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Comparative Study of Biological Activity and Phytochemical Composition of Selected Extracts from Libyan and Imported Garlic (*Allium sativum* L)

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دراسة مقارنة للنشاط البيولوجي والتركيب الكيميائي النباتي لمستخلصات مختارة من الثوم الليبي والمستورد (Allium sativum L)

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الملخص:
قيّمت هذه الدراسة النشاط المضاد للبكتيريا والتركيب الكيميائي النباتي لمستخلصات بصيلات الثوم الليبي والصيني **(Allium sativum L.)** التي تم الحصول عليها باستخدام مذيبات مختلفة. تم تقييم النشاط المضاد للبكتيريا باستخدام طريقتي الانتشار بالحفر **(Well diffusion)** والانتشار بالأقراص **(Disc diffusion)** ضد أربعة أنواع بكتيرية أظهرت مستخلصات الهكسان أعلى نشاط مضاد للبكتيريا، خاصة ضد بكتيريا المكورات العنقودية **(Staphylococcus)** والإشريكية القولونية**(E. coli)** ، مقارنة بمستخلصات الإيثانول وأسيتات الإيثيل. كما أظهر مستخلص الهكسان للثوم الصيني أقوى تأثير تثبيطي بين جميع المستخلصات المختبرة، في حين لوحظ نشاط محدود أو عدم وجود نشاط في مستخلصات المذيبات الأخرى. أظهر الفحص الكيميائي النباتي أن مستخلصات الهكسان كانت غنية بالمركبات غير القطبية، بما في ذلك الدهون، والستيرويدات، والتربينويدات، والمركبات العضوية الكبريتية .أما مستخلصات أسيتات الإيثيل فقد احتوت على مستويات متوسطة من المركبات الفينولية والفلافونويدات، في حين أظهرت مستخلصات الإيثانول بتركيز 70% أعلى مستويات من المركبات النباتية القطبية، بما في ذلك الفينولات، والفلافونويدات، والصابونينات ، وتشير هذه النتائج إلى أن قطبية المذيب تؤثر في استخلاص المركبات النشطة حيويًا وفي القدرة المضادة للبكتيريا لمستخلصات الثوم.

الكلمات الدالة: الثوم (Allium sativum L) ؛ الثوم الليبي؛ الثوم الصيني؛ النشاط المضاد للبكتيريا؛ الفحص الكيميائي النباتي.

Abstract

This study evaluated the antibacterial activity and phytochemical composition of Libyan and Chinese garlic (*Allium sativum* L.) bulb extracts obtained using different solvents. The antibacterial activity was assessed by well diffusion and disc diffusion methods against four bacterial species. Hexane extracts showed the highest antibacterial activity, particularly against *Staphylococcus* and *E. coli*, compared with ethanol and ethyl acetate extracts. The Chinese hexane extract exhibited the strongest inhibitory effect among all tested extracts, while limited or no activity was observed in other solvent extracts. Phytochemical screening revealed that hexane extracts were rich in non-polar compounds, including lipids, steroids, terpenoids, and organosulfur compounds. Ethyl acetate extracts contained moderate levels of phenolics and flavonoids, whereas 70% ethanol extracts showed the highest levels of polar phytochemicals, including phenolics, flavonoids, and saponins. These findings indicate that solvent polarity influences the extraction of bioactive compounds and the antibacterial potential of garlic extracts.

Keywords: Garlic (Allium sativum L.); Libyan garlic; Chinese garlic; Antibacterial activity; Phytochemical screening.

Introduction

Garlic (*Allium sativum* L.) is a vital medicinal and culinary plant in the family Amaryllidaceae (El-Saadony et al., 2024; Ozma et al., 2023). Cultivated for thousands of years, garlic is valued for its nutritional benefits, distinct flavor, and broad biological activities such as antioxidant, antimicrobial, anti-inflammatory, and cardioprotective effects (Grijalva et al., 2023). Originating in **Central and South Asia**, it spread to Europe, Africa, and beyond through trade and agriculture (El-Saadony et al., 2024). Today, garlic is widely grown in temperate and subtropical regions and remains an economically significant crop with growing demand in food and pharmaceutical industries (FAO, 2023).

