested against the aforementioned Gram-positive and Gram-negative bacterial strains.



Pure cultures were grown on nutrient agar, incubated at 37 °C for 24 hours, and subsequently stored at 4 °C. These strains were periodically re-cultured to ensure their viability throughout the study.

#### Preparation of Sensitivity Discs (Punching Method):

Sensitivity discs are small filter paper discs used in antibiotic susceptibility testing. To prepare them using the punching method:

1. **Filter Paper Selection:** Use sterile Whatman No. 1 filter paper.
2. **Sterilization:** Autoclave the paper or sterilize it using dry heat.
3. **Punching:** Use a sterile paper punch to cut uniform discs (usually 6 mm in diameter).
4. **Storage:** Store the discs in a sterile, airtight container until use.

#### 4. Results

The results obtained from the analysis using Gas Chromatography-Mass Spectrometry (GC/MS) indicated that the leaves of the *Allium orientale* plant contain a total of 13 different fatty acids. Among these, palmitic acid was found to be the most abundant, accounting for 29% of the total fatty acid content. This was followed by oleic acid, which constituted 26%, and then linoleic acid, which made up 22%. While analysis of the plant root extract identified nine distinct chemical compounds. Among them, linoleic acid was the most abundant, comprising 39% of the total composition, making it the primary fatty acid present in the extract. Oleic acid was the second most prevalent compound, accounting for 18% of the total. The results obtained are shown in **Tables 1 and 2**, and **Figs 3 and 4**.

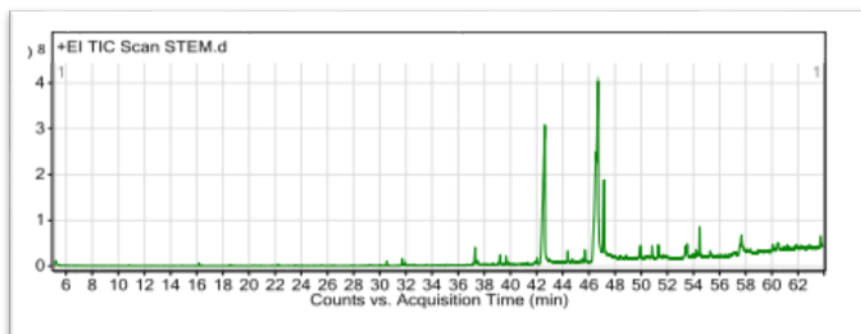
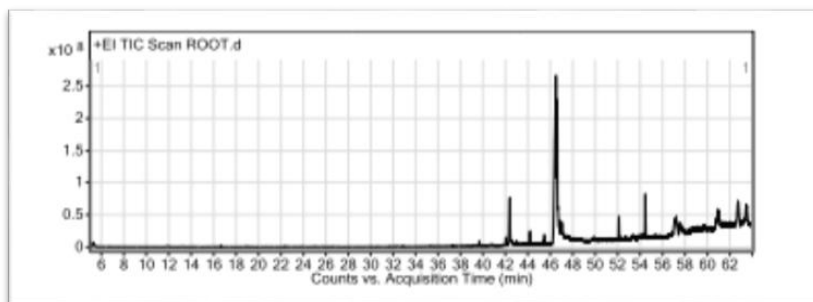


Fig. (3): GC/MS Chromatogram of fatty acid methyl ester leaves of *A. orientale*

**Table 1: GC/MS data of fatty acid methyl ester leaves of *A. oriental***

Peak No.	Fatty acid	Relative %
1	Lauric	0.61
2	Myristic	1.65
3	Pentadecanoic	2.44
4	Palmitic	29.16
5	Rumelenic	0.96
6	Linoleic	22.19
7	Alpha-linolenic	4.57
8	Oleic	26.54
9	cis-Vaccenic	7.7
10	Pristanic	0.68
11	cis-13-Eicosenoic	1.98
12	Butyl linolenic	0.36
13	Behenic	0.85



**Fig. (4): GC/MS Chromatogram of fatty acid methyl ester root of *A. oriental***

**Table 2: GC/MS data of fatty acid methyl ester root *A. oriental***

Peak No.	Fatty acid	Relative %
1	Palmitic	6.8
2	Linoleic	39
3	-linolenic	12
4	cis-Vaccenic	7.88
5	Oleic A	18
6	cis-13-Eicosenoic docosa-13,16-	21.5
7	dienoic	4.57
8	Erucic	0.45
9	Heptatriacotanoic	7.7

### Evaluation of the Antibacterial Activity of Hexane, Methanol, and Acetone Extracts from Roots and Flowers of the Plant

The results show that the hexane extract of the garlic root exhibited the strongest antimicrobial activity against both *E. coli* and *B. subtilis*, with inhibition zones of 3.2 cm and 2.3 cm respectively, and moderate activity against *C. albicans*. This suggests that non-polar compounds in the root are highly effective, especially against *E. coli*. On the other hand, the acetone extract of the root showed very weak activity against all tested microorganisms, indicating a lower concentration or weaker antimicrobial compounds in this fraction. The methanol root extract showed moderate inhibition, less than hexane but more than acetone.

