Effects of Akaka *Allium akaka* Gmel. extracts on the control of bacterial growth

Abdul-Ghany Omer Ismaeel Sarmamy*

الخلاصة

لمعرفة تاثير مستخلص الاجزاء الخضرية لنبات اللوشة (أكاكا) في السيطرة على نمو بعض انواع البكتريا الممرضة للانسان *Erwinia caratovora* و *Xanthomonas campestris* والنباتات(*Escherichia coli*) و (*Staphylococcus aureus*) استخلصت الاجزاء الخضرية لنباتات اللوشة (أكاكا) بالماء المقطر والايثانول(90%) وتم تحضير المستخلص بتراكيز 500و1000و5000و 10000 مكغم/قرص و مكغم/مل بالاضافة الى الماء المقطر المعقم كمعاملة ضابطة. طبقت التجارب بالتصميم العشوائي الكامل باربعة مكررات وتم تحليل النتائج بواسطة برنامج SPSS وقورنت متوسطات المعاملات بواسطة اختبار دنكن متعدد الحدود على مستوى 0.05 من المعنوية ودلت النتائج على ان مستخلص الايثانؤل كان فعالاً بتركيز5000 مكغم/قرص و10000 مكغم/مل في السيطرة في نمو *Staphylococcus aureus, Xanthomonas campestris* و *Erwinia caratovora* والمستخلص المائي اثرت بتركيز 10000 مكغم/مل فقط في *Staphylococcus aureus*. لم تتاثر *Escherichia coli* باي من التراكيزالمستخدمة في الدراسة. طريقة Agar disc diffusion technique كانت ادق واوضح في اظهار تاثيرات المستخلص النباتي في نمو البكتريا من طريقة disc saturation technique .

Abstract

Several experiments were conducted, to determine the effects of extracts of vegetative parts of Lusha (*Allium akaka*) on growth of some human and plant pathogenic bacteria such as *Staphylococcus aureus, Escherichia coli, Xanthomonas campestris* and *Erwinia caratovora*. Plant vegetative parts were extracted (crude extraction) using distilled water and ethanol (90%). Concentrations of 500, 1000, 5000 and 10000μg disc^{-1} and μg ml^{-1} were tested. Sterilized distilled water used as control. CRD was applied with four replications, the results analyzed statistically using SPSS and the means were compared using Duncan Multiple Range Test at 0.01 level.

*College of Science-Biology Dept. /University of Salahaddin-Erbil/Iraq

Results showed that ethanol extracts were effective at concentration of 5000μg disc^{-1} and 10000μg ml^{-1} against *Staphylococcus aureus, Xanthomonas campestris* and *Erwinia caratovora* while aqueous extract affected on *Staphylococcus aureus* at 10000 μg ml^{-1} only. *Escherichia coli* were not affected by the plant extract at all concentrations used in this study. Agar disc diffusion technique was more effective than disc saturation method for determination of plant extract effects on the growth of bacteria.

Introduction

Akaka *Allium akaka* is a medical plant, belongs to Liliaceae plant family, naturally grown in the central sector of alpine region of Iraq on the mountains of Helgurd, Kodo and Qendil (1). It reproduced by bulbs mainly and by seeds, distributed throughout the Kwestan lawns of the northern parts of Erbil governorate in Kurdistan Region-Iraq. Vegetative parts of the plant is used in food making and used also in folk medicine for treating cases of high blood pressure and for regulating blood cholesterol. The plant contains sulphur compounds with an onion flavor when added to the diet on a regular basis it helps in reducing blood cholesterol levels; it also acts as a tonic to the digestive system and also tonify the circulatory system. Plant bulbs - raw or cooked were used as an onion substitute. The whole of the young plant is said to be a great delicacy and it's used as an addition to rice in a pilau(traditional Kurdish cooked rice). Leaves - raw or cooked and flowers - raw used as a garnish on salads. The growing plant is said to repel insects and moles (2). Published literatures in this field had demonstrated the effects of such a plant extracts on fungal and bacterial growth (3, 4, 5).

This study was aimed to determine the main group of chemical compounds and the effects of plant extract on control of some pathogenic bacteria.