The increasing scientific interest in garlic is mainly due to its rich phytochemical profile and diverse biological activities (Tudu et al., 2022). Garlic bulbs are composed of multiple cloves wrapped in a papery outer layer and contain a variety of primary and secondary metabolites. These compounds play a crucial role in the plant's nutritional, pharmacological, and industrial value (Okoro et al., 2023). The concentration and makeup of these metabolites are affected by genetic factors, environmental conditions, cultivation methods, storage, and processing techniques (Martins et al., 2016).

Chemically, garlic is characterized by a high content of organosulfur compounds, which are considered the principal bioactive constituents responsible for many of its medicinal properties. Fresh garlic contains alliin (S-allyl-L-cysteine sulfoxide) (Melguizo et al., 2022), a stable sulfur-containing amino acid derivative that is converted into allicin by the enzyme alliinase when the cloves are crushed, chopped, or otherwise damaged. Allicin is an unstable but highly reactive compound that subsequently decomposes into numerous sulfur-containing metabolites, including diallyl sulfide, diallyl disulfide, diallyl trisulfide, ajoene, and S-allyl cysteine. These compounds are largely responsible for the characteristic aroma of garlic and its biological activities (El-Saadony et al., 2024; Sen et al., 2025).

Garlic is rich in organosulfur compounds, which are key bioactive constituents responsible for its medicinal properties. Fresh garlic contains alliin (S-allyl-L-cysteine sulfoxide) (Melguizo et al., 2022), a stable sulfur-containing amino acid derivative. When garlic cloves are crushed or damaged, the enzyme alliinase converts alliin into allicin, an unstable but highly reactive compound. Allicin then breaks down into several sulfur-containing metabolites, including diallyl sulfide, diallyl disulfide, diallyl trisulfide, ajoene, and S-allyl cysteine. These compounds contribute to garlic's distinctive aroma and its biological activities (El-Saadony et al., 2024; Sen et al., 2025).

In addition to sulfur-containing compounds, garlic contains a variety of phytochemicals such as flavonoids, phenolic acids, saponins, tannins, polysaccharides, amino acids, vitamins, and minerals. Important phenolic compounds identified in garlic include gallic acid, caffeic acid, ferulic acid, and p-coumaric acid, which contribute to its antioxidant capacity (Melguizo-Rodríguez et al., 2022; Tudu et al., 2022). Garlic is also a valuable source of essential minerals such as selenium, calcium, potassium, phosphorus, magnesium, and iron. The synergistic

interaction among these constituents enhances the biological effectiveness of garlic and increases its value as a functional food and medicinal plant (Verma et al., 2023).

Another significant biological impact of garlic is cardiovascular prevention. By lowering blood pressure, enhancing lipid profiles, preventing oxidative stress, and offering vascular protection, garlic extracts and sulfur-containing substances have been shown to promote cardiovascular health (Ried, 2016). These characteristics demonstrate garlic's ability to lower the risk of cardiovascular illnesses and improve vascular health (Lu et al., 2024).

Garlic-derived chemicals have been shown in recent studies to have anti-inflammatory, antidiabetic, neuroprotective, and anticancer properties. Organosulfur compounds have the ability to alter signaling pathways related to cellular proliferation, immunological control, apoptosis, and inflammation. Garlic-based functional foods, nutritional supplements, and pharmaceutical formulations targeted at controlling or preventing chronic diseases have been developed as a result of these effects (Jain et al., 2025).

The present study was designed to compare the antibacterial activity of different local and imported garlic samples. Three different extracts were prepared from each type of garlic using appropriate extraction methods. The antibacterial efficacy of the prepared extracts was evaluated in order to determine possible variations in biological activity between local and imported garlic samples.

