

A comparative Evaluation of Antibacterial Potential of Aqueous and Ethanolic Extracts of Yemeni and Indian *Allium cepa* against Some Human Pathogenic Bacteria

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Abstract: In Yemen, many medicinal plants have been used for treatment of various diseases for thousands of years. The onion plant (*Allium cepa*) was one of these medicinal plants. The concept of the current study was based on the antimicrobial activity of different concentrations (12.5, 25, 50, 100, 200 $\mu\text{g mL}^{-1}$) of Aqueous and Ethanolic extracts of Yemeni (local) and Indian (Imported) *A. cepa* against five pathogenic Gram negative and Gram positive bacteria, *Escherichia coli*, *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Staphylococcus aureus* using agar well diffusion method. Agar dilution method was used for determination of Minimum Inhibitory Concentration (MIC). The current study detected that the Yemeni *A. cepa* achieved antibacterial activity higher than the activity of Indian *A. cepa*, and the ethanolic extracts were stronger than the aqueous extracts for both onions. Therefore, the highest inhibition activity achieved by the aqueous and ethanolic extracts of Yemeni *A. cepa* was detected for *K. pneumonia* (20.5 \pm 0.5 mm) and *B. cereus* (22.5 \pm 0.5 mm), respectively, while for the aqueous and ethanolic extracts of Indian *A. cepa*, the highest inhibitory activity was detected for *S. aureus* (14.0 \pm 2.0 mm) and *K. pneumonia* (20.0 \pm 0.0 mm), respectively. The difference in the antibacterial activities was significant ($p < 0.05$) except in application of aqueous Yemeni extracts against *P. aeruginosa*. Moreover, *S. aureus* was not inhibited by all concentrations of aqueous Yemeni and ethanolic Indian *A. cepa*, and it was inhibited weakly by other extracts. MIC values were different, the lowest value (12.5 $\mu\text{g mL}^{-1}$) was recorded by Yemeni aqueous extract against *E. coli* and *B. cereus*, while the lowest value (25 $\mu\text{g mL}^{-1}$) was recorded by Yemeni ethanolic extract against *K. pneumonia*, *B. cereus* and *S. aureus*. Finally, the researchers recommend to conduct extensive and comprehensive studies about the effects of Yemeni *A. cepa* on fungi and other harmful organisms.

Key words: *Allium cepa*; medicinal; antibacterial; ethanolic extracts; *Escherichia coli*.

1. Introduction:

Yemeni medicinal plants have been used for many years in the treatment of a number of human diseases by the community, specifically in traditional medicine. They are considered the main source of new, natural, and safe drugs to be utilized in managing diseases as an effective and harmless alternative medicine [1]. Onion (*Allium cepa*) is one of the most important condiment edible plants that are placed under the family Amaryllidaceae which contain the powerful sulfur and other numerous phenolic compounds [2]. Alliums have had an important nutritional and therapeutic role for thousands of years, and can be provided in the form of a capsule, powder, oil and cooked *A. cepa* extract [3], [4].

A. cepa is one of the most popular food ingredients widely used all over the world. They contain high concentrations of folic acid, vitamin B6, magnesium, calcium, potassium, and phosphorus as well as vitamins and minerals [2], [4]. The pungence of alliums is caused by the large number of sulfur compounds. One of their most potent and active, powerful antibiotic component is the organosulfur-containing compound called allicin [5]. The enzyme allinase is released when the tissues of mature, intact cysteine sulfoxides containing alliums are chopped, converting the cysteine sulfoxides into the thiosulfinates. Flavonoids such as kaempferol and quercetin, alk(en)yl cysteine sulfoxides including S-propyl cysteine sulfoxide, S-methyl cysteine sulfoxide, cycloalliin, thiosulfinates, and sulfides are the

main compounds of the plant and most are reactive, volatile, lachrymatory and odor producing [6] [7], [8], [9].

Alliums are widely used as an antimicrobial agent, and showed anticancer, antidiabetic, antioxidant, antiplatelet, antihypertensive, and antidepressant effects and neuroprotective, anti-inflammatory, and antiparasitic effects. Alliums have beneficial effects on the digestive, circulatory, and respiratory systems, as well as on the immune system [10], [11], [12], [13]. *A. cepa* extracts have been shown to prevent and reduce hyperlipidemia, hypertension, thrombosis, atherosclerosis and hepatitis [14], [15], [16], [17]. Consumption of the onion and its products is associated with the cold and flu prevention [18], and they have been traditionally used as diuretic, carminative, expectorant, stomachic, antibacterial, antifungal, antiviral and insecticidal agents [19].

Allium cepa has been identified in the treatment of infectious diseases. Due to the contained bioactive components, they exhibited a broad antibacterial activity against a panel of both gram negative and gram positive bacteria including species of bacteria such as: *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, *Streptococcus*, *Shigella*, *Bacillus cereus*, *B. cerea*, *Clostridium*, *Salmonella enteritidis*, *S. typhimurium*, *Klebsiella*, *Proteus*, *Helicobacter pylori*, *Listeria monocytogenes* and *Pseudomonas aeruginosa* [20], [21], species of yeasts such as: *Saccharomyces cerevisiae*, *Candida albicans*, *C. tropicalis* [22], and species of fungi such as: *Fusarium oxysporum*, *Penicillium cyclopium*, and *Aspergillus Niger* [23].

In Yemen, the herbal medicine is employed widely for treatment of various diseases. Recent investigations were published during the last decade about the antimicrobial, pharmacological and chemical properties of a minority of traditionally used medicinal herbal plants in Yemen [1], [24], [25], [26], [27]. Considering to the importance of these untapped research area, the aim of this study was to evaluate the in vitro potential of one traditionally used medicinal plant (*A. cepa*) grown in Yemen, and their aqueous and ethanolic extracts as antibacterial agents. This research was growing on these plants as cheap, safe, and more acceptable for people than synthetic antibiotics.