Materials and Methods

Preparation of plant samples: Plant samples were collected from kwestan sites, of the mountains of north of Erbil governorate, in May 15, 2010 and dried in the laboratory. Plant parts were milled by electrical grinder, passed through 2mm mesh, and extracted by macerating 100gm

of the powder in 200ml of distilled water or ethanol 90% in volumetric flask, renounced for 24 hours on an electric shaker. Plant extracts were filtered by passing it through four folded layers of gauze and filtered by Buchnner apparatus. Water and ethanol extracts were concentrated by Rotary Vacuum Evaporator at 40 ° C. (6, 7) and sterilized by passing it through bacterial filter (Seitz). Five grams of conc. raw extracts were poured into a flask, 5 ml of dimethyl sulphoxide (DMSO) and 45 ml of sterilized distilled water were added, to prepare a solution of 100000μg ml^{-1} as a stock solution. Different plant extract concentrations were then prepared from the stock solution.

Preparation paper discs: Paper discs were made from thick filter paper using paper piercing instrument with a diameter of 7mm and sterilized by keeping them in an incubator at 70 ^{0}C for 24 hours. Paper disks placed in beakers containing extract solutions of different concentrations for 10 hrs and then placed in an incubator for 6 hrs at 40°c until drying. While in another method the requested amounts of different concentrations for each treatment were placed on the paper discs using micropipettes of different volumes (8). Nutrient broth and Nutrient agar were prepared according to (9).

Pathogenic bacteria: *Staphylococcus aureus and Escherichia coli* were obtained from the laboratories of Rizgary Hospital-Erbil while the plant pathogenic bacteria *Xanthomonas campestris and Erwinia caratovora* were obtained from the Labs.of department of plant protection/ College of Agriculture University of Salahaddin-Erbil.

Detection of groups of chemical compounds from plant extract (10, 11)**:**

Preparation of reagents and indicators:

Dragendroffs reagent:

Solution (**A**): 2 ml of concentrated hydrochloric acid (HCl) was added to a container containing 10 ml of distilled water and 0.6 g of bismuth sub nitrate.

Solution (**B**): 6 g of Potassium iodide was added to 10 ml D.W.

To solutions A and B, 7 ml of conc. HCl was added, mixed well together, and the volume was completed to 400ml using D.W.

Mayer reagent (Indicator):

Solution (A): 0.5 gm of mercuric chloride was dissolved in 60 ml of D.W.

Solution (B): 5 gm of potassium iodide was dissolved in 10 ml of D.W.

Solutions A and b were mixed together and the volume completed to1000ml using D.W.

Fehling reagent:

Solution (A): 35 g of copper sulphate was dissolved in 100 ml of D.W. and the volume was completed to 500 ml using D.W.

Solution (B):7 g of sodium hydroxide (NaOH) was mixed with 175 g of Rochelle's salt (Sodium potassium tartarate), dissolved in 100 ml of D.W. and the volume was completed to 500 ml D.W.

Equal volumes of solutions A and B were mixed together for detection of chemical constituents.

Detection of Alkaloids:

1 gm of plant powder was dissolved in 10 ml of distilled water. 0.2 ml of plant extract added to 5 ml of HCl (1%), and placed in water bath. Then 1 ml of the supernatant was treated with 1-3 drops of the following reagents:

a- Dragendorff's: appearance of orange precipitant is indicator for existence of alkaloids.

b- Mayer reagent: appearance of white precipitant is indicator for existence of alkaloids.

Tannins: 1 ml of plant powder was mixed with 10 ml of D.W., heated until boiling, the solution filtrated, cooled and separated to two parts:

a- To the first part 1% lead acetate was added, appearance of jelly precipitate was indicator for the existence of Tannins.

b- For the second part 1% ferric chloride was added, the appearance of blue green color indicating the presence of Tannins.

Glycosides: Equal parts of Fehling reagent and filtrate water extracts of the plant powder were mixed, allowed in a boiling water bath for 10 min. the appearance of red precipitant indicates the presence of glycosides.

Flavonoid:

Solution (A): 1 g of plant powder was added to 10 ml of ethanol (95%) leaved in a boiling water bath for 2 min.

Solution (B): 5 ml of ethanol (50%) was added to 5 ml of potassium hydroxide (KOH 50%). After the mixing of the two solutions, the appearance of yellow color indicator for presence of Flavonoid.
Coumarin: 0.5 mg of plant powder was dissolved in a test tube with 1 ml of alcohol. The test tube was covered with a filter paper wetted with diluted solution of sodium hydroxide in a boiling water bath for 5 min. then the filter paper exposed to ultraviolet light. Green yellow color appearance indicating surely the presence of Coumarin.