Materials and Methods

Collection and Preparation of Garlic Samples

Fresh garlic samples were collected from two different sources for comparative analysis. A total of 500 g of locally grown Libyan garlic was obtained from agricultural farms in the Jalu region, a town located southeast of Benghazi, Libya. In addition, 500 g of imported Chinese garlic was purchased from local commercial markets.

The garlic bulbs from both sources were manually peeled to remove the outer dry scales and non-edible layers. The peeled cloves were thoroughly washed with distilled water to eliminate adhering dust, soil particles, and other contaminants. Subsequently, the cloves were spread on clean trays and allowed to air-dry at room temperature until all surface moisture had evaporated.

After drying, representative portions of the garlic samples were prepared for extraction. Approximately 100 g of fresh garlic cloves from each source were accurately weighed using an analytical balance. The weighed cloves were then crushed and homogenized using a mortar and pestle in the laboratories of the Faculty of Science, University of Ajdabiya, Libya, to obtain a uniform paste. Homogenization was carried out to increase the surface area of the plant material and facilitate the release and extraction of bioactive constituents during subsequent solvent extraction procedures (Harborne, 1998; Houghton and Raman, 2012).

The resulting homogenized garlic paste was immediately subjected to sequential solvent extraction for the isolation of phytochemical constituents.

Preparation and Sequential Solvent Extraction of Garlic Samples

Fresh garlic samples were subjected to sequential solvent extraction using solvents of increasing polarity according to the phytochemical extraction procedures described by Harborne (1998) and Sasidharan et al. (2011). Briefly, 100 g of homogenized garlic paste was immersed in n-hexane and kept for 72 h at room temperature with continuous stirring to facilitate the extraction of non-polar bioactive compounds (Harborne, 1998).

After extraction, the mixture was filtered through a Büchner funnel under vacuum. The filtrate was collected and concentrated by solvent evaporation in a drying oven at a temperature below 40°C to avoid degradation of thermolabile compounds (Azwanida, 2015). The residue remaining after hexane extraction was subsequently extracted with ethyl acetate using the same procedure for 72 h with continuous stirring (Sasidharan et al., 2011).

Following filtration and concentration of the ethyl acetate extract, the residual plant material was extracted with 70% ethanol for 72 h under continuous stirring. The mixture was filtered through a Büchner funnel, and the filtrate was concentrated at a temperature below 40°C to obtain the crude ethanolic extract (Houghton & Raman, 2012).

This sequential extraction process yielded three crude extracts (hexane, ethyl acetate, and 70% ethanol extracts) from Libyan garlic and three corresponding extracts from Chinese garlic. All extracts were transferred into sterile amber glass bottles and stored at 4°C until further use (Sasidharan et al., 2011).

Qualitative Phytochemical Screening

The qualitative phytochemical screening of the garlic extract was performed using standard procedures. Terpenoids were detected by the Salkowski test using chloroform and concentrated sulfuric acid. Steroids were identified by the Liebermann–Burchard test with acetic anhydride and concentrated sulfuric acid. Lipids and oils were detected using the Sudan III test. Phenolic compounds were determined by the Ferric chloride test (5% $FeCl_3$). Flavonoids were identified by the Shinoda test using magnesium turnings and concentrated hydrochloric acid. Alkaloids were detected by Dragendorff's test, while saponins were identified using the Froth test (Evans, W. C. 2009).

Test Microorganisms

Clinical isolates of Staphylococcus spp., Streptococcus spp., Klebsiella spp., and E. coli spp., were obtained from the Microbiology Laboratory of Care Laboratory, Benghazi, Libya. The isolates were identified using conventional microbiological methods, including Gram staining, colony morphology, and standard biochemical tests.

Preparation of Bacterial Inoculum

Fresh bacterial cultures were sub cultured on appropriate media and incubated at 37°C for 18–24 h. A few well-isolated colonies were suspended in sterile normal saline, and the turbidity was adjusted to the 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) using visually.