2. Materials and methods:

2.1. Plant materials:

A. cepa bulbs were collected from Hadhramout farms (Local, Bafteem), and Indian *A. cepa* tubers (Imported) were purchased from the local markets of Hadhramout, Yemen in April 2020. The bulbs were free from any chemical pre-harvest treatment, and free from any deformities. The selected bulbs of *A. cepa* were peeled, washed, dried in room temperature, milled by an electric grind and stored at 4°C. The extraction was carried out using 80% ethanol (BDH chemical) and de-ionized water prepared by Milli-Q Plus system (Millipore, Bedford, USA) [1].

2.2. Bacterial strains:

In vitro antimicrobial studies were mediated against five human pathogenic Gram positive and Gram negative bacteria, *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. The strains were provided from National Center

for Public Health Laboratories, Mukalla branch, and stored at 4°C in the refrigerator. The researchers confirmed the type of bacteria in the Microbiology Laboratory of the Faculty of Environmental Sciences and Marine Biology, Hadhramout University.

2.3. Media used:

Nutrient Agar and broth, peptone water, MacConkey-Agar, Eosin methylene blue Agar, *Pseudomonas* Isolation Agar, Brain–Heart Infusion Agar and broth and Muller Hinton Agar were purchased from HiMedia – Mumbai – India, and used for preparation of inocula, confirmatory identification and preservation of test bacteria and evaluation of antibacterial potential.

2.4. Preparation of inoculum:

The bacterial inoculum was prepared using Brain Heart Infusion broth (BHI) and it was growing at 37°C for 24 h in an incubator (J.P. Selecta, Spain). Using peptone water, the actively growing inocula were prepared to a density of 108 cfu/ml [28], [29] as initial cell counts.

2.5. Preparation of aqueous extracts:

Ninety grams of fresh tuber powder of local and Indian *L. cepa* were mixed separately with deionized water (200 ml), blended for 15 min, then the mixtures were filtered using sterile gauze to remove the solids, this was followed by centrifugation at 4500 rpm for 30 min at 20 °C. The resulting supernatant was filtrated using vacuum pump (Heidolph Instruments GmbH, Germany) through Whatman filter paper no. 1. Finally, the separately collected extract was stored in a refrigerator at 4°C [1], [30].

2.6. Preparation of ethanolic extracts:

Amount of fifty grams of each powder was extracted using 200 ml of 80% ethanol and left for 24 h in a closing conical flask. Whatman filter paper no. 1 was used to filter the resulting liquid four times. The filtrates were concentrated in a rotary evaporator (IKA-WERKE, Germany) at 45°C to eliminate ethanol. The water was removed from the aqueous residue by drying the samples in an oven 45°C (Daihan Lab-Tech Co.) for about 48 h [1].

2.7. Determination of antibacterial activity:

Agar well diffusion method was used to determine the antibacterial activities of the previously prepared extracts of local and imported *L. cepa* depending on the measurement of the Inhibition Zone Diameter (IZD). A BaSO₄ turbidity (0.5) McFarland standard was used for standardization of the inoculum density of the five bacterial species. Each prepared bacterial suspension was uniformly spread using a sterile cotton swab on Muller Hinton agar (MHA) (HiMedia, Mumbai, India), separately. Six wells were bored in the inoculated plates using a 6 mm diameter sterile borer. Dimethyl sulfoxide, DMSO (1%) (BDH chemical) was used for preparation of five serial dilutions for the final product of each extract yielding concentrations of, 12.5, 25, 50, 100, 200 µg mL⁻¹. Using a micropipette, 90µl from each extract dilution were poured into each well in the inoculated plates. Sterile aqueous DMSO (1%) was used as a negative control, and the Ofloxacin antibiotic disk, 5µg (HiMedia, India) was used as a positive control using Kirby-Bauer disk diffusion technique depending on the guidelines of the Clinical and Laboratory Standard Institute (CLSI) [31]. The inoculated plates were incubated for 24 h at 36°C ± 1°C under aerobic

conditions. The previous procedure was performed in duplicate. After 24 h incubation, the bacteria had grown. Using a ruler, the inhibition of bacterial growth representing by IZD was measured in millimeters [1], [32], [33]. The Minimum Inhibitory Concentration, MIC (The lowest concentration of the antibacterial plant extract which completely inhibited the microbial growth) was determined using the Agar Dilution Method under the above conditions. The results were expressed in micrograms per milliliter [34], [35].

Statistical analysis:

The measured growth inhibition zone diameters achieved by the crude extracts were expressed as mean value \pm standard deviation. Statistical differences between the antibacterial activities of aqueous and ethanolic extracts were detected using the Statistical Package for Social Sciences (SPSS) software version 26.0 (SPSS, Chicago, IL, USA).

The analysis of variance (One way-ANOVA) followed by Tukey Test were conducted to test the differences between the antibacterial activities of each type of bacteria, and to test the dimensional comparisons between the aqueous and ethanolic extract concentrations. P value lower than 0.05 ($p < 0.05$) was considered significant.

Results:

3.1. Determination of the average inhibition zone diameters induced by the aqueous and ethanolic extracts of local and imported *A. cepa*:

The current study achieved distinct differences in the inhibition activities for the different concentrations of *A. cepa* extracts against *E. coli*. Extracts of *A. cepa* inhibited the growth of *E. coli*, whereas the aqueous extracts of the local and imported *A. cepa* had a better inhibitory effect more than that of an ethanolic extract. However, the aqueous extract of local *A. cepa* induced higher inhibition effect than the aqueous extract of imported *A. cepa* (Figure 1 and Table 1).