Results and Discussion

Effects of alcoholic raw extracts of *Allium akaka* on control of some pathogenic bacteria using disc diffusion technique method.

Table 1 shows that plant alcohol extract at concentrations of 500 and 1000 μg disc^{-1} has no effecte on control of *Xanthomonas campestris* and Erwinia caratovora but at concentrations 5000 and 10000μg disc^{-1} caused significant reduction in growth of the two bacteria. No effects on the growth of *E. coli* were observed at all concentrations however it significantly affected the growth of *Staphylococcus aureus* at all concentrations. *E. coli* was shown to be resistant the effects of plant extracts, whereas *Staphylococcus aureus* has been shown to be sensitive to plant extracts and Streptomycin.Figure 1 shows that the plant extract was not effective (as average) against studied bacteria at con-centrations of 500 and 1000μg ml^{-1}. The effects began at 5000μg ml^{-1} and increased significantly at 10000μg ml^{-1}.

Effects of raw extracts of *Allium akaka* on control of some pathogenic bacteria using disc saturation method.

Alcohol extracts

Table 2 shows the effects of alcohol raw extracts at concentrations of 500, 1000, 5000 and 10000μg ml^{-1} on the growth of bacteria. Alcohol extracts showed no effect on growth of *E. coli* but at concentration of 10000µg ml^{-1}had a significant effect on growth of Xanthomonas *campestris* and *Erwinia caratovora*, the Inhibitional Growth Circle Diameter (IGCD) were 2.95 and 2.83 mm respectively compared with 1.0 mm for control. The alcohol extract affected on the growth of *Staph. aureus* at concentrations of 1000, 5000 and 10000 μg ml^{-1} significantly

and gave the IGCD of 2.83, 2.93 and 3.0 mm respectively in compare to 1.0 and 4.7 mm for control and streptomycin respectively. Streptomycin has been shown to be effective against the tested bacteria.

Figure 2 Shows that the average effects of alcoholic plant extracts on the growth of studied bacteria with a significant effect at concentration of 10000μg ml^{-1} only.

Effects of aqueous extracts

Table 3 shows the effects of aqueous raw extracts at concentrations of 500, 1000, 5000 and 10000μg ml^{-1} on the growth of bacteria. Aqueous extracts has not shown effects on the growth of *E. coli*, *Xanthomonas campestris* and *Erwinia caratovora,* whereas it affected on *Staph. aureus* growth at concentrations of 5000 and 10000μg ml^{-1} by reducing its growth significantly with an IGCD of 2.25 and 2.83 mm respectively compared with 1.0 mm and 4.7 mm for control and streptomycin respectively.

Figure 3 shows that aqueous extract at concentrations of 500, 1000, 5000 and 10000μg ml^{-1} has not shown any significant effect against studied bacteria (in average).

Groups of chemical compounds detected in plant extract

Table 3 shows that plant extracts contains alkaloids, Glycosides, Phenolic compounds, flavonoids, Tannins and Saponins. All these chemicals are physiologically active chemicals which can be of valuable advantages for use in different purposes. Alcohol and aqueous extracts did not affect the growth of *E. coli* at different concentrations (Tables 1, 2 and 3) this can be due to the complexicity of cell wall of *E. coli* or maybe the lack of receptor for these chemicals on the bacterial cell. The bioactivity of a chemical components appear when its site of action is present in the entire cell or the chemical constituents of akaka has no effect on *E. coli*. Akaka extracts excreted its effect on the growth of *Staphylococcus aureus* and reduced its growth significantly, because akaka extracts containing bioactive components such as alkaloids, glycosides and saponins (Table 4) and all these chemical groups are biologically active against microorganisms such as bacteria and acts as antibacterial agents (12). Akaka extracts effects on *Xanthomonas campestris* and *Erwinia caratovora*. These two bacteria showed

resistance more than *Staphylococcus aureus* did, this may be due to their cell wall composition or their genotypes constituents. Plant alcoholic extracts were more effective against bacterial growth than aqueous extracts (Tables 2 and 3) which could be due to solubility of chemical constituents. Alkaloids are more soluble in organic solvents such as alcohol than in aqueous solvents (water in present study) therefore the alcohol extracts were more active against microbial growth than aqueous extracts (13, 8, 3). Agar disc diffusion techniques were shown to be more effective in the determination of plant extract effects than disc saturation methods because in agar disc diffusion technique exact amounts of plant extracts laid on each disc by using a micropipette while in disc saturation techniques, the discs were immersed in beakers which contain the solutions.