Antibacterial Activity Assay

The agar well diffusion method was used to evaluate the antibacterial activity of the garlic extracts, whereas the Kirby–Bauer disc diffusion method was employed to determine the susceptibility of the bacterial isolates to the reference antibiotic (Amikacin) and compered with antibacterial activity of the garlic extracts (Wayne, 2011)..

Mueller–Hinton agar plates (supplemented with 5% sheep blood for Streptococcus spp., where applicable) were inoculated uniformly with standardized bacterial suspensions using sterile cotton swabs. Wells measuring 6 mm in diameter were punched aseptically into the agar, and 100 µL of each extract was introduced into the respective wells (Valgas, et al., 2007).

Commercial amikacin (30 µg) discs (Oxoid, UK) were used as the positive control served as positive controls, while the extraction solvent served as the negative control. The plates were incubated at 37°C for 18–24 hours, after which the diameters of the inhibition zones were measured in millimeters (mm)

Reset

Phytochemical Comparison of Libyan and Chinese Garlic Extracts

The distribution of phytochemical elements differed according to solvent polarity, as shown by a comparative phytochemical screening of Chinese and Libyan garlic extracts produced by successive extraction using hexane, ethyl acetate, and 70% ethanol. Both garlic samples' hexane extracts showed moderate to high concentrations of non-polar substances, such as lipids, steroids, terpenoids, and volatile. The ethyl acetate extracts' intermediate polarity was indicated by the moderate levels of flavonoids and phenolic chemicals. On the other hand, polar phytochemicals, including phenolic compounds, flavonoids, saponins, and components associated to antioxidants, were most abundant in the 70% ethanol extracts. Both Chinese and Libyan garlic showed similar phytochemical patterns, albeit there were minor differences in several components' intensities between the two samples. Overall, the results indicate that solvent polarity plays a significant role in determining the extraction efficiency and distribution of phytochemical constituents. As shown in Table 1.

Table (1): shows the Phytochemical Comparison of Libyan and Chinese Garlic Extracts

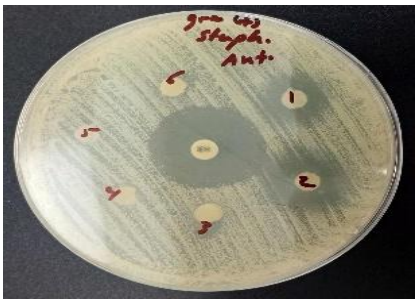
Phytochemical Group	Hexane (Libyan)	Hexane (Chinese)	Ethyl acetate (Libyan)	Ethyl acetate (Chinese)	70% Ethanol (Libyan)	70% Ethanol (Chinese)
Terpenoids	++	++	+	+	-	-
Steroids	++	++	+	+	-	-
Lipids & Oils	+++	+++	-	-	-	-
Phenolics	-	-	++	++	+++	+++
Flavonoids	-	-	+	+	++	++
Alkaloids	-	-	+	+	+	+

Antibacterial Activity of Libyan and Chinese Garlic (*Allium sativum* L.) Bulb Extracts