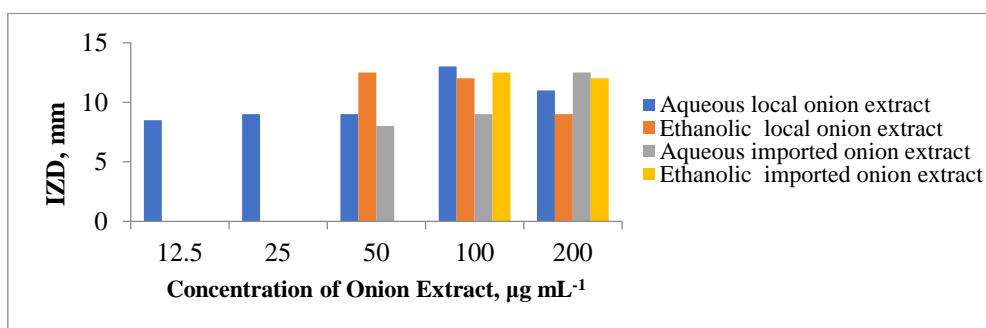


Figure 1. The effect of the concentration of the aqueous and ethanolic extract of the local and imported *A. cepa* on the average inhibition zone diameters of *E. coli*. IZD : Inhibition Zone Diameter.

For the local *A. cepa* extracts, the aqueous extract showed apparent inhibitory activity against *E. coli* at all concentrations, especially the concentration of 100 µg mL⁻¹, which inhibited the growth more (13.0 mm) than all other concentrations. The lowest inhibition activity (8.5 mm) was achieved by the concentration of 12.5 µg mL⁻¹. For the imported *A. cepa* extracts, there was no inhibition activity at concentrations of 12.5 and 25 µg mL⁻¹ for the aqueous and ethanolic extracts. While the rest concentrations of aqueous and ethanolic extracts were active giving the highest inhibition activity (12.5 mm) at 200 and 100 µg mL⁻¹,

respectively. The concentration 50 µg mL⁻¹ showed the lowest inhibition activity (8.0 mm) for aqueous extract and no inhibition activity for ethanolic extract (Figure 1 and Table 1). *A. cepa* extracts inhibited the growth of *P. aeruginosa*, whereas the statistical averages indicated that the aqueous extracts of the local *A. cepa* recorded a better inhibitory effect than ethanolic extracts, as well as the ethanolic extracts of the imported *A. cepa* were better and more inhibiting than the aqueous extracts. However, the aqueous extracts of local *A. cepa* recorded inhibition effect better than the aqueous extract of Imported *A. cepa* against *P. aeruginosa* (Figure 2 and Table 1).

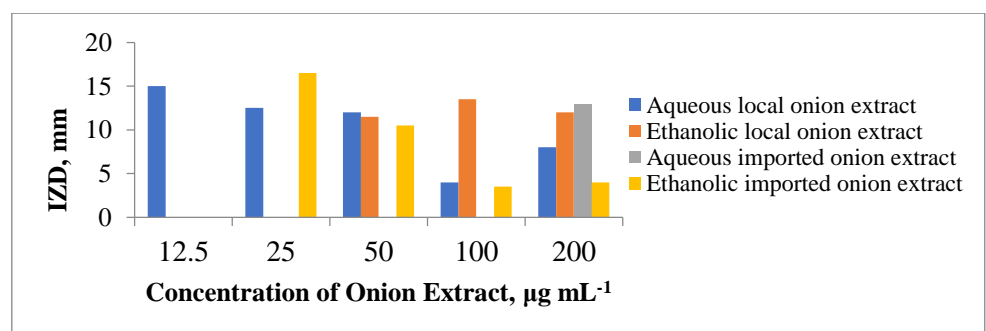


Figure 2. The effect of the concentration of the aqueous and ethanolic extract of the local and imported *A. cepa* on the average inhibition zone diameters of *P. aeruginosa*. IZD : Inhibition Zone Diameter.

The previous figure indicated that all extracts of *A. cepa* did not inhibit the growth of *P. aeruginosa* at a concentration of $12.5 \mu\text{g mL}^{-1}$ except for the aqueous extract of local *A. cepa*, which gave an inhibition activity of 15.0 mm. At the concentration of $25 \mu\text{g mL}^{-1}$, the ethanolic extract of the imported *A. cepa* induced the highest inhibition value of 16.5 mm, and the aqueous extract of the local *A. cepa* gave 12.5 mm, while the ethanolic extracts of the local *A. cepa* and the aqueous extracts of the imported *A. cepa* did not affect. The extracts of the ethanolic imported *A. cepa* ($100 \mu\text{g mL}^{-1}$), aqueous local *A. cepa* ($100 \mu\text{g mL}^{-1}$), and the ethanolic imported *A. cepa* ($200 \mu\text{g mL}^{-1}$) exhibited the lowest inhibition activities of 3.5, 4.0 and 4.0 mm, respectively (Figure 2 and Table 1).

Regarding *K. pneumonia*, the researchers observed that the

inhibition activity of all *A. cepa* extracts increased with the increasing of their concentrations. Table (1) recorded that the aqueous extract of the local *A. cepa* achieved antibacterial activity against *K. pneumonia* higher than that of its ethanolic extract, as well as the ethanolic extract of the imported *A. cepa* was more inhibiting than its aqueous extract, thus we found that the ethanolic extract of the imported *A. cepa* exhibited an antibacterial activity higher than that of the aqueous extract of the local *A. cepa* against *K. pneumonia*. It was indicated that in the case of the ethanolic extracts of local *A. cepa* and the aqueous extracts of imported *A. cepa* there was no inhibition activity at the concentration of $12.5 \mu\text{g mL}^{-1}$. Also, at the concentration of $25 \mu\text{g mL}^{-1}$, there was no inhibition activity of the ethanolic extract of the local *A. cepa* (Figure 3 and Table 1).