Table 1: Effects of alcohol raw extracts on control of some pathogenic bacteria.

treatments	*Staph. aureus*	*E. coli*	Xanth. campestris	*Erwinia caratovora*
	$\bar{X}$ ± Std.e	$\bar{X}$ ± Std. e	$\bar{X}$ ± Std. e	$\bar{X}$ ± Std. e
Control	1.0 ±.00a	1.00± .00a	1.00 ±.00a	1.00 ±.00a
500μg disc^{-1}	6.0 ±.16b	1.00±.00a	1.00 ±.00a	1.00 ±.00a
1000μg disc^{-1}	6.65±.10b	1.00±.00a	1.00 ±.00a	1.00 ±.00a
5000μg disc^{-1}	6.80 ±.29b	1.00±.00a	11.30±.56b	9.30 ±.29b
10000μg disc^{-1}	10.70 ±.33c	1.00±.00a	14.00 ±.20c	14.70 ±.24c
Streptomycin	18.00 ±.74d	12.30±.58b	20.30±0.29d	14.30 ±.29c
$\bar{X}$	8.19 ±1.09c	2.88± .88a	8.10±1.58c	6.9±1.50b

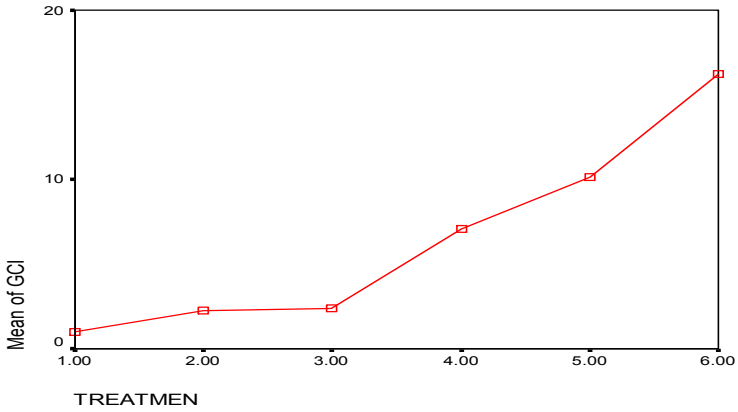

Figure 1: Effects of treatments on growth circle diameter of studied bacteria.

Table 2: Effects of alcoholic raw extracts on control of some pathogenic bacteria.

treatments	*Staph. aureus*	*E. coli*	*Xan. campestris*	*Erwinia caratovora*
	$\bar{X}$ ± Std. e	$\bar{X}$ ± Std. e	$\bar{X}$ ± Std. e	$\bar{X}$ ± Std. e
Control	1.00 ± .00a	1.00 ± .00a	1.00 ±.00a	1.00 ± .00a
500μg ml^{-1}	1.00 ± .00a	1.00 ± .00a	1.00 ±.00a	1.00 ± .00a
1000μg ml^{-1}	2.83±.12b	1.00 ± .00a	1.00 ±.00a	1.00 ± .00a
5000μg ml^{-1}	2.93± .22b	1.00 ± .00a	1.00 ±.00a	1.00 ± .00a
10000μg ml^{-1}	3.00± .07b	1.00 ± .00a	2.95± .21b	2.83 ± .14b
streptomycin	4.70 ±.12c	4.80 ±.15 b	3.80± .11c	4.58 ± .19c
$\bar{X}$	2.58 ± .39a	1.63 ± .38a	1.79 ±.32a	1.89 ± .38a