Regarding the flower extracts, the hexane fraction was effective mainly against *E. coli* and *C. albicans*, while the acetone extract showed the highest activity against *B. subtilis*, indicating that polar compounds in the flowers might be responsible for inhibiting this Gram-positive bacterium. Methanol flower extracts displayed the least antimicrobial effect overall.

These findings are summarized in Table 3, which presents the inhibition zones measured during the assay.

**Table (3): Antimicrobial activity of different extracts of *A. orientalis*.**

Extract	<i>E. coli</i>	<i>B. subtilis</i>	<i>C. albicans</i>
H. root	3.2cm	2.3cm	1.4cm
A. root	0.6cm	0.7cm	0.2cm
M. root	2.1cm	1.6cm	0.8cm
H. flowers	2.5cm	1.3cm	1.6cm
A. flowers	1.4cm	2.6cm	0.4cm
M. flowers	1.1cm	0.5cm	0.7cm

### 5. Discussion

The GC/MS (Gas Chromatography–Mass Spectrometry) analysis of the roots and leaves of *Allium A. orientalis* (wild garlic) revealed a high concentration of fatty acids, particularly both saturated and unsaturated fatty acids. Among these, oleic acid (C18:1) and linoleic acid (C18:2) were found to be the most abundant components. The presence of these fatty acids indicates that the plant, particularly its roots and leaves, could have nutritional and medicinal value, especially in promoting heart health and potentially exhibiting anti-

inflammatory or antioxidant properties. These results are consistent with previous studies on other *Allium* species, which have also reported the predominance of similar fatty acids. For instance, *Allium sativum* (garlic) and *Allium cepa* (onion) have been shown to contain significant levels of linoleic and oleic acids, with linoleic acid being a major component in both species' lipid profiles [23]. Similarly, a study on *Allium tuberosum* indicated that linoleic acid was the dominant fatty acid, accounting for over 35% of the total fatty acid content. The presence of these unsaturated fatty acids, known for their antioxidant and anti-inflammatory properties, further supports the nutritional and therapeutic relevance of *Allium orientale*. These comparative findings suggest that *A. orientale*, like its relatives, holds promising potential for nutraceutical applications [24].

Garlic (*Allium oriental*) has been extensively studied for its potent biological properties, particularly its antimicrobial activity. Numerous studies have demonstrated that garlic exhibits strong antibacterial effects against various pathogenic bacteria, including Gram-positive and Gram-negative strains. Our investigation confirmed that garlic possesses high biological activity, significantly inhibiting bacterial growth across multiple tested species. The antibacterial effect is primarily attributed to the presence of allicin, a sulfur-containing compound that is formed when garlic cloves are crushed or chopped. Allicin is known for its ability to interfere with lipid synthesis in bacterial cell membranes, leading to cell lysis and death.

Several previous studies support these findings. For instance, a study by Choo et al (2020) [25] reported that allicin exhibits broad-spectrum antimicrobial activity, effectively inhibiting the growth of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Similarly, research published by Sunil et al (2025) [26] showed that garlic extracts significantly reduced the viability of oral pathogens, suggesting its potential use in dental hygiene applications.

## 6. Conclusion

The findings from both chemical and biological analyses highlight the significant antimicrobial potential of *Allium orientale*, particularly its root extracts. GC/MS analysis revealed that the leaves are rich in palmitic, oleic, and linoleic acids, while the roots are predominantly composed of linoleic acid, followed by oleic acid.

These fatty acids, especially in their non-polar form, appear to contribute to the observed bioactivity.

The hexane extract of the root demonstrated the highest antimicrobial activity, notably against *E. coli* and *B. subtilis*, indicating that non-polar compounds play a key role in the plant's antimicrobial effect. In contrast, acetone and methanol extracts showed weaker activity, suggesting a lower presence or efficacy of polar compounds.

Overall, the variation in antimicrobial activity among extracts is closely linked to the polarity of the solvent and the plant part used. These results support the potential use of *Allium orientale* root extracts, particularly the non-polar fractions, as a natural source of antimicrobial agents. Further investigations, including compound isolation and mechanism studies, are warranted to fully explore and validate these promising.

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