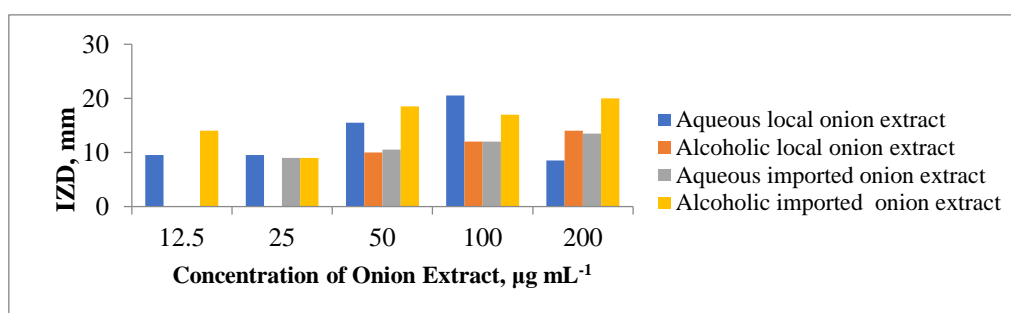


Figure 3. The effect of the concentration of the aqueous and ethanolic extract of the local and imported *A. cepa* on the average inhibition zone diameters of *K. pneumonia*. IZD : Inhibition Zone Diameter.

Figure 3 showed that the inhibitory effect of the aqueous extract of local *A. cepa* reached a value (25.0 mm) at a concentration of $100 \mu\text{g mL}^{-1}$ greater than the inhibitory effect of its ethanolic extract (20.0 mm) at a concentration of $200 \mu\text{g mL}^{-1}$. Also, the ethanolic extract of imported *A. cepa* achieved an inhibition activity (20.0 mm) at a concentration of $200 \mu\text{g mL}^{-1}$ and inhibited the growth of *K. pneumonia* more than that of its aqueous extract (13.5 mm) at the same concentration. The study also showed that the aqueous extract of the local *A. cepa* inhibited the growth of *K. pneumonia* with an inhibition activity higher than the ethanolic extract of the imported *A. cepa*.

The growth of *B. cereus* bacteria was not inhibited by the local and imported *A. cepa* extracts at all concentrations, especially 12.5 , 25 and $50 \mu\text{g mL}^{-1}$, except the aqueous extract of local *A. cepa* which inhibited the growth (13.5 mm) at concentration $12.5 \mu\text{g mL}^{-1}$ and the ethanolic extract of local *A. cepa* (10.5 mm) and the aqueous extract of imported *A. cepa* (9.5 mm) at $50 \mu\text{g mL}^{-1}$ and the ethanolic extract of local *A. cepa* (22.5 and 19.0 mm) at 100 and $200 \mu\text{g mL}^{-1}$, and the ethanolic extract of imported *A. cepa* (18.0 and 8.0 mm) at 100 and $200 \mu\text{g mL}^{-1}$, respectively (Figure 4 and Table 1).

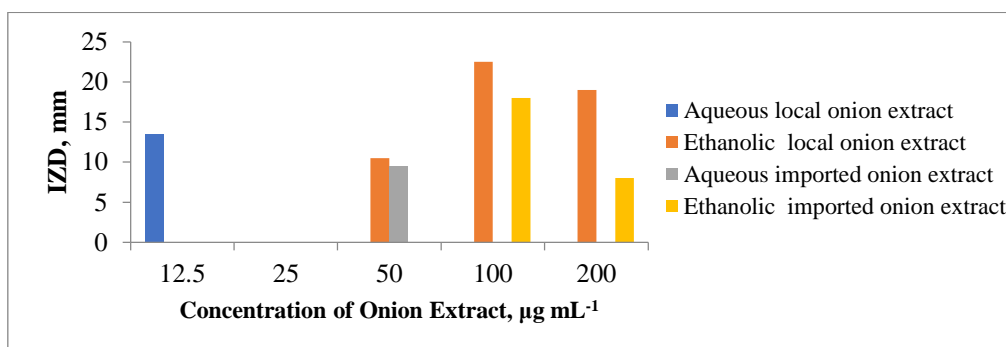


Figure 4. The effect of the concentration of the aqueous and ethanolic extract of the local and imported *A. cepa* on the average inhibition zone diameters of *B. cereus*. IZD : Inhibition Zone Diameter.

The most effective inhibition activity was achieved by the ethanolic extract of the local *A. cepa* (22.5, 19.0 and 10.5 mm at concentrations of 100, 200 and 50 $\mu\text{g mL}^{-1}$, respectively against *B. cereus*.

The ethanolic extract of the local *A. cepa* induced an

inhibitory effect against *S. aureus* higher than that of its aqueous extract, as well as the aqueous extract of the imported *A. cepa* was more inhibiting than its ethanolic extract and more inhibiting than the aqueous extract of the local *A. cepa* against *S. aureus* (Figure 5 and Table 1).

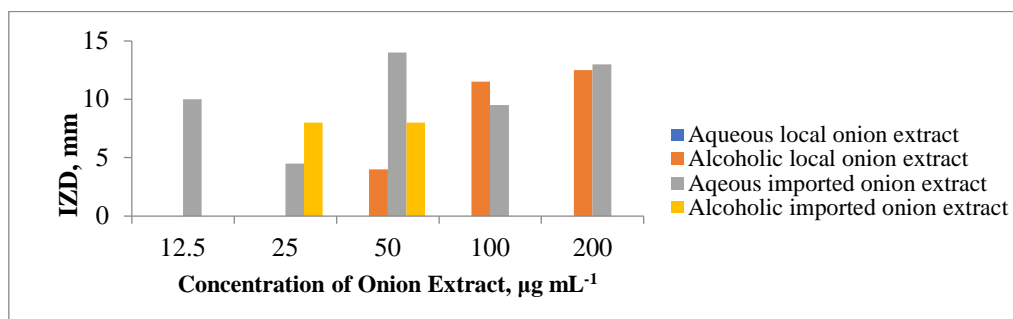


Figure 5. The effect of the concentration of the aqueous and ethanolic extract of the local and imported *A. cepa* on the average inhibition zone diameters of *S. aureus*. IZD : Inhibition Zone Diameter.