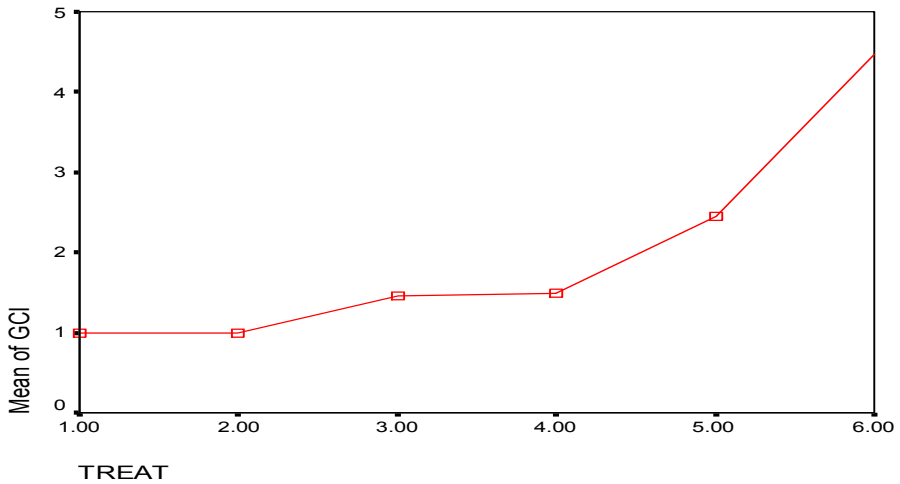

Figure 2: Mean effects of treatments on the growth of studied bacteria.

Table 3: Effects of aqueous raw extracts on control of some pathogenic bacteria.

treatments	*Staph. aureus*	*E. coli*	*Xan. campestris*	*Erwinia caratovora*
	$\bar{X}$ ± Std. e	$\bar{X}$ ± Std. e	$\bar{X}$ ± Std. e	$\bar{X}$ ± Std. e
Control	1.00 ± .00a	1.00 ± .00a	1.00 ±.00a	1.00 ± .00a
500μg ml^{-1}	1.00 ± .00a	1.00 ± .00a	1.00 ±.00a	1.00 ± .00a
1000μg ml^{-1}	1.00 ± .00a	1.00 ± .00a	1.00 ±.00a	1.00 ± .00a
5000μg ml^{-1}	2.25 ±.14b	1.00 ± .00a	1.00 ±.00a	1.00 ± .00a
10000μg ml^{-1}	2.83 ±.12c	1.00 ± .00a	1.00 ±.00a	1.00 ± .00a
streptomycin	4.70 ±.12d	4.80 ±.15b	3.80± .11b	4.58 ± .19b
$\bar{X}$	2.13 ± .39a	1.63 ±.38b	1.47 ± .32 b	1.60 ± .38 b

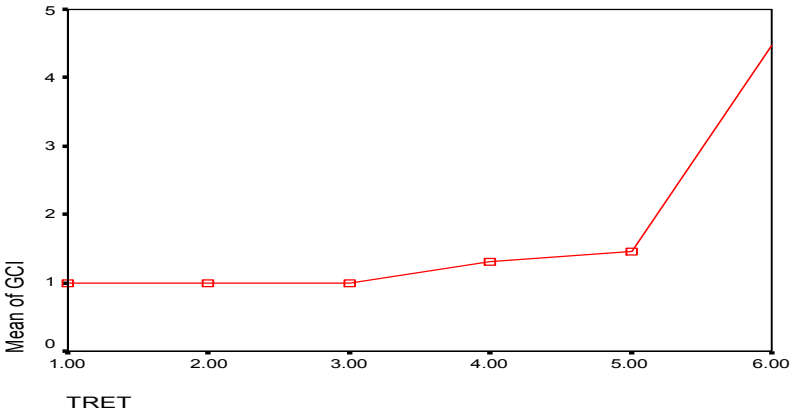

Figure 3: Effects of treatments on the growth of studied bacteria.

Table 4: Groups of chemical compounds detected in plant extract.

Chemical groups and compound	Reagent used	Color	*Allium Akaka* extracts
-Alkaloids -Glycosides -Carbohydrate	Dragenandroffs Benedict α-naphthol added to aqueous extract with H_2SO_4	Orange precipitate Red precipitate Purple ring	+* + +
-phenolic compounds -Flavonoids -tannins	ferric chloride 1% Ethanol (50%) KOH (50%) added to ethanol extract Lead acetate	Blue green colour Yellow colour White gelatinous precipitate	+ + +
saponin	Ferric chloride	White gelatinous precipitate	+

- + Means that plant extract containing the chemical group compound and
□ means no chemical group compound in the plant extract

Conclusions

Alcohol solvents showed to be suitable for preparing plant extracts used for controlling bacterial growth because the physiologically active chemicals present in the plant tissues are soluble in alcohol more than in water. Agar disc diffusion technique is more effective than disc saturation technique when used for bacterial control. Akaka extracts has been shown to be effective as a bacterial control agents as it contains active physiological constituent chemicals.

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