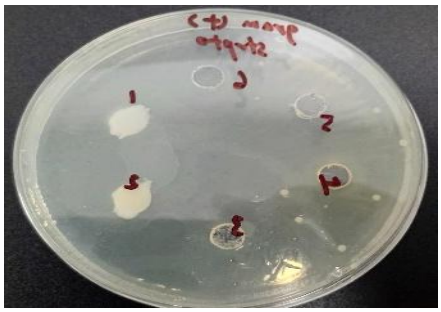
The results presented in Table 2 and Figure 1 showed that the hexane extracts of both Libyan and Chinese garlic (*Allium sativum* L.) exhibited the highest antibacterial activity among all tested extracts. The Chinese hexane extract produced the largest inhibition zones against *Staphylococcus* (30 mm) and *E. coli* (28 mm), followed by the Libyan hexane extract with inhibition zones of 25 mm and 20 mm, respectively. Both hexane extracts showed moderate activity against *Klebsiella* (15 mm), whereas no antibacterial activity was detected against *Streptococcus*. The ethanol extracts of both garlic samples showed no antibacterial activity, except for weak activity (<10 mm) of the Libyan ethanol extract against *Staphylococcus* and *Klebsiella*. Similarly, the ethyl acetate extracts exhibited no activity against the tested bacteria, except for the Chinese ethyl acetate extract, which showed an inhibition zone of 15 mm against *Staphylococcus*. Overall, the hexane extracts demonstrated the greatest antibacterial activity compared with the ethanol and ethyl acetate extracts.

Table (2): shows the antibacterial activity of all Libyan and Chinese garlic (*Allium sativum* L.) bulb extracts against the four tested bacterial species, as determined by the Well Diffusion Method..

Extract	Staphylococcus	Streptococcus	Klebsiella	E. coli
1. Libyan Hexane	25mm	R	15mm	20mm
2.Chinese Hexane	30mm	R	15mm	28mm
3. Libyan Ethanol	R<10	R	R<10	R
4.Chinese Ethanol	R	R	R	R
5. L. Ethyl Acetate	R	R	R	R
6.Ch.Ethyl Acetate	15	R	R	R



1- Staphylococcus



2- Streptococcus

3- Klebsiella

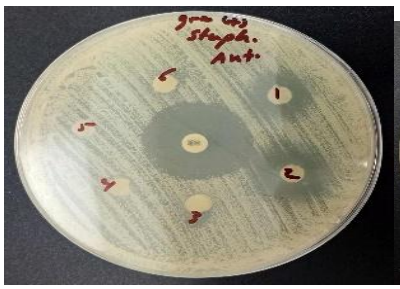
4- E. coli

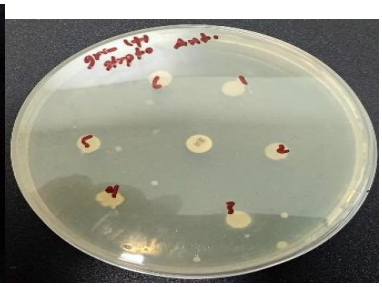
Figure (1): Antibacterial Activity of the all Extracts of Libyan and Chinese Garlic (Allium sativum L.) Bulbs Against Staphylococcus, Streptococcus, Klebsiell and , E. coli by the Well Diffusion Method

Meanwhile, the antibacterial activity of Libyan and Chinese garlic (*Allium sativum* L.) bulb extracts was evaluated using the disc diffusion method. The results showed that the hexane extracts exhibited the highest antibacterial activity among all tested extracts. The Chinese hexane extract produced the largest inhibition zones against *E. coli* (40 mm) and *Staphylococcus* (20 mm), while the Libyan hexane extract showed inhibition zones of 10 mm and 15 mm, respectively. Weak activity (<10 mm) was observed only for the Chinese hexane extract against *Klebsiella*, whereas no activity was detected against *Streptococcus*. In contrast, the ethanol and ethyl acetate extracts of both Libyan and Chinese garlic showed no antibacterial activity against any of the tested bacterial species, as shown in Table 3 and Figure 2.

Table (3): shows the antibacterial activity of all Libyan and Chinese garlic (*Allium sativum* L.) bulb extracts against the four tested bacterial species, as determined by the disc diffusion method.