The previous figure showed that all the concentrations of the aqueous extract of local *A. cepa* did not inhibit the growth of *S. aureus*, as well as its ethanolic extract at the tow concentrations of 12.5 and 25 $\mu\text{g mL}^{-1}$, as well as the ethanolic extract of imported *A. cepa* at concentrations of 12.5, 100 and 200 $\mu\text{g mL}^{-1}$. It was noticed that the highest

inhibition activity (14.0 mm) among all extracts was for the aqueous extract of the imported *A. cepa* at a concentration of 50 $\mu\text{g mL}^{-1}$, followed by (13.0 mm) at a concentration of 200 $\mu\text{g mL}^{-1}$, followed by the inhibition activity of the ethanolic extract of the local *A. cepa* (12.5 mm) at a concentration of 200 $\mu\text{g mL}^{-1}$, finally followed by (11.5 mm) at a concentration of 100 $\mu\text{g mL}^{-1}$.

Table 1. Average* inhibition zone diameters (antibacterial activity), mm, induced by the aqueous and ethanolic extracts of local and imported *A. cepa*. Different superscripts denote significant differences among the antibacterial activities achieved by all concentrations of aqueous local, aqueous imported, ethanolic local and ethanolic imported *A. cepa* extracts, separately at $p < 0.05$

Bacteria	<i>A. cepa</i> extract	Inhibition zone Diameter IZD, mm									
		Concentration of <i>A. cepa</i> extract, $\mu\text{g mL}^{-1}$									
		12.5		25		50		100		200	
		Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract	Aqueous extract	Ethanolic extract
<i>E. coli</i>	Local onion	8.5 ^a ±0.5	0 ^a	9.0 ^a ±0.0	0 ^a	9.0 ^a ±0.0	12.5 ^{bc} ±0.5	13.0 ^b ±0.0	12.0 ^c ±0.0	11.0 ^{ab} ±1.0	9.0 ^d ±1.0
	Imported onion	0 ^a	0 ^a	0 ^a	0 ^a	8.0 ^{bc} ±0.0	0 ^a	9.0 ^c ±0.0	12.5 ^b ±1.5	12.5 ^c ±0.5	12.0 ^{ab} ±1.0
<i>P. aeruginosa</i>	Local onion	15.0 ^a ±0.0	12.0 ^a ±1.0	12.5 ^a ±2.5	13.5 ^a ±1.5	12.0 ^a ±1.0	11.5 ^{bcd} ±1.5	4.0 ^a ±4.0	0 ^{cd}	8.0 ^a ±0.0	0 ^d
	Imported onion	0 ^a	0 ^a	0 ^a	16.5 ^b ±1.5	0 ^a	10.5 ^{ab} ±0.5	0 ^a	3.5 ^{ab} ±3.5	13.0 ^b ±5.0	4.0 ^{ab} ±4.0
<i>K. pneumonia</i>	Local onion	9.5 ^a ±0.5	0 ^a	9.5 ^a ±0.5	0 ^a	15.5 ^{ab} ±3.5	10.0 ^{bc} ±0.0	20.5 ^b ±0.5	12.0 ^{cd} ±1.0	8.5 ^a ±0.5	14.0 ^d ±1.0
	Imported onion	0 ^a	20.0 ^a ±0.0	9.0 ^{bcd} ±1.0	17.0 ^a ±1.0	10.5 ^{cde} ±0.5	18.5 ^{bc} ±1.5	12.0 ^{de} ±1.0	9.0 ^{ad} ±0.0	13.5 ^c ±1.5	14.0 ^b ±1.0
<i>B. cereus</i>	Local onion	13.5 ^a ±2.5	0 ^a	0 ^{bcd}	0 ^a	0 ^{cde}	10.5 ^b ±0.5	0 ^{de}	22.5 ^c ±0.5	0 ^e	19.0 ^d ±1.0
	Imported onion	0 ^a	0 ^a	0 ^a	0 ^a	9.5 ^b ±0.5	0 ^a	0 ^a	18.0 ^b ±2.0	0 ^a	8.0 ^c ±0.0
<i>S. aureus</i>	Local onion	0 ^a	0 ^a	0 ^a	0 ^a	0 ^a	4.0 ^{abc} ±4.0	0 ^a	11.5 ^{bc} ±0.5	0 ^a	12.5 ^c ±0.5
	Imported onion	10.0 ^a ±1.0	0 ^a	4.5 ^b ±4.5	8.0 ^b ±0.0	14.0 ^c ±2.0	8.0 ^b ±0.0	9.5 ^a ±0.5	0 ^a	13.0 ^a ±1.0	0 ^a

*Average (n=2) ± standard deviation (SD). The mean difference is significant at the 0.05 level. DMSO solvent showed no growth inhibition for the tested organisms.

3.2.The susceptibility of test bacteria toward the negative and positive controls

All growing test bacteria were not inhibited when the sterile aqueous DMSO (1%) was applied, but clear inhibition zones were observed in the growth of all test bacteria induced by the effect of Ofloxacin antibiotic (5 µg) when added. The antibacterial activity of Ofloxacin against bacteria *E. coli*, *P. aeruginosa*, *K. pneumonia*, *B. cereus* and *S. aureus* was varied depending on the type of bacteria: 32.0, 41.0, 25.0, 34.0 and 21.0 mm, respectively. All bacterial strains were susceptible to Ofloxacin comparing to the standard IZDs.

3.3.Determination of the Minimum Inhibitory Concentration (MIC) of aqueous and ethanolic extracts of local and imported *A. cepa*

Table 2 indicated that the aqueous extract of local *A. cepa* was more inhibiting for *E. coli* and *B. cereus* by a value of MIC equals 12.5 µg mL⁻¹, and its ethanolic extract was more inhibiting *K. pneumonia*, *B. cereus* and *S. aureus* by a value of MIC equals 50 µg mL⁻¹. Thus, we conclude that the aqueous extract of the local *A. cepa* is more inhibitory than its ethanolic extract. The aqueous and ethanolic extracts of imported *A. cepa* were inhibiting for *K. pneumonia* and *S. aureus* by a value of MIC equals 25 µg mL⁻¹, while the aqueous extract inhibited the growth of *E. coli*, *P. aeruginosa* and *B. cereus* at low MIC values. The aqueous extract of the imported *A. cepa* is more inhibitory than its ethanolic extract.