Extract	Staphylococcus	Streptococcus	Klebsiella	E. coli
1. Libyan Hexane	15	R	R	10
2. Chinese Hexane	20	R	R<10	40
3. Libyan Ethanol	R	R	R	R
4. Chinese Ethanol	R	R	R	R
5. L. Ethyl Acetate	R	R	R	R
6.Ch.Ethyl Acetate	R	R	R	R





1- Staphylococcus 2- Streptococcus

3- Klebsiell 4- E. coli

Figure (2): Antibacterial Activity of the all Extracts of Libyan and Chinese Garlic (Allium sativum L.) Bulbs Against Staphylococcus, Streptococcus, Klebsiell and , E. coli by the Disc Diffusion Method

Discussion

The phytochemical screening results indicate that solvent polarity had a significant effect on the extraction of bioactive compounds from both Libyan and Chinese garlic. Hexane extracts were rich in non-polar compounds, including lipids, steroids, terpenoids, and organosulfur compounds, consistent with previous reports (Batiha et al., 2020; Zainudin et al., 2019). Ethyl acetate extracts contained moderate amounts of phenolics and flavonoids, demonstrating its effectiveness in extracting semi-polar compounds (Gopinath et al., 2022). In contrast, the 70% ethanol extracts showed the highest levels of phenolics, flavonoids, saponins, and antioxidant-related compounds, confirming that polar solvents are more efficient for extracting hydrophilic phytochemicals. Overall, both Libyan and Chinese garlic exhibited similar phytochemical profiles, with minor differences likely related to geographical origin and growing conditions.

Regarding the biological activity, the hexane extract exhibited the highest antibacterial activity, particularly against *Staphylococcus* spp. and *Escherichia coli*, whereas the ethanol and ethyl acetate extracts demonstrated weak or no inhibitory effects against most bacterial isolates.

The superior antibacterial activity of the hexane extract may be attributed to the presence of lipophilic organosulfur compounds in garlic, including diallyl sulfide, diallyl disulfide, diallyl trisulfide, and ajoene, all of which possess well-documented antimicrobial properties. These compounds are more readily dissolved in non-polar solvents such as hexane, resulting in a higher concentration of biologically active constituents in the extract. Once released, these sulfur-containing compounds can disrupt bacterial cell membranes, inhibit essential metabolic enzymes, and interfere with protein synthesis, ultimately suppressing bacterial growth.

With respect to bacterial susceptibility, *Staphylococcus* spp. were the most susceptible organisms among the tested isolates, whereas *Streptococcus* spp. exhibited complete resistance to nearly all extracts. Among the Gram-negative bacteria, susceptibility varied, with *Escherichia coli* showing greater sensitivity than *Klebsiella* spp. These differences may be explained by variations in bacterial cell envelope structure. Gram-positive bacteria possess a thick peptidoglycan cell wall but lack an outer membrane, allowing antimicrobial compounds to penetrate more readily. In contrast, Gram-negative bacteria possess an additional outer membrane composed of lipopolysaccharides, which serves as a permeability barrier and limits the entry of many antimicrobial agents.

The observed antibacterial activity is consistent with previous studies demonstrating that garlic exhibits broad-spectrum antimicrobial activity, with its efficacy largely influenced by the extraction method and the composition and stability of sulfur-containing compounds. Non-polar solvents have been reported to extract greater amounts of lipophilic organosulfur constituents, thereby enhancing antibacterial activity, whereas the instability of compounds such as allicin and differences in solvent polarity can significantly affect the antimicrobial potential of garlic extracts (Verma et al., 2023; Jikah et al., 2024).

Conclusion

Overall, the findings demonstrate that solvent polarity plays a critical role in determining the phytochemical composition and antibacterial activity of garlic extracts. Both Libyan and Chinese garlic exhibited comparable phytochemical profiles, with 70% ethanol producing the highest yield of polar bioactive compounds, while hexane was more effective in extracting non-polar organosulfur constituents. The hexane extracts displayed the strongest antibacterial activity, particularly against *Staphylococcus* spp. and *Escherichia coli*, highlighting the antimicrobial potential of lipophilic sulfur-containing compounds. These results emphasize the importance of selecting an appropriate extraction solvent to maximize the recovery of biologically active constituents and support the potential use of garlic as a natural source of antimicrobial agents.

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