Table 2. Average* Minimum Inhibitory Concentration (MIC) of aqueous and ethanolic extracts of local and imported *A. cepa* against some bacteria

Bacteria	Values of Minimum Inhibitory Concentration (MIC), µg mL ⁻¹			
	Local <i>A. cepa</i>		Imported <i>A. cepa</i>	
	Aqueous Extract	Ethanolic Extract	Aqueous Extract	Ethanolic Extract
<i>E. coli</i>	12.5±0.5	200±14	50±4	200±10
<i>P. aeruginosa</i>	100±7	200±16	50±7	100±3
<i>K. pneumonia</i>	200±19	50±2	25±4	25±3
<i>B. cereus</i>	12.5±0.6	50±5	50±1	200±10
<i>S. aureus</i>	-	50±4	25±6	25±2

*Average (n=2) ± standard deviation (SD) at the 0.05 level. The sign (-) means absence of inhibition at all studied concentrations.

3.4.The statistical differences among the inhibition activities of the aqueous extracts and among the inhibition activities of the ethanolic extracts of local and imported *A. cepa*

Oneway-ANOVA was conducted to analyze the differences

between the antibacterial activity of the aqueous extracts of local and imported *A. cepa* in addition to the differences between the antibacterial activity of the ethanolic extracts of local and imported *A. cepa* against the tested bacteria (Table 3).

Table 3. Explanation of the statistical differences of the antibacterial activities achieved by the aqueous and ethanolic extracts of local and imported *A. cepa* against the tested bacteria using Oneway-ANOVA

Bacteria	Aqueous Extract of						Ethanolic Extract of					
	Local <i>A. cepa</i>			Imported <i>A. cepa</i>			Local <i>A. cepa</i>			Imported <i>A. cepa</i>		
	df	F	Sig.	Df	F	Sig.	df	F	Sig.	df	F	Sig.
<i>E. coli</i>	4	14.200	0.006	4	636.00	0.000	4	0.784	0.000	4	69.308	0.000
<i>P. aeruginosa</i>	4	4.022	0.080	4	6.760	0.030	4	0.924	0.000	4	7.020	0.028
<i>K. pneumonia</i>	4	10.075	0.013	4	31.250	0.001	4	0.904	0.000	4	22.294	0.002
<i>B. cereus</i>	4	29.160	0.001	4	361.00	0.000	4	2.184	0.000	4	79.000	0.000
<i>S. aureus</i>	-	-	-	4	2.608	0.161	4	2.184	0.010	-	-	-

Notice: The tabulated F value at the degree of freedom (4) and the significance level ($\alpha \leq 0.05$) equals 6.287

Table (3) indicated that the antibacterial activity of the aqueous extracts of local and imported *A. cepa* against all studied bacteria was statistically significant at the level of ($\alpha \leq 0.05$) except for *P. aeruginosa* (Sig. 0.080) with the local *A. cepa* extract, and *S. aureus* which was not inhibited by it, and *S. aureus* with imported *A. cepa* extract (Sig. 0.161), which refers to absence of statistically significant differences. In comparison, we found that all calculated F values of the rest of the bacteria were greater than the tabulated F values, and

accordingly there were statistically significant differences in the antibacterial activity of the aqueous extracts of local and imported *A. cepa*. Table (3) indicated also that the antibacterial activity of the ethanolic extracts of local and imported *A. cepa* against all studied bacteria was statistically significant at the level of ($\alpha \leq 0.05$) except for *Staphylococcus* which was not inhibited with the extract of imported *A. cepa*. In a comparison, we found that all calculated F values were smaller than the tabulated F values for the ethanolic extracts

of local *A. cepa*, and accordingly there were no differences in the inhibition effect. On the other hand, we found that all the calculated F values were greater than the tabulated F values of the ethanolic extracts of imported *A. cepa* referring to the presence of differences in their antibacterial activity (Table 3).

Discussion:

The susceptibility of the studied bacteria toward the *A. cepa* extracts differed regardless whether the bacteria were Gram positive or Gram negative, contrary to the results of Santas et al. [20] who reported that the Gram positive bacteria were more sensitive to *A. cepa* extracts than Gram negative bacteria.

The antibacterial potential of the *A. cepa* extract varied depending on the type of bacteria and the origin of plant, where the aqueous extract showing the best antibacterial activity was originated from the local *A. cepa* against *E. coli*, *P. aeruginosa* and *K. pneumonia*. The ethanolic extract showing the best antibacterial activity was originated from the local *A. cepa* against *B. cereus* and *S. aureus*. The aqueous extract showing the best antibacterial activity was originated from the imported *A. cepa* against *E. coli* and *S. aureus*, the ethanolic extract showing the best antibacterial activity was originated from the imported *A. cepa* against *P. aeruginosa*, *K. pneumonia* and *B. cereus*.

For the local *A. cepa*, the aqueous extract had the highest antibacterial activity, but in case of the imported *A. cepa*, the ethanolic extract was the best. The aqueous extract of local *A. cepa* was potential for Gram negative bacteria more than Gram positive bacteria which were highly sensitive to the ethanolic extract of local *A. cepa*, this result is consistent with the founding of Shinkafi and Dauda [36] and not consistent with the founding of Santas et al. [20]. The aqueous extract of imported *A. cepa* was more inhibitory to *E. coli* and *S. aureus* more than the ethanolic extract which showed antibacterial potential against *P. aeruginosa*, *K. pneumonia* and *B. cereus*.

The aqueous and ethanolic extracts of local *A. cepa* showed the highest inhibition activity against *K. pneumonia* (20.5 ± 0.5 mm) and *B. cereus* (22.5 ± 0.5 mm), respectively, and the least inhibition activity was observed against *P. aeruginosa* and *S. aureus* (4.0 ± 0.40 mm), respectively. It was observed that, the antibacterial activities achieved by the aqueous and ethanolic extracts of imported *A. cepa* were different, whereas the highest antibacterial activity was shown against *S. aureus* (14.0 ± 2.0 mm) and *K. pneumonia* (20.0 ± 0.0 mm), respectively, and the least antibacterial activities were observed against *S. aureus* (4.5 ± 4.5 mm) and *P. aeruginosa* (3.5 ± 3.5 mm), respectively. By comparing the results of Etikala et al. [37] who studied the antibacterial activity of *Allium cepa* extracts on *B. cereus*, we found that the resulting inhibition zones had the largest diameters.

The aqueous extract of the local *A. cepa* inhibited the growth of *E. coli* significantly with a concentration $100 \mu\text{g mL}^{-1}$, while its ethanolic extract affected the growth significantly with the concentrations 100 and $200 \mu\text{g mL}^{-1}$. In contrast to the aqueous extracts of the imported *A. cepa*, they inhibited the growth of *E. coli* significantly with the concentrations 50 , 100 and $200 \mu\text{g mL}^{-1}$, while the ethanolic extracts of imported *A. cepa* were inhibiting at 100 and $200 \mu\text{g mL}^{-1}$, this means that the antibacterial activity of the aqueous extracts of imported *A. cepa* exceeded the antibacterial activity of the four extracts of

the local and imported *A. cepa*. Oyawoye et al. [38] reported that the ethanolic extract of *A. cepa* L. showed the best antibacterial activity against *E. coli*, 12.5 mm higher than the aqueous extract. There was a direct proportion between the concentration of the aqueous extract of local *A. cepa* with the achieved antibacterial activity against *E. coli*, in agreement with the findings of Tahiruddin and Indriastuti [39].

There was no significant difference in the antibacterial activity achieved by the aqueous extracts of local *A. cepa* against *P. aeruginosa* with all concentrations ($p > 0.05$), while the antibacterial activities achieved by the ethanolic extracts of local *A. cepa* were different significantly at concentrations of 50 , 100 and $200 \mu\text{g mL}^{-1}$. In contrast, the growth of *P. aeruginosa* was inhibited by the aqueous extract of the imported *A. cepa* with a concentration $200 \mu\text{g mL}^{-1}$. The ethanolic extract of the imported *A. cepa* inhibited the growth with a concentration $12.5 \mu\text{g mL}^{-1}$. Therefore, the antibacterial activity achieved by the ethanolic extract of the local *A. cepa* exceeded the antibacterial activity achieved by the rest extracts, such results agree with the findings of Al-Mussawi and Al-Jaber [40] study.

The powerful antibacterial activity of *A. cepa* extracts inhibiting *K. pneumonia* is attributed to the aqueous extracts of local *A. cepa* (20.5 ± 0.5 mm) and to the ethanolic extracts of imported *A. cepa* (20.0 ± 0.0 mm). Significant differences ($p < 0.05$) were observed in the antibacterial effects, whereas the *K. pneumonia* showed sensitivity to the ethanolic extract of imported *A. cepa* greater than the aqueous extract of local *A. cepa* depending on Tukey Test. Enejiyon et al. [41] showed maximum activity against *K. pneumonia* (21.2 ± 0.5 mm) closest to our result.

The antibacterial activities achieved by the aqueous extracts of local *A. cepa* against *B. cereus* showed significant differences at concentrations of 12.5 , 25 , 50 and $100 \mu\text{g mL}^{-1}$. The ethanolic extract of the local *A. cepa* inhibited the growth of *B. cereus* with all concentrations, unlike the aqueous extract of imported *A. cepa* which affected the growth at concentrations of 100 and $50 \mu\text{g mL}^{-1}$. It was noticed that, most concentrations of all extracts showed antibacterial activity, especially the aqueous extract of the local *A. cepa*. This investigation agree with the study of Sagar and Pareek [42] who indicated that *B. cereus* was inhibited by the extract of pink skin of *A. cepa*, while Bakht et al. [43] recorded that *Bacillus subtilis* was the most susceptible bacteria inhibited by all extracts of *A. cepa*.

S. aureus was not inhibited by all concentrations of the aqueous extracts of local *A. cepa*, and similarly it was not inhibited by the ethanolic extracts of the imported *A. cepa*. *S. aureus* was inhibited by the ethanolic extract of the local *A. cepa* with the higher concentrations 50 , 100 and $200 \mu\text{g mL}^{-1}$ with significant differences, in agreement with the results of Benkeblia [23], unlike the aqueous extract of imported *A. cepa* which inhibited the growth of *S. aureus* without significant differences at all concentrations. In general, the antibacterial activities of the aqueous and ethanolic extracts of local and imported *A. cepa* were weak against *S. aureus*. Bag and Chattopadhyay [44] discovered different findings, *S. aureus* had an inhibitory impact larger than that of *E. coli*. The higher concentrations of the studied extracts of *A. cepa* inhibited the growth of *S. aureus* more than the lower

concentrations, in agreement with the results of Tahiruddin and Indriastuti [39]. Oyawoye et al. [38] found that the zone of inhibition of each ethanolic section ranged from 3 to 12.5 mm, while the aqueous extracts ranged from 4 to 10 mm.

The MIC values were different, indicating that the type of bacteria, the type of solvent, and the origin of the *A. cepa* plant have a great influence in the determination of the MIC values. The MIC reached the lowest value ($12.5 \mu\text{g mL}^{-1}$) when the aqueous extract of local *A. cepa* was applied against *E. coli* and *B. cereus*, while the ethanolic extract of the local *A. cepa* reached the lowest MIC value ($25 \mu\text{g mL}^{-1}$) when applied to *K. pneumonia*, *B. cereus* and *S. aureus*. Enejiyon et al. [41] reported that all extracts of *A. cepa* showed MIC ranged between 10 and 20 mg/mL against the test bacteria as a result close to this study. Bag and Chattopadhyay [45] reported a distant result, they showed that the MIC of *A. cepa* extract against *E. coli* equals $93.8 \pm 44.2 \text{ g mL}^{-1}$.

Conclusion

The susceptibility of the five human pathogenic Gram positive and Gram negative bacteria toward the Aqueous and Ethanolic extracts of Yemeni and Indian *Allium cepa* was different regardless whether the bacteria were Gram positive or Gram negative depending on the bacteria type, solvent type and plant origin. For the Yemeni *A. cepa*, the aqueous extract exhibited the most effective antibacterial activity against *K. pneumonia* ($20.5 \pm 0.5 \text{ mm}$), *E. coli* and *P. aeruginosa*, while the ethanolic extract exhibited the most effective antibacterial activity against *B. cereus* ($22.5 \pm 0.5 \text{ mm}$) and *S. aureus*. For the Indian *A. cepa*, the aqueous extract exhibited the most effective antibacterial activity against *S. aureus* ($14.0 \pm 2.0 \text{ mm}$) and *E. coli*, the ethanolic extract exhibited the most effective antibacterial activity against *K. pneumonia* ($20.0 \pm 0.0 \text{ mm}$), *P. aeruginosa* and *B. cereus*. The aqueous extracts of Yemeni *A. cepa* achieved the more efficient antibacterial activity than that of the aqueous extracts of Indian *A. cepa*, while the most effective ethanolic extract was that of the Indian *A. cepa*. The aqueous Yemeni extracts inhibited the Gram negative bacteria more than Gram positive bacteria which were highly sensitive to the ethanolic Yemeni extracts. The MIC values were different, the lowest value ($12.5 \mu\text{g mL}^{-1}$) was recorded by the Yemeni aqueous extracts against *E. coli* and *B. cereus*, while the lowest value ($25 \mu\text{g mL}^{-1}$) was recorded by the Yemeni ethanolic extracts against *K. pneumonia*, *B. cereus* and *S. aureus*.

The researchers recommend to continue the study for other plants, and they recommend to conduct extensive and comprehensive studies against parasites and other organisms harmful to the human body.

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Conflicts of Interest

The authors declare that they have no conflicts of interest to report regarding the present study.

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دراسة نشاط المستخلصات المائية والإيثانولية للبصل اليمني والهندي المضاد لبعض البكتيريا المسببة لأمراض الإنسان

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المخلص: يستخدم اليمنيون منذ آلاف السنين عدداً كبيراً من النباتات الطبية، من أهمها نبات البصل الأحمر (*Allium cepa*) اعتمدت هذه الدراسة على تقدير النشاط التثبيطي لتراكيز مختلفة (12,5، 25، 50، 100، 200 ميكروجرام لكل مليلتر من المستخلصات المائية والإيثانولية للبصل اليمني ومقارنته بنفس المستخلصات للبصل الهندي ضد خمسة أنواع من البكتيريا السالبة الجرام والموجبة الجرام، الإشريكية القولونية، الكلبسيلا الرئوية، الزائفة الزنجارية، العصوية الشمعية و المكورة الذهبية العنقودية باستخدام طريقة الانتشار في حفر الأجار. استخدمت طريقة التخفيف على الأجار لتحديد قيمة التركيز التثبيطي الأدنى (MIC) واستنتجت الدراسة أن مستخلصات البصل اليمني سجلت نشاطاً تثبيطياً أكبر من مستخلصات البصل الهندي، وأن المستخلصات الإيثانولية أقوى من المستخلصات المائية لكلا النوعين من البصل. وقد أظهرت المستخلصات المائية والإيثانولية للبصل اليمني نشاطاً تثبيطياً أكبر ضد بكتيريا الكلبسيلا الرئوية ($0,5 \pm 20,5$ مم) و بكتيريا العصوية الشمعية ($0,5 \pm 22,5$ مم)، على التوالي، بينما المستخلصات المائية والإيثانولية للبصل الهندي أظهرت نشاطاً تثبيطياً أكبر ضد المكورة الذهبية العنقودية ($2,0 \pm 14,0$ مم) والكلبسيلا الرئوية ($0,0 \pm 20,0$ مم)، على التوالي. اتضح وجود فروقات معنوية بين النشاطات التثبيطية لجميع المستخلصات ($P > 0,05$) ماعدا عند تطبيق المستخلصات المائية للبصل اليمني ضد الزائفة الزنجارية. أشارت الدراسة أن جميع تراكيز المستخلصات المائية للبصل اليمني وتراكيز المستخلصات الإيثانولية للبصل الهندي لم تثبط نمو المكورة الذهبية العنقودية الذي كان تثبيطه ضعيفاً جداً باستخدام بقية المستخلصات. وأشارت الدراسة الحالية إلى اختلاف قيم التراكيز التثبيطية الدنيا (MIC) للمستخلصات المختلفة حيث حقق المستخلص المائي للبصل اليمني أصغر قيمة (12,5 ميكروجرام لكل مليلتر) ضد الإشريكية القولونية والعصوية الشمعية، وحقق المستخلص الإيثانولي للبصل اليمني أصغر قيمة (12,5 ميكروجرام لكل مليلتر) ضد الكلبسيلا الرئوية، العصوية الشمعية والمكورة الذهبية العنقودية. وأخيراً، يوصي الباحثون باستمرار البحث وإجراء دراسات مكثفة وشاملة حول قوة وطبيعة النشاط التثبيطي للبصل اليمني ضد الفطريات وأنواع مختلفة من الكائنات الدقيقة الضارة الأخرى.

الكلمات المفتاحية: البصل الأحمر، طبي، مضاد للبكتيريا، المستخلص الإيثانولي، الإشريكية القولونية.